EXPLORING THE DRIVERS OF CO-OCCURRING MULTIPLE NON-NATIVE FISH ASSEMBLAGES WITHIN AN INVADED AND FLOW-MODIFIED AFRICAN RIVER SYSTEM

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (Science)

by

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ABSTRACT

Globally, there is growing concern on the negative impacts of species invasions and habitat disturbance because these have been shown to have the potential to disrupt native community structure and function. In some instances, these two stressors can occur in concert, such as in river systems associated with inter-basin water transfer (IBWT) schemes. The Great Fish River in the Eastern Cape, South Africa, is an example of a system affected by both habitat modification and multiple fish invasions largely because of an IBWT scheme. The opening of the Orange-Fish IBWT, which transfers water from the Orange River to the Great Fish River, modified the latter's natural flow regime from irregular seasonal to perennial. In addition, the IBWT facilitated translocations of five fish species from the Gariep Dam (Orange River system) into the Great Fish River system. Proliferation of these non-native fish species, along with that of other fish species introduced for angling and biological control, raise questions on the mechanisms facilitating their existence within this highly modified river system. This thesis explored mechanisms associated with co-occurrences of these multiple non-native fishes within the Great Fish River.

A comparison of historical and contemporary records on the ichthyofauna of the Great Fish River revealed that, of the 11 non-native fishes reported in this system, seven have established successfully, three have failed to establish and the status of one was uncertain. The Orange-Fish IBWT and angling were the main vectors of these invasions, accounting for 36% and 46%, respectively. The study also found that most established non-native fish species were large sized, had high longevity and wide habitat tolerance.

Trait-based approaches were employed to investigate the role of functional diversity of non-native and native fishes in relation to their composition, distribution and environmental relationships. Although considerable interspecific variation in body morphology-related functional traits among species were observed, there was no clear distinction in these traits between native and non-native fish assemblages on a trait-ordination space. Furthermore, there were weak species-trait-environment relationships, suggesting that environmental filtering was less plausible in explaining the occurrence patterns of these fishes.

Stable isotope-based trophic relationships were evaluated in three invaded sections: the upper (UGFR) mainstem sections of the Great Fish River; and lower (LGFR) mainstem sections of the Great Fish River; and its tributary, the Koonap River. It was observed that native and non-native fish assemblages exhibited variation in isotopic diversity typified by low isotopic diversity overlaps in UGFR and Koonap River, whereas the LGFR was characterised by high isotopic diversity overlap. Within the invaded sections, non-native fishes were found to have isotopic niches characterised by variable isotopic niche sizes and were more isotopically dissimilar with propensity towards trophic differentiation within the UGFR and Koonap River but were mostly characterised by high isotope niche overlaps in the LGFR. Overall, these results provided evidence of trophic niche differentiation as a probable mechanism associated with the co-occurrences of the non-native fishes. However, mechanisms facilitating these co-occurrences within the invaded sections appears to be complex, context-specific and, in some cases, unclear.

Lastly, machine learning techniques, boosted (BRT) and multivariate (MRT) regression trees, revealed that the flow-disturbed habitats were invaded by multiple non-native species, whereas the non-disturbed headwaters remained invasion free. In addition, non-native species were predicted to co-occur with native species within the mainstem and large tributary sections of the Great Fish River system. Thus, the IBWT-disturbed mainstem sections were predicted to be more prone to multiple invasions compared to undisturbed headwater tributaries.

KAKARETSO (SESOTHO)

Tlhaselo ka mefuta ya diphoofolo-tsa-matswantle (non-native species), ha mmoho le phetolo/tsenyehelo ya bodulo ba diphoofolo-tsa-lehae (native species), di nkuwa ele tse pedi tsa tse kgolo ka ho fetisisa hara ditshoso tse kgahlanong le paballo kapa tshireletso ya diphoofolo-tsa-lehae tse phelang dinokeng kapa metsing. Maemong a mang, dikgatello tsena tse pedi dika etsahala ka nako e le nngwe, jwalo ka dinokeng tseo di amanang le maano a ho fetisa/tsamaisa metsi pakeng tsa dinoka tse fapa-fapaneng (IBWT). Enngwe ya dinoka tse jwalo, ke noka e bitswang ka Great Fish River, e fumanehang Kapa-Botjabela (Eastern Cape) ka hara naha ya Afrika Borwa (South Africa). Noka ena ya Great Fish River e angwa ke tshenyehelo ya bodulo ba ditlhapi-tsa-lehae, ha mmoho le tlhaselo ya tsona ka ditlhapi-tsa-matswantle. Tsena di etsahala hahololo ka lebaka la morero kapa leano la phepelo ya metsi le bitswang Orange-Fish IBWT, leo lona le ileng la fetola phallo ya tlhaho ya metsi a Great Fish River. Ho feta moo, leano lena la phephelo yametsi, Orange-Fish IBWT, le entse hore ho be bonolo ho fetisetswa ha mefuta e mehlano ya ditlhapi-tsa-matswantle ho tloha letamong le bitswang Gariep Dam, hoya kena ka hara noka ya Great Fish River. Ditla morao tsa tsena tsohle, ebile ho ata ha mefuta e mengata ya ditlhapi-tsa-matswantle ka hara noka ya Great Fish River. Ho ata hona ha ditlapi-tsa-matswantle ka hara noka ena ya Great Fish River, ho hlahisa dipotso mabapi le mekgwa e bebofatsang ho phela ha ditlhapi tsena tsa matswantle ka hara noka ena; hore ana ebe diphela jwang ka hara noka ya Great Fish River? Ka hona, sepheo le merero wa thuto ena ke ho phuputsa mekgwa e bebofatsang ho phela ha mefuta ena e fapaneng ya ditlhapi-tsa-matswantle ka hara noka ya Great Fish River.

Dipheto tsa diphuputso di hlalosa hore, ha jwale, ka hara noka ena ya Great Fish River, hona le ditlhapi-tsa-matswantle tse leshome le motso o mong (11). Bosupa (7) ba tsona di phela ka

katleho, ha tse tharo di hlolehile ho theha (3), mme e le nngwe (1) boemo ba teng ha bo hlake. Hare lekola hore ke efeng mekgwa e amanang le ho ata ha ditlhapi-tsa-matswantle ka hara Great Fish River, re fumana hore leano la phephelo ya metsi la Orange-Fish IBWT ka 36%, ha mmoho le boithapollo ba ho tshwasa ditlhapi (angling) ka 46%, ene ele tsona tsela tsa ho kena ha ditlhapi-tsa-matswantle ka hara Great Fish River, tse ka sehlohong. Re fumantsha hape hore katleho ya ditlhapi-tsa-matswantle e amahangwa le hore di boholo bo bokae, le hore diphela nako e ka kang. Mohlala, ditlhapi tse kgolo tse phelang nako etelele ka tlhaho ya tsona, di amahangwa le katleho ya ho theha ka hara noka ena. Ha tseo tse phelang nako e kgutshwanyane tsona disa amahangwe leho atleha ka hara noka ena.

Tse ding tsa dipheto di hlalosa hore, ditlhapi-tsa-lehae le ditlhapi-tsa-matswantle, ka karolelano, hadi fapane haholo ka dibopeho tsa mmele, dihlopa tsena tse pedi diya tshwana.

Re fumantsha hape hore dihlopa tsena tse pedi tsa ditlapi dija mefuta e fapaneng ya dijo. Eleng engwe ya dintho tse netefatsang katleho ya ditlhapi-tsa-matswantle ka hara noka ena ya Great fish river. Hona keka lebaka la hore, dihlopa tsena tse pedi hadi bakisane dijo, empa di phela ka mefuta e fapaneng ya dijo.

Hare phethela, re fumantsha hore mefuta e fapafapaneng ya ditlhapi-tsa-matswantle e fumaneha feela ka hara madulo a amahangwang le phethoho ya phallo ya metsi (flow alteration), madulo asa amahangwang le phetoho ya phallo ya metsi ona ane a hloka ditlhapi-tsa-matswantle. Sena se bolela hore phetolo ya phallo ya metsi ya Great Fish River, ka lebaka la Orange-Fish IBWT, e fokoditse matla a noka ena ho lwantsha tlhaselo ya ditlhapi-tsa-matswantle. Ka hona, ho bobebe hore ditlhapi-tsa-matswantle di thehe ka katleho ka hara noka ena. Tsena tsohle keka baka la phetolo ya phallo ya metsi a Great Fish River e bakilweng ke leano la phephelo ya metsi la Orange-Fish IBWT.

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PREFACE

This thesis is divided into seven chapters; a general introduction (Chapter 1), description of study area and sampling protocols (Chapter 2), four research chapters (Chapters 3 - 6) and a general discussion or synthesis (Chapter 7). Because the research chapters are written as stand-alone segments, that have either been submitted or intended for peer review submission, there might be some degree of repetition or overlap between them.

Additional outputs completed during this thesis:

Peer reviewed papers

- **Mpopetsi PP**, Kadye WT. 2023. Colonisation theory and invasive biota: the Great Fish River case history, 35 years later. *African Journal of Aquatic Science.* https://doi.org/10.2989/16085914.2022.2142508

Conference presentations

- **Mpopetsi PP,** Kadye WT. Colonisation theory and invasive biota: The Great Fish River case history, 35 years later (Abstract and oral presentation). *Society of Freshwater Science 2021-Virtual Meeting*. (23 – 28 May 2021).
- **Mpopetsi PP,** Kadye WT. The role of functional traits in facilitating multiple non-native species within an invaded African river system (Abstract and oral presentation). *59th Annual Congress of the Southern African Society of Aquatic Scientists (SASAqS)*. (26 – 30 June 2022).

ETHICS STATEMENT

Ethical approval was granted by Rhodes University Animal Research Ethics Committee (Approval number: 2020-2822-4866). Sampling permission was granted by Eastern Cape's Department of Economic Development, Environmental Affairs and Tourism (South Africa, Permit number: CRO 130/19CR).

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DEDICATION

I dedicate this thesis to my two boys

and to my parents.

DECLARATION

I, **Pule Peter Mpopetsi (g08m0225)**, hereby declare that this thesis constitutes my own original work and that it has not been submitted to any learning institution other than Rhodes University, either in part or in its entirety, for the award of any other degree.

Signed: Mpopetsi

Date: …………………14/02/2023…….…………….

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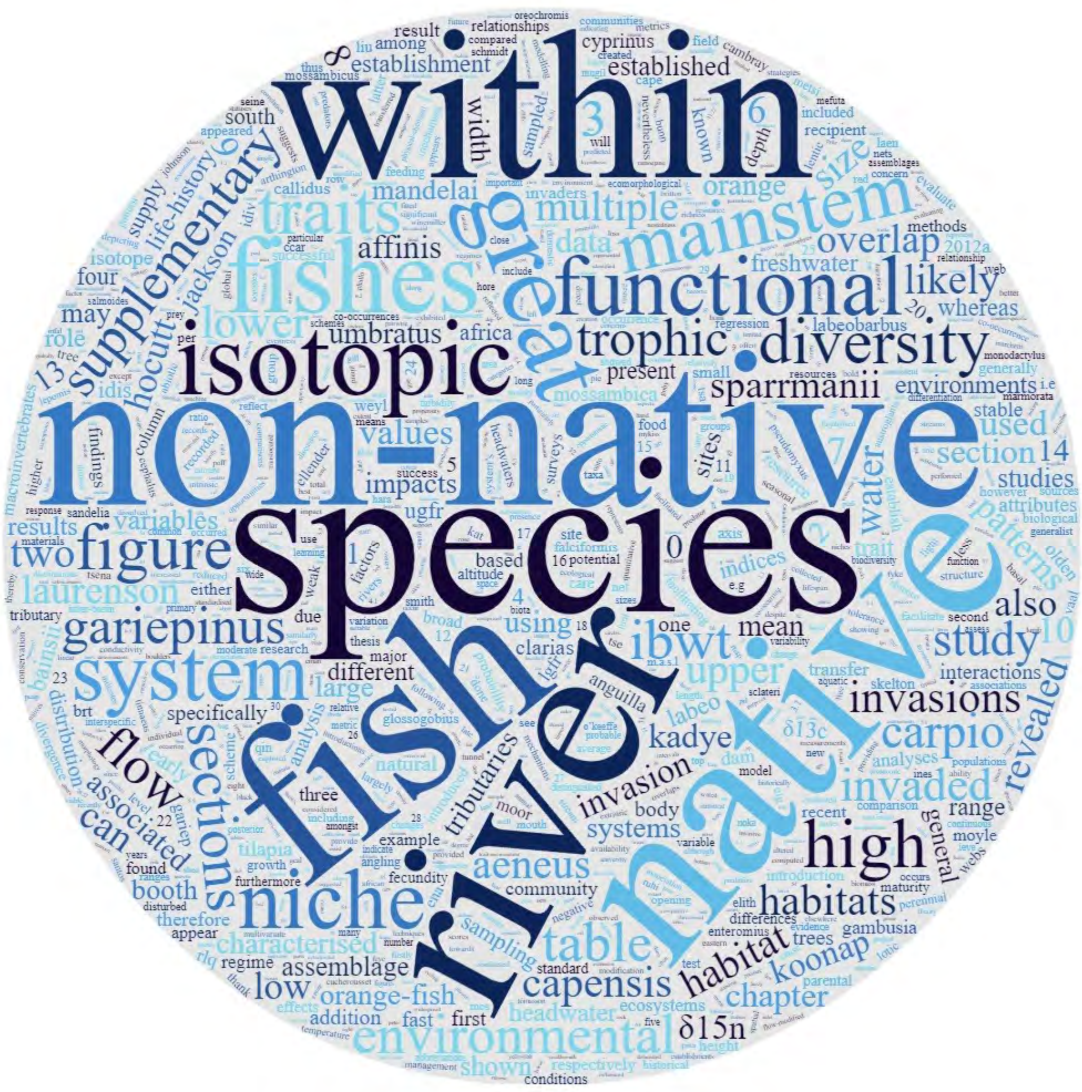

Thesis word cloud representing most used words scaled by size.

CHAPTER 1

GENERAL INTRODUCTION

Global freshwater resources and the role of inter-basin water transfer schemes

The global demand for fresh water as a resource to meet societal needs in many of the dryland environments, especially those characterised by low and unpredictable rainfall, has led to a surge in the number of impoundments and inter-basin water transfer (IBWT) schemes designed to curb this exigency (Ashton 2002, 2007; Nilsson et al. 2005; Ruhi et al. 2019). In these parts of the world, water transfers from areas of surplus to water-scarce regions have become common, whereby they are considered the most viable solution to the uneven water distribution (Snaddon et al. 1999; Ashton 2007; Gupta and Van der Zaag 2008). However, these interventions often involve changes in natural flow regimes of streams and rivers, as well as facilitation of movement of species between historically disjunct river systems, often with consequential impacts on native biodiversity of both the donor and the recipient systems (Laurenson and Hocutt 1986; Petitjean and Davies 1988; Slabbert 2007; Kadye and Booth 2012a, 2013; Gallardo and Aldridge 2018).

Southern Africa is considered a “dryland” zone, with scarce water resources (Midgley et al. 1994; Snaddon et al. 1999; Ashton 2007). In particular, South Africa has a low conversion of mean annual precipitation to mean annual runoff, along with an uneven and poorly predictable rainfall distribution (Petitjean and Davies 1988; Ashton 2007). Furthermore, water resources are unevenly distributed, with 66% of the total runoff being yielded by rivers of the eastern escarpment, whereas one-third of the country is estimated to yield 1% of the total runoff (Petitjean and Davies 1988; Davies et al. 1992). To augment freshwater supply and meet the country’s societal needs associated with freshwater demands (such as agricultural, domestic and industrial needs), dams, reservoirs and IBWTs have been constructed (Snaddon and Davies 1998; Ashton 2007).

Inter-basin water transfer refers to mass transfer of water from one geographically distinct river catchment, or basin, to another (Petitjean and Davies 1988; Davies et al. 1992). Currently, over 22 IBWT schemes have been constructed in Southern Africa (Table 1), and most river systems have been used to construct several dams and reservoirs at various points along their lengths (Snaddon et al. 1999; Ashton 20007; Slabbert 2007). However, constructions of these projects present a threat to the native biota of both the donor and recipient river systems through altering their physico-chemical conditions (including habitat and flow regimes), translocation of non-native species between historically disconnected catchments or river systems and through mixing of genetic material from different populations, from isolated river systems, of the same species (Schmidt et al. 2019; Nagelkerke et al. 2018; Ramoejane et al. 2021). This is because natural flow regimes determine physical habitat availability, species composition and the structure and function of riverine ecosystems (Poff et al. 1997; Bunn and Arthington 2002; Lytle and Poff 2004). Furthermore, some studies have shown natural/near natural flow regimes as deterrent to the persistence of non-native species while promoting proliferation of native species (Marchetti and Moyle 2001; Ellender et al. 2011). Therefore, changes in the natural flow regime of a system, as a result of IBWT, has the potential to impact its native biodiversity.

Besides altering flow regimes, IBWTs can provide opportunities for species to disperse between historically isolated river basins (Gallardo and Aldridge 2018). Often, species transfers between systems involve translocations of more than one taxon, thereby promoting co-occurrences of multiple non-native species (e.g. Nagelkerke et al. 2018; Kadye and Booth 2020). In systems associated with both species invasions and IBWTs, the combined impacts of these two stressors could prove detrimental, especially to the recipient system’s native biodiversity (Gallardo and Aldridge 2018).

Table 1.1. Some of the extant inter-basin water transfer (IBWT) schemes in Southern Africa. Also included are donor and recipient rivers or systems for each of the schemes. Modified from Snaddon et al. (1999).

Project or scheme	Donor river/system	Recipient river/system	Purpose
1 = Amatole	Toise and Kubusi	Yellowwoods & Nahoon	Domestic
2 = Caledon-Modder Transfer	Caledon	Modder	Domestic, Industrial
3 = Eastern National Water Carrier	Swakop	Windhoek area	Domestic, General
4 = Grootdraai Emergency Augmentation	Vaal	Vaal	Emergency supply
5 = Lesotho Highlands Water Project	Katse Dam	Vaal	General supply
6 = Orange-Fish Project	Orange	Great Fish	Irrigation
	Great Fish	Sundays	Irrigation
7 = Orange-Riet	Vanderkloof Dam	Riet	Domestic, Irrigation
8 = Orange-Vaal Transfer Scheme	Orange and Vaal	Vaal-Orange confluence	Irrigation
9 = Keerom Diversion	Olifants	Berg	General supply
10 = Komati Water Scheme	Komati	Olifants	Power station cooling
11 = Molatedi Dam - Gaborone	Molatedi Dam	Gaborone Botswana	Domestic, General
12 = Mooi-Mgeni Transfer	Mooi	Mpofana	General supply
13 = North-South Carrier Water	Shashe	Eastern Botswana	General supply
14 = Palmiet River	Palmiet River	Steenbras Dam	General supply
15 = Riviersonderend – Berg-Eerste River	Riviersonderend	Eerste	General supply
16 = Slang River Government Water	Slang	Perdewaterspruit	Power station cooling
17 = Tugela - Vaal Scheme	Tugela	Vaal	General supply
18 = Tugela-Mhlathuze Transfer	Tugela	Mhlathuze	General supply
19 = Umzimkulu - Umkomaas - Illovo	Mzimkulu	Mkomaas	Domestic
20 = Usuthu-Vaal	Vaal	Olifants	Electricity generation
	Assegaai (Usuthu)	Vaal	General supply
21 = Vaal-Olifants	Grootdraai Dam	Olifants Management Area	Industrial and power
22 = Zambezi Aqueduct	Zambezi	Botswana and Vaal	General supply

The state of freshwater fish invasions in South Africa

Globally, invasions by non-native freshwater fishes have caused direct and indirect adverse impacts on native recipient fish communities (Rahel 2002; Strayer 2010; Bernery et al. 2022). Direct impacts include competition for resources and predation by non-natives on native species (Collares-Pereira et al. 1999; Weyl and Lewis 2006), whereas indirect impacts include modification of habitats in the recipient ecosystems, which may render unfavourable conditions for native species (Miller and Crowl 2006; Zambrano et al. 2010). South Africa is no exception to the challenge of invasions by non-native fish species. Specifically, it is considered to be a fish invasion hotspot (Leprieur et al. 2008), with 55 species (28 alien and 27 extralimital) either introduced or translocated outside their native range (Ellender and Weyl 2014). In this context, alien species refer to those that have been introduced from outside the boundaries of South Africa, whereas extralimital species are defined as those whose distribution range has been extended into adjacent systems they did not naturally occur. Although many of these introductions were deliberate, such as through angling and recreational fisheries, aquaculture and biological control, others were introduced unintentional, such as through IBWTs (Ellender and Weyl 2014). Examples of fish species unintentionally introduced outside their native ranges include the rock catfish *Austroglanis sclateri* (Boulenger, 1901), sharptooth catfish *Clarias gariepinus* (Burchell, 1822), smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822) and the Orange River mudfish *Labeo capensis* (Smith, 1841) from the Orange River into the Great Fish River through the Orange-Fish River IBWT (Cambray and Jubb 1977; Laurenson and Hocutt 1984, 1986). There is also some potential of unintentional IBWT translocations of non-native fish species in other regions. For example, it is probable that the Caledon-Modder transfer scheme could serve as a conduit for unintentional translocation of common carp *Cyprinus carpio* Linnaeus, 1758 and mosquitofish *Gambusia affinis* (Baird and Girard, 1853) from the Caledon River into the Modder River. Similarly, there is a potential risk of translocation of fishes, in particular *L. aeneus* from east flowing rivers, such as the Tugela and Usuthu rivers into the Vaal River system. Such translocations have been hypothesised to likely have negative repercussions to native biodiversity, including fish populations, of the recipient systems (Davies et al. 1992), and there have been evidence to support this notion (e.g. Kadye and Booth 2012a; Ramoejane et al. 2020).

On the other hand, intentional introductions are mainly those associated with recreational angling and they include common carp, bluegill *Lepomis macrochirus* Rafinesque, 1819, smallmouth bass *Micropterus dolomieu* (Lacepède, 1802), largemouth bass *Micropterus salmoides* (Lacepède, 1802), spotted bass *Micropterus punctulatus* (Rafinesque, 1819), rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), brown trout *Salmo trutta* Linnaeus 1758 and banded tilapia *Tilapia sparrmanii* (Smith, 1840) (De Moor and Bruton 1988; Ellender and Weyl 2014). In particular, *M. salmoides* has been predicted to have a broad habitat suitability in South Africa (Khosa et al. 2019), which could exacerbate its impacts on native biodiversity. Other intentional introductions include the silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) and the highly invasive Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) introduced for aquaculture enhancement, as well as *G. affinis* and grass carp *Ctenopharyngodon idella* (Valenciennes, 1844), which were introduced for biological control purposes (Ellender and Weyl 2014). These introductions, whether intentional or not, have caused, or have potential to cause, considerable negative impacts on the native biota. Notable impacts include the change in behaviour of Berg River redfin *Pseudobarbus burgi* (Boulenger, 1911) in the presence of *O. mykiss* (Woodford and Impson 2004) and the extirpation of native fish species by *M. salmoides* from the stream reaches in the Blindekloof River (Ellender et al. 2011). Other impacts include range contractions of Cape galaxias *Galaxias zebratus* (Castelnau, 1861), Cape kurper *Sandelia capensis* (Cuvier, 1829) and *P. burgi* in the Berg River, as well as the local extinctions of native freshwater fishes in some mountain tributaries attributed to the presence or introductions of non-native species such as *M. dolomieu*, *M. punctulatus* and *O. mykiss* (Shelton et al. 2008; Clark et al. 2009). Despite these impacts, most studies investigating invasive fishes in South Africa have focused mainly on single species or their associated impacts on recipient systems (Ellender and Weyl 2014). Of these, only few have attempted to assess the co-occurrences and impacts of multiple non-native species on the recipient system, although many freshwater systems are known to have been invaded by multiple non-native species (e.g. Clark et al 2009, Weyl et al. 2009; Ellender et al. 2011; Kadye and Booth 2013; Guareschi et al 2021).

Elsewhere, there has been increasing interest on the co-occurrence of multiple non-native species and their potential impacts on recipient freshwater ecosystems (Johnson et al. 2009; Jackson and Britton 2013; Sagouis et al. 2015; Liu et al. 2018; Jackson 2015). Certain co-occurring non-native species have been reported to interact synergistically to facilitate their invasion that could, in turn, result in broader ecological impact within the invaded communities (Jackson and Britton 2013; Braga et al. 2018; Hohenadler et al. 2018). Nevertheless, other studies have observed either negative or no interactions amongst co-occurring non-native species (Griffen et al. 2008; Johnson et al. 2009; Liu et al. 2018). However, the mechanisms facilitating these co-occurrences within invaded systems appears to be complex, context-specific and, in some cases, unclear. For example, anthropogenic flow disturbance (Kadye and Booth 2020), increased propagule pressure (Cassey et al. 2018), trophic niche differentiation (Jackson and Britton 2013; Jackson et al. 2014), lack of natural enemies for the invading species (Shea and Chesson 2002) and invasional meltdown (Hohenadler et al. 2018), can facilitate the co-occurrences of multiple species in aquatic environments. The Great Fish River system, South Africa, is one such system invaded by multiple non-native fish species.

The Great Fish River system case study

The Great Fish River is one of the largest river basins in the Eastern Cape province of South Africa (See Figure 2.1) (Midgely et al. 1994). In 1977, an 82.45 km tunnel (Orange-Fish IBWT), which connected the Orange River with the Great Fish River was opened to supply water from the former into the water-scarce regions of the Eastern Cape, mainly for irrigation purposes (Laurenson and Hocutt 1984; O'Keeffe and De Moor 1988). The opening of the IBWT, however, changed the natural flow regime of the Great Fish River from irregular seasonal to perennial flow (O'Keeffe and De Moor 1988). This has resulted in an increased annual flow, mainly in the upper reaches, and a reduction in seasonal flow variation in the lower reaches (O'Keeffe and De Moor 1988). The now permanent water flow in the Great Fish River, which also increased its turbidity, modified pre-existing habitats and created new ones (Laurenson and Hocutt 1986).

In addition to water transfer, the Orange-Fish IBWT also facilitated the transfer of the Orange River biota into the Great Fish River. The once restricted animal movements between the two rivers, by the natural land barrier, were now made possible by the Orange-Fish IBWT (Laurenson and Hocutt 1984, 1986, Weyl et al. 2009). As a result, some species expanded their distributional range by invading the Great Fish River (Weyl et al. 2009). Five fish species are known to have been transferred into Great Fish River system through the Orange-Fish IBWT. These are *A. sclateri*, *C. gariepinus*, *L. aeneus*, *L. capensis* and moggel *Labeo umbratus* (Smith, 1841) (Laurenson and Hocutt 1986, Ellender and Weyl 2014). Although *L. umbratus* is indigenous to both river systems, a population of the Orange River has been shown to have made it into the Great Fish River system (Ramoejane et al. 2020) most likely through the Orange-Fish IBWT. In addition, there are other introduced non-native fishes such *C. carpio*, *M. salmoides* and *T. sparrmanii*, which were introduced through angling, and *G. affinis*, which was introduced for the biological control of mosquitoes. It does appear that the major threats to native biota of the Great Fish River system are not only limited to changes in its physico-chemical conditions (particularly changes in flow regime and habitat modification) but they also include co-occurrences of multiple non-native species. The establishment of multiple non-native species within the Great Fish River has the potential to lead to positive interactions amongst its invaders, thereby enhancing their invasions and net effects (Simberloff and Von Holle 1999; Jackson et al. 2014). This could, in turn, further weaken its invasion resistance and facilitate future invasions. For example, *C. carpio* is known to reduce water clarity through its bottom feeding behaviour which could cause loss of habitat for many native species (Zambrano et al. 2006). Likewise, *C. gariepinus* has been documented to prey on native invertebrates and fishes (Potts et al. 2008; Kadye and Booth 2012a) and being a generalist predator, it has the potential to compete with native species for food resources. Introductions of *L. capensis* and *L. umbratus* populations from the Orange River into the Great Fish River could result in mixing of genetic material with *L. umbratus* population from latter system. Firstly, a recent study uncovered unique lineages between the Orange River and the Great Fish River populations of *L. umbratus* (Ramoejane et al. 2021). Therefore, translocations of *L. umbratus* populations between these two river systems could cause interbreeding. In addition, although *L. capensis* and *L. umbratus* co-occur naturally within the Orange River, hybrids of the two species have been detected in lentic environments, outside their native ranges, the Hardap Dam, Namibia (Orange River system) and Darlington Dam, Eastern Cape, south Africa (Great Fish River system) (Gaigher and Bloemhoef 1975; Ramoejane et al. 2020). It is possible that similar situation can occur within the Great Fish River where non-native *L. capensis* from the Orange River hybridises with *L. umbratus* population of the Great Fish River. Furthermore, the establishment of *L. capensis* within the Great Fish River threaten the Great Fish population of *L. umbratus* through competition as both species have similar breeding and environmental conditions (Tómasson et al. 1984; Skelton, 2001). All these impacts have the potential to occur in concert within the Great Fish River system thereby exacerbating the net effects of multiple non-native species on native biota. Therefore, understanding mechanisms facilitating the co-occurrences of these non-native fishes can help mitigate their net impacts on the native species of the Great Fish River.

Thesis outline

The overarching aim of this thesis was to explore ecological mechanisms likely responsible for the co-occurrences of multiple non-native fish species within the Great Fish River system. To achieve this, the thesis was divided into seven chapters. Chapter 1 provides a brief general introduction and Chapter 2 describes the study area and general sampling methods. In Chapter 3, historical and contemporary literature on non-native fishes is used to assess current state of invasions and probable mechanisms facilitating establishment of these non-native fishes within the Great Fish River system. It was hypothesised that non-native fishes would reflect life-history traits and environmental association attributes that are consistent with adaptability to disturbed and flow-modified habitats conditions. Trait-based approaches were employed in Chapter 4 to investigate the role of functional diversity of non-native and native fishes in relation to their composition, distribution and environmental relationships. It was hypothesised that native and non-native fish species would have different functional trait diversity and would exhibit different patterns within the species-trait-environment relationship. Chapter 5 examined trophic diversity and niche patterns of non-native and native fish species within the Great Fish River system using stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$. It was hypothesised that native and non-native fish assemblages would have variable trophic functional diversity patterns that likely reflected broad resource use patterns, and that non-native fish species would exhibit broad trophic niche patterns that would likely reflect trophic generalisation. In Chapter 6, machine learning techniques were used to quantitatively predict non-native fish distributions and the associated assemblage patterns within the system in order to identify native fishes that are likely prone to the impacts of non-native fish invasion. A synthesis of the findings is provided in the final chapter (Chapter 7).

CHAPTER 2

STUDY AREA AND GENERAL SAMPLING METHODS

Study area

The Great Fish River system (Figure 2.1), with a catchment area of 30 243 km^2 and a longitudinal axis of 650 km, is one of the largest river basins in the Eastern Cape, South Africa. It generally flows southeast-wards and discharges into the Indian Ocean. In 1975, the Great Fish River was connected with the Orange River system through an 82.45 km tunnel (Orange-Fish IBWT) to supply water from the Orange River system into the water-scarce regions of the Eastern Cape, mainly for irrigation purposes (Laurenson and Hocutt 1984; O'Keeffe and De Moor 1988; Davies et al. 1992). The tunnel draws water from the Gariep Dam (Orange River system) and discharges into the headwater tributaries of the Great Fish River, the Teebus River and Great Brak River, and then into the upper mainstem section of the Great Fish River (O'Keeffe and De Moor 1988).

The Orange River, which is the IBWT donor system, is South Africa's longest river (approximately 2400 km) with a catchment area of approximately 974 000 km^2 (Keulder 1979). It flows west-wards into the Atlantic Ocean. Its main landmark, the Gariep Dam, is South Africa's largest impoundment covering a surface area of approximately 360 km^2 and has a shoreline of about 400 km (Keulder 1979; Hamman 1981). It is a highly turbid impoundment estimated to have a mean and a maximum depth of 16.3 m and 50 m at full water level, respectively (Hamman 1981, Tómasson et al. 1984). The ichthyofauna of the impoundment comprises eight native and four non-native fishes (Cambray et al. 1978). The native ichthyofauna in the Gariep Dam is generally distinct from that of the Great Fish River. Although historical data indicated close affinities in taxa such as *Labeo umbratus* (A. Smith, 1841) and *Enteromius anoplus* (Weber, 1897) between the two systems, subsequent research has shown that these also represent distinct lineages and separate species (Ramoejane et al. 2020; Kambikambi et al. 2021).

The opening of the Orange-Fish IBWT changed the natural flow regime of the Great Fish River from irregular seasonal to perennial flow (O’Keeffe and De Moor 1988). Before the opening of the tunnel, seasonal flow variation in the Great Fish River was high, resulting in the river occasionally getting reduced to series of unconnected pools in dry seasons (O’Keeffe and De Moor 1988). The continuous inflow of water from the Orange River system has increased turbidity of the Great Fish River system. The inflow also increased the annual flow and caused a reduction in seasonal flow variations in the lower reaches (O’Keeffe and De Moor 1988).

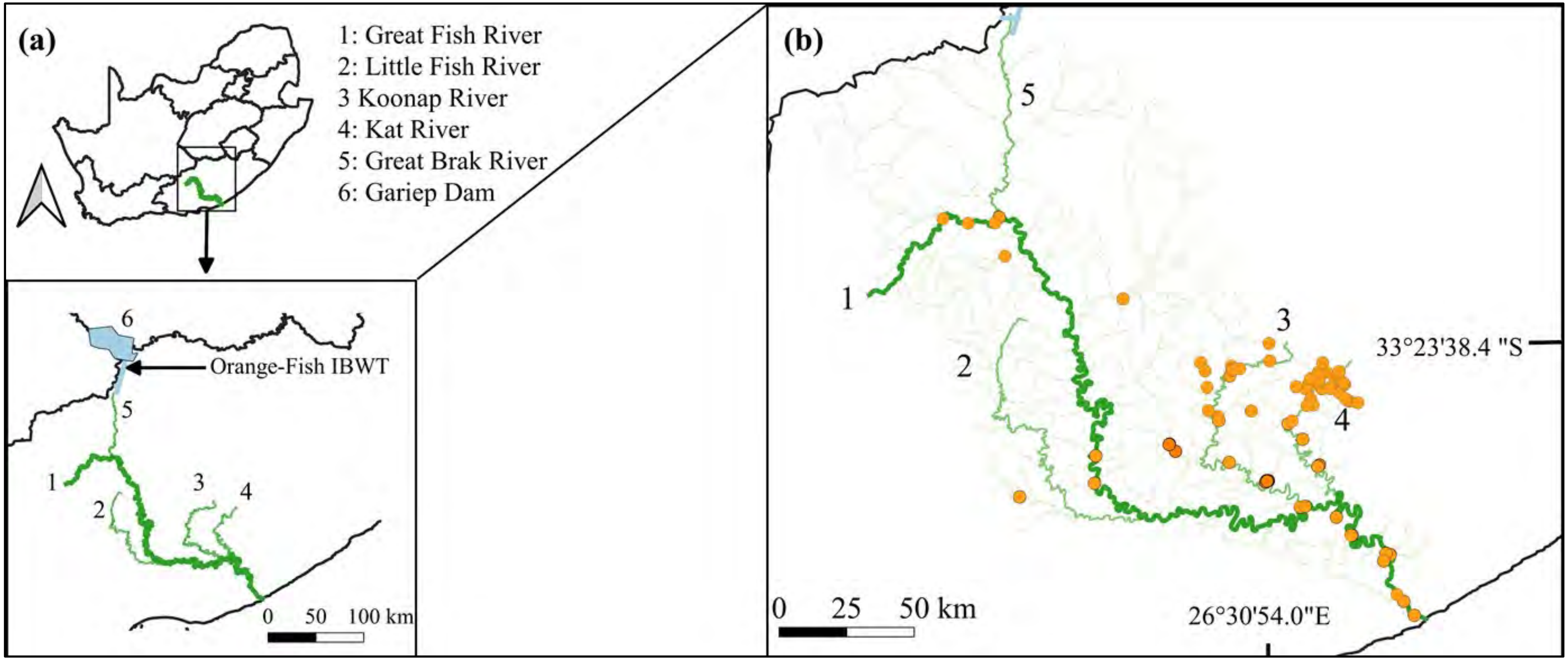

Figure 2.1. Great Fish River system (a) showing recent sampling sites (b) in orange circles. The system is comprised of the Great Fish river mainstem (1) and four major tributaries (2 – 5). Also shown on the map are the Gariep Dam (6) in the Orange River system, and the Orange-Fish inter-basin water transfer scheme (IBWT) linking the Orange River to the Great Fish River system.

Non-native fish introductions into the Great Fish River system

The Great Fish River system is known to host an ichthyofauna assemblage that comprises both native and non-native species. The native fish assemblage included primary freshwater taxa such as *E. mandelai* and the critically endangered *Sandelia bainsii* Castelnau, 1861, which were mostly confined to headwater tributaries. In comparison, the mainstem comprised mostly medium- to large-sized species with the upper section comprising two native species, *L. umbratus* and *Anguilla mossambica* Peters, 1852, whereas the lower section was characterised by secondary division freshwater (those that are generally restricted to freshwater and have a limited tolerance to brackish waters) and estuarine taxa. The non-native fish assemblage comprised some species known to have been transferred into the system through the Orange-Fish IBWT (Laurenson and Hocutt 1986; Ellender and Weyl 2014), such as *Austroglanis sclateri* (Boulenger, 1901), *Clarias gariepinus* (Burchell, 1822), *Labeobarbus aeneus* (Burchell, 1822), and *Labeo capensis* (A. Smith, 1841). In 1977, two years after the tunnel was completed, *L. capensis* (A. Smith, 1841) and *L. aeneus* (Burchell, 1822) were caught in the Great Fish River (Laurenson and Hocutt 1986). These were followed by *C. gariepinus* and *A. sclateri* in 1978 and 1983, respectively (Laurenson and Hocutt 1984, 1986). Prior to the opening of the tunnel, none of these species had been recorded in the Great Fish River (Laurenson and Hocutt 1986). In addition, other non-natives have been recorded within this system, which were introduced either for biocontrol, such as *Gambusia affinis* (Baird and Girard, 1853), or for angling purposes including *Cyprinus carpio* Linnaeus, 1758 and *Tilapia sparrmanii* A. Smith, 1840.

General sampling

A total of 68 (67 sites in lotic habitats and one lentic habitat) sites were sampled from both the mainstem and tributaries of the Great Fish River (Figure 2.1b, Supplementary Table 1). The surveys were conducted at different periods; January-May 2017 and October 2019-March 2020. Additional data for the study were obtained from surveys conducted from 2009 to 2011 (Kadye and Booth 2013) and 2017 (Sifundza et al. 2021). At each sampling site, physico-chemical variables and habitat conditions were recorded (Supplementary Table 1). Temperature (°C), pH and dissolved oxygen (DO; mg/L and DO%) were measured using YSI Pro20 meter. Conductivity (µS cm^{-1}) and total dissolved solids (TDS; ppm) were measured using Hanna HI98129 Combo meter, whereas turbidity (NTU) was measured using HANNA HI98703 turbidity meter. Depth and habitat characteristics, including substratum type, stream width, depth, canopy cover and macrophyte presence were also recorded at each site. For depth data, 30 measurements were taken randomly using a graduated pole and the measurements were then averaged to get a mean depth for that particular site. Substratum type and presence of macrophytes were assessed with a radius of 25 cm around each of the 30 random points at each site. Substratum was categorised following a modified Wentworth (1922) scale as silt (< 0.05 mm), sand (0.05-2 mm), gravel (2-100 mm), cobble (100-300 mm), boulder (300-500 mm) and bedrock (> 500 mm in diameter), and each category was expressed as a proportion (percentage) of the total measurements. Similarly, canopy cover was assessed overhead from each of the sampled points and expressed as a proportion based on the total number of points that were assessed. Stream width was recorded as an average of three transects measurements taken perpendicular to flow. Where width measurements were difficult to measure in the field, they were measured using the GPS coordinates that were recorded at each site. Stream width was used along with sampling length to determine sampling area. Altitude at each site was measured using GPS.

Several sampling methods, including electrofishing, fyke netting, gill netting, hand netting and seine netting, were used to capture fishes from both the tributaries and the mainstem sections of the Great Fish River. For electrofishing, a 12V battery-powered SAMUS backpack electrofisher in combination with a stop net (4 mm mesh net) were used to sample shallow rocky sites in the headwaters, where neither seine nor fyke nets were efficient. Stunned fish were collected with a hand net. A stop net, secured to the streambed, was used downstream to block electrocuted fish that were missed by hand netting. To standardise the sampling effort, three-pass electrofishing was conducted for 15 minutes per site. Marginal and shallow habitats, in headwaters and tributaries, were sampled using an 8 m long seine net with 4 mm mesh size. Two to four seine hauls (average of three) were performed at each site where seine netting was conducted. Mainstem sites were sampled using experimental gill nets, fyke nets (single and double ended) and the seine net. Experimental gill nets were 30 m long with three 10 m panels of mesh sizes of 50, 75 and 100 mm. Fyke nets had an 8 m guiding net and a first ring diameter of 55 cm and a 10 mm mesh size. One impoundment, the Mankazana Dam, which is on a tributary of the lower Great Fish River, was sampled using six experimental gill nets and a 25 m long seine net with 4 mm mesh size. Experimental gill nets and fyke nets were deployed overnight, from late afternoon/evening to morning, with an average soak time of 14 hours.

CHAPTER 3

COLONISATION THEORY AND INVASIVE BIOTA: THE GREAT FISH RIVER CASE HISTORY, 35 YEARS LATER

Introduction

Laurenson and Hocutt (1986) proposed a hypothetical framework to predict mechanisms that facilitate successful fish invasions in environments modified by inter-basin water transfer schemes (IBWT), which involve the transfer of water from a geographically isolated river system to another. Using the Great Fish River, South Africa, as a case study, their framework highlighted two broad aspects: the biological characteristics of successful invaders (intrinsic factors): and the properties of the invaded habitats that enhanced invasion success (extrinsic factors) (Laurenson and Hocutt 1986). Intrinsic factors are largely the invaders' biological characteristics related to their phenotypic and life-history attributes (Laurenson and Hocutt 1986; Sakai et al. 2001; Catford et al. 2009). By comparison, extrinsic factors are environmental characteristics of the recipient system (biotic and abiotic) that facilitate its invasion by non-native species (Laurenson and Hocutt 1986; Moyle and Light 1996a,b). These factors include the physico-chemical attributes, the recipient system's community structure, propagule pressure of the invading species, and the interactions between introduced species and native species (Shea and Chesson 2002; Lockwood et al. 2005; Ruesink 2005; Cassey et al. 2018).

For IBWT-facilitated invasions, studies elsewhere have shown the importance of intrinsic and extrinsic factors in facilitating invasion success (Nagelkerke et al. 2018; Qin et al. 2020; Schmidt et al. 2019). For example, within IBWT-modified environments, successful invaders have been shown to possess certain intrinsic biological attributes, such as propensity towards "*r*-strategy" (Grabowska and Przybylski 2015; Qin et al. 2020). These attributes are usually characteristic of species with small body size, faster growth, short lifespan, early maturity and increased fecundity (MacArthur and Wilson 1967; Noakes and Balon 1982). Species with these attributes are often associated with invasion of frequently disturbed environments (Winemiller and Rose 1992; Olden et al. 2006) where they generally adapt to novel habitats by maximising resource utilisation towards reproduction and recolonising (Adams 1980; Laurenson and Hocutt 1986; Nagelkerke et al. 2018; Schmidt et al. 2019; Qin et al. 2020). Other studies have, nevertheless, shown that large body size, late maturity, greater longevity, and high parental care, which are typical of "K-strategy" species (MacArthur and Wilson 1967; Noakes and Balon 1982), are attributes that may also promote the proliferation of non-native species in anthropogenically altered flow regime habitats (Vila-Gispert et al. 2005; Liu et al. 2017). Specifically, such species have been reported to predominate in benign and predictable environments (Pianka 1970; Laurenson and Hocutt 1986) within which they maximise their fitness by utilising a wide range of resources towards long-term survival (Adams 1980). However, successful invaders appear to exhibit wide-ranging life-history attributes along the *r*-K continuum (Pianka 1970; Adams 1980; Noakes and Balon 1982; Winemiller and Rose 1992). This underscores the importance of evaluating the role of intrinsic and extrinsic factors in mediating the success or failure of non-native species in novel environments.

The IBWT-modified habitats are generally predominantly characterised by structural changes in their physico-chemical conditions due to altered flow regimes. This exposes native species to novel environmental conditions, while creating conditions conducive for the survival and establishment of non-native species (Poff et al. 1997; Marchetti and Moyle 2001; Byers 2002; Vila-Gispert et al. 2005; Dudgeon et al. 2006; Mims and Olden 2013; Ruhi et al. 2016). Specifically, the IBWTs not only act as invasion pathways but usually cause physical and ecological changes that often weaken the invasion resistance of the recipient system due to the negative effects such as reduced competitive ability of native biota (Baltz and Moyle 1993; De Moor 1996; Ruhi et al. 2016; Comte et al. 2021). This is because most native species would have evolved life-history and survival strategies highly depend on natural flow regimes (Moyle and Light 1996b; Bunn and Arthington 2002; Lytle and Poff 2004). Furthermore, extrinsic biotic factors of recipient ecosystems, such as species richness, number of predators or competitors, and resource availability may also play a major role in determining the success of non-native species in new environments (Laurenson and Hocutt 1986; Shea and Chesson 2002; Levine 2004). For example, species-rich systems may have stronger biotic resistance, which is the ability of the community to withstand invasions, due to fewer niche opportunities for the invading species than species-poor systems (Elton 1958; MacArthur 1970; Hierro et al. 2005; Jeschke 2014; Escoriza and Ruhi 2016). By comparison, species-poor ecosystems are likely to represent unsaturated environments with under-utilised niches that may be occupied by the several invading species (Hierro et al. 2005) due to the occurrence of fewer native species that may be competitive and functionally similar to the non-native invaders (Elton 1958; Shea and Chesson 2002).

The Great Fish River, South Africa, represents a classic case study of a system that is heavily modified system because it is connected to the Orange River by an IBWT. Before the opening of the Orange-Fish IBWT, the Great Fish River had highly seasonal flow, with the river occasionally occurring as series of unconnected pools in dry seasons (O’Keeffe and De Moor 1988). The opening of this IBWT, thus, changed the natural flow regime of the Great Fish River from seasonal to perennial flow (O’Keeffe and De Moor 1988; Rivers-Moore et al. 2007). This has not only resulted in increased annual flow, mainly in the upper reaches, but has also reduced the seasonal flow variations in the lower reaches of the mainstem habitats (O’Keeffe and De Moor 1988). The IBWT-driven changes in abiotic factors, such as altered flow regime from seasonal to perennial, and the creation of large mainstem habitats were postulated to have enhanced the Great Fish River's susceptibility to invasions by non-native species (Laurenson and Hocutt 1986; Laurenson et al. 1989). Similarly, extrinsic biotic factors have been hypothesised to have weakened the biotic resistance of this system thereby making it more prone to invasions by non-native species (Laurenson and Hocutt 1986; Laurenson et al. 1989). This is because, similar to rivers in the adjacent Cape Fold Ecoregion, the Great Fish River is characterised by high endemism, few indigenous fish fauna and few natural generalist predators (Laurenson and Hocutt 1986; De Moor 1996; Kadye and Booth 2020). In their framework, Laurenson and Hocutt (1986) reported that the non-native fishes constituted 38% (10 out of 26 species) of the Great Fish River ichthyofauna. These non-native fishes encompassed a wide range of life-history strategies and environmental requirements, with the latter aspect having been recently explored (e.g. Kadye and Booth 2020). Over the years, there have been further introductions of non-native species into the Great Fish River, with only a few dedicated studies focussing on individual species (e.g. Weyl et al. 2009; Kadye and Booth 2012a, 2013). This raises questions on the current state of invasions and the mechanisms that have facilitated the adaptations of the non-native fishes in this river system. The objective of this study was to evaluate the extent to which Laurenson and Hocutt's (1986) colonisation theory of non-native fishes within the Great Fish River is supported. To achieve this, this study used extensive literature on intrinsic and extrinsic factors related to the non-native fishes and associated this information with the most recent distribution records of these fishes in the Great Fish River. This study hypothesised that these non-native fishes would reflect life-history traits and environmental association attributes that are consistent with adaptability to disturbed and flow-modified habitats conditions.

Materials and methods

Data collection and analysis

A combination of extensive literature and data from both the historical records and recent surveys were used to test Laurenson and Hocutt's (1986) proposed framework regarding the non-native species that were likely to establish within the Great Fish River. Historical records, which consistent with Laurenson and Hocutt (1986), were obtained from the South African Institute for Aquatic Biodiversity (SAIAB) in Makhanda, South Africa. Recent survey data included those from Kadye and Booth (2013), Sifundza et al. (2021) and field surveys that were undertaken between 2019 and 2020 during the current study (Figure 2.1: Chapter 2). For the recent field surveys, fishes were captured using a variety of sampling methods, including electrofishing, fyke netting, gill netting, hand netting and seine netting (detailed description is provided in Chapter 2). All captured fishes were identified to species following Skelton (2001). Firstly, the study documented fishes of the Great Fish River, including both native and non-native species (Table 3.1). Secondly, all of the officially reported non-native fish species within the system and their modes of introduction were identified. In addition, an assessment of current invasion statuses of these non-native species within the Great Fish River was carried out. Fish species composition collected from the Great Fish River before or just after the opening of the tunnel (i.e. from Laurenson and Hocutt (1986)) was compared to that from recent surveys of the system (i.e. from Kadye and Booth (2013), Sifundza et al. (2021) and 2019/2020 surveys). Changes in taxonomy, in any of these species, which occurred between 1985 and 2020 were noted and such species were referred by their latest accepted scientific name, following published regional literature (i.e. Skelton 2001 and Kambikambi et al. 2021) (Table 3.1).

To distinguish which intrinsic biotic attributes of invaders best influence their success within the Great Fish River, published literature was used to identify life-history traits of non-native fish species found within this system. These included species' growth, age at maturity, maximum size (total length), fecundity and parental care. Ecological attributes such as environmental and habitat tolerance as well as feeding patterns (diet) of each species were also documented. In addition, each of these life-history and environmental attributes were further distinguished using fuzzy coding (Table 3.2). Four clustering algorithms were then used to compare whether there were any similarities in life-history and environmental attributes among the different non-native fishes to discern which attributes were associated with successful invaders. The clustering algorithms were single linkage, complete linkage, average linkage (unweighted pair group method with arithmetic mean) and Ward's method. Cophenetic correlations, which measure how a dendrogram preserves the pairwise distances between the original unmodeled data points (Saraçli et al. 2013), were used to evaluate the best fitting clustering algorithm. The average linkage clustering performed best and was used in the subsequent interpretation of the results. The appropriate number of conglomerates were evaluated using silhouette plots and Mantel's optimum number of clusters, which were evaluated based on Pearson's correlation coefficients. All these analyses were performed in R environment (R Core Team 2022).

Results

Thirty-three fish species from fifteen families were recorded based on both the historical data and recent surveys from the Great Fish River (Table 3.1). Twenty-two of these were native, whereas 11 were non-native. Historical data comprised 26 species that included 16 native and 10 non-native species. By comparison, in the recent surveys, 24 species were recorded, which included 17 native and seven non-native species (Table 3.1). Despite general similarities in number of species between the two periods, there was, nonetheless, a mismatch amongst the native species with the two periods having different species composition (Table 3.1). This mismatch was largely related to coastal and estuarine species, which included species that were either recorded in historical data, such as *Acanthopagrus berda* (Forsskål, 1775), *Anguilla bengalensis labiata* Peters, 1852, *Anguilla bicolor bicolor* McClelland, 1844, *Liza richardsonii* (Smith, 1846) and *Pomadasys commersonnii* (Lacepède, 1801) or recorded in the recent surveys, such as *Glossogobius callidus* (Smith 1937), *Planiliza macrolepis* (Smith, 1846), *Psammogobius knysnaensis* (Smith, 1935), *Redigobius dewaali* (Weber 1897), *Rhabdosargus holubi* (Steindachner, 1881) and *Valamugil robustus* (Günther, 1861). Four native species *Enteromius mandelai* Kambikambi, Kadye and Chakona, 2021, *Enteromius pallidus* A. Smith, 1841, *P. macrolepis* and *Pseudomyxus capensis* (Valenciennes, 1836) and four non-native species *A. sclateri*, *Glossogobius giuris* (Hamilton-Buchanan, 1822), *L. aeneus* and *Oncorhynchus mykiss* (Walbaum, 1792) have since been taxonomically revised.

Table 3.1. Historical and contemporary records of native and non-native fishes of the Great Fish River and the current invasion statues of non-native species. The species names in parenthesis refers to old species that have been taxonomically revised subsequent to Laurenson and Hocutt (1986). Historical records are from Laurenson and Hocutt (1986) and current records are from surveys conducted between 2012 and 2020. The symbols "+" and "-" refer to presence and absence, respectively, of a species from either historical, Laurenson and Hocutt (1986), or recent surveys.

	Species	**Laurenson and Hocutt (1986)**	**Recent surveys**	**Invasion status**
Native	*Acanthopagrus berda*	+	-	Native
	Anguilla bengalensis labiata	+	-	Native
	Anguilla bicolor bicolor	+	-	Native
	Anguilla marmorata	+	+	Native
	Anguilla mossambica	+	+	Native
	Enteromius mandelai (*Barbus anoplus*)	+	+	Native
	Enteromius pallidus (*Barbus pallidus*)	+	+	Native
	Gilchristella aestuaria	+	+	Native
	Glossogobius callidus	-	+	Native
	Labeo umbratus	+	+	Native
	Planiliza macrolepis	-	+	Native
	Liza richardsonii	+	-	Native
	Monodactylus falciformis	+	+	Native
	Mugil cephalus	+	+	Native
	Pseudomyxus capensis	+	+	Native
	Oreochromis mossambicus	+	+	Native
	Psammogobius knysnaensis	-	+	Native
	Pomadasys commersonnii	+	-	Native
	Redigobius dewaali	-	+	Native
	Rhabdosargus holubi	-	+	Native
	Sandelia bainsii	+	+	Native
	Valamugil robustus	-	+	Native
Non-native	*Austroglanis sclateri*	+	-	Not established
	Clarias gariepinus	+	+	Established
	Cyprinus carpio	+	+	Established
	Gambusia affinis	-	+	Established
	Glossogobius giuris (*Glossogobius tenuiformis*)	+	-	Uncertain
	Labeo capensis	+	+	Established
	Labeobarbus aeneus (*Barbus aeneus*)	+	+	Established
	Lepomis macrochirus	+	-	Not established
	Micropterus salmoides	+	+	Established*
	Oncorhynchus mykiss (*Salmo gairdneri*)	+	-	Not established
	Tilapia sparrmanii	+	+	Established

* This species was captured within a lentic habitat.

Table 3.2. Fuzzy coding of non-native fishes' life-history traits and habitat requirements. Growth: 1 = fast, 2 = slow. Maturity: 1 = early, 2 = late. Size: 1 = small, 2 = large. Longevity: 1 = short, 2 = moderate, 3 = long. Fecundity: 1 = low, 2 = high. Parental care: 1 = reduced, 2 = high. Feeding: 1 = predator, 2 = omnivore, 3 = benthivore. Habitat: 1 = narrow, 2 = broad.

	Growth	**Maturity**	**Size**	**Longevity**	**Fecundity**	**Parental care**	**Feeding**	**Habitat**
Austroglanis sclateri	1	1	1	1	1	1	1	1
Clarias gariepinus	1	1	2	3	1	1	2	2
Cyprinus carpio	1	2	2	3	1	1	2	2
Labeo capensis	2	2	2	3	1	1	3	2
Labeobarbus aeneus	2	2	2	3	2	1	2	2
Gambusia affinis	1	1	1	1	1	2	1	1
Lepomis macrochirus	1	1	1	3	1	1	2	1
Micropterus salmoides	1	1	2	3	2	2	1	1
Oncorhynchus mykiss	1	1	2	2	1	1	1	1
Tilapia sparrmanii	1	1	1	3	1	2	2	1

Overall, the Orange-Fish IBWT and angling were the primary vectors of invasions, accounting for 36% and 46% of the non-native species, respectively, into the Great Fish River (Table 3.3). For the recent surveys data, three species, *C. gariepinus, L. aeneus* and *L. capensis*, introduced through the Orange-Fish IBWT, have become established in the lotic habitats of this river system. Other established non-native fishes include *Cyprinus carpio* Linnaeus, 1758, *Micropterus salmoides* (Lacepède, 1802) and *Tilapia sparrmanii* A. Smith, 1840, which were introduced through angling (forage fish and illegal stockings), and *Gambusia affinis* (Baird and Girard, 1853), which was first introduced in 1936 for the biological control of mosquitoes. *Clarias gariepinus* and *L. aeneus* were the most widespread and common non-native fishes within this river system (Figure 3.1). By comparison, *G. affinis*, *L. capensis, M. salmoides* and *T. sparrmanii* were either less common or had restricted distribution. Specifically, *G. affinis* and *T. sparrmanii* were localised, occurring mainly in the lower sections of the river system, with former occurring mainly in the mainstem whereas the latter occurred within both the mainstem and tributaries (Figure 3.1). *Labeo capensis* was characterised by incidental collections in the middle mainstem sections of the river system, whereas *C. carpio* was restricted to the middle and lower sections of the mainstem. *Micropterus salmoides* was captured within the Mankazana Dam, which was the only lentic habitats sampled in the Great Fish River system. Three primary freshwater non-native species, which were historically recorded, have failed to establish in the Great Fish River system (Table 3.3): *Lepomis macrochirus* Rafinesque, 1819 and *Oncorhynchus mykiss* (Walbaum, 1792), which were introduced through angling; and *A. sclateri*, which was introduced through the IBWT. The invasion status of *G. giuris*, an estuarine species, was uncertain because this species was not recorded in the recent surveys.

Cluster analysis grouped all the non-native fishes into four classes (Figure 3.2a). The major cluster comprised four species (*C. gariepinus*, *C. carpio*, *L. aeneus* and *L. capensis*) that have become established within the Great Fish River system. These were generally large-sized species, characterised by low parental care, high longevity and habitat generalists (Figure 3.2b). Other established non-native species were *G. affinis and T. sparrmanii,* which were characterised by traits associated with opportunistic life-history strategy such as small body size, fast growth, early maturity and relatively high reproductive investment (Figure 3.2b).

Table 3.3. Introduction pathways, life-history traits and current invasion statuses of recorded non-native fish species introduced or translocated within the Great Fish River, South Africa. IBWT is an abbreviation for inter-basin water transfer scheme. Small and large sizes refer to species which attains less than 500 and more than 500 mm in standard length (adults) respectively.

Species	Common name	Invasion pathway	Current invasion status	Life-history attributes	Source/reference
Austroglanis sclateri (Boulenger, 1901)	Rock catfish	IBWT	Not established	Fast growth, early maturity, small size, short lifespan, moderate fecundity, reduced parental care. Predator. Narrow environmental tolerance.	Niehaus 1996; Skelton 2001
Clarias gariepinus (Burchell, 1822)	Sharptooth catfish	IBWT	Established, widespread	Fast growth, early maturity, large size, long lifespan, high fecundity, reduced parental care. Generalist predator. Broad environmental tolerance	De Moor and Bruton 1988; Kadye and Booth 2012a; Kadye and Booth 2013, 2020; Skelton, 2001; Weyl et al. 2016
Cyprinus carpio Linnaeus, 1758	Common carp	Angling	Established, abundant	Fast growth, delayed maturity, large size, long lifespan, high fecundity, reduced parental care. Omnivorous. Habitat generalist.	Britton et al., 2007; Koehn, 2004; Skelton, 2001; Weber and Brown, 2009; Winker et al., 2011; Zambrano et al. 2006,
Gambusia affinis (Baird and Girard, 1853)	Mosquitofish	Biological control	Established, localised	Fast growth, early maturity, small size, short lifespan, low fecundity per spawning event, high parental care. Predator. Broad environmental tolerance.	Pyke 2005; Pyke, 2008; Skelton 2001
Glossogobius giuris (Hamilton-Buchanan, 1822)	Tank goby	Marine environment dispersal	Not recorded in recent surveys	Fast growth, early maturity, small size, High fecundity. Carnivorous.	Islam 2004; Qambrani et al. 2015
Labeo capensis (A. Smith, 1841)	Orange River mudfish	IBWT	Established, less common	Relatively slower growth, delayed maturity, large size, long lifespan, high fecundity, reduced parental care. Detrital feeding. Broad environmental tolerance.	De Moor and Bruton 1988; Skelton 1986; Tómasson et al. 1984; Winker et al. 2012

Species	Common name	Invasion pathway	Current invasion status	Life-history attributes	Source/reference
Labeobarbus aeneus (Burchell, 1822)	Smallmouth yellowfish	IBWT	Established, widespread	Relatively slower growth, delayed maturity, large size, long lifespan, moderate fecundity, reduced parental care. Omnivorous. Broad environmental tolerance.	De Moor and Bruton 1988; Ellender et al. 2016; Gerber et al. 2012; Kadye and Booth 2020; Skelton 2001; Tómasson et al. 1984; Weyl et al. 2009;
Lepomis macrochirus Rafinesque, 1819	Bluegill sunfish	Angling (fodder)	Not established	Fast growth, early maturity, small size, long lifespan, high fecundity, high parental care. Omnivorous. Tolerate broad environmental conditions.	Lee 1980; De Moor and Bruton 1988; Belk and Hales 1993; Skelton 2001; Aday et al. 2006; Hossain et al. 2013; Ndaleni et al. 2018
Micropterus salmoides (Lacepède, 1802)	Largemouth bass	Angling	Established in lentic habitats	Fast growth, early maturity, large size, long lifespan, moderate to high fecundity, high parental care. Generalist predator. Habitat specific.	Beamish et al. 2005; Heidinger 1976; Skelton 2001;
Oncorhynchus mykiss (Walbaum, 1792)	Rainbow trout	Angling	Not established	Fast growth, early maturity, large size, moderate lifespan, high fecundity, reduced parental care. Predator. Reduced habitat and environmental tolerance.	Cambray 2003; Candiotto et al. 2011; Shelton et al. 2016
Tilapia sparrmanii A. Smith, 1840	Banded tilapia	Angling (fodder)	Established, localised (in tributaries)	Fast growth, early maturity, small size, longer lifespan, low to moderate fecundity per spawning event, high parental care. Omnivorous. Wide habitat and environmental tolerance.	Noakes and Balon 1982; Skelton 2001; Zengeya and Marshall 2007

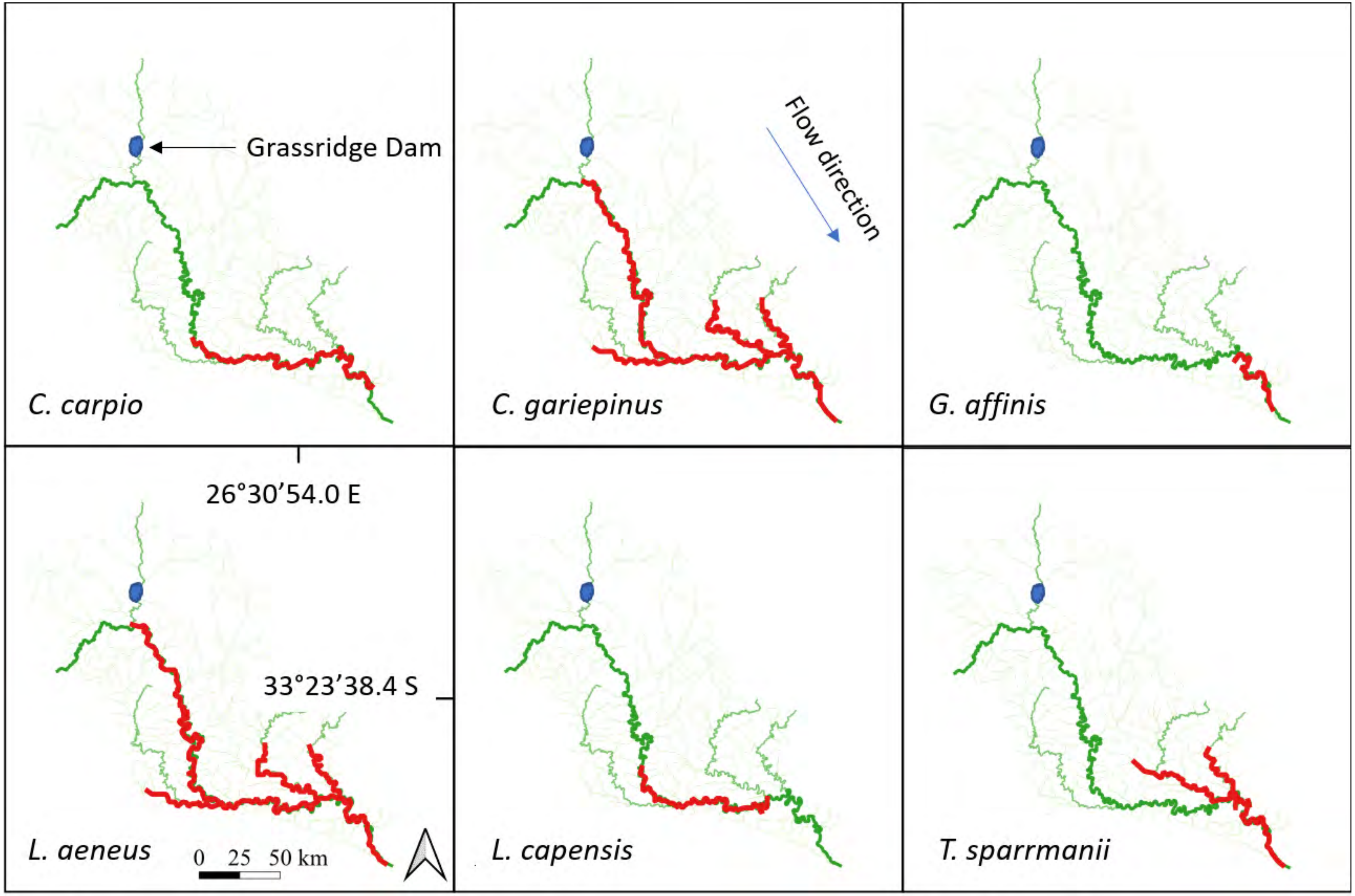

Figure 3.1. Distribution of established non-native fish species within the Great Fish River, based on data from from Kadye and Booth (2013), Sifundza et al. (2021) and field surveys that were undertaken between 2019 and 2020. For a detailed map of the Great Fish River system see Figure 2.1. (—) indicates potential extent of invasion.

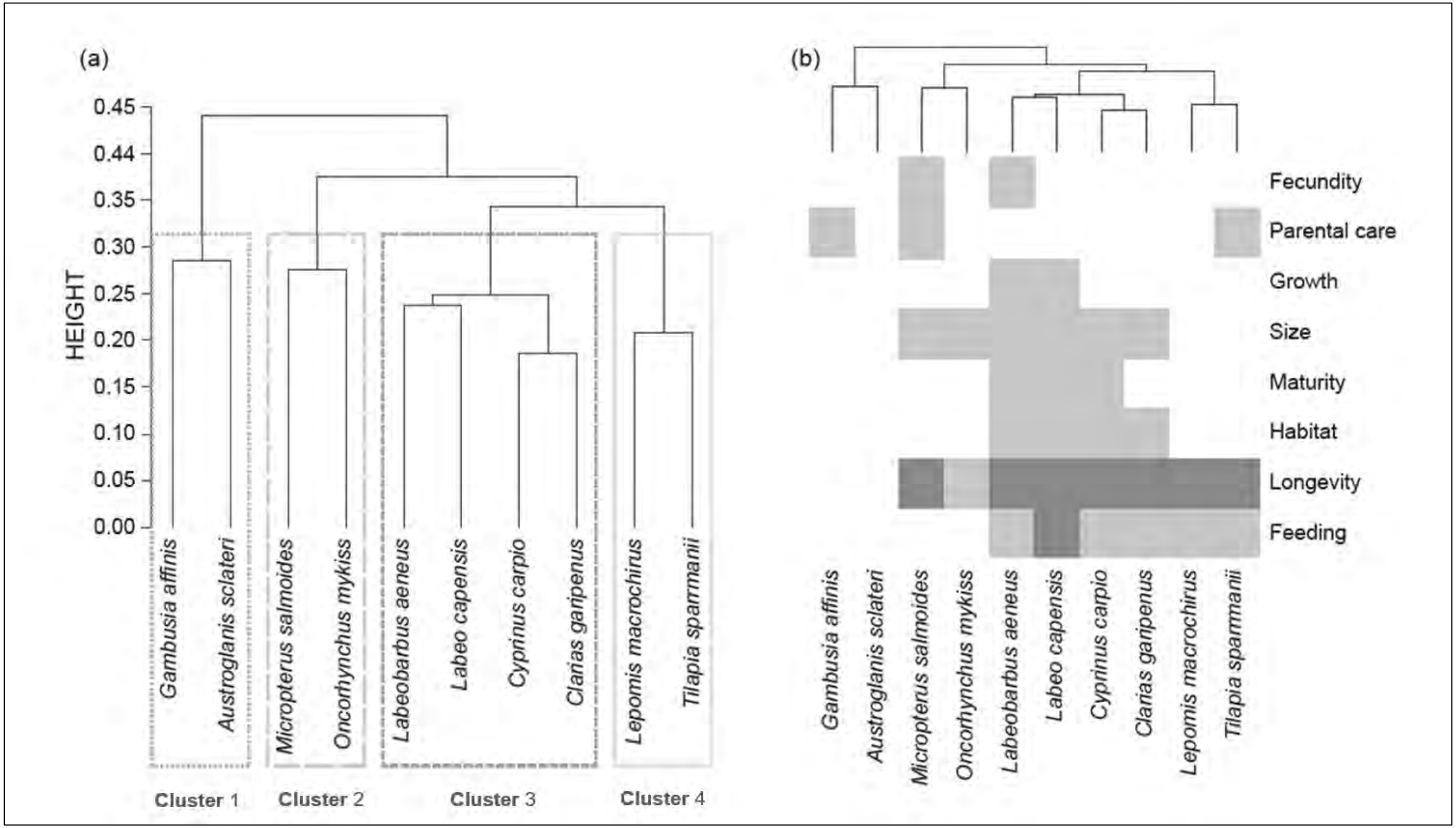

Figure 3.2. Cluster analysis of non-native fishes in the Great Fish River, South Africa. The cluster analysis is based on (a) the average method together with (b) the heat map showing fish species and the associated life-history and environmental attributes.

Discussion

Historical research on the invasion of the Great Fish River showed the occurrence of 10 non-native fishes, with four of these invading the river through the Orange-Fish IBWT (Cambray and Jubb 1977; Laurenson et al. 1989). Evidence from the present study revealed that, currently, seven non-native species have become established in the Great Fish River, with three of these invading through the IBWT. Three species, which were previously recorded during the historical research, have either failed to establish or were undetected in recent surveys.

Four of the six established non-native fishes (*C. gariepinus*, *C. carpio*, *L. aeneus* and *L. capensis*), in the present study, were generally large-sized species, characterised by high longevity, high fecundity, no parental care and were habitat generalists. These traits were characteristic of typical periodic life-history strategists (Winemiller and Rose 1992). Periodic life-history strategists are species that are characterised by large body size, late maturity, high fecundity per spawning event, low parental care and juvenile survivorship (Winemiller and Rose 1992; Olden et al. 2006). Periodic-strategist fishes are often assumed to typically inhabit highly variable and seasonal environments but they can nonetheless adapt to either perennial or seasonally stable environments (Winemiller and Rose 1992; Olden et al. 2006). The establishment of these four species could be an indication that their life-history traits may have facilitated their successful establishment in the Great Fish River, which has been anthropologically disturbed by IBWT. Their ability to adapt to variable environments could have also assisted their successful establishment in the Great Fish River. For example, Ellender et al. (2016) found that the establishment of *L. aeneus* to Gariep Dam was largely due to its periodic life-history strategy being suitable to establish in this large turbid impoundment. This is despite these strategists (periodic) being predicted to inhabit highly variable and seasonal environments (Winemiller and Rose 1992; Olden et al. 2006). The above findings are in contrast to those from other studies that have found that increased flow constancy, coupled with reduced flow variability, favoured equilibrium strategists in rivers across the United States of America (Mims and Olden 2013). Altered flow regimes of the Great Fish River appear to have created permanent and more stable habitats due to a shift from seasonal to perennial flow (Laurenson and Hocutt 1986). Specifically, the establishment of *L. aeneus* and *L. capensis*, which had limited distribution at the inception of the IBWT, appear to have been facilitated primarily through the creation of permanently flowing habitats in the mainstem section of the Great Fish River.

Similar to other established non-native fishes, the establishment of *G. affinis* and *T. sparrmanii* was likely due to their life-history strategies, coupled with the creation of new and favourable habitats in the Great Fish River system. In the present study, these two species have established in the lower section of the mainstem, with the latter species also occurring in lower sections of major tributaries. *Gambusia affinis* and *T. sparrmanii* appeared to exhibit opportunistic invader traits such as small body size, fast growth and early maturation. Generally, opportunistic life-history strategists are characteristic of species with small body size, early maturation, high reproductive investment, and low fecundity per spawning event but high fecundity overall through continuous spawning (Winemiller and Rose 1992; Olden et al. 2006). These life-history traits are assumed to confer advantage in highly disturbed and unpredictable environments (Winemiller and Rose 1992; Olden et al. 2006), and have also been shown to facilitate invasions in both IBWT-associated systems and other novel habitats elsewhere (Schmidt et al. 2019; Qin et al. 2020; Tarkan et al. 2021). For example, this life-history strategy was postulated to have favoured the establishment of tubenose goby, *Proterorhinus semilunaris,* in novel environments characterised by high resource availability (Grabowska et al. 2021). The above observations appear to support the hypothesis that non-native fish species with small body size, multiple spawning (iteroparous), short generation times and early maturity, which are characteristics of typical r-selected/opportunistic life-history strategy, may be successful in IBWT environments (Laurenson et al. 1985; Schmidt et al. 2019).

In addition to its opportunistic life-history strategy, *T. sparrmanii*, which is known to exhibit parental care by guarding newly hatched eggs (Skelton, 2001), had typical characteristic of equilibrium life-history strategy that enhances offspring survival (Winemiller and Rose 1992). Equilibrium strategists are characterised by small to medium body size, moderate maturation, low fecundity per spawning event, and high juvenile survivorship as a result of increased parental care (Olden et al. 2006). These species are predicted to typically thrive in stable environments. An example is Shimofuri goby (*Tridentiger bifasciatus*) that invaded Lake Nansi, which is part of the South-to-North Water Transfer Project of China. The Shimofuri goby's invasion success was attributed to a combination of its opportunistic and equilibrium life-history traits of reduced life span and high fecundity (opportunistic) and parental care (equilibrium), which suggested life-history plasticity (Qin et al. 2020). It does appear that *T. sparrmanii* exhibited life-history trait plasticity between opportunistic and equilibrium strategies, which are attributes that have been reported as drivers of invasion success in other studies (e.g. Fox and Copp 2014; Qin et al. 2020). For example, successful establishments of the tubenose goby (Top et al. 2018), round goby *Neogobius melanostomus* (Hôrková and Kováč 2014) and pumpkinseed *Lepomis gibbosus* (Tarkan et al. 2021) were attributed to their increased adaptive capacities resulting from high plasticity in life-history traits.

The present study showed that most habitat-generalist fishes were successful in establishing viable populations within the Great Fish River. This appears to be consistent with the view that flow modification, as a result of the Orange-Fish IBWT, created new niche opportunities that benefitted these habitat-generalist non-native species (Laurenson and Hocutt 1986). Studies elsewhere have also revealed that anthropogenically altered flow regimes can lead to creation of new environments, which could ultimately create new niche opportunities for invasions by non-native species (Bunn and Arthington 2002; Light and Marchetti 2007; Ruhi et al. 2016; Comte et al. 2021). This suggests that non-natives that can overcome environmental barriers of the Great Fish River, in particular abiotic factors, are likely to establish viable populations within this system. Recently, Kadye and Booth (2020) showed that abiotic factors, which influenced species distribution, were likely the key determinants of establishment of non-native fishes within the Great Fish River. Specifically, Kadye and Booth (2020), using ordination techniques (outlying mean index analysis), found that non-native fish species in the Great Fish River generally had high environmental tolerance and broad environmental niches, supporting the niche opportunity hypothesis. They also found that most of these non-native species, particularly *L. aeneus* and *C. gariepinus*, were less constrained by environmental conditions and that, overall, the established non-natives formed a functional assemblage in this river system. Similarly, Weyl et al. (2009) attributed the success of *L. aeneus* in the Great Fish River to the availability of feeding opportunities and suitable spawning habitats due to perennial flow, which led to successful reproduction, whereas Kadye and Booth (2013) attributed the proliferation of *C. gariepinus* to habitat modification and altered flow regimes. The role of abiotic properties in providing resistance to invasions has been studied extensively elsewhere. For example, in Californian streams, abiotic factors were predicted to play a major role in determining fish invasion success, with non-native fish species being found to have a high probability of invading suitable environments (Moyle and Light 1996a). Similarly, in Australian freshwater ecosystems, proliferation of two alien fish species, *C. carpio* and *G. affinis*, was associated with flow modification (reviewed in Bunn and Arthington 2002). Collectively, these findings highlight the importance of abiotic factors in acting as a facilitator to colonisation and establishment of non-native species (Moyle and Light 1996a,b; Catford et al. 2009).

Studies have shown that species-poor systems are likely to have weaker biotic resistance compared to species-rich systems (Elton 1958; Jeschke 2014). This is because systems with fewer resident species, or low species diversity, are likely to be associated with the availability of vacant niches that may be occupied by non-native invaders (Shea and Chesson 2002; Leuven et al. 2009). Comparisons can be drawn for the Great Fish River whose freshwater habitats were historically dominated by few species. Specifically, the upper and middle mainstem section of this river were historically characterised by few native species *Anguilla mossambica* Peters, 1852, *E. mandelai* and *Labeo umbratus* A. Smith , 1841 (Cambray and Jubb 1977; Laurenson and Hocutt 1986; Skelton 2001). These sections have now become dominated by non-native species (Kadye and Booth 2020).

This study showed that four non-native fishes (*M. salmoides*, *O. mykiss*, *L. macrochirus,* and *A. sclateri*) were undetected from recent surveys, or failed to establish viable populations, in the lotic habitats of the Great Fish River system. Some of these failed establishments may be explained by extrinsic and intrinsic factors. For example, *M. salmoides*, which is known to prefer lentic environments with clear and standing or slow flowing waters (Heidinger 1976; Essington and Kitchell 1999; Skelton 2001), was captured in Mankazana Dam, the only standing water habitat that was sampled during this study. By comparison, the Great Fish River system's lotic and lentic habitats appeared to be less favourable for *O. mykiss*, which is known to prefers clear, cool and well aerated waters and is limited by warm water temperatures (Skelton 2001). In addition, these species are visual predators, which renders the turbid mainstem sections of the Great Fish River unsuitable habitats (Laurenson and Hocutt 1986; Kadye and Booth 2013). It is, however, noteworthy that much of the sampling effort in recent surveys was concentrated more in lotic than lentic environments, the latter being the most preferred by *M. salmoides* (Skelton 2001; Van Der Walt et al. 2016). While *L. macrochirus* is tolerant of wide range of environmental conditions and is a generalist predator, it has nevertheless failed to establish. This species has a narrow habitat tolerance, preferring quiet and slow-flowing water with aquatic vegetation (Lee 1980; Skelton 2001). The upper and middle mainstem of the Great Fish River are characterised by constant flow (Laurenson and Hocutt 1986), which makes it less optimal for this species to establish, whereas the relatively slow-flowing lower section, which potentially have suitable habitats, is characterised by high turbidity. Although the IBWT created permanent and flowing habitats suitable for rheophilic species such as *A. sclateri*, its unsuccessful establishment in the Great Fish River could be attributed to both its scarcity and environmental requirement mismatch. *Austroglanis sclateri* has specific habitat requirements, preferring rapids and rocky habitats in flowing water (Niehaus 1996; Skelton 2001). In addition, the species is relatively rare in its natural distribution, especially near the Orange-Fish tunnel inlet in Gariep Dam (Niehaus 1996; Ellender and Weyl 2014). It is likely, therefore, that only a small number of individuals of this species invade the Great Fish River through the Orange-Fish tunnel, and these individuals are unlikely to sustain a viable population. However, because of habitat specificity for *A. sclateri* (i.e. fast flowing rocky habitats), there is need for concerted sampling effort particularly in the upper mainstem sections to ascertain whether this species is present. Currently, the high flow in habitats that are likely suitable for this species in the upper mainstem section of the Great Fish River precludes efficient sampling.

This study revealed that angling and IBWT were the primary invasion pathways for the non-native fishes in the Great Fish River. Specifically, the established angling taxa included two global invaders, *C. carpio* and *M. salmoides*, which are non-native in South Africa, and *T. sparrmanii*, which was reported to be present in the Great Fish River prior to the IBWT (Laurenson and Hocutt 1986). In comparison, three established species, *C. gariepinus*, *L. aeneus* and *L. capensis*, which are native to the Orange River system (Cambray et al. 1978), appear to have successfully invaded the Great Fish River through the IBWT. Laurenson and

Hocutt (1986) further indicated the need to explore the possible translocations of *L. umbratus* and *E. anoplus* populations from the Orange River into the Great Fish River, which historically were considered to likely represent different populations in the two rivers. Recent evidence indicated that the Orange River system comprise different lineages and species for these taxa, and partly highlighted some of Laurenson and Hocutt (1986)'s concerns. Specifically, recent molecular research on *L. umbratus*, which revealed that the Orange River and Great Fish River populations are distinct lineages, provided evidence of the occurrence of hybrids of these two lineages within the IBWT-transformed environments (Ramoejane et al. 2020, 2021). This indicates that the IBWT-facilitated successful translocation of the Orange River lineage of this species into the Great Fish River. This research, which evaluated mitochondrial (mtDNA) cytochrome *b* (cyt *b*) on *L. umbratus* from the Orange River and Great Fish River systems, recommended that the these genetically distinct lineages should be managed as separate evolutionarily significant units (ESUs) to avoid the loss of the species genetic diversity (Ramoejane et al. 2021). In comparison, the recent taxonomic revision of the *Enteromius anoplus* species complex revealed the occurrence of distinct species in the two rivers (Kambikambi et al. 2021). Specifically, this taxonomic revision, which revealed the occurrence of *E. mandelai* and *E. oraniensis* in the Great Fish and Orange rivers, respectively, found no evidence of the translocation of the latter into the Great Fish River (Kambikambi et al. 2021). In addition, although *E. oraniensis* occurs within the Gariep Dam (Ellender et al. 2010), it is relatively rare within the vicinity of the Orange-Fish IBWT inlet in Gariep Dam (Ellender and Weyl 2014). Therefore, very few individuals, if any, are likely translocated into the Great Fish River. The latter is supported by the absence of this species from recent extensive sampling of the Great Fish River system (Kambikambi et al. 2021).

To conclude, the opening of the Orange-Fish IBWT broke the geographic barrier that existed between the fishes of the Orange River and the Great Fish River systems. The IBWT provided an introduction pathway and facilitated the translocation of the Orange River fish species into the Great Fish River, in addition to modifying habitat within the latter system. This study showed that both intrinsic and extrinsic factors were important characteristics facilitating the establishment of non-native species within the Great Fish River. Almost 35 years later, seven species have established successfully within the Great Fish River system, six occurring mostly in the lotic habitats, with three of these invading through the Orange-Fish IBWT. The introduction and subsequent establishment of some non-native species, along with habitat modification due to change in flow regime, have altered species composition of this system. The establishment of these multiple non-native species present a great threat to the biodiversity of the Great Fish River through competition with, and predation on, native species, as well as through loss of genetic diversity. Future studies should aim to investigate mechanisms facilitating the co-existence of these multiple non-native species and their net effects within the Great Fish River.

CHAPTER 4

EVALUATING THE ROLE OF FUNCTIONAL DIVERSITY IN FACILITATING NON-NATIVE SPECIES WITHIN A FLOW-MODIFIED AFRICAN RIVER SYSTEM

Introduction

Species invasions and habitat modification are considered two of the greatest threats to native biota in freshwater ecosystems where they have the potential to disrupt native community structure and function (Dudgeon et al. 2006; Rahel 2007; Comte et al. 2016; Shuai et al. 2018; Ruhi et al. 2019). Furthermore, these two processes may interact synergistically to negatively impact native communities (Angulo-Valencia et al. 2022). An example where such interactions may occur is in river systems influenced by inter-basin water transfer schemes (IBWTs), which involve mass transfer of water between historically disconnected river systems. Globally, these IBWTs are considered a major cause of habitat modification and species invasions in aquatic environments (Nagelkerke et al. 2018; Schmidt et al. 2019). This is because, in addition to altering the physico-chemical conditions of the recipient ecosystems, mainly through changing their natural flow regime, IBWTs may inadvertently facilitate species translocations and successful establishments of non-native taxa into new environments (Laurenson and Hocutt 1986; Ellender and Weyl 2014). Often, IBWTs facilitate translocations of more than one species, thereby promoting co-occurrences of multiple non-native species, which raises concern over their potential impact within the invaded systems (e.g. Laurenson and Hocutt 1986; Nagelkerke et al. 2018; Kadye and Booth 2020).

Recent studies have highlighted how the impact of multiple co-occurring non-native species can be additive and may exert different pressures on recipient communities (Beggel et al. 2016; Balzani et al. 2020; Guareschi et al. 2021). For example, general studies on multiple non-native species have shown that their co-occurrence may lead to synergistic effects, in which positive interactions amongst invaders may facilitate their invasion success (Simberloff and Holle 1999; Simberloff 2006, 2011; Braga et al. 2018; Hohenadler et al. 2018). This may occur when the multiple non-native invaders have co-adapted and can jointly influence aspects such as distribution, abundance, and resource use patterns of the native biota (Ricciardi 2001). Synergistic interactions may have broader impact on the ecosystem by compounding the net effects of multiple non-native species on native communities, such as through altering ecosystem structure and function (Johnson et al. 2009; Jackson et al. 2014). Other studies have also shown that in certain systems with multiple non-native species, some single invaders may act as ecosystem engineers that exert strong interspecific interactions on aspects such as species-environment relationships, nutrient cycling and trophic interactions, which can result in ecosystem-wide effects on all species (De Vanna et al. 2011). Therefore, understanding mechanisms facilitating co-occurrence of multiple non-native species and their net effects within introduced communities is crucial for managing biodiversity in invaded ecosystems.

Historically, biodiversity patterns or species responses to habitat degradation and invasions have largely been assumed to be reflected by taxonomic diversity indices, such as through assessments of species richness and abundances (Jackson et al. 2001; Kuczynski et al. 2018). However, within the context of biological invasions, biodiversity patterns that reflect impacts ought to account for the ecological role of different species in the ecosystem (Villéger et al. 2011; Zhao et al. 2019; Takács et al. 2021), an aspect that is often missing when applying taxonomic diversity indices. To account for the ecological roles, empirical evidence has shown that species functional traits, which are the phenotypic attributes that strongly reflect their performance or fitness in a given environment (McGill et al. 2006; Weiss and Ray 2019), are robust in unravelling how species interact with their environment (i.e. fitness) and in showing linkages between ecological structure and ecosystem functioning (Kaushik et al. 2022).

Consequently, species functional traits have recently been used to understand community responses to disturbances, including changes in environment as well as the response to biological invasions (Ribeiro et al. 2016; Shuai et al. 2018; Lin et al. 2021; Angulo-Valencia et al. 2022).

The use of trait-based approaches, which broadly describe the functional diversity of communities, present a more relevant and better description of community patterns because these approaches consider both species functional traits and their role in an ecosystem (Mouillot et al. 2011; Kaushik et al. 2022). Thus, there has been an increase in the use of functional diversity in biological invasion studies to elucidate attributes associated with species invasions and their impacts, with most of these revealing that the success of invasive species into new environments is generally associated with specific functional traits (e.g. Thuiller et al. 2012; Shuai et al. 2018; Milardi et al. 2019; Takács et al. 2021; Angulo-Valencia et al. 2022; Kaushik et al. 2022). This is not unexpected as species' habitat associations and ability to exploit resource are generally determined by functional attributes that confer fitness and competitive advantage (Montaña and Winemiller 2010). Nevertheless, some studies have unravelled discrepancies regarding the most important traits in facilitating the establishment success of non-native fishes (e.g. Vila-Gispert et al. 2005; Grabowska and Przybylski 2015; Liu et al. 2017; Qin et al. 2020). Furthermore, the impacts of species invasions on local functional diversity appears to be context dependent. For example, while some studies have revealed that invasions are associated with general decrease in aspects of native functional diversity through the loss of native species with distinctive functional traits (e.g. Lin et al. 2021), other studies have shown that invasions were associated with either no change or positive effects on native functional richness (De Vanna et al. 2011; Zhao et al. 2019; Pereira et al. 2021). Therefore, identifying context-specific functional traits conferring potential advantage for non-native species in disturbed environments can better assist to inform conservation management of these ecosystems (Weiss and Ray 2019).

The Great Fish River in the Eastern Cape, South Africa, is characterised by multiple non-native fish species, mainly in the mainstem habitats (Laurenson and Hocutt 1986; Kadye and Booth 2020). Their co-occurrences with native species in these habitats raise questions on mechanisms facilitating these relationships. This is because these non-native species, which include global invaders such as common carp *Cyprinus carpio* Linnaeus, 1758, African sharptooth catfish *Clarias gariepinus* (Burchell, 1822) and mosquitofish *Gambusia affinis* (Baird and Girard, 1853), are likely to pose threat to the native biota of the Great Fish River. Previous research suggested that these non-native species likely form a functional assemblage that is sustained by anthropogenic flow disturbance caused by the Orange-Fish IBWT (Kadye and Booth 2020). Specifically, the Orange-Fish IBWT, which has altered the natural flow regime of the Great Fish River from seasonal to perennial, has been postulated to have promoted the successful establishment of some non-native species in this system (Weyl et al. 2009; Kadye and Booth 2013, 2020). However, it remains unclear how the species' intrinsic attributes contribute to these successful establishments and to the formation of non-native species functional assemblage as previously hypothesized. The overarching aim of this study was, therefore, to assess the role of functional diversity in facilitating the co-occurrence of these non-native fish species within the Great Fish River. Specifically, the present study sought to: (1) examine fish functional trait diversity using functional diversity indices and compare their variation between native and non-native fishes; (2) evaluate the relationship between species functional traits and the environment; and, (3) explore whether there were specific functional traits that could be used as predictors of species invasions. It was hypothesized that species functional traits would reflect broad differences in species, their distribution patterns and habitat associations within the Great Fish River system. It was further predicted that native and non-native fish species would have different functional trait diversity and exhibit different patterns within the species-trait-environment relationship.

Materials and methods

Data collection

A total of 67 sites were sampled from both the mainstem and tributaries of the Great Fish River, with each site being sampled once (Figure 2.1). At each sampling site, physico-chemical variables and habitat conditions were recorded. Fish were captured using variety of sampling methods. (Please refer to Chapter 2 for detailed description regarding the sampling of fish and environmental variables). Captured fish were identified to species using Skelton (2001), counted and either released back alive after recovering from handling stress or sacrificed for voucher specimens to be used for ecomorphological measurements. For the latter part, fish were sacrificed by bathing in an overdose of clove oil. Thereafter, fish were preserved by fixing in 10% formalin in the field and later transferred to 50% and then 10% ethanol in the laboratory.

Trait data collection

Eighteen functional traits that described two broad ecological categories (habitat use and feeding) were evaluated from 13 fish species, eight natives and five non-natives, following methods by Pease et al. (2012), Pessanha et al. (2015) and Santos et al. (2019) (Table 4.1). These traits, partitioned into 38 attributes (Table 4.1), were chosen because they have been shown to represent a wide range of functional diversity attributes, such as feeding preference, habitat use and swimming/locomotion in fishes (Winemiller 1991; Pease et al. 2012). The 38 attributes were divided into two sets of traits based on the scale of measurement. The first set consisted of traits, ratios, determined from standard linear measurements (SLM traits) of fish specimens (see Supplementary Table 2: Eleven functional ecomorphological traits (SML) used in the present study). The second set was populated from published regional literature (PRL traits) (i.e. from Skelton 2001) (Table 4.1). For SLM traits, a total of 101 specimens from 13 species, 4-12 specimens per species, were used for ecomorphological trait data (Table 4.2). The numbers of specimens used in the present study were selected to cover the full spectrum of the captured size classes and were largely determined by their availabilities. Some species were either endangered, were not captured from recent surveys or were rare and difficult to capture in the field. *Enteromius mandelai* and *S. bainsii* specimens were loaned from the South African Institute for Aquatic Biodiversity (SAIAB) located in Makhanda, South Africa. Digital callipers, 0.01 mm precision, and a dissecting microscope (Labomed CZM4, Labomed, USA) were used to record standard linear morphometric measurements from the left side of each specimen. For larger specimens, a combination of tape measure and callipers were used to record measurements.

Table 4.1. Eighteen functional traits evaluated from 13 species, eight natives and five non-natives. Traits and categories were adapted following methods by Pease et al. (2012), Pessanha et al. (2015) and Santos et al. (2019).

Source	Trait	category	Code
Standard linear measurements (SML)	Compression body index	Maximum body height / Maximum body width	CBI
	Relative body height	Maximum body height / Standard length	RBH
	Relative caudal peduncle length	Caudal peduncle length / Standard length	RCPL
	Caudal peduncle compression index	Caudal peduncle height / Caudal peduncle width	CPCI
	Relative head length	Head length / Standard length	RHL
	Relative eye position	Eye height / Head height	REP
	Relative mouth width	Mouth width / Maximum body width	RMW
	Relative mouth height	Mouth height/maximum body height	RMH
	Mouth aspect ratio	Mouth height/Mouth width	MAR
	Fineness coefficient	Standard length / √Maximum body height *Maximum body width	FC
Regional literature (RL)	Body size	Small (<250 mm)	BS1
		Medium (250 - 499 mm)	BS2
		Large (>500 mm)	BS3
	Diet	Omnivores	DT2
		Carnivores	DT3
		Detritivores	DT4
		Omnivores-detritivores	DT5
	Feeding strata	Water column	FS2
		Benthic	FS3
		Surface-mid water	FS4
		Surface-mid water-benthic	FS5
	Body morphology	Fusiform	BM1
		Compressed	BM2
		Elongated	BM4
		Moderate	BM5
		Depressed-elongated	BM6
		Fusiform-moderate	BM7
	Barbels	Present	BB1
		Absent	BB2
	Migration	Present	SM1
		Absent	SM2
	Mouth position	Superior	MP1
		Terminal	MP2
		Subterminal	MP3
	Temperature preference	Cooler(mean <24°C)	TP1
		Warmer (mean >24°C)	TP2
		Cool-warm	TP3

Table 4.2. Species, and their distributional statuses, used in ecomorphological trait analyses in the present study. Also reflected is their size (SL) in cm. * Species occurs naturally in both the Great Fish River and Orange River systems. SL and SD refer to standard length and standard error, respectively.

Species	***n***	**Mean SL (±SD)**	**Range SL**	**Status**
Anguilla mossambica	04	146.05 (±105.34)	86.92 – 303.74	Native
Clarias gariepinus	10	164.9 (±111.39)	53.61 – 285.85	Extralimital
Cyprinus carpio	12	172.36 (±144.53)	47.29 – 519.70	Exotic
Enteromius mandelai	10	42.88 (±10.97)	30.01 – 64.16	Native
Gambusia affinis	10	30.65 (±1.90)	26.77 – 32.58	Exotic
Glossogobius callidus	10	52.43 (±4.57)	43.44 – 59.80	Native
Labeo umbratus	06	111.74 (±64.32)	38.20 – 232.25	Native*
Labeobarbus aeneus	09	296.15 (±81.29)	162.88 – 394.98	Extralimital
Monodactylus falciformis	05	53.14 (±4.66)	47.98 – 59.55	Native
Pseudomyxus capensis	05	80.22 (±8.62)	72.07 – 93.53	Native
Oreochromis mossambicus	10	76.05 (±10.26)	59.65 – 93.66	Native
Sandelia bainsii	05	95.99 (±31.71)	62.06 – 128.18	Native
Tilapia sparrmanii	05	64.3 (±4.32)	57.85 – 68.61	Extralimital

Data analyses

To compare native and non-native fish species' functional diversity patterns, functional trait data were used to compute five functional diversity indices for each group (native and non-native fishes) using the function dbFD in the R package FD (Laliberté et al. 2014). These indices were: functional richness (FRic) index, which represents functional space occupied by the community in a trait space; functional evenness (FEve), which describes the evenness or regularity in species distribution in a trait space; functional divergence (FDiv), which reflects the divergence of species abundance from the centre of community functional trait space; functional dispersion (FDis), which represents individual species mean distance from the centroid of all species in a functional trait space; and, Rao's quadratic entropy (Rao), which uses functional trait dissimilarity to reflect relationship between species relative abundances and their paired distances (Villéger et al. 2008; Laliberté and Legendre 2010; Mouillot et al. 2013). Means and variances were compared, using *t*-test and Levene's test respectively, between native and non-native species for each of the five functional diversity indices. Assumptions of the *t*-test were based on Bartlett for equality of variance and Shapiro-Wilk test for normality of residuals. Where the assumptions were violated (i.e. $\alpha < 0.05$), the data were log-transformed (in this case, this was done for FEve values). To further explore functional trait variation among the native and non-native species, a factor analysis of mixed data (FAMD) was performed based on the eight categorial (PRL) and 11 quantitative (SML) trait variables using the function FAMD in the R package *FactoMineR* (Lê et al. 2008).

Species functional trait and environment relationships were evaluated using quantitative data on the environmental variables, species distributions and their ecomorphological traits. Because the environmental variables (Supplementary Table 1), primarily microhabitat and physico-chemical, were based on different scales of measurements, they were standardised prior to data analyses. Physico-chemical variables were transformed into *z*-scores (i.e.

$z = (x_i - \overline{x}_i / s_i)$ where x_i is the individual measurement of the variable, $\overline{x}_i$ and s_i are the variable mean and standard deviation, respectively. Physical habitat variables, in the form of proportional data (%), were arcsine-transformed. To test for multicollinearity among environmental variables, a generalised linear model was fitted and the variance inflation factor (VIF) for each variable was computed (Dormann et al. 2013). Only variables with a VIF value $\leq$ 5 were retained and used for subsequent analyses. Trait data consisted of a mixture of eight categorial (PRL) and 11 quantitative variables (SML) (Table 4.1). Categorical trait variables (PRL) were coded as factors, whereas the quantitative variables (SML) were standardised ratios of measured morphological traits (see Supplementary Table 3: Trait data for the 13 species used in the trait-environment association analyses). Because of differences in their ranges, the 11 quantitative trait variables (SML) were [log(x +1)] transformed before analyses. Because different fish capturing methods were used in this study, species data were based on presence/absence records of fishes at each sampling site.

The RLQ and fourth-corner analyses were used to investigate the relationships among species distributions, their functional traits and environmental variables (Dolédec et al. 1996; Legendre et al. 1997). The RLQ evaluates main co-structures between traits and environmental variations while fourth-corner method provides test for the significance of the bivariate relationships (Dray et al. 2014). These approaches are considered optimal solutions to the assessment of trait-environment relationships because, unlike traditional methods that use a two-step analysis, they provide direct assessment of trait-environment relationships by analysing species, environmental and species trait data simultaneously (Dolédec et al. 1996; Dray and Legendre 2008; Brind'Amour et al. 2011; Santos et al. 2019). Both analyses use three input matrices (R, L and Q), which, respectively, represent environmental variables, species response and species traits. The first matrix (R) contained environmental variables for each site. The second matrix (L) was based on the presence/absence data of species at each site. The last matrix (Q) described trait data of species and this was based on functional traits for both the SLM and PRL attributes. Matrix R was ordinated by PCA on standardised environmental data. Matrix L was ordinated by correspondence analysis (CA) on species absence/presence data, whereas the L matrix was ordinated by Hill-Smith ordination (HS) on a mixture of qualitative and standardised quantitative variables. Significance testing of ordinations was performed using Monte Carlo randomisation test at $\alpha = 0.05$ (×999 permutations). The RLQ and fourth-corner analyses were performed using functions rlq and fourthcorner, respectively, in the R package *ade4* (Dray and Dufour 2007). All statistical analyses were performed in R program version 4.2.2 (R Core Team 2022). Statistical significance was considered at $p < 0.05$.

Results

Non-native fishes were mostly distinguished by having higher values for functional dispersion (FDis) and Rao's quadratic entropy (Rao), and lower values for functional richness (FRic) than native fishes (Figure 4.1). However, there were no significant differences in the mean values for most functional diversity indices (*t*-test, $p > 0.05$) between native and non-native species except for functional evenness (FEve) whereby native species had higher FEve compared to non-native species (*t*-test, $p = 0.02$) (Figure 4.1, Table 4.3). Similarly, there were no significant differences in the variances for most diversity indices except for functional evenness whereby non-native species were more variable than native species (Levene's test, $F_1 = 12.45$, $p = 0.002$) (Figure 4.1, Table 4.3). The first two FAMD axes explained approximately 38% of variation in the functional traits among species (Figure 4.2). The first axis largely reflected differences in functional traits such as body depth, eye size and mouth height. Specifically, laterally compressed and deep bodied species with big eyes, such as *Monodactylus falciformis* Lacepède, 1801, *Oreochromis mossambicus* (Peters, 1852), *S. bainsii* and *T. sparrmanii* had high and positive scores on the first axis, whereas more elongated and cylindrical species, such as *Anguilla mossambica* (Peters, 1852), *Glossogobius callidus* (Smith, 1937) and *C. gariepinus* had high negative scores on this axis. The second axis appeared to differentiate species largely based on mouth aspect ratio, mouth position and preferred feeding position along the water column. This axis distinguished *G. affinis* from the rest of the species. Broadly, however, there was no discernible distinction in functional traits between non-native and native species, except for *G. affinis* (Figure 4.2).

Table 4.3. Functional diversity indices' results for native and non-native fishes of the Great Fish River. *Significant difference at $p < 0.05$.

Functional indices	**Sample sizes**		**Levene's test**			**T test**		
	native	**non-native**	**df**	***F***	***p*-value**	**df**	**t value**	***p*-value**
Functional richness (FRic)	15	5	1,18	0.54	0.47	18	-0.02	0.99
Functional evenness (FEve)	15	5	1,18	12.45	0.002*	18	2.46	0.02*
Functional divergence (FDiv)	15	5	1,18	0.009	0.93	18	-0.22	0.83
Functional dispersion (FDis)	26	12	1,36	5e-04	0.98	36	-1.75	0.09
Rao's quadratic entropy (Rao)	26	12	1,36	0.02	0.89	36	-1.75	0.08

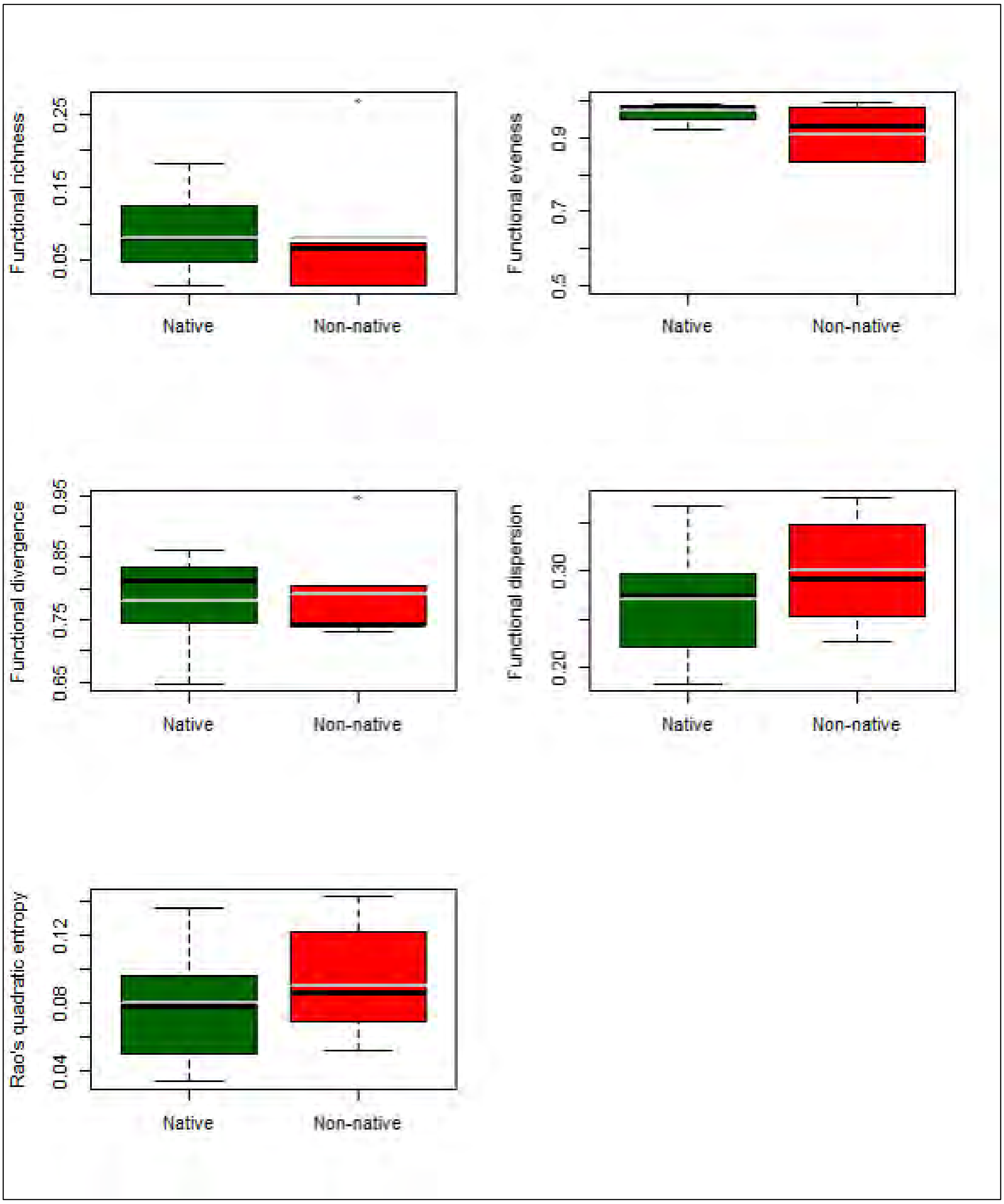

Figure 4.1. Functional diversity indices for native (green) and non-native (red) fishes in the Great Fish River (see Table 4.3 for statistical results). In the box plots, the black and grey lines indicate the median and the mean, respectively.

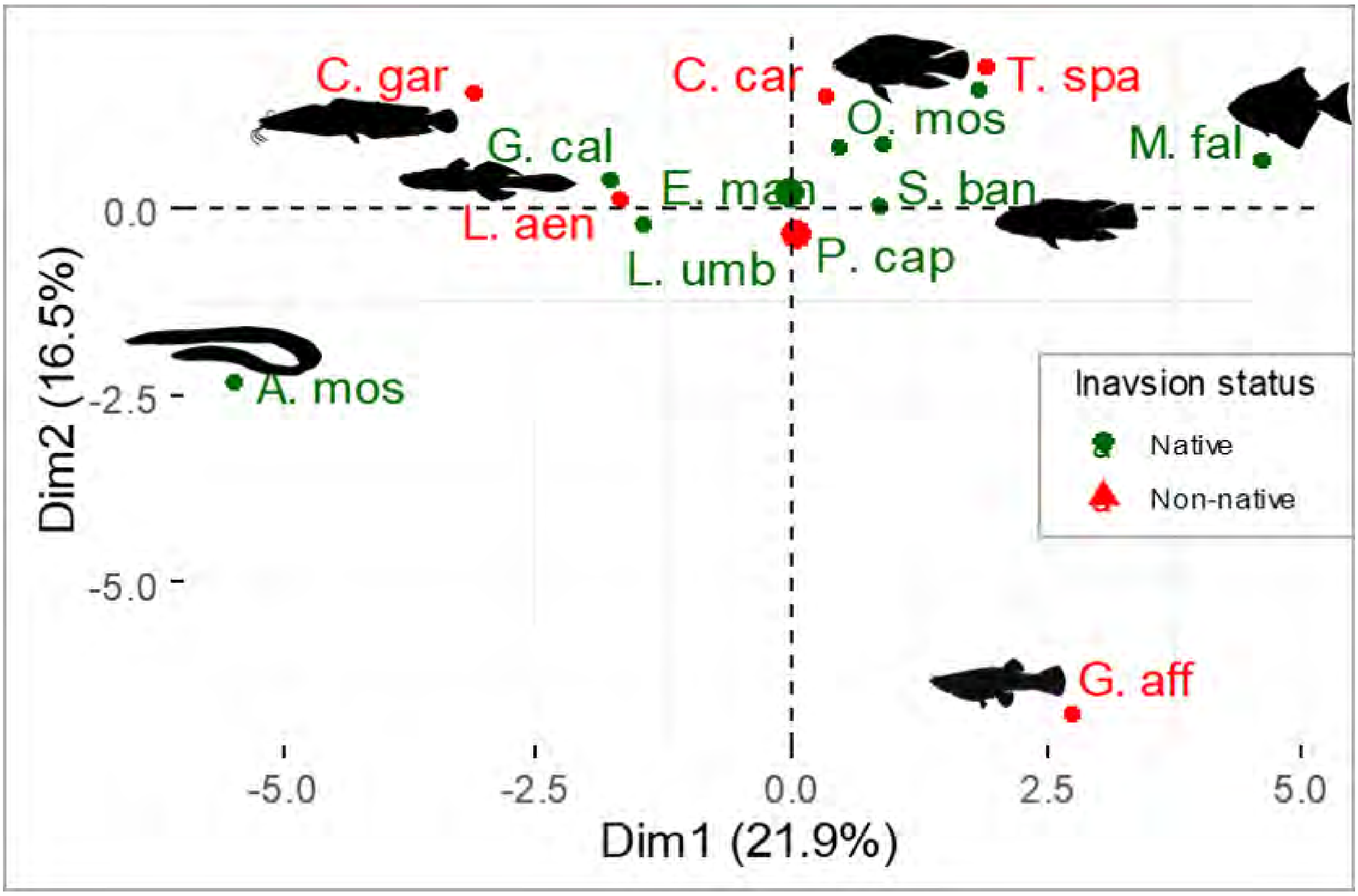

Figure 4.2. Trait-ordination space based on species functional ecomorphological traits. A. mos = *Anguilla mossambica*, C. gar = *Clarias gariepinus*, C. car = *Cyprinus carpio*, E. man = *Enteromius mandelai*, G. aff = *Gambusia affinis*, G. cal = *Glossogobius callidus*, L. umb = *Labeo umbratus*, L. aen = *Labeobarbus aeneus*, M. fal = *Monodactylus falciformis*, O. mos = *Oreochromis mossambicus*, P. cap = *Pseudomyxus capensis*, S. ban = *Sandelia bainsii*, and T. spa = *Tilapia sparrmanii*.

The first two axes of the RLQ analysis explained 87.74% (axis 1 = 72.25%, axis 2 = 15.49%) of the covariance among species functional traits, environmental factors, and species distribution data (Table 4.4). The RLQ analysis indicated that species distribution was correlated with the variation in the environment (model 2, $p < 0.01$), but was independent on functional traits (model 4, $p = 0.87$) (Table 4.4). These findings showed a weak correlation between species functional traits and the environmental variables. The first axis (72.25%) was negatively associated with stream width, depth and TDS and positively associated with altitude (Figure 4.3). Based on the environmental factors (R) scores, this axis distinguished the mainstem from the tributaries. Specifically, this pattern was reflected by a positive environmental factor score for altitude that represented headwater tributaries, whereas negative scores for large stream/river width, deep habitats and high total dissolved solids represented mainstem sections. The trait (Q) scores depicted that for RLQ axis 1, traits such as low temperature preference (TP1) and moderate body morphology (BM5) had positive scores, and these traits were associated with species such as *E. mandelai*, *S. bainsii* and the non-native *T. sparrmanii* that generally occurred in the headwater streams and the major tributaries (Figure 4.3). By comparison, traits with negative scores along RLQ axis 1 included medium body size (BS2), superior mouth (MP1) and feeding on water column (FS2), which were consistent with species that occurred in the mainstem, such as *G. affinis*, *M. falciformis, O. mossambicus* and *Pseudomyxus capensis* (Valenciennes, 1836). Furthermore, this axis had negative scores on traits such as large-sized individual (BS3), subterminal mouth (MP3), benthivory (FS3) and detritivory (DT4 and DT5). These traits were associated with species such as *A. mossambica, C. gariepinus, L. aeneus* and *Labeo umbratus* (A. Smith, 1841). The second RLQ axis (15.49%) separated the species along the longitudinal gradient of the mainstem, with positive scores being associated with lower sections and negative scores depicting the upper sections. The lower sections, which had relatively wider stream widths, with boulders forming the large part of the substrata, were distinguished by the presence of secondary freshwater species, such as *G. affinis*, *G. callidus, M. falciformis, O. mossambicus and P. capensis*. The upper mainstem sections were characterised mainly by the presence of both native and non-native primary freshwater fishes which included *C. carpio*, *C. gariepinus*, *L. aeneus and L. umbratus*. The results of the fourth-corner analysis revealed no significant associations ($p > 0.05$) between species traits and environmental variables (see Supplementary figure 1: Results of fourth-corner analysis revealing no significant association between species functional traits and environmental variables).

Table 4.4. Summary of the RLQ analysis showing the first two axes extracted. The association between species distribution and environment is tested by model 2, whereas the relationship between species distribution and functional traits is tested with model 4.

Total inertia	6.884				
Model 2	$p = 0.001$				
Model 4	$p = 0.866$				
Eigen values	**Axis 1**	**Axis 2**			
	4.97	1.07			
Projected inertia(%)	**Axis 1**	**Axis 2**			
	72.26	15.49			
Cumulative inertia (%)	**Axis 1**	**Axis 2**			
	72.25	87.74			
Eigenvalues	Eig	Covariance	sdR	sdQ	Correlation
eig 1	4.97	2.23	1.81	1.87	0.66
eig 2	1.07	1.03	1.35	2.13	0.36
Inertia and coinertia R	Inertia	Max	Ratio		
eig 1	3.28	3.49	0.94		
eig 12	5.10	5.87	0.87		
Inertia and coinertia Q	Inertia	Max	Ratio		
eig 1	3.51	6.96	0.50		
eig 12	8.06	11.34	0.71		
Correlation L	Correlation	Max	Ratio		
eig 1	0.66	0.88	0.75		
eig 2	0.36	0.68	0.53		

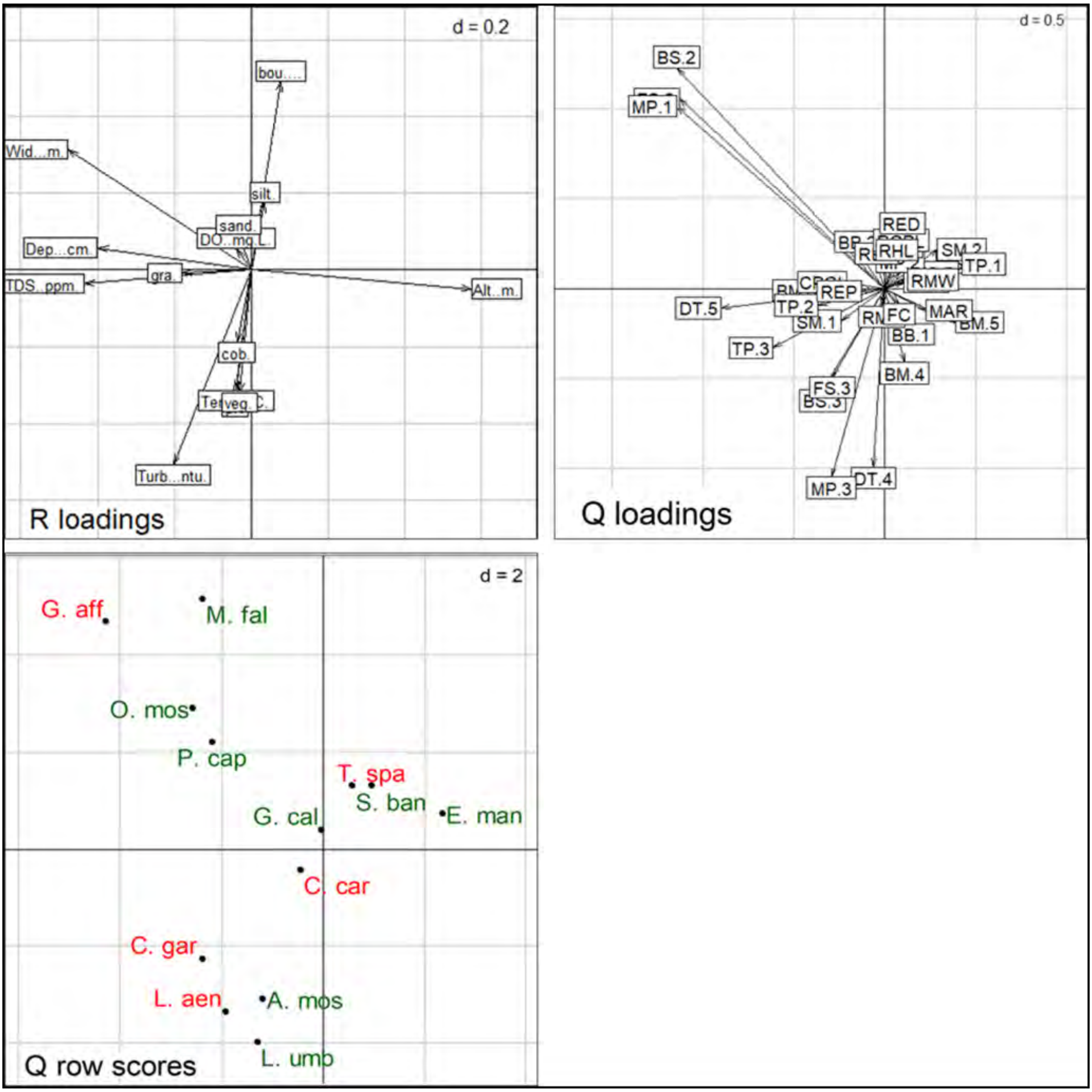

Figure 4.3. Plots of RLQ results; showing environmental (top left), trait (top right) and species ordinations (bottom left). Green and red colours indicate native and non-native species, respectively. A. mos = *Anguilla mossambica*, C. gar = *Clarias gariepinus*, C. car = *Cyprinus carpio*, E. man = *Enteromius mandelai*, G. aff = *Gambusia affinis*, G. cal = *Glossogobius callidus*, L. umb = *Labeo umbratus*, L. aen = *Labeobarbus aeneus*, M. fal = *Monodactylus falciformis*, O. mos = *Oreochromis mossambicus*, P. cap = *Pseudomyxus capensis*, S. ban = *Sandelia bainsii*, and T. spa = *Tilapia sparrmanii*.

Discussion

Species functional traits can be used to better understand fish assemblage structure (Pease et al. 2012; Walsh et al. 2022), quantify the trait-environment relationships (Brind'Amour et al. 2011; dos Santos et al. 2017; Santos et al. 2019) and assess the response of ecosystem to disturbances, including the introduction of non-native species into novel environments (Dala-Corte et al. 2016; Lin et al. 2021; Miiller et al. 2021; Angulo-Valencia et al. 2022). Consistent with the first hypothesis that functional traits would reflect broad differences in species and their habitat associations within the Great Fish River system, the results of the present study revealed evidence of interspecific variation in functional traits, which appeared to reflect spatial differences in the distribution patterns and species-environmental relationships. Specifically, at the assemblage level, multivariate ordination revealed broad differences among species based on ecomorphological functional traits, such as body depth, eye size and mouth height. From a species-trait-environment relationship perspective, results revealed spatial differences that distinguished high latitude tributary habitats that were characterised by the occurrence of species such as *E. mandelai*, *S. bainsii* and *T. sparrmanii*, which depicted traits such as moderate body morphology and affinity for low temperatures. By comparison, mainstem habitats, which had wider and deeper habitats with high turbidity, reflected the occurrence of two species groups. The first group comprised species that occurred in the upper mainstem section. These were primary freshwater taxa, such as *A. mossambica, C. carpio, C. gariepinus, L. aeneus* and *L. umbratus*, which depicted traits such as large body size, benthic feeding, detritivory, and subterminal mouth position. The second group comprised species that occurred in the lower mainstem section. This group was dominated by secondary freshwater species such as *G. affinis, G. callidus, M. falciformis, O. mossambicus* and *P. capensis* that were represented by traits such as medium body size, superior mouth position and feeding mainly along the water column.

In contrast to the second hypothesis that native and non-native fish species would have different functional trait diversity, the results of the present study revealed no discernible differences in functional traits between non-native and native fishes. This study showed that non-native fishes were present in different sections of the invaded range where they co-occurred with native species that appeared to share similar functional traits. Specially, no differences in functional diversity indices were found between these two groups except for functional evenness (differences in both means and variances), which revealed that non-native species had lower functional evenness and were characterised by functional traits that were more irregularly distributed compared to native species. This suggests that the non-native species group comprised taxa that were more functionally dissimilar compared to native fishes. However, the observed pattern is most likely due to *G. affinis,* which was more variable compared to other non-native species. Furthermore, results from RLQ ordination revealed that despite broad spatial patterns in species-trait-environmental relationships, native and non-native fishes co-occurred in different invaded sections of the system, suggesting the likely importance of shared functional traits among taxa from the different groups. For example, some sections of major tributaries were characterised by the presence of non-native *T. sparrmanii* and the two native species, *S. bansii* and *E. mandelai* that shared some functional traits such as moderate body morphology and affinity for low temperatures. Similarly, the upper mainstem section of the Great Fish River were dominated by three non-native fishes, *C. gariepinus*, *L. aeneus* and *C. carpio,* which co-occurred with *L. umbratus* and *A. mossambica*. The most important shared traits among these taxa included large body size, benthic feeding, detritivory, and subterminal mouth position. The findings from this study are in contrast to other studies that have found significant differences in functional traits between native and non-native species in fish communities (Shuai et al. 2018; Angulo-Valencia et al. 2022). Successful establishments of non-native species in other aquatic environments have been linked to their functional differences with native species (Olden et al. 2006; Liu et al. 2017; Qin et al. 2020). The results of the present study, which found no evidence of differences in functional traits between the native and non-native species, may therefore suggest that functional traits used in the present study cannot account for establishment success of non-native fishes within the Great Fish River. These findings can also mean that other functional traits beside the ones used in this study may be responsible for successful establishment of non-native species. Overall, these findings may suggest that, at an assemblage level, there was a general absence of specific ecomorphological traits that distinguished non-natives and native species within the Great Fish River.

The potential for shared functional traits between *T. sparrmanii* and *S. bainsii*, such as moderate body morphology and affinity for low temperature, is of major concern because this could result in negative interspecific interactions for resources, such as potential prey and habitat associations if the two species do co-occur. *Sandelia bainsii* is an endangered species comprising three allopatric lineages (Chakona et al. 2020), with the Great Fish River lineage showing evidence of range reduction in invaded habitats (Sifundza et al. 2021). Despite co-occurring in some tributaries, particularly in the Koonap River and sections of the Kat River, evidence from this study suggests that *T. sparrmanii* has not extended its range into most habitats where *S. bainsii* occurs. Therefore, the tributaries within which *S. bainsii* occurs, which are already designated as sanctuary for this species under the South Africa’s National Freshwater Ecosystem Priority Areas (NFEPA) (Nel et al. 2011), should continue to be prioritised to mitigate against invasion by *T. sparrmanii*. In addition to potential interspecific interactions, there is concern that the presence of non-native fishes, particularly piscivores such as *C. gariepinus,* likely compromises the dispersal ability of *S. bansii* by limiting connectivity among different populations and therefore hindering gene flow (Chakona et al. 2020). The other major concern involves the co-occurrence and sharing of functional traits between the native *A. mossambica* and *L. umbratus* with the three non-natives *C. carpio, C. gariepinus* and *L. aeneus* in the upper mainstem sections, as this has the potential to lead to competitive interaction amongst these species. In addition to competing with native species, the co-occurrence of non-natives *C. carpio* and *C. gariepinus* has the potential to cause deleterious impacts within the Great Fish River. For instance, *C. carpio* and *C. gariepinus* are known to cause ecological impacts in systems in which they invaded, largely due to their benthivorous and predatory feeding behaviours (Gehrke et al. 1999; Zambrano et al. 2006; Kadye and Booth 2012a; Weyl et al. 2016). However, it is likely that interspecific interactions between these two groups may likely be ameliorated by potential resource opportunities resulting from the opening of the Orange-Fish IBWT, such as the creation of new and permanent habitats in the mainstem. Studies elsewhere have shown that disturbances that result in high resource availability or resource opportunity may facilitate the coexistence of different fish functional groups (Cardoso et al. 2019).

Despite the general broad spatial patterns, there were no significant species-trait-environmental relationships in this study. Specifically, the results of the fourth-corner analyses, which indicated non-significant species-trait-environmental relationship, suggest that species functional traits were not major drivers of fish community structure in the Great Fish River. These findings are in contrast to other studies that have found significant associations between environmental variables and fish species traits, highlighting the role and importance of species functional traits in shaping fish community structure in aquatic environments (Erős et al. 2009; Brind'Amour et al. 2011; Pease et al. 2012; Ribeiro et al. 2016; Miiler et al. 2021). The lack of significant trait-environment association in the present study may suggest that environmental filtering was a weak determinant of functional trait structure (Asefa et al. 2017), and that functional traits used in this study were a poor indicator of resource use patterns for fishes of the Great Fish River. The results may further suggest that new niche opportunities created by the change in natural flow regime do not favour any particular suite of species traits within this system. Changes in natural flow regime of rivers have been suggested to weaken the relationship between species traits and environment. For example, in some systems, natural flow regimes have been associated with limited persistence of non-native species (Marchetti and Moyle 2001; Ellender et al. 2011), whereas changes in natural flow have been found to promote invasions by limiting invasion resistance of recipient communities (Moyle and Light 1996a). In the present study, the poor trait-environment association could be attributed to weaker environmental filtering resulting from a change in natural flow regime of the mainstem. Natural flow regime is an important abiotic factor in aquatic environments and has been linked to species life-history processes in stream ecosystems (Bunn and Arthington 2002; Lytle and Poff 2004; Comte et al. 2021). In addition, disturbances usually result in resource release (Shea and Chesson 2002). In the same way, change in natural flow regime of the Great Fish River could have resulted in resource opportunities within this system. It is likely, therefore, that the change in natural flow regime may have created new resource opportunities, including feeding and large permanent habitats that likely favour the proliferation of the multiple non-native species within the mainstem sections of the system.

In conclusion, this study provided evidence of interspecific variation in ecomorphological traits among all fish species within the Great Fish River system. Although the non-native and natives exhibited some differences in aspects of functional diversity, there was no clear assemblage distinction in both functional diversity and ecomorphological traits between these two groups. Furthermore, this study found weak species-trait-environment relationships, suggesting that environmental filtering may be a poor predictor of functional relationships within this system. Consistent with previous research and studies elsewhere (e.g. Schmidt et al. 2019; Kadye and Booth 2020), it is likely that the anthropogenic modification of this river system, particularly through altered flow, may have resulted in a functional assemblage that has acclimatised to these conditions. This assemblage appears to largely reflect ichthyofauna groups with shared functional traits in different invaded sections, including the tributaries where non-native *T. sparrmanii* co-occurs with native species, the upper and lower mainstem sections where most non-native species co-occur with native species. Thus, functional relationships observed in this study likely reflect species responses to the prevailing environmental conditions of this system.

CHAPTER 5

CAN TROPHIC DIVERSITY AND NICHE PATTERNS EXPLAIN CO-OCCURRENCES OF MULTIPLE NON-NATIVE FISHES IN A HUMAN-DISTURBED RIVER SYSTEM?

Introduction

Biological invasions and habitat degradation have the potential to undermine community structure and function of freshwater ecosystems (Collen et al. 2014; Comte et al. 2016). This is particularly so for freshwater ecosystems that are vulnerable to invasions, such as those invaded by keystone non-native species (Ligtvoet et al. 1991; Johnson et al. 2006; Ficetola et al. 2007; De Vanna et al. 2011), characterised by high local endemism and low invasion resistance (Tedesco et al. 2012; Collen et al. 2014; Weyl et al. 2014; Jordaan et al. 2020), characterised by the occurrence of multiple non-native invaders (Jackson 2015; Pyšek et al. 2020; Guareschi et al. 2021) and subject to high habitat-related anthropogenic modifications (Laurenson and Hocutt 1986; Ruhi et al. 2016; Liu et al. 2017). Understanding how non-native species integrate and impact the invaded environments is therefore crucial for managing species invasions in these ecosystems (Comte et al. 2016). One way to understand this is by exploring trophic patterns and energy flow among native and non-native species (Copp et al. 2017). From a trophic ecology perspective, non-native species have the potential to disrupt community structure and function either directly through predation and competition (Britton et al. 2010; Kadye and Booth 2012a; Bašić et al. 2019; Rogosch and Olden 2020; Murphy et al. 2021) or indirectly by negatively influencing aspects of native biota’s life-history attributes, including growth and reproduction (Britton et al. 2010; de Araújo et al. 2022), and ecological attributes such as habitat associations and behaviour of the native taxa (Vander Zanden et al. 1999; Kadye et al. 2020).

Classic research on the influence of non-native taxa on the trophic structure and function of recipient ecosystems has provided some important insights on invasion impacts. Examples from these studies include: the near collapse of the lentic food web in Lake Victoria, East Africa, following the dramatic biomass reduction and localised extirpation of haplochromine cichlids due to predation by the introduction of the Nile perch *Lates niloticus* (Witte et al. 1992; Downing et al. 2012); the competitive exclusion of kokanee sockeye salmon *Oncorhynchus nerka* by introduced opossum shrimp *Mysis diluviana* in the Flathead Lake, Montana, USA, which led to the decrease in the biomass of the former and the associated ecosystem-wide negative impacts on other predators, such as birds and bears that relied on salmon for prey (Spencer et al. 1991); and the trophic cascade-induced changes in primary production and algal biomass due to heavy predation on grazing invertebrates by non-native salmonids and high nutrient cycling by crayfish in lotic habitats in Australia, New Zealand and the USA (Crowl et al. 1992; Flecker and Townsend, 1994; Nyström et al. 2001; Herbst et al. 2009). Recently, there have been increasing concerns on the co-occurrence of multiple non-native species and their potential impacts on recipient freshwater ecosystems' trophic dynamics (Johnson et al. 2009; Jackson and Britton 2013; Sagouis et al. 2015; Liu et al. 2018). Specifically, there has been concern on the interactions among non-native invaders, which could potentially result in either independent additive or facilitative and complementary effects on the structure and function of food webs in invaded systems (Jackson et al. 2014; Jackson 2015). This is because multiple invasive species occurring in sympatry can have broader ecological impact within invaded communities due to the potential of such species to occupy multiple or different trophic levels (Johnson et al. 2009; Jackson and Britton 2013). Similarly, non-native species with high diet plasticity and/or broad trophic niches, such as opportunistic omnivorous and generalist predators, could also prey on native organisms across multiple trophic levels thereby exacerbating their impacts within the invaded communities (Olsson et al. 2009; Jackson and Britton 2013; Liu et al. 2018). Other studies have shown potential synergistic impacts among invaders. Examples of such studies include the facilitation of non-native bullfrog *Lithobates catesbeiana* by non-native bluegill *Lepomis macrochirus* due to the reduction of the biomass of predatory invertebrates thereby enhancing the survival of tadpoles (Adams et al. 2003), and the predation by non-native crayfish *Orconectes rusticus* on native snails (*Physa* and *Lymnaea* spp.), which potentially facilitated the establishment of non-native snail *Bellamya chinensis* due to a reduction in competitive interactions between the native and non-native snails (Johnson et al. 2009). Despite the emerging evidence on the impacts of multiple invasions (Jackson and Britton 2013; Sagouis et al. 2015), what's unclear is the broad nature of complex trophic interactions amongst co-occurring non-native species outside their native ranges since these interactions are likely to be context-dependent (Jackson 2015). For example, there have been reports of different interspecific interactions, ranging from overt predation and competition impacts (Liu et al. 2018), weak or no negative effect on one another (Griffen et al. 2008; Johnson et al. 2009) to non-native species facilitating each other's establishment success (Hohenadler et al. 2018). Therefore, understanding these complex interactions could assist to mitigate the impacts of co-occurring multiple non-native species on native communities (Liu et al. 2018; Balzani et al. 2020).

Stable isotopes have become an important tool to examine community trophic or food web structures (Layman et al. 2007; 2012), and in assessing the ecological impacts of anthropogenic environmental changes (Cucherousset et al. 2012a; Alp and Cucherousset 2022). Specifically, because they provide temporally-integrated assessment of consumer diet (Hershey et al. 2007), stable isotope analyses (SIA) of $\delta^{13}C$ and $\delta^{15}N$ can be used to assess the energy sources and trophic positions of organisms, respectively (Post 2002; Anderson and Cabana 2007; Vander

Zanden et al. 2016). Thus, they can be used to trace energy flow from basal sources (primary producers and detrital sources) through primary to secondary and tertiary consumers (Hershey et al. 2007; Layman et al. 2012). Consequently, stable isotope analyses have been extensively used to evaluate biological invasions-related trophic ecology of aquatic organisms in freshwater ecosystems (Vander Zanden and Fetzer 2007; Jackson et al. 2020), including the assessment of trophic interactions between co-occurring native and non-native fishes (Pennock et al. 2021; Top-Karakuş et al. 2021) and broad trophic impacts of these introductions on recipient communities (Vander Zanden et al. 1999; Kadye and Booth 2012a; Cucherousset et al. 2012aa; Fink and Harrod 2013; Jackson and Britton 2013; Bašić et al. 2019). In addition to providing robust estimates of trophic interrelationships, applications of SIA have recently been extended to provide quantitative assessments of stable isotope-based trophic and functional diversity indices, which facilitate comparisons of food webs among different ecosystems (Layman et al. 2007; Cucherousset and Villéger 2015; Rigolet et al. 2015; De Cáceres et al. 2019). Besides the general applications of these diversity indices to assess the community structure and functioning of freshwater ecosystems (Comte et al. 2016; Frossard et al. 2020), they have also been used to evaluate the role of anthropogenic disturbances, including the impact of biological invasions within these ecosystems (Cucherousset and Villéger 2015; Jackson et al. 2020).

The Great Fish River, South Africa, has become synonymous with the establishment of multiple non-native fishes and habitat modification due to the opening of the Orange-Fish inter basin water transfer scheme (IBWT), which transfer water from the Orange River (Gariep Dam) into the Great Fish River system. Previous research on species-environment relationships indicated that the non-native species assemblage was characterised by taxa with high environmental tolerance, suggesting propensity towards niche opportunism (Kadye and Booth

2020). The previous chapter (Chapter 4) further revealed that the multiple non-native fish species within this river system exhibited unclear functional morphological patterns that were inconsistent with environmental niche partitioning. It was, therefore, postulated that these co-occurring non-native fishes were likely to reflect trophic niche partitioning as a probable mechanism that facilitate their potential coexistence. The present study used SIA-based approaches, as proxy for trophic ecology, to ascertain trophic diversity and niche patterns of non-native and native fish species within the Great Fish River system. To achieve this, firstly, this study provided a broader overview of the Great Fish River system's food webs. Specifically, this study identified sections that were invaded by non-native fish species and used these to investigate isotopic diversity patterns of non-native and native fishes. Secondly, this study explored the isotopic niche patterns of non-native fishes to investigate whether trophic niche patterns were a probable driver of resource utilisation within this river system. Due to the variable ecomorphological traits exhibited by different species (see Chapter 4), it was hypothesized that native and non-native fish assemblages would have high trophic functional diversity patterns that likely reflected broad resource use. It was further hypothesized that due to the likely propensity towards niche opportunism for non-native fishes (Kadye and Booth 2020), these species would exhibit broad trophic diversity patterns and would be characterised by low niche overlap that would reflect potential trophic niche partitioning.

Materials and methods

Data collection and sample preparation

Sampling was done to collect carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope data in the Great Fish River mainstem, and its major two tributaries, the Kat and Koonap rivers (Figure 5.1a, Supplementary Table 1). In the Great Fish River mainstem (hereafter Great Fish River), sampling was conducted during summer from October 2009 to April 2010, and October 2014 to March 2015. In the Koonap and Kat rivers, sampling was done in October 2018 to March 2019. Samples for stable isotope analysis were collected from fishes and all potential prey that included macroinvertebrates and basal sources (Supplementary Table 4). Fishes were collected using variety of sampling methods (fish sampling protocols and methods are outlined in Chapter 2). Fish were identified to species level using regional literature. A sample of a maximum of 15 individuals per site was euthanised by a lethal dose of clove oil after which a small piece of the dorsal muscle tissue was taken using a clean scalpel blade for stable isotope analysis, and the samples were transferred to Eppendorf tubes for storage. For *Sandelia bainsii,* which is endangered, a small piece of the anal fin was taken using a clean pair of scissors.

Macroinvertebrates were collected from instream substratum and submerged vegetation. At each sampling site, stream substrates were disturbed by kick sampling, two minutes per sample, and samples were collected using a 250 µm hand-held scoop net, positioned downstream of the sampling site. The net was then progressively moved upstream to catch dislodged macroinvertebrate samples. Additional samples were collected by brushing and rinsing pebbles and macrophytes into the net. More samples were collected from riparian vegetation, which comprised C_3 and C_4 plants, by hand. Collected macroinvertebrate samples were rinsed with distilled water and then transferred into a collection tray for sorting. Coarse particulate matter (CPOM), which included large organic matter debris, was either handpicked or dislodging from the substratum and filtered through a hand-held net. Fine particulate organic matter (FPOM) was collected by filtering 25 litres of stream water through a 100 µm net. Epilithic algae were scrapped from coarse substratum (boulders and bedrock) with a scalpel blade and rinsed with distilled water. Free-floating filamentous algae, macrophytes and other organic matter were collected by hand, rinsed and transferred into collection bottles.

All samples for stable isotope analysis were kept on ice in the field and transported to the laboratory, Rhodes University, Makhanda, South Africa, for further processing. For macroinvertebrates, a reference sample was stored in 70% ethanol.

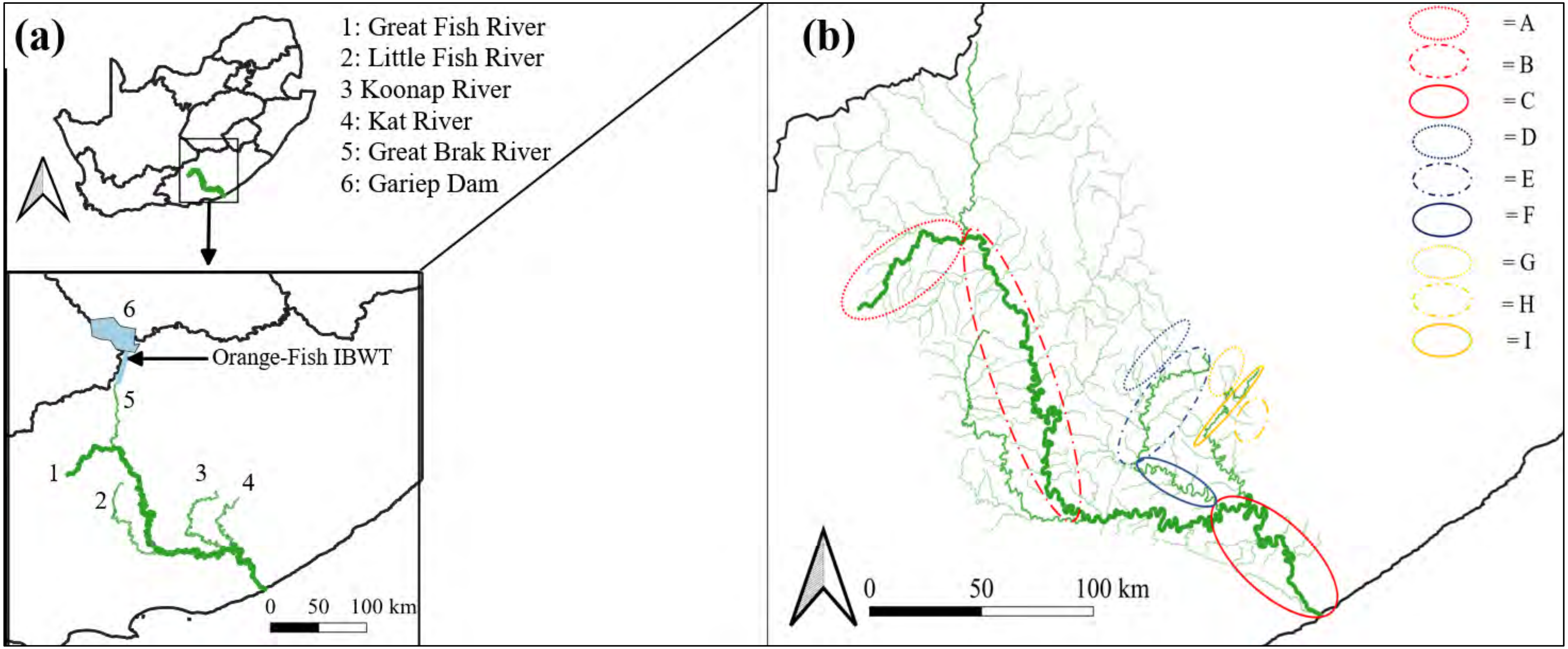

Figure 5.1. The Great Fish River system and its major tributaries (a). The study system was delineated into nine sections (A – I) based on the presence or absence of non-native species (b) (see text below for full details). A = The Great Fish River headwaters, B = the upper Great Fish River (UGFR), C = The lower Great Fish River (LGFR), D = The Koonap River headwater tributaries, E = the Koonap River upper mainstem, F = The lower Koonap River, G – H = the Kat River headwater tributaries and I = the Kat River upper mainstem section.

At the laboratory, all samples of macroinvertebrates were separated into sites, thawed and then identified to either generic or the lowest taxonomic level possible using a dissecting microscope (Labomed CZM4, Labomed, USA) and regional identification guides (Day et al. 2001a,b; Day and De Moor 2002a,b; Day et al. 2002; Gerber and Gerber 2002; Day et al. 2003a,b). All samples, grouped as species per site, were thawed and oven-dried at 60°C for 48-72 hours after which they were ground into a fine homogenous powder, using a pestle and mortar. Plant material, grouped as either C_3 or C_4 plants, were also thawed, dried and ground into a fine powder under similar conditions for macroinvertebrates explained above. The sample material was weighed (1 ± 0.05 mg for animal tissue and 3 ± 0.5 mg for plant tissue) and packed into 8 × 5 mm tin capsules. Stable isotope analysis was done at the Rhodes University and the University of Pretoria, South Africa. At Rhodes University, stable isotope analysis of carbon and nitrogen was performed using Europa Scientific INTEGRA isotope ratio mass spectrometer at the IsoEnvironmental Lab, Makhanda. At the University of Pretoria, the isotopic analysis was done on a Flash SEA 1112 series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via ConFlo IV system (ThermoFischer, Bremen, Germany). The $\delta^{13}C$ and $\delta^{15}N$ were obtained in parts per thousands (‰) by comparisons to Vienna Pee Dee Belemnite and atmospheric nitrogen standards, respectively. The following formula was used:

δ^{13} C or δ^{15} N = $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Precision of isotopic composition was evaluated using the following laboratory standards: Merck Gel: $\delta^{13}C = -20.26 \pm 0.07‰$, $\delta^{15}N = 7.89 \pm 0.07‰$, C% = 41.28, N% = 15.29, DL-Valine: $\delta^{13}C = -10.57 \pm 0.06‰$, $\delta^{15}N = -6.15 \pm 0.06‰$, C% = 55.50, N% = 11.86, and Casein: $\delta^{13}C = -26.98 \pm 0.12‰$, $\delta^{15}N = 5.94 \pm 0.05‰$, C% = 48.8, N% = 13.5.

Data analyses

Firstly, the broad food web patterns were explored using the $\delta^{13}C$ and $\delta^{15}N$ biplots. Consequently, the study system was delineated into different sections based on the presence or absence of non-native species (Figure 5.1b). Thus, the river system was delineated into the following sections. (A) The Great Fish River headwaters, which comprised an uninvaded section where one native species, *Enteromius mandelai*, occurred. (B) The upper Great Fish River (UGFR), which comprised an invaded upper section of the mainstem. This section had two native fishes, *Anguilla mossambica* and *Labeo umbratus*, and four non-native fishes, *Clarias gariepinus*, *Cyprinus carpio*, *Labeobarbus aeneus* and *Labeo capensis*. (C) The lower Great Fish River (LGFR) that constituted the invaded lower reaches of the mainstem characterised by both primary freshwater and estuarine fishes. This section was invaded by six non-native fishes, *C. gariepinus*, *C. carpio*, *Gambusia affinis*, *L. aeneus*, *L. capensis* and *Tilapia sparrmanii* and had 14 native species, *Anguilla marmorata, A. mossambica, Gilchristella aestuaria, Glossogobius callidus, Planiliza macrolepis, Labeo umbratus, Pseudomyxus capensis, Mugil cephalus, Monodactylus falciformis, Oreochromis mossambicus, Pomadasys commersonnii, Psammogobious knysnaensis, Redigobius dewaalii* and *Rhabdosargus holubi*. (D) The Koonap River headwater tributaries and (E) the Koonap River upper mainstem that were both uninvaded and had one native species, *E. mandelai*. (F) The lower Koonap River where two native fishes, *E. mandelai* and *L. umbratus*, and three non-native fishes, *C. carpio*, *C. gariepinus* and *T. sparrmanii* occurred. Only the uninvaded sections of the Kat River were sampled. This was because the invaded portion of the Kat River comprised sections that were either dominated by silviculture (due to citrus plantations) or heavily polluted by sewage, specifically the section downstream of the Fort Beaufort town. Therefore, the sampled sections of the Kat River included (G) the headwater tributaries where *E. mandelai* occurred, (H) the headwater tributary where *E. mandelai* and *Sandelia bainsii* occurred, and (I) the upper mainstem section where *E. mandelai*, *S. bainsii* and *G. callidus* occurred. (Figure 5.1b).

Secondly, to evaluate and compare trophic diversity patterns for non-native and native fish assemblages within the invaded sections, isotopic diversity indices were computed following Cucherousset and Villéger (2015) and Rigolet et al. (2015). The isotopic diversity indices were thus computed for three invaded sections, the upper (UGFR) and lower (LGFR) Great Fish River mainstem sections, and the lower Koonap River. The isotopic diversity metrics were derived by standardising the $\delta^{13}C$ and $\delta^{15}N$ multidimensional δ-space whereby each isotopic axis was scaled to the same range of 0 – 1 (Cucherousset and Villéger 2015). The isotopic diversity indices were computed as follows;

1. Isotopic richness (IRic), which provides a quantitative estimate of the isotopic space for the whole community or assemblage. Low IRic values (close to 0) may indicate low functional diversity, possibly due to species loss, limited use of resources and low buffering from disturbances, whereas high IRic values (close to 1) may indicate high use of the trophic space.
2. Isotopic divergence (IDiv), which describes how the isotopic space is occupied based on the degree of isotopic spacing by community members. Low IDiv values (close to 0) likely reflects a community characterised by trophic generalists, whereas high IDiv values (close to 1) likely indicates a high degree of trophic specialisation for the community.
3. Isotopic dispersion (IDis), which reflects the extent to which community members differ in their stable isotope values. Low IDis values (close to 0) indicates that community members have similar stable isotope values, whereas high IDis values (close to 1) reflect dissimilar stable isotope values.

4. Isotopic evenness (IEve), which provides a quantitative estimation of the regularity in the distribution of the stable isotope values among community members. Low IEve values (close to 0) reflects high clustering among community members, which may be consistent with high trophic redundancy and competition, whereas high IEve values (close to 1) may indicate high use of trophic resources by community members.
5. Isotopic uniqueness (IUni), which indicate the degree of isotopic dissimilarity among community members. Low IUni values (close to 0) indicate that most of the weight belongs to isotopically similar organisms, whereas high IUni values (close to 1) indicate that most of the organisms are isolated in the stable isotope space.

In addition, isotope overlap between the non-native and native fish assemblages was evaluated based on isotopic similarity (ISim) and nestedness (ISim) (Cucherousset and Villéger 2015). Isotopic similarity provides a quantitative measure of the degree of intersection between two groups, with values close to 0 indicating separation, whereas values close to 1 indicate that the two groups have the similar isotopic diversity patterns. Isotopic nestedness provides a quantitative estimate of the degree to which one group or community is a subset of the other. Low nestedness indicates no isotopic overlap, whereas high nestedness, which is consistent with high isotopic overlap, reflect that one group is a subset of the other.

Lastly, to evaluate the trophic niche patterns and interspecific interactions of non-native fishes, a Bayesian isotope niche analysis was conducted following Swanson et al. (2015). This was based on a two-pronged approach. Firstly, the isotope niche size for each species was computed based on the joint probability distributions of scaled (0 – 1) δ^{13}C and δ^{15}N values, resulting in probability distributions ($\alpha = 95\%$) that reflect the most plausible isotopic trophic niche sizes from a multi-dimensional isotopic-space. This was expressed as: $P(Y \in N_R)$, where Y is the

δ^{13}C and δ^{15}N data matrix for a particular species and N_R is the probable isotopic niche region for a particular species. Secondly, interspecific interactions between two species were determined based on the extent of isotopic niche overlap as: $O_B^A = P(Y_A \in N_R(B))$, where O_B^A is the probability of species A overlapping onto the isotopic niche of species B (Swanson et al. 2015). The isotope niche overlaps were based on Bayesian posterior distributions (posterior means and 95% credible intervals) and were estimated based on Monte Carlo simulations with 1,000 iterations. All statistical analyses were performed using R software program version 4.2.2 (R Core Team 2022). Isotopic diversity indices were computed using published R code provided by Cucherousset and Villéger (2015). Isotopic niche patterns were computed using the R package *nicheROVER* (Lysy et al. 2021).

Results

Isotopic food web patterns

The Great Fish River and its tributaries were characterised by a shift in the isotopic food webs from the headwaters to the lower sections, which appeared to be consistent with food web complexity (Figure 5.2, Table 5.1). In the Great Fish River, this shift was mostly characterised by differences in the δ^{15}N ranges for the communities, which were small in the headwater section (from algae δ^{15}N = 6.94‰ to *E. mandelai* δ^{15}N = 12.21 ± 0.44‰), intermediate in the UGFR (from CPOM δ^{15}N = 4.75‰ to *A. mossambica* δ^{15}N = 14.92 ± 0.81‰) and large in the LGFR (from C_3-CPOM δ^{15}N = 0.58‰ to *A. marmorata* δ^{15}N = 18.38 ± 0.4‰). Similarly, in the Koonap River, the isotopic food webs were characterised by differences in δ^{15}N, which were smaller in the tributaries (from algae δ^{15}N = 1.55‰ to *E. mandelai* δ^{15}N = 11.22 ± 0.60‰) and upper sections (from C_3 δ^{15}N = – 0.35 ± 0.44‰ to *E. mandelai* δ^{15}N = 11.98 ± 0.61‰) than the lower section (from C_3 δ^{15}N = 1.78 ± 2.88‰ to *T. sparrmanii* δ^{15}N = 15.11 ± 0.68‰). In comparison, in addition to upstream to downstream differences in $\delta^{15}N$ ranges, the Kat River food webs were mostly distinguished by a large breadth in $\delta^{13}C$ values. Specifically, there was a wide breadth in $\delta^{13}C$ for macroinvertebrates in the headwater tributaries (from *Afronurus* $\delta^{13}C$ = –28.43‰ to *Cheumatopsyche* $\delta^{13}C$ = – 18.12‰), whereas the macroinvertebrates in the mainstem section had both wide $\delta^{13}C$ breadth (from Plecoptera $\delta^{13}C$ = – 25.88 ± 0.02‰ to *Potamonautes* $\delta^{13}C$ = – 19.19 ± 0.23‰) and high $\delta^{15}N$ range (from Oligochaeta $\delta^{15}N$ = 6.71‰ to *Cheumatopsyche* $\delta^{15}N$ = 11.94 ± 0.10‰). Within the invaded UGFR section, non-native fishes were distinguished by having intermediate $\delta^{15}N$ values compared to the two native fishes, *A. mossambica*, which had the highest ($\delta^{15}N$ = 14.91 ± 0.81‰) and *L. umbratus,* which had the lowest ($\delta^{15}N$ = 11.57 ± 0.63‰) values (Figure 5.2b, Table 5.1). Furthermore, in this section, non-native fishes had a wider breadth in $\delta^{13}C$ values (-26.72 to -22.67 ‰) compared to either the LGFR (- 26.98 to -23.71 ‰) or the lower Koonap River (- 26.10 to -24.72 ‰). By comparison, within the invaded LGFR, the non-native fishes generally had lower $\delta^{15}N$ values than most native fishes (Figure 5.2c, Table 5.1). In contrast, all non-native fishes were distinguished by having higher $\delta^{15}N$ values than native fishes within the invaded lower Koonap River (Figure 5.2f).

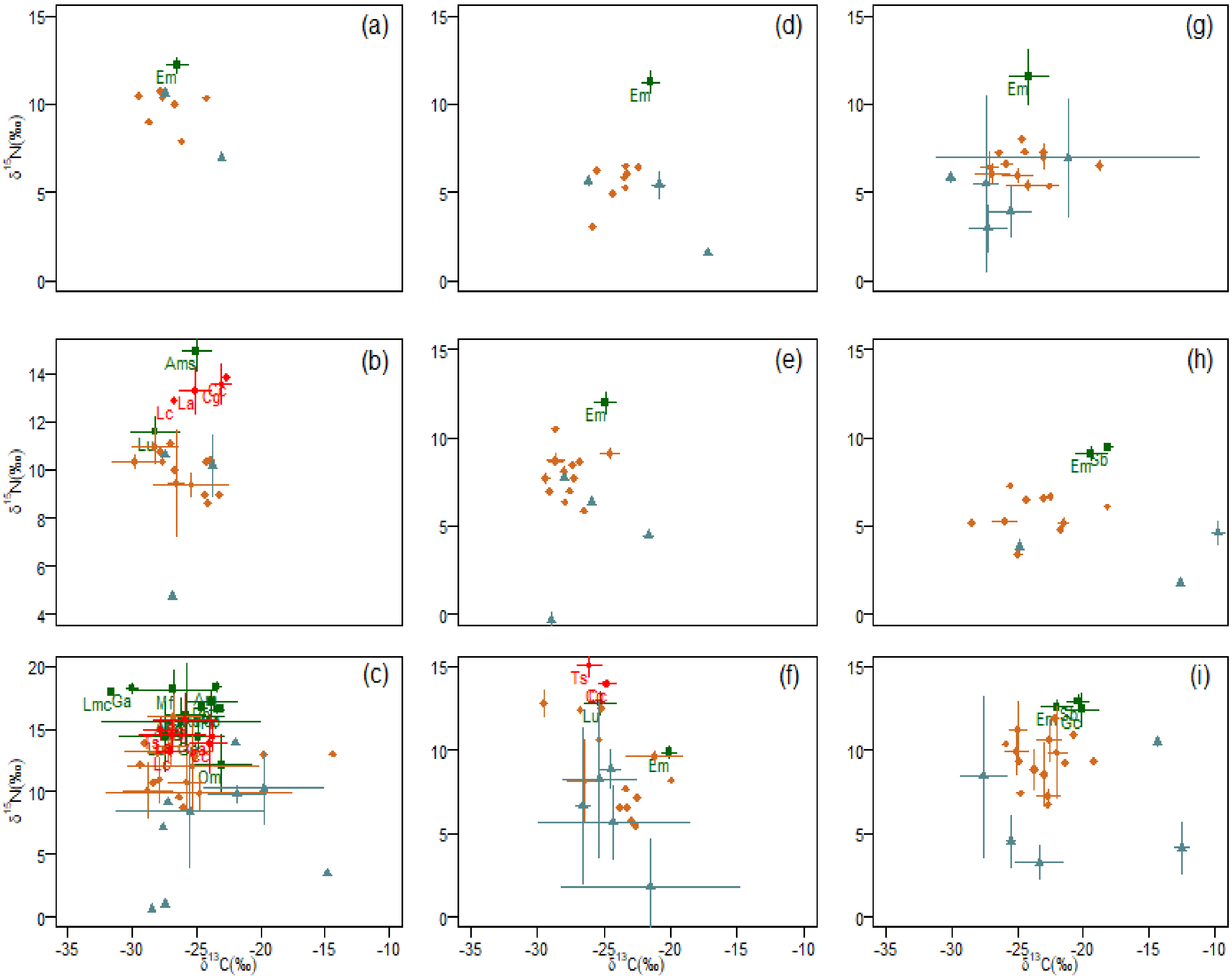

Figure 5.2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope biplots for fishes (native = green squares, non-native = red diamonds), macroinvertebrates (brown diamonds) and basal sources (cadet blue triangles) within the Great Fish River headwater section (a), upper mainstem section (b), lower mainstem section (c), the Koonap River headwater (d), upper (e) and lower (f) mainstem, and the Kat River tributaries (g and h) and upper mainstem (i). See Supplementary Table 4 for abbreviations.

Table 5.1. Mean values of carbon and nitrogen stable isotopes and range values (in parentheses) for fishes (native and non-native), macroinvertebrates and basal sources within the Great Fish River headwater section (A), upper Great Fish River mainstem section (B)*, lower Great Fish River mainstem section (C)*, the Koonap River headwater (D), upper (E) and lower (F)* mainstem, and the Kat River tributaries (G and H) and upper mainstem (I). * indicate invaded sections.

		Great Fish River			**Koonap River**			**Kat River**		
		A	B*	C*	D	E	F*	G	H	I
Basal	$\delta^{13}C$	-25.25 (-27.45,-23.50)	-29.84 (-41.28, -23.76)	-23.86 (-28.51, -14.85)	-21.41 (-26.19, 17.17)	-26.14 (-28.96, -21.65)	-24.42 (-26.54, -21.49)	-26.30 (-30.09, -21.18)	-15.69 (-24.82, -9.69)	-20.66 (-27.61, -12.47)
	$\delta^{15}N$	8.78 (6.94, 10.62)	8.68 (4.75, 10.62)	7.07 (0.58, 13. 88)	4.19 (1.55, 5.62)	4.53 (-0.35, 7.69)	6.24 (1.79, 8.86)	5.02 (2.96, 6.96)	3.39 (1.74, 4.61)	6.16 (3.24, 10.46)
Invertebrates	$\delta^{13}C$	-27.24 (-29.50,-24.23)	-26.07 (-29.79, -23.17)	-25.96 (-29.32, -14.36)	-23.92 (-25.79, -22.42)	-27.69 (-29.35, -24.57)	-24.05 (-29.47, 19.92)	-24.31 (-27.11, -18.68)	-23.60 (-28.43, -18.13)	-23.06 (-25.88, -19.19)
	$\delta^{15}N$	9.82 (7.80, 10.76)	9.97 (8.61, 11.11)	12.04 (8.70, 15.97)		7.99 (5.87, 8.67)	8.73 (5.46, 12.79)	6.59 (5.35, 8.00)	5.69 (3.38, 7.27)	9.34 (6.72, 11.94)
Native fish	$\delta^{13}C$	-26.52 (-26.52, -26.52)	-26.62 (-28.22, -25.02)	-25.72 (-31.63, -23.05)	-21.49 (-21.49, -21.49)	-24.87 (-24.87, -24.87)	-22.69 (-25.28, -20.10)	-24.14 (-24.14, -24.14)	-18.74 (-19.39, -18.08)	-20.83 (-21.99, -20.10)
	$\delta^{15}N$	12.21 (12.21,12.21)	13.24 (11.57, 14.92)	16.32 (12.14, 18.38)	11.22 (11.22, 11.22)	11.98 (11.98, 11.98)	11.33 (9.85, 12.81)	11.54 (11.54, 11.54)	9.28 (9.10, 9.46)	12.65 (12.43, 12.91)
Non-native fish	$\delta^{13}C$		-24.40 (-26.73, -22.67)	-25.88 (-27.78, -23.72)			-25.24 (-26.10, -24.72)			
	$\delta^{15}N$		13.39 (12.90, 13.85)	14.47 (13.24, 15.82)			14.36 (13.95, 15.11)			

Isotopic diversity patterns of non-native and native fishes

In the UGFR, the non-native and native fish assemblages exhibited no isotopic diversity overlap (Figure 5.3a). This indicated that the non-native and native fishes had different isotopic diversity patterns. Specifically, non-native fishes were most distinguished by having high isotopic divergence (IDiv = 0.9) and isotopic dispersion (IDis = 0.9) compared to native species (IDiv = 0.4; IDis = 0.5) (Table 5.2). This indicated that the non-native fishes were characterised by dissimilar isotopic values and had propensity towards trophic specialisation. By comparison, native fishes were most distinguished by high isotopic uniqueness (IUni = 0.8), indicating that the species had different stable isotope values, likely reflecting different resource utilisation. In contrast to the pattern observed in the UGFR, within the LGFR, non-native and native fish species exhibited high isotopic diversity overlap that was characterised by high isotopic nestedness (INes = 0.9) (Figure 5.3b). In particular, the non-native fish assemblage's isotopic diversity was low and appeared to be a subset of the native fish assemblage's isotopic diversity. This was further corroborated by higher isotopic richness for native (IRic = 0.6) than non-native (IRic = 0.1) species. However, overall, most isotopic diversity indices were comparable between the native and non-native fishes (Table 5.2). In addition, the relatively higher isotopic divergence for non-native (IDiv = 0.8) compared to native fishes' (IDiv = 0.6) suggest that the former group had had species with propensity towards trophic specialisation. In the Koonap River, there was a low isotopic diversity overlap between non-native and native fish assemblages (Figure 5.3c) and most of their isotopic diversity metrics were comparable (Table 5.2), indicating that the two assemblages were characterised by somewhat different isotopic diversity patterns. In addition, the two groups were characterised by high isotopic divergences (IDiv = 0.8) but low isotopic evenness (IEve < 0.4), which showed that both groups were likely trophic specialists with the possibility of high trophic redundancy.

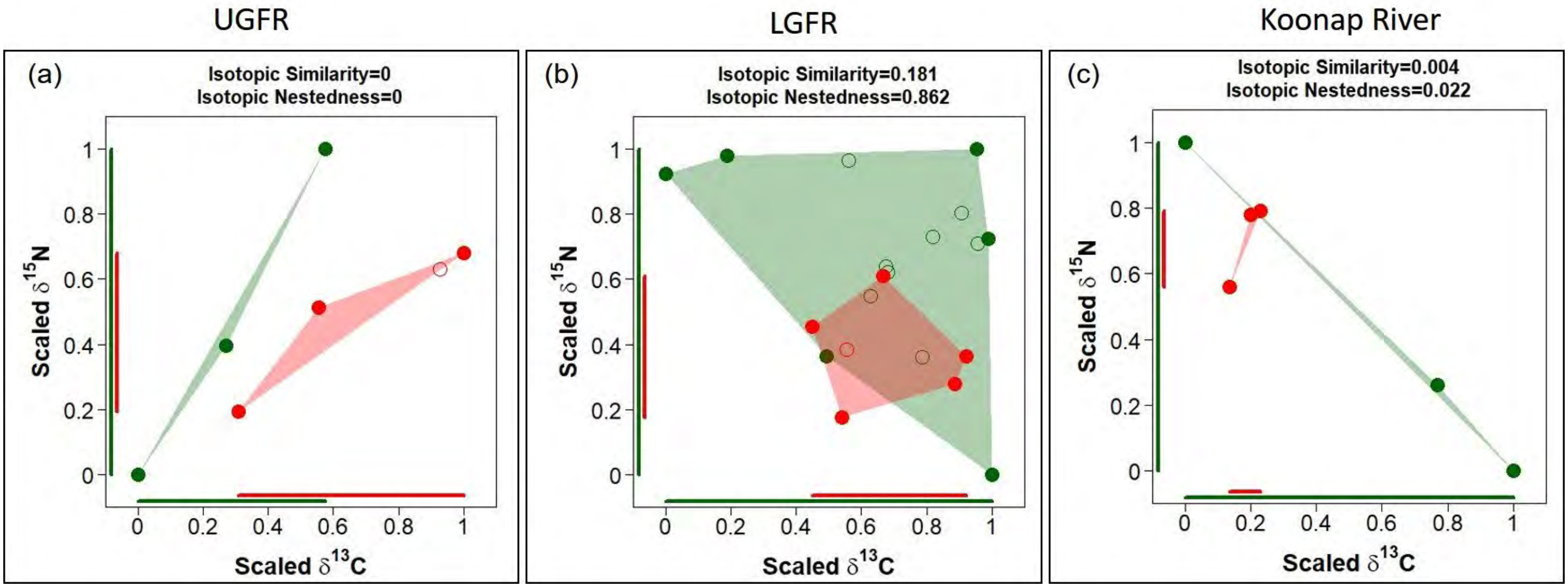

Figure 5.3. Isotopic diversity overlap metrics between native (green) and non-native (red) fish species of the three communities within the Great Fish River system; the upper Great Fish River (UGFR) mainstem (a), the lower Great Fish River (LGFR) mainstem (b) and the Koonap River (c). Also shown are two isotopic overlap metrics, isotopic similarity (ISim) and isotopic nestedness (INes), computed from scaled δ^{13}C and δ^{15}N values.

Table 5.2. Isotopic niche variability of native and non-native fishes based on isotopic diversity indices (IDIs) and isotopic overlap metrics (ISim and INes). * Two populations of *E. mandelai* were used to allow for computation of IDIs within the UGFR. Abbreviations are: *n* = number of species, IDiv = isotopic divergence, IDis = isotopic dispersion, IEve = isotopic evenness, IUni = isotopic uniqueness, ISim = isotopic similarity and INes = isotopic nestedness. UGFR and LGFR refer to upper Great Fish River and lower Great Fish River mainstem sections, respectively. For IDIs visual representations refer to Supplementary Figures 2 – 10.

Locality	**Community**	***n***	**Scaled δ^{13}C range**	**Scaled δ^{15}N range**	**IRic**	**IDiv**	**IDis**	**IEve**	**IUni**	**ISim**	**INes**	**Supplementary figure**
UGFR*	Assemblage	7	1.0	1.0	0.3	0.7	0.5	0.7	0.4	0	0	2
	Native	3	0.6	1.0	0.0	0.4	0.5	1.0	0.8			3
	Non-native	4	0.7	0.5	0.1	0.9	0.9	0.6	0.6			4
LGFR	Assemblage	20	1.0	1.0	0.6	0.6	0.3	0.6	0.3	0.2	0.9	5
	Native	14	1.0	1.0	0.6	0.6	0.4	0.7	0.4			6
	Non-native	6	0.5	0.4	0.1	0.8	0.7	0.8	0.6			7
Koonap	Assemblage	6	1.0	1.0	0.2	0.8	0.7	0.7	0.7	0	0.02	8
	Native	3*	1.0	1.0	0.0	0.8	0.7	0.4	0.6			9
	Non-native	3	0.1	0.2	0	0.8	0.9	0.3	0.5			10

Non-native fish isotopic niche patterns

In the UGFR, non-native fishes had variable isotopic niche sizes that ranged from being large for *L. aeneus* (scaled niche size (NS) = 0.95 ± 0.16‰^{2}), intermediate for *C. gariepinus* (NS = 0.31 ± 0.11‰^{2}) and small for *L. capensis* (NS = 0.08 ± 0.05 ‰^{2}) and *C. carpio* (NS = 0.05 ± 0.02‰^{2}) (Figure 5.4a, Table 5.3). In general, most pairwise comparisons revealed low isotopic niche overlap (mean overlap < 20%) (Figure 5.5a, Table 5.3). This showed the propensity for trophic niche differentiation for most species. Nevertheless, exceptions were shown by the high probabilities of isotopic niche overlaps for *C. carpio* onto the isotopic niche of *C. gariepinus* (mean overlap = 64.44%, 95% CI = 13.0 – 100%), *C. gariepinus* onto the isotopic niche of *L. aeneus* (mean overlap = 59.90%, 95% CI = 15.0 – 96.0%) and *L. capensis* onto the isotopic niche of *L. aeneus* (mean overlap = 78.43%, 95% CI = 26.0 – 100%). In the LGFR the three non-native species, *C. carpio*, *C. gariepinus* and *L. aeneus* had intermediate to higher niche sizes (NS range = 0.53 ± 0.19 – 0.68 ± 0.13‰^{2}) compared to those of *T. sparrmanii*, *G. affinis* and *L. capensis*, which had lower niche sizes (NS range = 0.06 ± 0.04 – 0.20 ± 0.09‰^{2}) (Figure 5.4b, Table 5.4). There were moderate to high probabilities of isotopic niche overlaps (mean overlap > 50%) for almost half (13 out of 30) of the pairwise comparisons (Figure 5.5b, Table 5.4). In general, all non-native species in this section had high probabilities of isotopic niche overlap (mean overlap > 60%) with both *C. gariepinus* and *L. aeneus*, which showed that these two species were likely dietary generalists (Table 5.4). In addition, *G. affinis* exhibited high probability of overlap onto the isotopic niche of *C. carpio* (mean overlap = 98.56%, 95% CI = 89 – 100%). By comparison, most species had low probabilities of isotopic niche overlap (mean overlap < 20%) with both *G. affinis* and *L. capensis* (Figure 5.5b, Table 5.4). Within the Koonap River, *T. sparrmanii* was characterised by having higher niche size (NS = 0.87 ± 0.19‰^{2}) compared to both *C. carpio* (NS = 0.12 ± 0.06‰^{2}) and *C. gariepinus* (NS = 0.10 ± 0.06‰^{2}) (Figure 5.4c, Table 5.5). Most pairwise comparisons revealed low to moderate isotopic niche overlap probabilities (mean overlap = 36.83 – 40.91%), an indication for propensity toward niche differentiation amongst species (Figure 5.5c, Table 5.5). These included *C. carpio* onto the isotopic niche of *T. sparrmanii* (mean overlap = 36.83%, 95% CI = 1 – 94%), *C. gariepinus* onto the isotopic niche of *C. carpio* (mean overlap = 40.91%, 95% CI = 3 – 95%). Nevertheless, there was a high probability of isotopic niche overlap by *C. gariepinus* onto the isotopic niche of *T. sparrmanii* (mean overlap = 58.27%, 95% CI = 2 – 100%) (Table 5.5).

Table 5.3. Pairwise posterior means (%) and 95% credible intervals (in parentheses) for the overlap metric, with niche size at 0.95, for four non-native species within the upper Great Fish River (see also Supplementary Figure 11). Posterior means >50% highlighted in bold. The overlap metric is one directional, such that it represents the mean probability (in %) that at least one individual from Species A (row) will be found in the niche of Species B (column). Also shown is mean size (± standard deviation) for each species, standardised to range between 0 and 1.

Species A	**Species B**				**Niche size (‰^{2})**
	Cyprinus carpio	*Clarias gariepinus*	*Labeobarbus aeneus*	*Labeo capensis*	
Cyprinus carpio	-	**64.44 (13, 100)**	15.76 (0, 80)	0.00	0.05 ± 0.02
Clarias gariepinus	12.20 (2, 33)	-	**59.90 (15, 96)**	0.00	0.31 ± 0.11
Labeobarbus aeneus	0.57 (0, 3)	10.77 (2, 27)	-	4.58 (1,15)	0.95 ± 0.16
Labeo capensis	0.00	0.00	**78.43 (26, 100)**	-	0.08 ± 0.05

Table 5.4. Pairwise posterior means (%) and 95% credible intervals (in parentheses) for the overlap metric, with niche size at 0.95, for the six non-native species within the lower Great Fish River (see also Supplementary Figure 12). Posterior means >50% highlighted in bold. The overlap metric is one directional, such that it represents the mean probability (in %) that at least one individual from Species A (row) will be found in the niche of Species B (column). Also shown is mean size (± standard deviation) for each species, standardised to range between 0 and 1.

Species A	**Species B**						**Niche size (‰^{2})**
	Cyprinus carpio	*Clarias gariepinus*	*Gambusia affinis*	*Labeobarbus aeneus*	*Labeo capensis*	*Tilapia sparrmanii*	
Cyprinus carpio	**-**	**76.14 (48, 97)**	39.93 (18, 67)	**64.08 (34, 90)**	4.97 (0, 23)	12.23 (0, 46)	0.53 ± 0.19
Clarias gariepinus	**56.11 (32, 84)**	-	19.26 (10, 34)	**68.71 (47, 89)**	3.82 (0, 14)	31.77 (12, 64)	0.68 ± 0.13
Gambusia affinis	**98.56 (89,**	**97.88 (89, 100)**	-	**93.10 (68, 100)**	5.04 (0, 41)	8.45 (0, 74)	0.11 ± 0.03
Labeobarbus aeneus	45.91 (22, 80)	**75.12 (50, 94)**	16.09 (6, 30)	-	12.74 (3, 33)	**50.65 (25, 81)**	0.58 ± 0.15
Labeo capensis	38.91 (1, 99)	**62.63 (12, 100)**	5.03 (0, 30)	**97.02 (72, 100)**	-	38.96 (0, 100)	0.06 ± 0.04
Tilapia sparrmanii	26.44 (1, 79)	**84.47 (52, 100)**	2.36 (0, 16)	**96.23 (77, 100)**	6.86 (0, 28)	-	0.20 ± 0.09

Table 5.5. Pairwise posterior means (%) and 95% credible intervals (in parentheses) for the overlap metric, with niche size at 0.95, for the three non-native species within the Koonap River (see also Supplementary Figure 13). Posterior means >50% highlighted in bold. The overlap metric is one directional, such that it represents the mean probability (in %) that at least one individual from Species A (row) will be found in the niche of Species B (column). Also shown is mean size (± standard deviation) for each species, standardised to range between 0 and 1.

Species A	**Species B**			
	Cyprinus carpio	*Clarias gariepinus*	*Tilapia sparrmanii*	**Niche size (‰^{2})**
Cyprinus carpio	-	37.77 (3, 89)	36.83 (1, 94)	0.12 ± 0.06
Clarias gariepinus	40.91 (3, 95)	-	**58.27 (2, 100)**	0.10 ± 0.06
Tilapia sparrmanii	4.24 (0, 19)	4.10 (0, 14)	-	0.87 ± 0.19

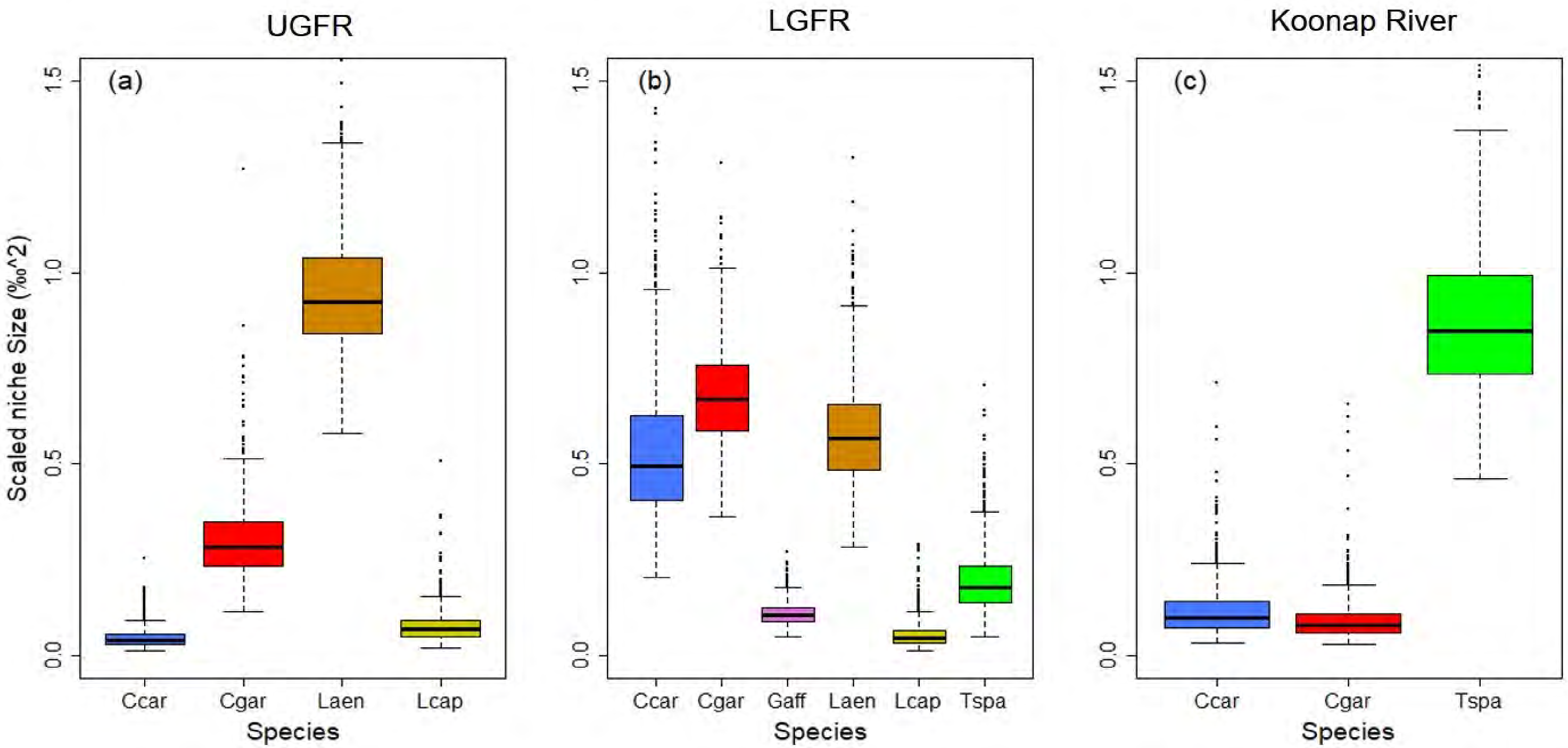

Figure 5.4. Isotopic niche sizes (in **‰**^{2}) for non-native fishes within the three invaded sections of the Great Fish River system, the upper (a) and the lower (b) Great Fish River mainstem sections and the Koonap River (c). Niche sizes were computed based on the joint probability distributions of scaled (0 – 1) δ^{13}C and δ^{15}N values. Species abbreviations are; Ccar = *Cyprinus carpio*, Cgar = *Clarias gariepinus*, Gaff = *Gambusia affinis*, Laen = *Labeobarbus aeneus*, Lcap = *Labeo capensis* and Tspa = *Tilapia sparrmanii*.

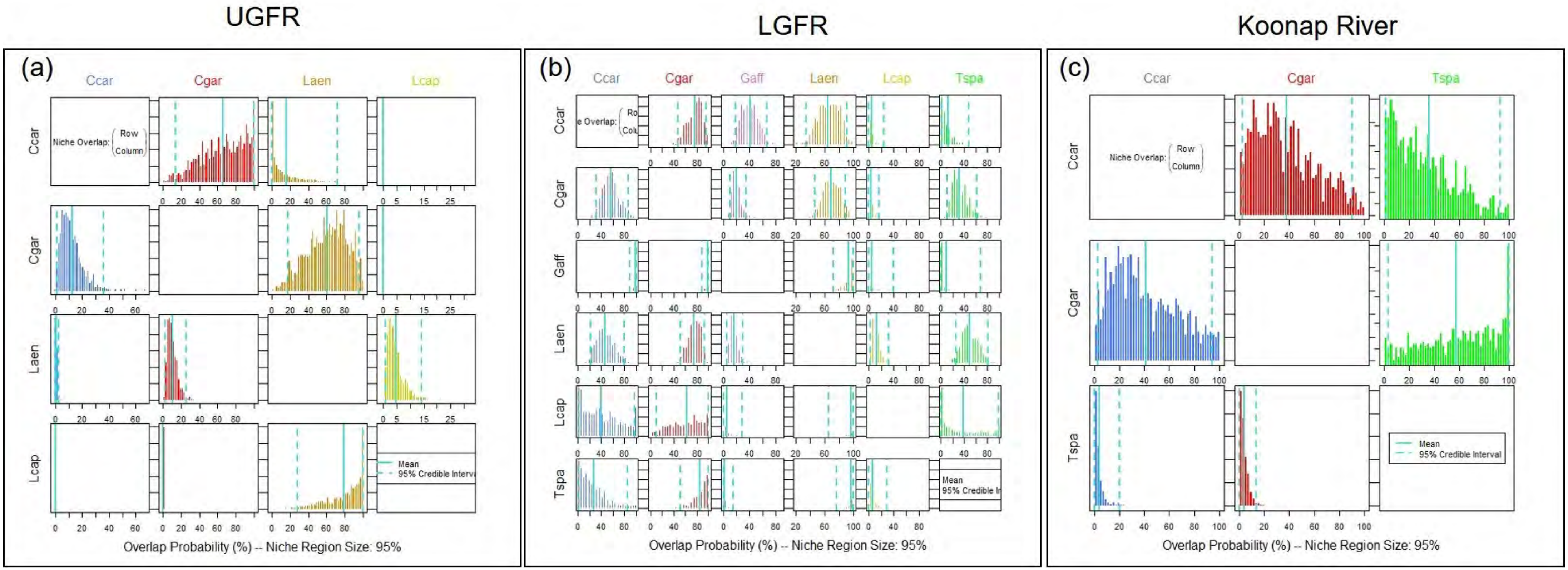

Figure 5.5. Mean niche overlap probabilities (%) of the non-native species within the three invaded sections, upper Great Fish River (a), lower Great Fish River (b) and the Koonap River (c). The overlap metric is directional, such that it represents the probability that an individual from a species (row) will overlap onto the isotope niche of the other species (column). The niche size (niche region) was defined as the 95% credibility intervals of isotopic space. Species abbreviations are Ccar = *Cyprinus carpio*, Cgar = *Clarias gariepinus*, Gaff = *Gambusia affinis*, Laen = *Labeobarbus aeneus*, Lcap = *Labeo capensis* and Tspa = *Tilapia sparrmanii*.

Discussion

The Great Fish River system's isotopic food webs were broadly distinguished into two categories; the uninvaded headwater sections dominated by either single or few native fish species (predominantly *E. mandelai* at most sites, and *S. bainsii* and *G. callidus* in the Kat River headwater sections) and the invaded mainstem and lower sections of the Koonap River. Despite the occurrence of common non-native fishes within the invaded sections, these species' isotopic values were variable with no discernible pattern in relation to those of the native species. Specifically, the non-native species exhibited variation that ranged from having intermediate δ^{15}N values and wider δ^{13}C breadth in the UGFR, relatively lower δ^{15}N values and narrower δ^{13}C breadth in the LGFR and higher δ^{15}N values but narrower δ^{13}C breadth in the Koonap River compared to native species. These broad differences appeared to be consistent with the first hypothesis that native and non-native fish assemblages would have high trophic functional diversity patterns that likely reflected broad resource use. From an isotopic diversity perspective, the non-native and native fish assemblages exhibited variation that was typified by low isotopic diversity overlaps in UGFR and Koonap River, whereas the LGFR was characterised by high isotopic diversity overlap. By comparison, the second hypothesis, non-native species would exhibit broad trophic diversity patterns and would be characterised by low niche overlap, provided some evidence of potential isotope niche differentiation among non-native fishes in the UGFR and Koonap River, a pattern that appeared to corroborate the isotopic diversity patterns. In the LGFR however, the non-native fishes were mostly characterised by high isotope niche overlaps, a pattern that appeared to be inconsistent with the second hypothesis. This suggests that both the trophic diversity and trophic niche patterns of non-native fishes were likely influenced by their resource use patterns, the nature of the invaded section and their probable interactions with the native fish assemblage within the respective invaded sections.

The lack of consistent and predictable trophic diversity and niche patterns for non-native fishes across the invaded sections in this study was likely a reflection of trophic plasticity, which is a common phenomenon related to environmental stochasticity, variation in prey availability and interspecific interactions coupled by niche opportunities that has been observed in other studies (Córdova-Tapia et al. 2015; Pennock et al. 2021). In the UGFR and the Koonap River, the low isotopic diversity overlaps between non-native and native species and the low isotopic niche overlaps amongst the constituents of non-native species suggest that trophic niche differentiation was a probable mechanism influencing resource use in these habitats. Specifically, the results of this study posited the possibility of trophic niche specialisation due to high isotopic divergence and diversion for the non-native species, and the high isotopic uniqueness for the native species. Furthermore, despite having large isotopic niche breadths, most non-native species generally showed low trophic niche overlaps, except for trophic generalists, such as *C. carpio* and *C. gariepinus* whose isotopic niche overlapped onto those of other non-native species. This appeared to be consistent with empirical studies elsewhere, which have also shown low isotopic overlaps to be a reflection of trophic niche differentiation among non-native species (e.g. Jackson and Britton 2013; Jackson et al. 2014). In the Great Fish River, it is likely that the creation of permanently flowing water body in the upper mainstem section as a result of the IBWT (Laurenson and Hocutt 1986) could have influenced resource availability that facilitated potential trophic niche differentiation, particularly by potentially increasing biomass of primary production and that of lower trophic secondary production, such as benthic invertebrates. For example, O'Keeffe and De Moor (1988) noted that following the creation of permanent flow in the Great Fish River, the system was characterised by a substantial shift towards the dominance of hydropsychid, chironomid and simuliid invertebrate species. These resources would likely sustain the proliferation of rheophilic taxa of non-native species such as the benthivorous *L. capensis* and the generalist invertivorous *L. aeneus* probably through reduced interspecific interactions with *C. carpio,* and the generalist piscivore *C. gariepinus*. Alternatively, it is likely that the low native species richness of the UGFR and Koonap River may have presented vacant niches that were exploited by the different non-native fishes with minimum competitive interactions. This is because species depauperate environments are more likely to be susceptible to invasions due to high availability of vacant niches (Shea and Chesson 2002; Hierro et al. 2005; Leuven et al. 2009; Jeschke 2014).

While trophic niche differentiation is generally considered a common mechanism for coexistence among fishes (Mason et al. 2008; Pilger et al. 2010; Jackson and Britton 2013) and appeared to be most probable in the UGFR and Koonap River, this mechanism appeared less plausible in the LGFR. This is because the non-native fish assemblage's isotopic diversity largely overlapped with, and appeared to be a subset of, that of the native fish assemblage. Furthermore, these non-native fishes exhibited high isotopic niche overlap. In contrast to native fishes, the relatively low non-native species' isotopic diversity within the LGFR suggested less trophic complexity within this species group. This contrasts with other studies that have found non-native species to generally have high isotopic diversity, which reflected that they exploited wide range of resources compared to native species in freshwater ecosystems (Sagouis et al. 2015; De Santis et al. 2022). On the other hand, the generally high isotopic diversity for the native fish assemblage may reflect high trophic complexity that characterises most downstream sections of large rivers, which is hypothesized to be maintained by weak consumer-resource interactions (Levin 2000; Kokkoris et al. 2002; Bellmore et al. 2015). On the other hand, these findings could be indication of native community's resilience to non-native trophic impact.

The occurrence of multiple non-native species with relatively low isotopic diversity but high isotopic niche overlap within the LGFR, suggests that weak trophic interactions among these species may likely facilitate or drive their occurrence in this invaded section. The establishment of multiple non-native species in this section, however, raises concern on their potential impact. This is because non-native species such as *C. gariepinus*, *C. carpio* and *L. aeneus*, which were found to be characterised by large isotope niche sizes, were likely to exert negative influences on the trophic dynamics of this system due to their foraging habits and possible facilitative interactions. For example, *C. carpio*, a global invader, is known to influence nutrient availability and to increase water turbidity (Zambrano et al. 2006; Parkos et al. 2011), which may confer an advantage to *C. gariepinus*, a generalist predator that does not require high visibility for foraging. There is therefore concern on the potential synergistic impacts on aspects such as direct predation by *C. gariepinus* on small- to medium-sized native species, such as the round herring *Gilchristella aestuaria*, oval moony, *Monodactylus falciformis* and *Pseudomyxus capensis*. Furthermore, there is potential indirect impacts through interspecific interference and probable competitive interaction for benthic resources by species such as *C. carpio* and *L. capensis*, and the invertivorous *L. aeneus* on species such as the native population of *L. umbratus* that feeds on soft sediments and detritus, *P. macrolepis*, which feeds on diatoms grubbed out of bottom sand and the *G. callidus* that feeds on bottom-living insects and small invertebrates.

General studies on trophic impacts by non-native fishes in lotic habitats have shown that these species can influence food webs by either increasing their trophic ranges, such as when there is invasion by non-native top predators (Post and Takimoto 2007; Cucherousset et al. 2012b; Walsworth et al. 2013), expanding the food web sizes, such as when there is invasion by taxa that are located on the edges of the food webs (Sagouis et al. 2015) or shrinking of the food webs and/or compression of the isotopic niche (Vander Zanden et al. 1999; Walsworth et al. 2013), such as when there is invasion by generalist predators and when there is elimination of certain taxa. In this study, some of these aspects appeared to have been reflected as well. For example, the UGFR and the Koonap River appeared to typify food web expansion due to the addition of non-native species, with high δ^{15}N values, on the top or edges of the food webs. Specifically, in the UGFR, the food web appeared to show a wider breadth in carbon sources for the fishes, which was typified by non-native species such as *C. carpio* with high δ^{13}C value and *L. aeneus* with low δ^{13}C value. In comparison, the Koonap River appeared to reflect an increase in the trophic range increase in its food web because all the non-native fishes (*C. carpio, C. gariepinus* and *T. sparmanii*) had higher δ^{15}N than non-native fishes.

In conclusion, results of the present study suggested that isotopic diversity patterns likely largely reflected trophic niche differentiation among native and non-native fishes. In addition, isotopic niche patterns suggested that trophic niche differentiation was a probable mechanism associated with co-occurrence of different non-native fishes. Nevertheless, these patterns appeared to vary across the different invaded sections. Specifically, the low isotopic diversity overlaps between native and non-native fishes and low isotopic niche overlap among most non-native fishes in the upper mainstem and invaded tributary sections posits the likelihood of exploitation of vacant trophic niches by the invaders. On the other hand, weak trophic interactions among non-native species were likely responsible for their co-occurrence within the LGFR. The results of this study add to the body of knowledge that seeks to understand how non-native species integrate into disturbed ecosystems and the mechanisms facilitating these integrations.

CHAPTER 6

PREDICTING THE ENVIRONMENTAL DETERMINANTS AND ASSEMBLAGE PATTERNS ASSOCIATED WITH MULTIPLE NON-NATIVE FISH INVASIONS

Introduction

Managing invasion impacts requires an understanding of the distribution and abundance patterns of the non-native invaders together with the factors that drive these patterns (Baquero et al. 2012; O'Neill et al. 2021). This can be achieved through continuous monitoring of the non-native species ranges (Uden et al. 2015; Bellard et al. 2016), exploring the potential drivers of their invasion success (Bellard et al. 2016), and evaluating the integrity and availability of critical habitats that could serve as sanctuaries for the native biota (Pyšek et al. 2003; Gallardo et al. 2017; Finlayson et al. 2018). Often, however, effective management into continuous monitoring and empirical-based experiments on invasions is limited by capacity and resource availability, which hampers the implementation of appropriate mitigation measures. In addition, conservation interventions derived from managers' or experts' experience often are not easily verifiable, or they lack systematic reviews of actions that provide the likelihood of success when the actions are employed in new and different contexts (Drolet et al. 2014, 2015). To address these challenges, there has been a focus on the applications of algorithm-based predictive models, such as species distribution modelling and machine learning techniques, as decision-making tools that can guide conservation planning and ecological management (Zengeya et al. 2013; Lübcker et al. 2014; Drake et al. 2015; Uden et al. 2015; Villero et al. 2017; Xiao et al. 2018). These types of methods provide relatively feasible and inexpensive contributions to management of biological invasions (Cote and Reynolds 2022).

Specifically, there has been growing interest in the applications of machine learning in ecological modelling approaches. This is largely because of their ability to model complex ecological patterns and processes on a wide range of data types without prerequisites of satisfying restrictive assumptions in comparison to most parametric statistical approaches (Phillips et al. 2006; Olden et al. 2008; Elith and Leathwick 2009; Escalante et al. 2013; Xiao et al. 2018). Furthermore, machine learning approaches have been demonstrated to typically perform better in certain circumstances than traditional modelling approaches (Elith et al. 2006; Leathwick et al. 2006; Olden et al. 2008). For example, applications of machine learning based techniques are often preferred where the available data are primarily based on species occurrence records collected in different time frames and using different collection methods (Elith et al. 2006; Hastie and Fithian 2013; Guillera-Arroita et al. 2015). Some of the most commonly used machine learning methods include boosted regression trees (BRT) and multivariate regression trees (MRT) (De'ath 2002; Elith et al. 2008).

Boosted regression trees are non-parametric statistical techniques that integrate boosting with decision trees (i.e. regression trees) to improve predictive accuracy or performance in modelling data (Friedman 2001; Elith et al. 2008). They have been applied in biodiversity conservation studies to model habitat suitability in plants (Damaneh et al. 2022; Rahmanian et al. 2022), distribution of amphibians (Cerasoli et al. 2017) and fish species richness and habitat suitability (Leathwick et al. 2006; Abdul et al. 2021). Furthermore, BRT techniques have been applied to model the current and future distributions of invasive species, as well as their habitat suitability (Hao et al. 2022; Sittaro et al. 2023). Similarly, MRT techniques have been widely employed in ecological modelling (e.g. Tatsuhara and Antatsu 2010; Clarkin et al. 2012; Sahuquillo and Miracle 2013; Salmon et al. 2016; Rodrigues et al. 2019; de la Escalera et al. 2021) including in conservational and invasion biology studies (Allen et al. 2009; Kadye and Booth 2013).

The aim of this chapter was to use BRT and MRT to provide a quantitative prediction of non-native fish distributions and the associated assemblage patterns in the Great Fish River system. Specific objectives were to: (1) evaluate the primary environmental predictors of multiple fish invasions; and (2) identify the fish assemblage associations to determine the native fishes that were likely vulnerable to non-native fish invasions.

Materials and methods

Data collection

This chapter is based on the fish distribution and environmental data that are described in Chapters 2 - 4. The fish distribution data comprised all common non-native (*Cyprinus carpio*, *Labeobarbus aeneus*, *Clarias gariepinus*, *Gambusia affinis* and *Tilapia sparrmanii*) and native fishes (*Anguilla marmorata*, *Anguilla mossambica*, *Enteromius mandelai*, *Labeo umbratus*, *Glossogobius callidus*, *Monodactylus falciformis*, *Mugil cephalus*, *Pseudomyxus capensis*, *Oreochromis mossambicus* and *Sandelia bainsii*) captured at 67 sites within the lotic habitats of the Great Fish River system. The environmental data included physico-chemical (altitude (recorded as meters above sea level [m.a.s.l]), temperature, conductivity, pH, turbidity and dissolved oxygen) and microhabitat (average depth, average width, and the proportion (%) of bedrock, boulders, cobbles, gravel, sand and silt at each sampled site). An additional nominal variable was used to distinguish each sampled site based on three categories, namely headwater tributary sites, large tributary sites and mainstem sites.

Data analysis

Firstly, a vector of invasion status was created based on binary response (0 for uninvaded and 1 for invaded). The uninvaded response represented sites where only native fishes were captured, whereas the invaded response represented sites where at least one non-native fish species was present. Secondly, to evaluate the determinants of multiple fish invasions, boosted regression trees (BRT, Elith et al. 2008) were used to model the invasion status in relation to environmental data. Boosted regression analysis is a machine-learning based ecological modelling approach for prediction (Elith et al. 2008). This modelling approach fits complex functions and interactions between variables without making assumptions about the fitted functions (De'ath 2007). Because the response was binary, the BRT models were constructed based on Bernoulli distribution using the function *gbm.stem* in the R statistical program. For model optimisation, several BRT models were built sequentially based on different values of tree complexity (*tc*), learning rate (*lr*) and bag fraction to achieve a minimum number of trees (*nt*) following the outline by Elith et al. (2008). The optimisation procedure was done with *tc* values ranging from 1 to 10, *lr* values ranging from 0.01 to 0.001 and bag fraction of 0.5 to 0.75 to achieve a minimum *nt* of 1000. Model performance was evaluated using a 10-fold cross-validation (*cv*). The model fit was based on the means of *cv* correlations, which measures the correlation between observed and fitted values, and the area under the receiver-operator curve (AUC) whose values range between 0.5 to 1, with high values indicating a better performing BRT. Variable importance was selected based on the relative influence of each predictor in the splitting process, and visualisation of the BRT fitted functions was done using partial dependence plots (Elith et al. 2008). To assess assemblage associations in relation to environmental variables, multivariate regression tree analysis (MRT, De'ath, 2002) was conducted. The MRT analysis was conducted to evaluate the assemblage associations of native fishes, non-native fishes and for the two groups combined. Each of the MRT model was conducted through recursive partitioning to identify the best predictive tree based on the lowest cross-validated relative error (CVRE). Prior to both BRT and MRT analyses, multicollinearity among predictor variables was evaluated based on variance inflation factors (VIF) obtained from a generalised linear model (see Chapter 4). Variables with VIF values > 5 were removed from further analyses. Statistical analyses were performed using the R software program version 4.2.2 (R Core Team 2022). The BRT analyses were done using the R packages *dismo* (Hijmans et al. 2015) and *gbm* (Ridgeway 2015). Multivariate regression trees were done using the R packages *mvpart* (Therneau and Atkinson 2014) and *MVPARTwrap* (Ouellette and Legendre 2013).

Results

The best BRT model was optimised to a minimum of 2500 trees based on *tc* of 7, *lr* of 0.001 and bag fraction of 0.5. Cross-validation results showed *cv* correlations of 0.90 ± 0.04 (mean ± standard error) and AUC scores of 0.97 ± 0.04, indicating that the adjusted models reasonably fitted the data. Altitude was ranked as the most important variable (92.1%) in explaining the occurrence of non-native fishes (Table 6.1). Other important variables were conductivity (2.5 %) and stream width (2.5%). Therefore, the results showed that within the Great Fish River system, probability of multiple invasions was generally high at low altitude sites with greater channel width and high conductivity (Figure 6.1). This was characteristic of the mainstem and large tributary sites of the river system.

Table 6.1. The relative contribution (%) of predictor variables for a boosted regression tree that was cross-validated based on tree complexity of 7, learning rate of 0.001, a bag fraction of 0.5 and a tree complexity of 2500.

Predictor variable	**Relative contribution (%)**
Altitude	92.08
Conductivity	2.53
Average width	2.51
River attribute	1.33
Boulders	0.37
Turbidity	0.29
pH	0.15
Dissolved oxygen	0.12
Vegetation	0.12
Bedrock	0.11
Average depth	0.10
Cobbles	0.10
Sand	0.08
Temperature	0.07
Gravel	0.05

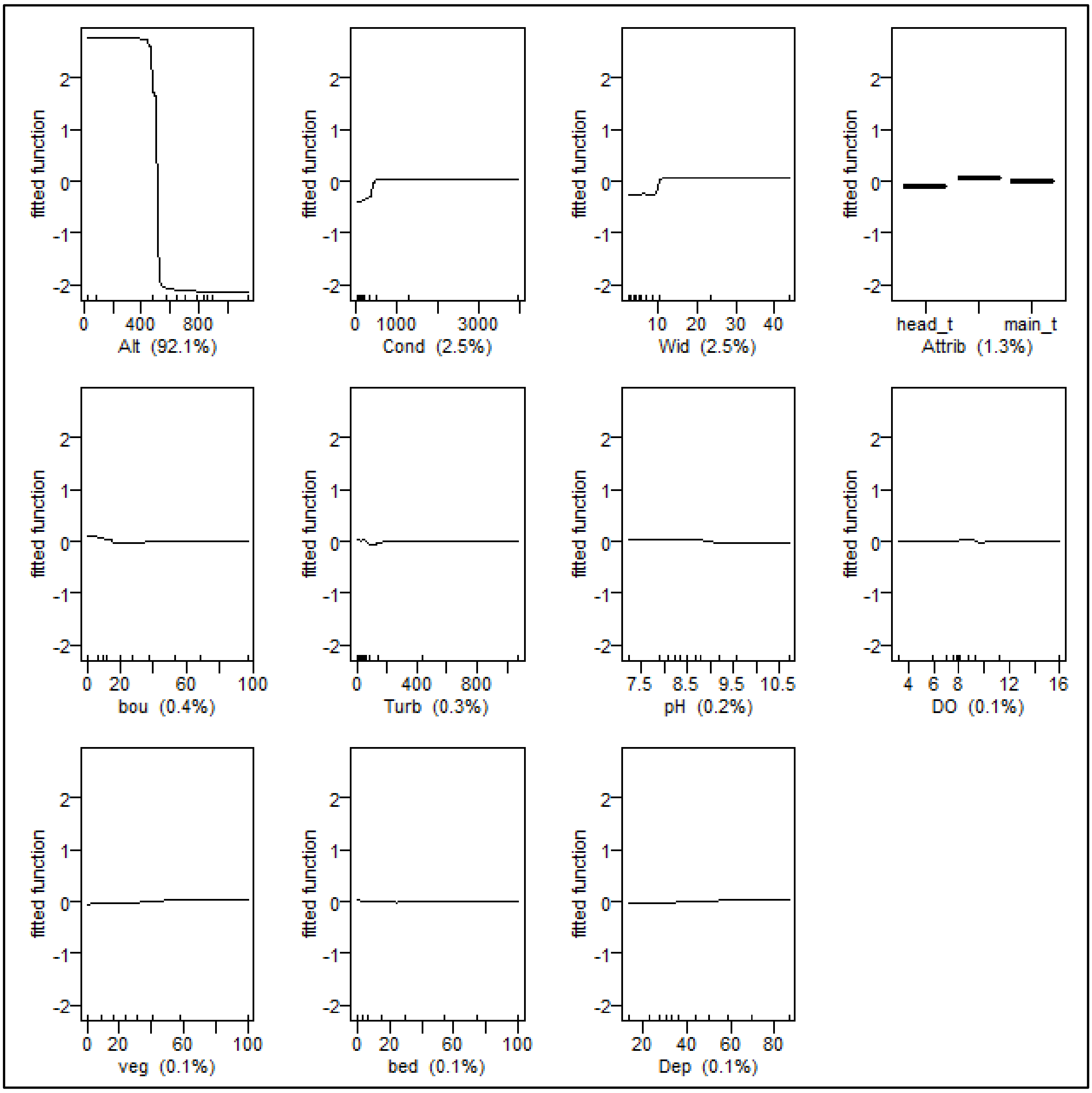

Figure 6.1. Partial dependence plots showing the probability of invasion (abscissa scale, point distance from the y-axis as scaled with the x-axis) as a function of the different predictor variables (ordinate). Abbreviations are Alt = altitude, Cond = conductivity, Wid = stream width, Attib = site category (i.e. headwater tributary sites, large tributary sites and mainstem sites), bou = boulder, Turb = turbidity, DO = dissolved oxygen, veg = vegetation cover, bed = bedrock and Dep = average depth.

Multivariate regression tree analysis of native fishes revealed assemblage associations that depicted changes in species composition from headwaters to downstream sections (Figure 6.2). These associations were shown by the occurrence of *Enteromius mandelai* and *A. mossambica* at high-altitude (> 715 m.a.s.l.) sites with high conductivity, the relative high frequency of occurrence of *S. bainsii* together with *E. mandelai* and *A mossambica* at high latitude sites with low conductivity, which were characteristic of headwater sites, and the general occurrence of *L. umbratus*, *A. mossambica*, *E. mandelai*, *G. callidus* and *S. bainsii* at middle altitude (715 < m.a.s.l. ≥ 489) sites, which was generally characteristic of mainstem sections of the major tributaries. Low-altitude sites (<489 m.a.s.l.) were represented by assemblages that were either dominated by freshwater fishes (*L. umbratus*, *G. callidus*, *O. mossambicus*, *E. mandelai* and *A. mossambica*) or comprised estuarine species, such as *M. falciformis*, *P. capensis* and *M. cephalus* (Figure 6.2).

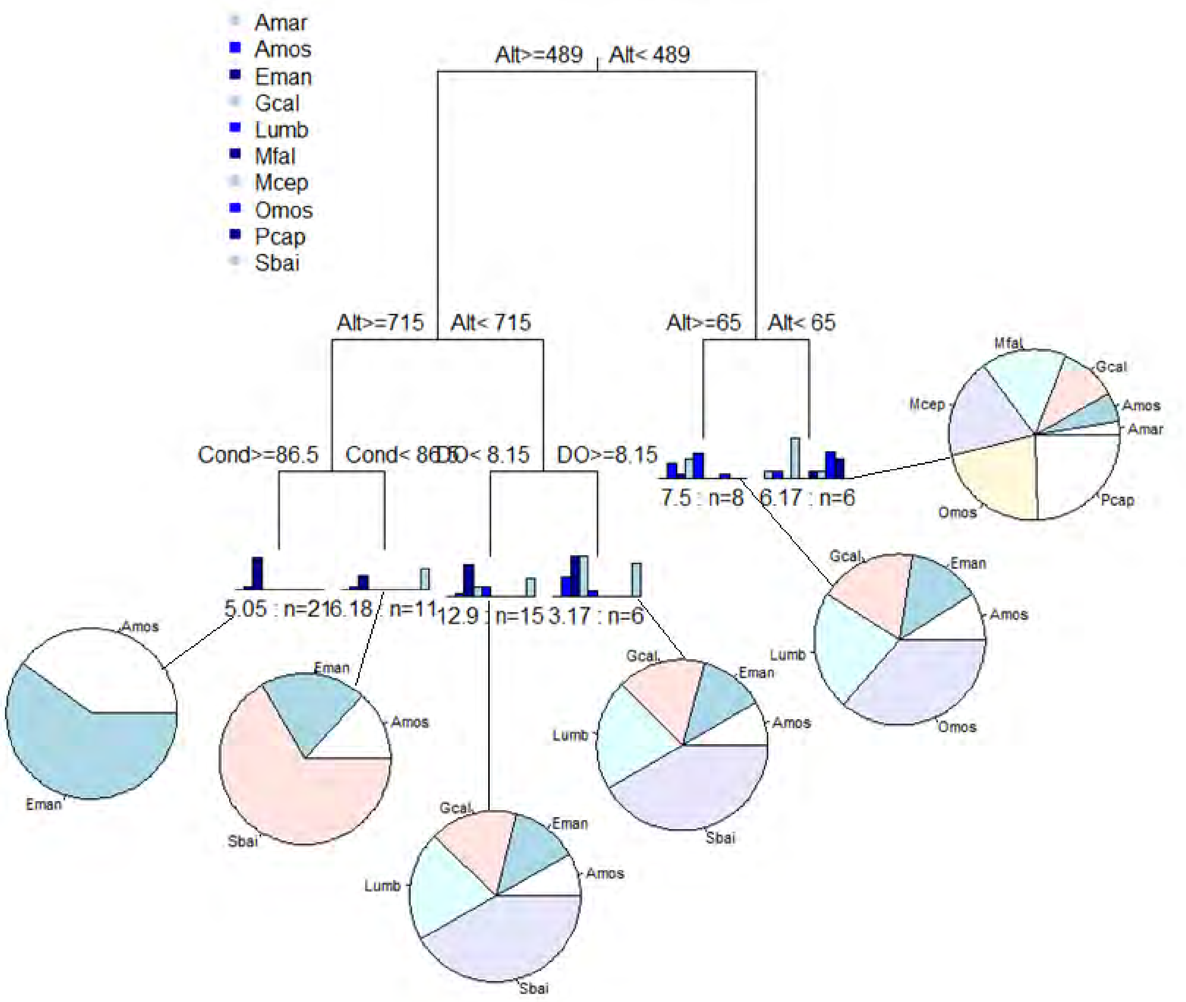

Figure 6.2. Multivariate regression trees depicting the relationship between native fishes and environmental variables. In the pie charts, the species are abbreviated as: Amar = *Anguilla marmorata*, Amos = *Anguilla mossambica*, Eman = *Enteromius mandelai*, Lumb = *Labeo umbratus*, Gcal = *Glossogobius callidus*, Mfal = *Monodactylus falciformis*, Mcep = *Mugil cephalus*, Pcap = *Pseudomyxus capensis*, Omos = *Oreochromis mossambicus* and Sbai = *Sandelia bainsii*.

Multivariate regression analysis of the non-native fishes showed assemblage associations that depicted spatial variation (Figure 6.3). These association included the predominance of *L. aeneus* and *C. gariepinus* at sites that were an altitude > 468 m.a.s.l., which was characteristic of upper mainstem sections of the Great Fish River. Low-altitude sites (< 468 m.a.s.l) comprised two assemblage associations. The first assemblage, which consisted of *L. aeneus*, *C. carpio*, *C. gariepinus* and *T. sparrmanii*, represented non-native fishes that occurred in the mainstem sections of the major tributaries. The second association consisted of all non-native fishes and was characteristic of the lower mainstem section of the Great Fish River (Figure 6.3).

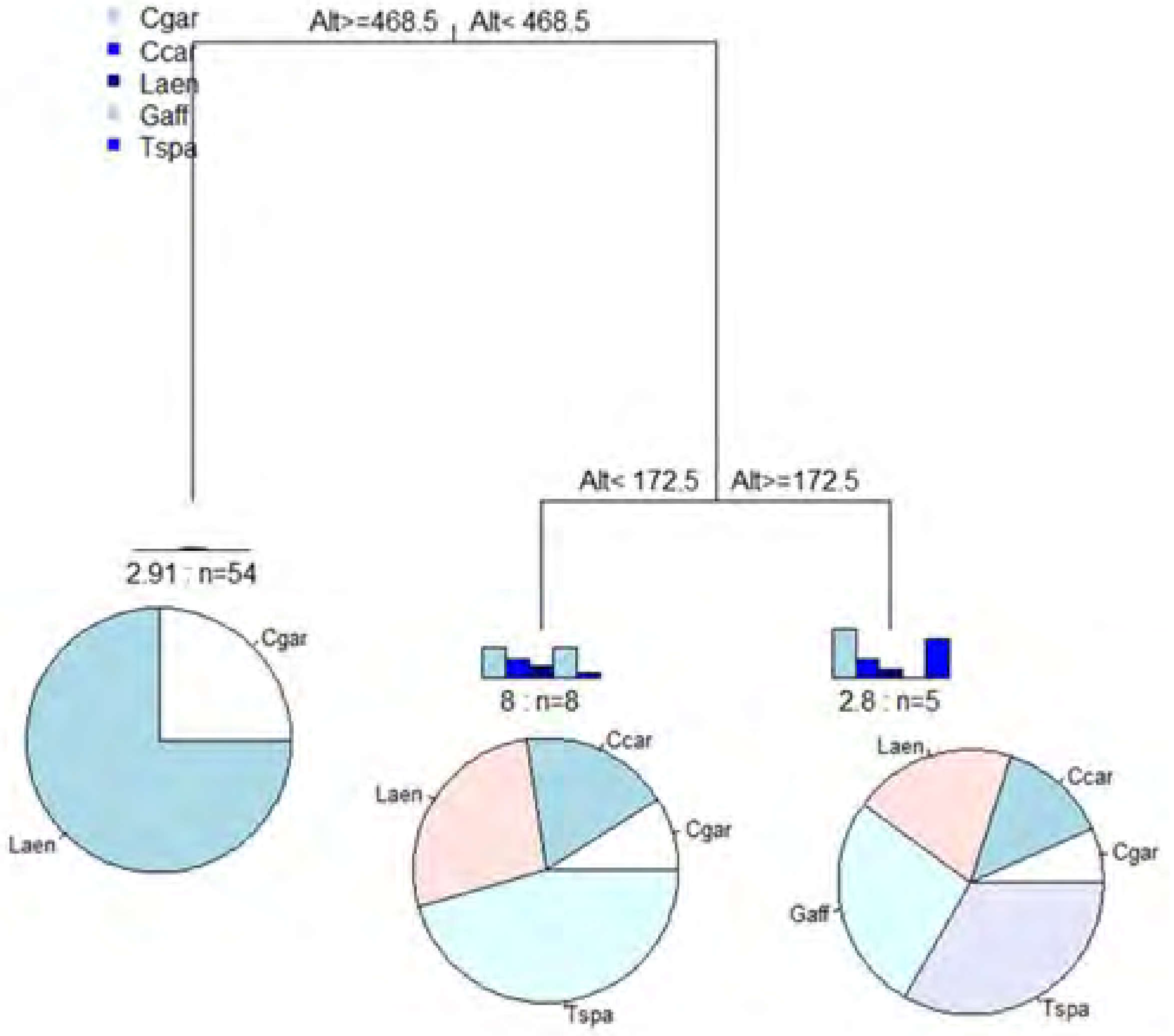

Figure 6.3. Multivariate regression trees depicting the relationship between non-native fishes and environmental variables. In the pie charts, the species are abbreviated as: Ccar = *Cyprinus carpio*, Laen = *Labeobarbus aeneus*, Cgar = *Clarias gariepinus,* Gaff = *Gambusia affinis* and Tspa = *Tilapia sparrmanii*.

For the combined fish groups, MRT analyses revealed two assemblage association patterns (Figure 6.4). The first pattern was represented by the associations of native species (*L. umbratus*, *A. mossambica*, *E. mandelai*, *G. callidus* and *S. bainsii*) that occurred at high altitude (>508 m.a.s.l.) sites. The second pattern revealed associations that included both native and non-native species at low altitude (<508 m.a.s.l.) sites. Two non-native species, *C. gariepinus* and *C. carpio* were most ubiquitous because they were present in all the invaded assemblage associations (Figure 6.4). Other non-native species included *G. affinis*, which was associated with assemblages in either deep habitats or in habitats with low dissolved oxygen, and *T. sparrmanii*, which was associated with assemblages in either shallow habitats or in habitats with low dissolved oxygen. *Labeobarbus aeneus* was associated with assemblages occurring in middle altitude sites, which were generally characteristics of the Great Fish River mainstem sections and lower sections of major tributaries.

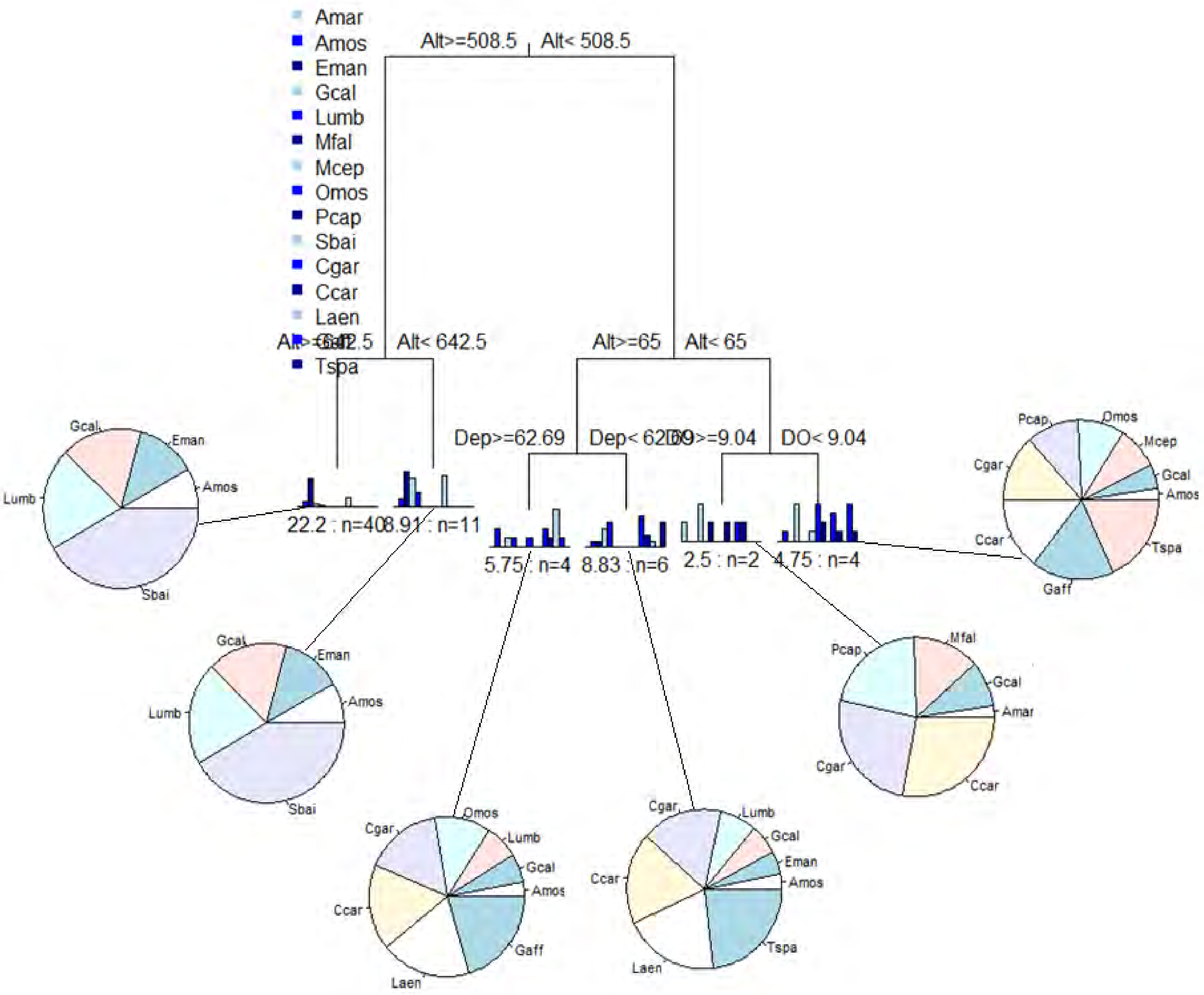

Figure 6.4. Multivariate regression trees depicting the relationship between all fishes and environmental variables. In the pie charts, the species are abbreviated as: Ccar = *Cyprinus carpio*, Laen = *Labeobarbus aeneus*, Cgar = *Clarias gariepinus*, Gaff = *Gambusia affinis*, Tspa = *Tilapia sparrmanii*, Amar = *Anguilla marmorata*, Amos = *Anguilla mossambica*, Eman = *Enteromius mandelai*, Lumb = *Labeo umbratus*, Gcal = *Glossogobius callidus*, Mfal = *Monodactylus falciformis*, Mcep = *Mugil cephalus*, Pcap = *Pseudomyxus capensis*, Omos = *Oreochromis mossambicus* and Sbai = *Sandelia bainsii*.

Discussion

Understanding non-native species' distribution and habitat suitable for their invasions within invaded habitats is important for mitigating the future spread and potential impacts associated with these invaders. The present study used machine learning techniques to model and predict non-native fish distributions and the associated assemblage patterns within the Great Fish River system. Results of the study revealed that altitude, conductivity and stream width were the primary predictors of species distribution and, in particular, multiple fish invasions within the Great Fish River system. Furthermore, the results also revealed spatial variation in assemblage of non-native species, which also co-occurred with some native species. However, the occurrences of these non-native species were limited to the upper and lower mainstem sections as well as the large tributary habitats.

The results of the BRT revealed that sites characterised by low altitude, low conductivity and wider stream channels had high probability of being invaded by multiple non-native species. These were the mainstem and large tributary sections, which were largely impacted by the change in flow regime caused by the opening of the Orange-Fish inter-basin water transfer (IBWT) tunnel (Laurenson and Hocutt 1986). There are important caveats regarding the potential co-occurrence of certain non-native species, as has been observed in other studies (e.g. Kuebbing et al. 2013; Jackson et al. 2014; Jackson 2015). For example, results of this chapter showed that the two global invaders, *C. carpio* and *C. gariepinus*, were ubiquitous as they occurred in all invaded sections. *Clarias gariepinus* is a generalised predator that is known to have a wide range of prey (Kadye and Booth 2012a). Its co-occurrence with native species is likely to result in competition with, and/or predation on, native species such as *A. mossambica, G. callidus, L. umbratus, M. falciformis, O. mossambicus* and *P. capensis* that occur in the mid to lower altitude. Similarly, *C. carpio*, through its bottom-grubbing feeding behaviour, can modify the habitat by increasing turbidity and nutrient suspension (Wahl et al.

2011; Vilizzi et al. 2015) with negative consequences for some native species. In contrast to the mainstem and large tributary sections, the high-altitude sites (i.e. headwater streams) were generally free of non-native species and were largely dominated by native minnows such as *E. mandelai* and the critically endangered *S. bainsii*. These sites were not affected by the change in flow regime posed by the Orange-Fish IBWT tunnel. These findings concur with previous studies that have shown the headwater streams to act as refuge habitats for native fishes against non-native species (Ellender et al. 2011; Kadye and Booth 2012b).

Although the headwater streams of the Great Fish River are generally unaffected by the change in natural flow (O'Keeffe and De Moor 1988) and remain largely free of invasions, there is still a possibility that they can be invaded under certain environmental conditions, especially during rainy seasons or periods of continuous flow (Kadye and Booth 2012b). This is because periodic invasions of headwater streams by non-native fish species have been shown to occur elsewhere (e.g. Ellender et al. 2011, 2015). In the present study, headwater streams appear to provide protection for vulnerable native species against potential threats posed by non-native fish species. Specifically, the critically endangered *S. bainsii* has limited distribution within the Great Fish River system headwaters where it is threatened by species invasions and habitat degradation (Chakona et al. 2020). Therefore, the headwater habitats where this species currently occurs, serve as *de facto* sanctuaries to its remaining populations within this river system. The potential invasion of non-native species such as *C. carpio, C. gariepinus* and *T. sparrmanii* into these headwaters, where *S. bainsii* occurs, will pose threats to its future existence. For instance, *T. sparrmanii*, which occurs in the major tributaries and is known to co-occur with *S. bainsii* could negatively impact the latter through competition for trophic resources and physical habitat (De Moor and Bruton 1988). Therefore, management interventions should prioritise conservation and protection of these headwaters from both habitat degradation and species introduction (Sifundza et al. 2021).

The results of this chapter also confirmed that only the flow-disturbed habitats were invaded by multiple non-native species, whereas the non-disturbed headwaters remained invasion free. Natural flow regime has been shown to plays a crucial role in deterring establishment of non-native species. In addition, altering system’s natural environment, such as changing its natural flow regime, has been hypothesised to weaken ecosystem’s resistance to invasions by non-native species and to facilitate the establishment and spread of non-native species in novel environments (Laurenson and Hocutt 1986; Baltz and Moyle 1993; Marchetti and Moyle 2001). Therefore, the findings of the present study appear to support the hypothesis that change in natural flow regime of the Great Fish River mainstem has weakened the system’s resistance to biological invasions, making it more susceptible to non-native fishes (Laurenson and Hocutt 1986). These findings have important implications to the general management of IBWT-modified systems within the context of biological invasions. This is because, in South Africa, there are several ongoing and planned IBWT schemes (Snaddon et al. 1999).

In conclusion, the findings of the present study predicted multiple non-native species to co-occur with native species within the mainstem and large tributary sections of the Great Fish River system, whereas the headwater streams were predicted to be invasion free. Therefore, in addition to the current ongoing conservation efforts, such as the creation of *S. bainsii* sanctuary in the Kat River headwaters, protection of this system’s headwater streams, which offer refuge against non-native species, should be considered a conservation priority.

CHAPTER 7

GENERAL DISCUSSION

Human-assisted movements of species into new environments, in which they previously did not occur, have accelerated species introduction in many ecosystems, including freshwater environments (Cambray and Jubb 1977; Ricciardi et al. 2007). In particular, inter-basin water transfer (IBWT) schemes are known to have facilitated movements of aquatic species between historically disjunct communities (Davies et al. 1992; Snaddon et al. 1999; Schmidt et al. 2019) and to be one of the main vectors of freshwater fish invasions in South Africa (Ellender and Weyl 2014). Despite this, mechanisms facilitating successful co-occurrences of these non-native species within IBWT-modified environments appear to be context-specific and, in some cases, remain unclear. Because there are several planned and ongoing IBWT schemes both locally and globally, there are general concerns on their potential ecological impacts on recipient ecosystems (Schmidt et al. 2019). Therefore, the Great Fish River serves as a critical example to unravel multiple fish invasion-related dynamics associated with IBWT-modified freshwater habitats, from which important lessons can be drawn.

This thesis provided evidence that multiple non-native fish species, owing to both intrinsic and extrinsic factors, have established successfully within the Great Fish River post the opening of the Orange-Fish IBWT (Chapter 3). At the inception of the IBWT, three non-native fish species (*Austroglanis sclateri*, *Labeo capensis* and *Labeobarbus aeneus*) were previously considered to have limited distributions, not extending beyond the Grassridge Dam, whereas *Clarias gariepinus* was deemed to occur in small numbers throughout its range within the Great Fish River (Laurenson and Hocutt 1986). Currently, however, these species, except *A. sclateri*, have since dispersed beyond the Grassridge Dam and further into the middle and the lower sections of the mainstem (Chapter 3). Specifically, *C. gariepinus* and *L. aeneus* were the most widespread and common of the non-native species, whereas *Labeo capensis* was characterised by incidental collections in the middle mainstem sections within the Great Fish River. In addition, other non-native fishes such as *C. carpio, Gambusia affinis* and *Tilapia sparrmanii,* introduced for angling and biological control, have also established successfully (Chapter 3). Overall, habitat-generalist species with broad environmental tolerance were able to establish successfully.

Differences in species functional traits have been suggested to facilitate co-occurrences of non-native and native species, and to facilitate the former's establishment success within invaded aquatic systems (Liu et al 2017; Qin et al. 2020; Angulo-Valencia et al. 2022). In the present study, no discernible differences were observed between native and non-native fish species in terms of functional traits (Chapter 4). Specifically, those native and non-native species that shared similar functional traits co-occurred within the invaded sections, underscoring the importance of shared (or having similar) functional traits between these two groups within the invaded habitats. The thesis also uncovered weak trait-environment relationships, which suggests that functional traits were not major drivers of fish community structure in the Great Fish River (Chapter 4). Interestingly, non-native species were inferred to have high trait divergence, and therefore likely had the potential to exploit or utilise wide range of resources.

The use of stable isotope-based analyses to ascertain species resource utilisation have become increasingly popular in ecology (Layman et al. 2007, 2012 Cucherousset et al. 2012a; Alp and Cucherousset 2022). Application of these techniques revealed propensity towards niche differentiation amongst non-natives species within some invaded sections (Chapter 5). This suggests trophic niche partitioning or differences in resource use amongst non-native species within the upper mainstem and the invaded tributary sections, which appears a plausible mechanism enabling the co-occurrences of these non-natives within these invaded sections. The resource partition observed is likely driven by vacant niches created by flow disturbance. This appeared to be consistent with studies elsewhere, which have inferred resource partitioning as a driver of co-existence between sympatric freshwater fishes, including between native and non-native fish species (Mason et al. 2008; Zambrano et al. 2010; Zengeya et al. 2011; Top-Karakuş et al. 2021). In contrast to the upper mainstem and the large tributary sections, weak trophic relations amongst non-natives were inferred as the probable mechanisms responsible for non-native species' co-occurrence in the lower mainstem section.

Application of machine-learning techniques confirmed that only the mainstem and large tributary sections were invaded, whereas the headwaters remained largely invasion free (Chapter 6). The mainstem was shown to be the most section susceptible to multiple fish invasions (Chapter 6). This finding implied that the headwaters appear to act as refuge for native species. Therefore, introductions of non-native species within these sections should be strongly discouraged. These findings are in line with others that have found that the headwaters provide refuge for native species (Ellender et al. 2011; Kadye and Booth 2013).

Based on the research undertaken after the opening of the Orange-Fish IBWT (e.g. Cambray and Jubb 1977; Laurenson and Hocutt 1984, 1986; Weyl et al. 2009; Ramoejane et al. 2020; Kadye and Booth 2013, 2020), it is apparent that the mitigating measures (reviewed in Cambray and Jubb 1977; Laurenson and Hocutt 1986) employed to prevent the translocations of species from the Orange River into the Great Fish River were ineffective. These measures included the following: introduction of "pepper-pot" valves to control the flow of water through the tunnel and direct the water against the concrete walls at high pressure in the recipient system; and the guarding of the tunnel's intake with grid of bars that can filter and prevent debris larger than 65 mm from entering the tunnel (Cambray and Jubb 1977; Laurenson and Hocutt 1986). Because of the inefficiencies of these introduced measures, the IBWT tunnel likely continues to be central in translocating and providing propagules of non-native species, such as *C. carpio*, *C. gariepinus*, *L. aeneus* and *L. capensis*, into the Great Fish River. Research shows that non-natives are likely to establish successfully in permanently altered river systems, with the most successful invaders being those that are able to adapt to the prevailing modified flow conditions (Moyle and Light 1996a, b; Bunn and Arthington 2002). It thus appears that the continuous flow of water from the Orange River may have affected the geomorphology of the Great Fish River, creating new and unoccupied habitats and providing vacant niches for non-native fishes. For example, continuous flow likely favoured species such as *L. capensis*, which is known to prefer fast flowing waters of large rivers (Skelton 2001). In addition, the Gariep Dam water has been described as highly turbid (Tómasson et al. 1984), and therefore, the transfer of this water into the Great Fish River has increased the latter’s turbidity (O'Keeffe and De Moor 1988) and likely favoured species such as *C. carpio* and *C. gariepinus* which do not require visual cues for survival. Natural flow is the fundamental determinant of species habitats in streams and its alteration is likely to facilitate successful establishment of non-native species in rivers (Bunn and Arthington 2002). For example, reduction in seasonal flow variation and a more stable flow regime have been suggested to have facilitated establishment of global invaders *C. carpio* and *G. affinis* elsewhere (reviewed in Bunn and Arthington 2002). Because these are conditions similar to those found in the mainstem section of the Great Fish River, it is likely that these types of conditions also facilitated establishment of non-natives species with broad environmental tolerance such as *C. gariepinus*, *C, carpio*, *G. affinis*, *L. aeneus* and *L. capensis*. All the above-mentioned scenarios likely enhance establishment of non-native species within the Great Fish River via increased propagule pressure, natural flow alteration and habitat modifications, as well as creation of niche opportunities for non-native species. Thus, the invasion of mainstem section of the Great Fish River underscores the importance of flow alteration in facilitating invasions in fluvial ecosystems.

Although no official extirpation of native species has been documented in the Great Fish River as a direct result of the opening of the Orange-Fish IBWT, research has shown some negative impacts associated with translocated species including predation by the generalist piscivore *C. gariepinus* (Kadye and Booth 2012a). Furthermore, although *L. umbratus* and its congeneric *L. capensis* co-occur naturally within the Orange River system, a recent study has highlighted probable interspecific hybridisation between the two species outside their native range (Ramoejane et al. 2020). Their introduction within the Great Fish River can thus provide opportunity for these two species to hybridise.

Perhaps the major concerns emanating from this thesis is that non-native co-occurred with native species in the mainstem and large tributary sections, and that mainstem was predicted to be susceptible to multiple fish invasions (Chapter 6). These non-natives are likely to have negative effects on range restricted native species occurring in the mainstem sections, such as those occurring in the lower section like *Gilchristella aestuaria* and *Glossogobius callidus*. In addition, introductions of these non-natives within the headwaters should be deterred, especially in those habitats where the critically endangered *Sandelia bainsii* occurs. The lack of discernible difference in functional traits between native and non-native fishes (Chapter 4) implies the risk of direct competition for resources between these two groups, particularly within the lower Great Fish River section where they have been shown to have a high isotopic overlap (Chapter 5). Similar concerns are expressed in the headwaters, where if *T. sparrmanii* and the critically endangered *S. bainsii* co-occur it could lead to direct resource competition between these species.

Conclusion

The opening of the Orange-Fish IBWT has facilitated the translocation and subsequent establishment of multiple non-native fish species within the Great Fish River system. In contrast to earlier predictions (e.g. Cambray and Jubb 1977; Laurenson and Hocutt 1986), the Grassridge Dam does not appear to act as an environmental buffer to the dispersal of some species as this study revealed that most fish species have extended their ranges downstream of the Grassridge Dam. This thesis has shown that the change in natural flow regime of the Great Fish River, as a result of the Orange-Fish IBWT, has rendered this system more susceptible to fish invasions. The IBWT has heavily impacted the Great Fish River, creating suitable habitats for invasions by multiple non-native fishes. In particular, the mainstem, which is the most modified, has become more susceptible to multiple fish invasions while the headwaters provide protection for natives.

The results of this thesis also suggest that both intrinsic and extrinsic factors can explain the invasion of non-native fishes within the Great Fish River. Both the periodic and opportunistic life-history strategies can facilitate establishment success of non-native species within the Great Fish River, and possibly in other similarly disturbed aquatic ecosystems. From the functional diversity perspective, the higher variability in functional trait diversity observed amongst non-native species, as reflected by functional diversity indices and the trait ordination, suggests wider exploitation of resources by these species. Change in natural flow regime has likely weakened the species trait-environmental relationships, an indication that environmental filtering was a poor determinant of functional structure within the Great Fish River. Therefore, it appears that weak trophic interactions amongst non-native species, coupled with trophic niche differentiation in some sections are probable mechanisms associated with co-occurrences of non-native fishes within the invaded sections of Great Fish River.

Petitjean and Davies (1988) reported that long-term ecological impacts associated with the Orange-Fish IBWT were not considered, or at least were ignored, before its opening. As evidenced from this thesis and other studies (e.g. O'Keeffe and De Moor 1988; Kadye and Booth 2012a), the Orange-Fish IBWT certainly had negative ecological consequence on the Great Fish River system, particularly within the mainstem. Likewise, future planned IBWT could facilitate the dispersal of aquatic species, including fish and macroinvertebrates, into environments or river systems in which they previously did not occur. They can also change the physico-chemical conditions of the recipient systems, thereby weakening their invasion resistance and making them more prone to invasions by non-native species. In light of these, it is prudent that planned future IBWT projects explore and consider the long-term ecological consequences prior to their construction, in order to minimize their ecological impacts on both the donor and the recipient systems. In addition, the efficacy of mitigating measures proposed to prevent potential translocations of species between the connected systems, should be fully tested prior to their implementations. Furthermore, as it was shown that the headwaters provide protection for the native species, regular monitoring of these habitats should be prioritised.

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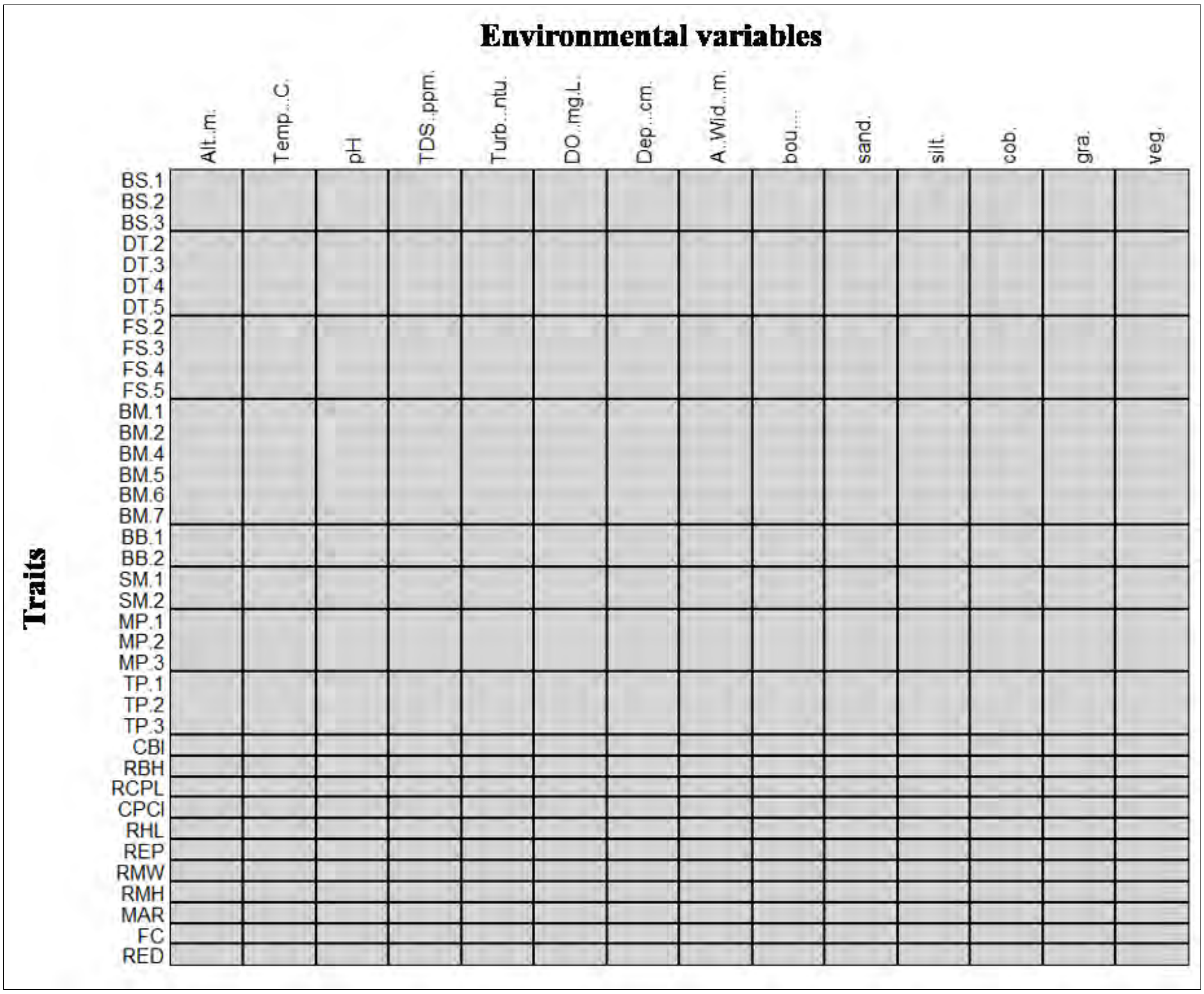
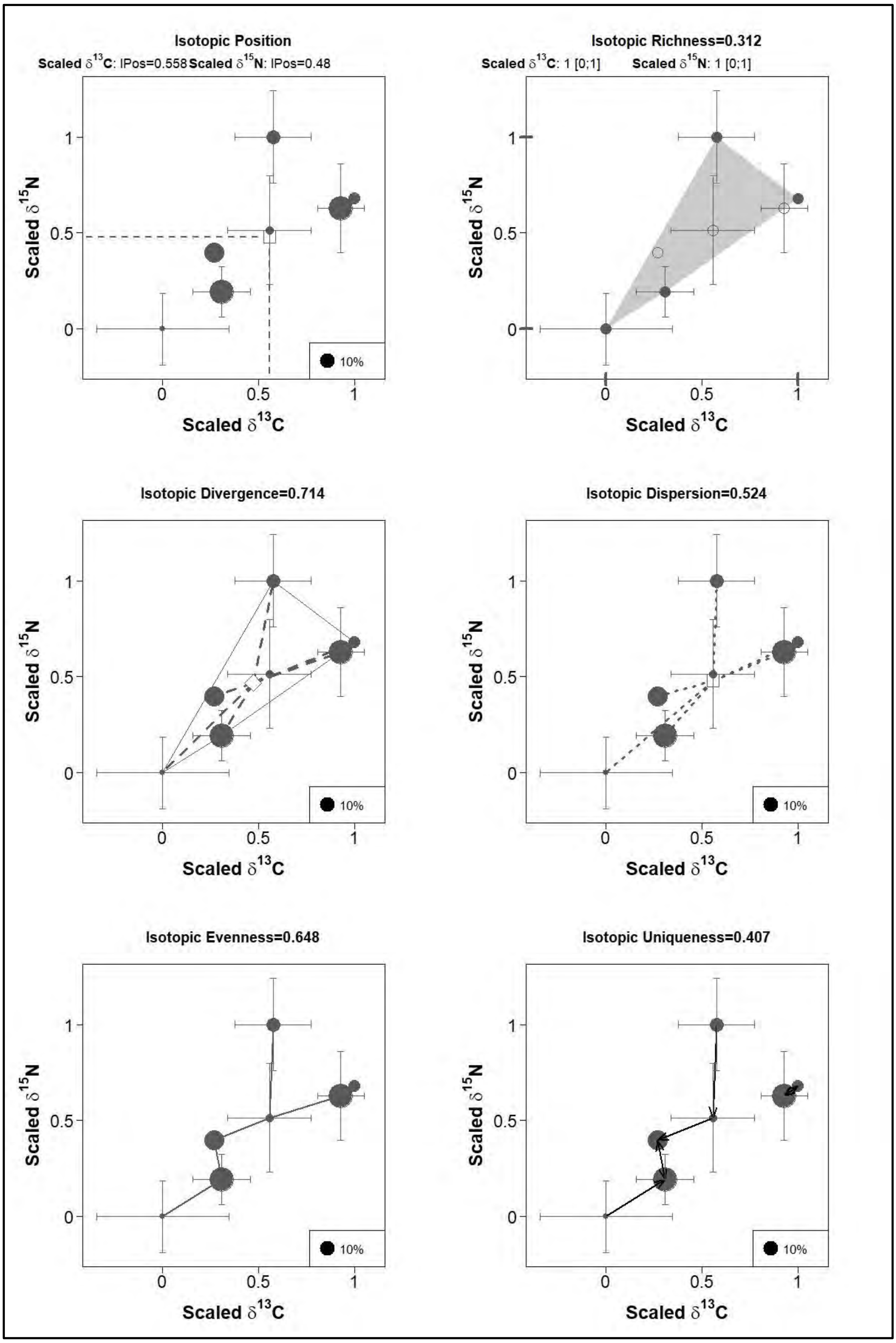
SUPPLEMENTARY MATERIAL

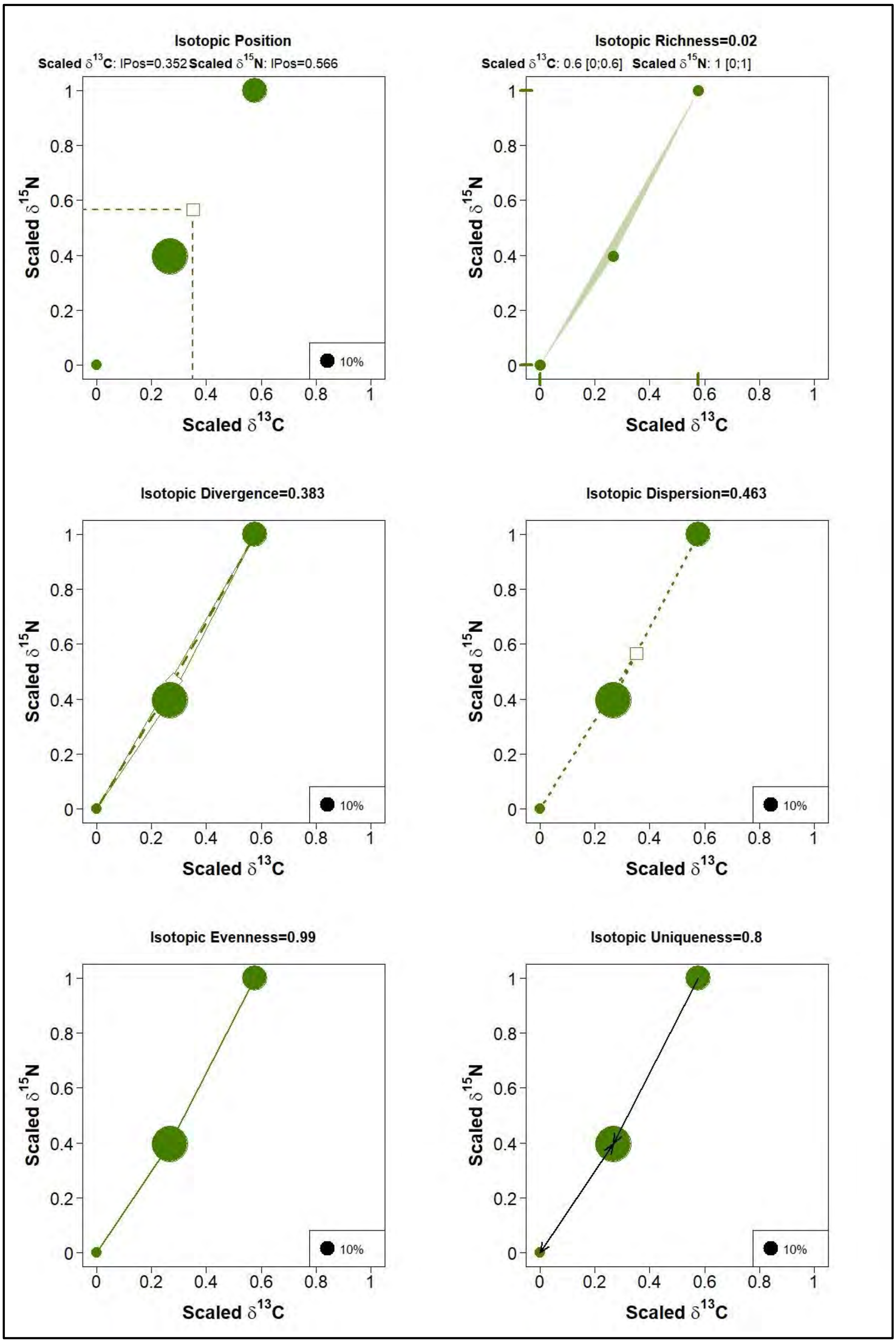
Supplementary figures



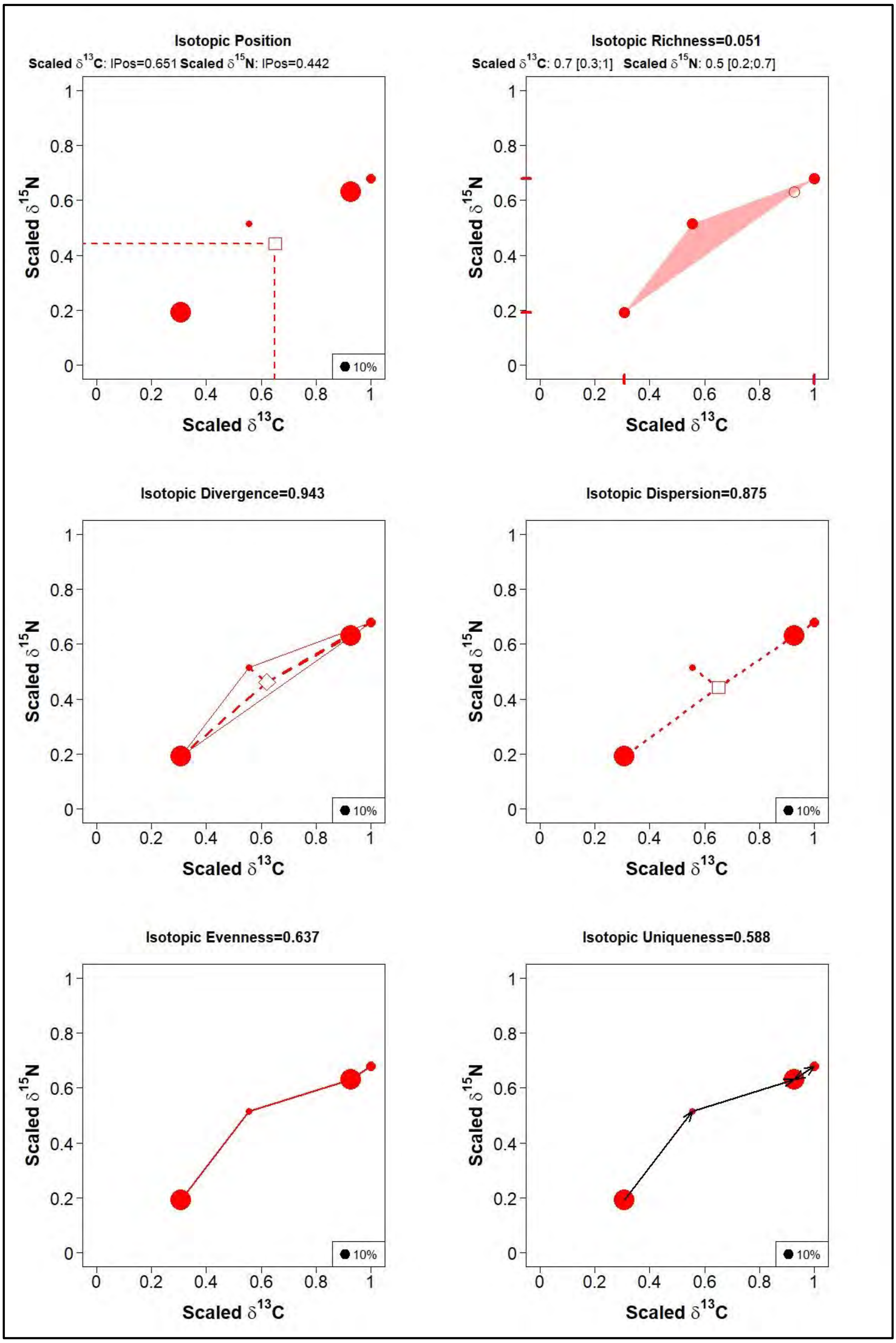
Supplementary Figure 1. Results of fourth-corner analysis revealing no significant association between species functional traits and environmental variables. Trait and environmental abbreviations are given on Table 4.1 and Supplementary Table 1, respectively.



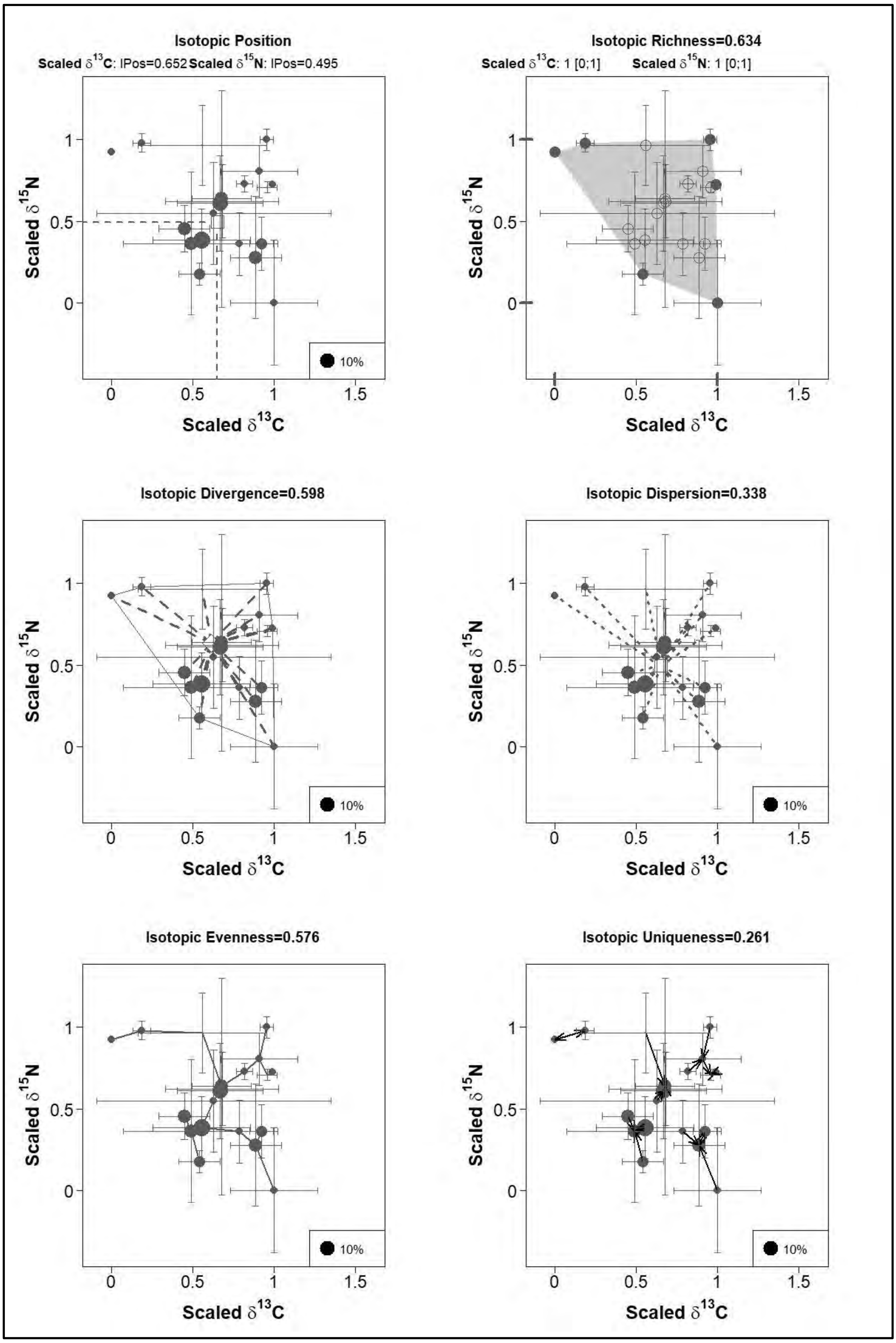
Supplementary Figure 2. Isotopic niche variability of fish assemblage (native and non-native species) within the upper Great Fish River (UGFR) based on isotopic diversity indices. Two populations of *E. mandelai* were used to allow for computation of IDIs within the UGFR.



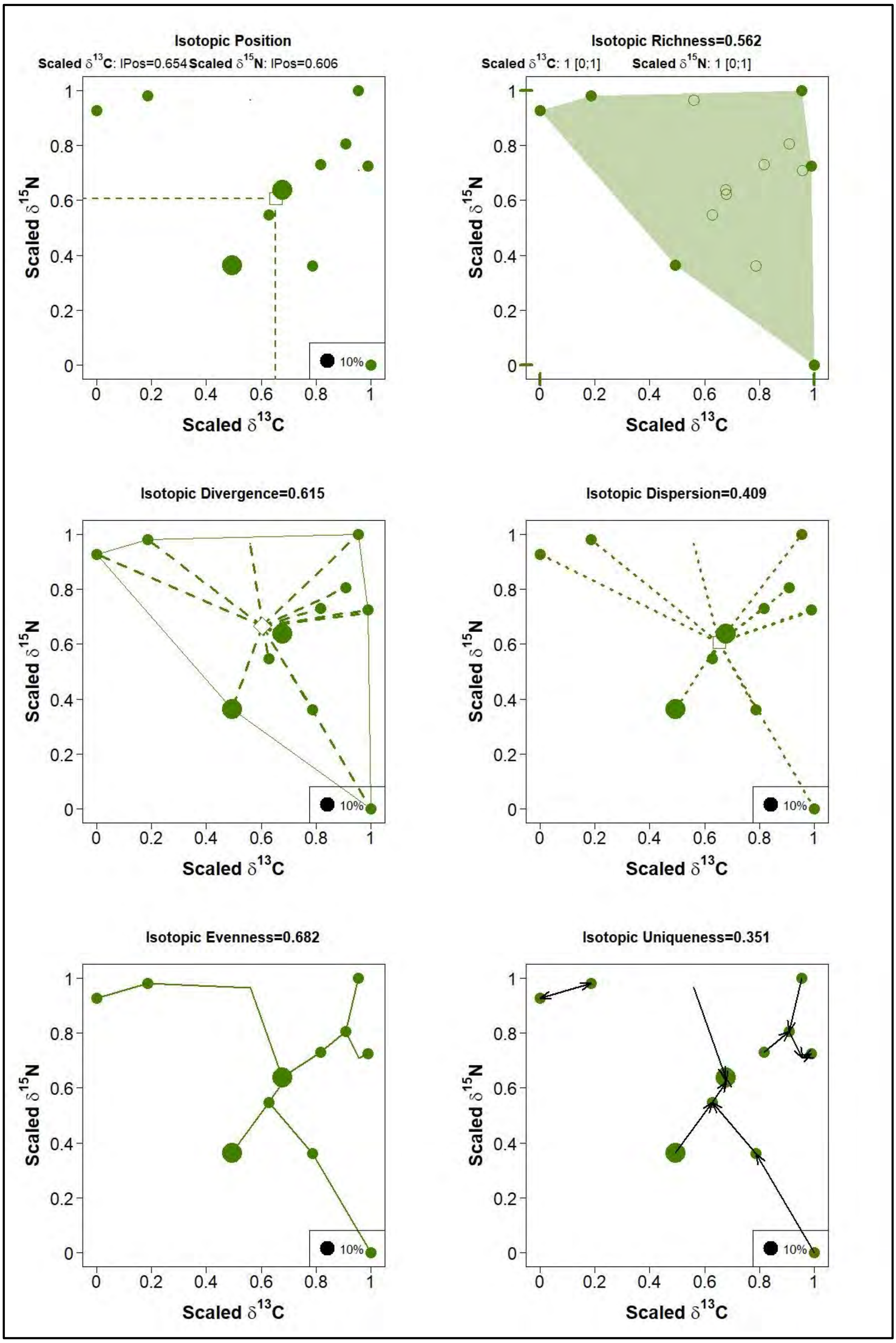
Supplementary Figure 3. Isotopic niche variability of native fish species within the upper Great Fish River (UGFR) based on isotopic diversity indices. Two populations of *E. mandelai* were used to allow for computation of IDIs within the UGFR.



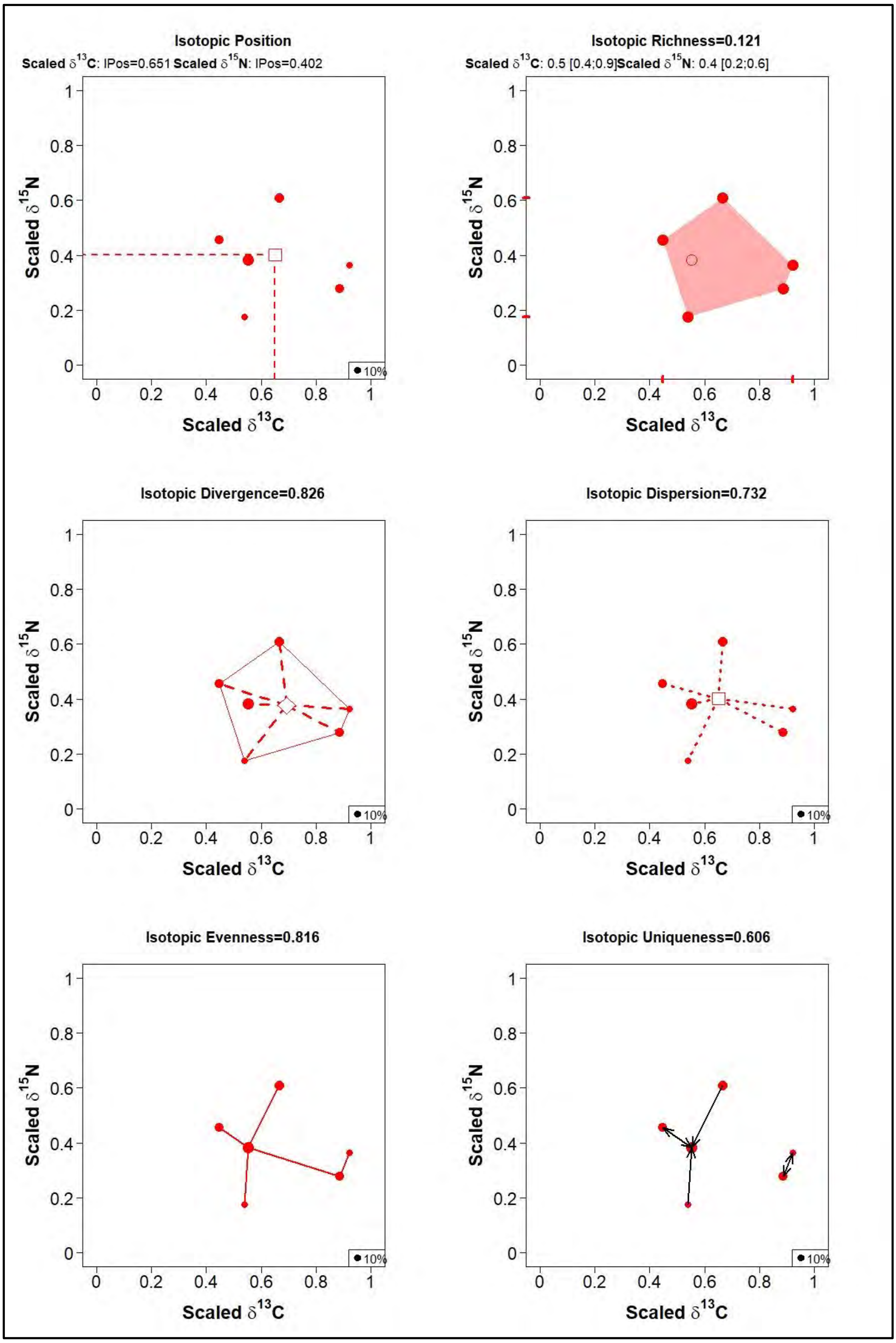
Supplementary Figure 4. Isotopic niche variability of non-native fish species within the upper Great Fish River (UGFR) based on isotopic diversity indices.



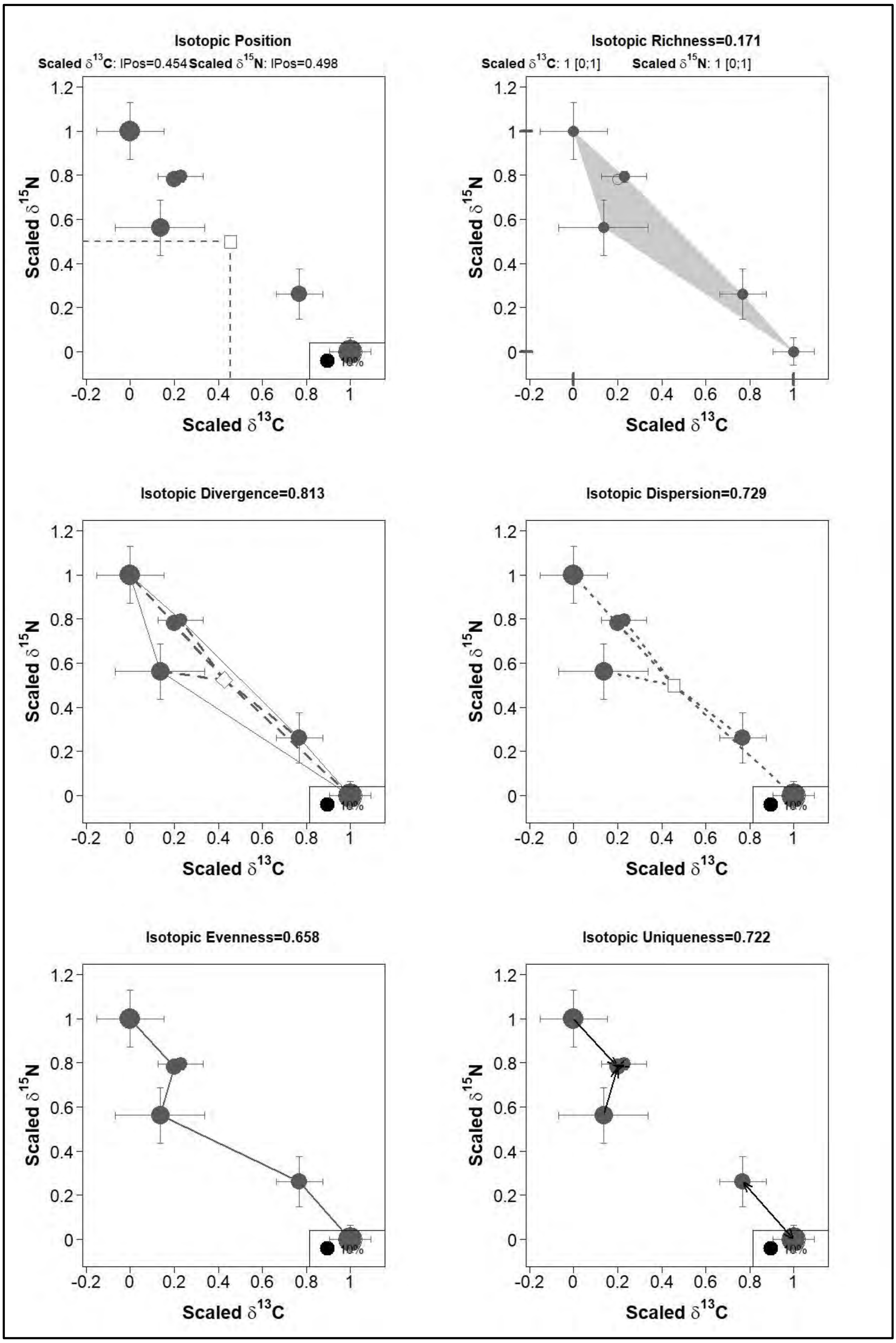
Supplementary Figure 5. Isotopic niche variability of fish assemblage (native and non-native species) within the lower Great Fish River (LGFR) based on isotopic diversity indices.



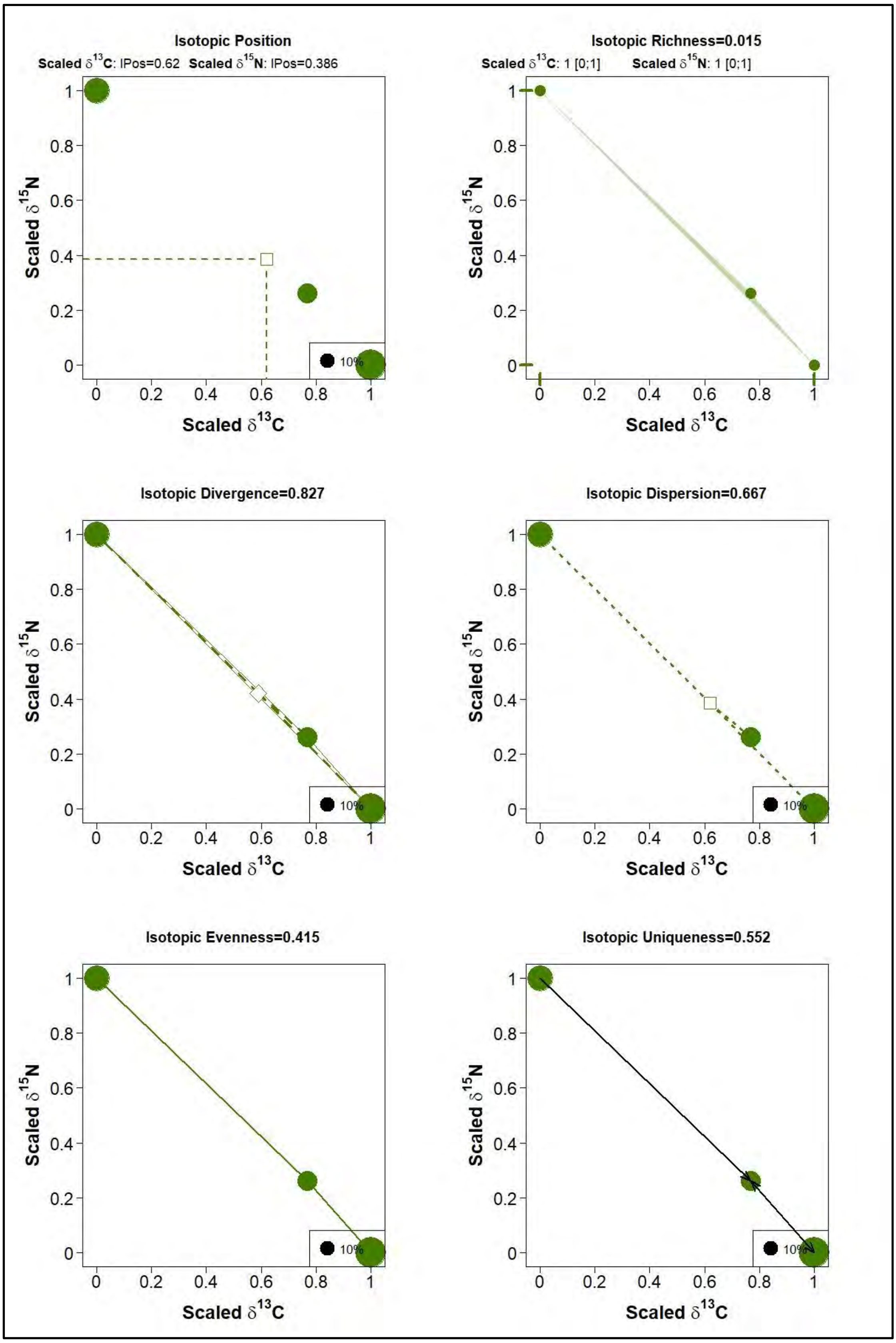
Supplementary Figure 6. Isotopic niche variability of native fish species within the lower Great Fish River (LGFR) based on isotopic diversity indices.



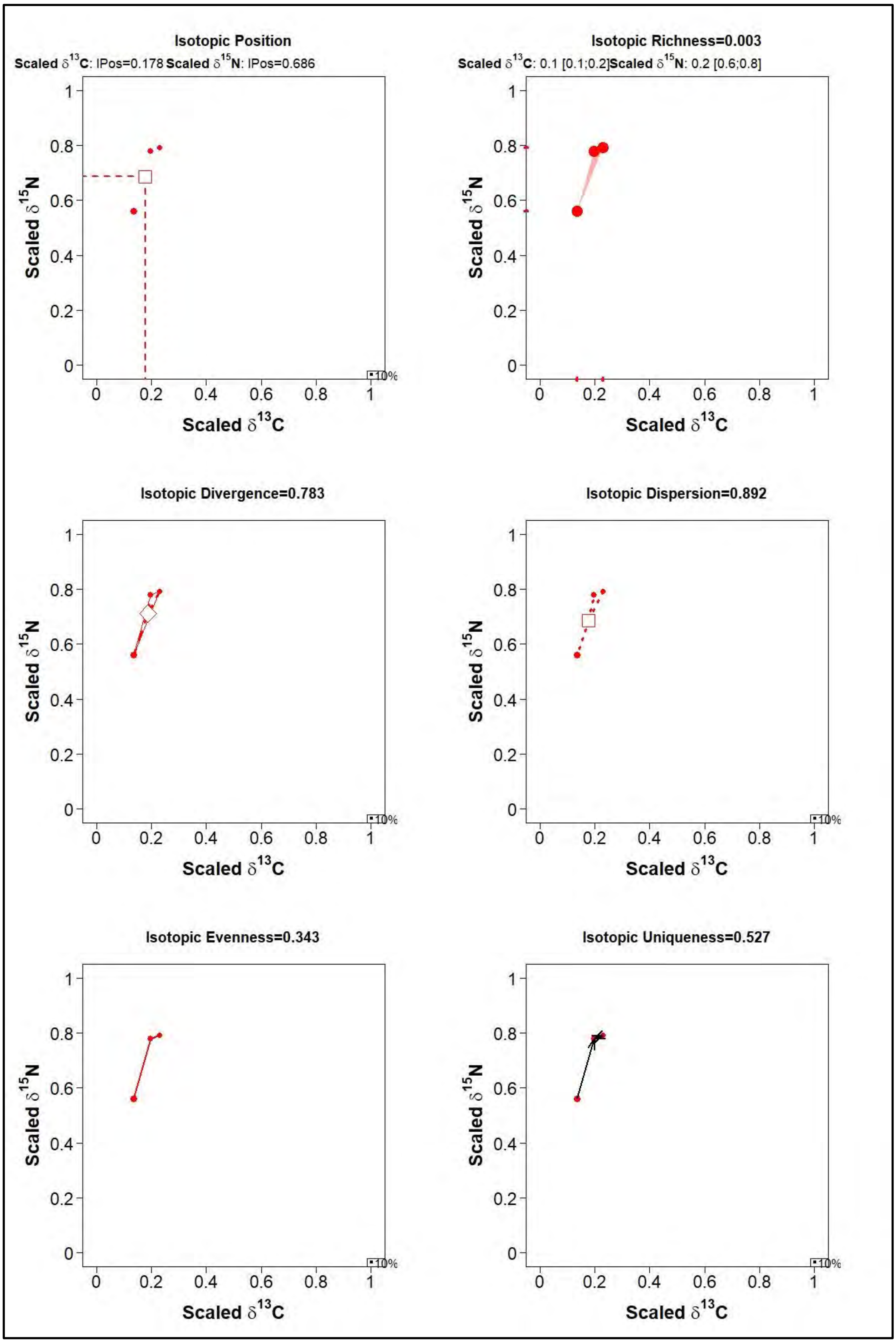
Supplementary Figure 7. Isotopic niche variability of non-native fish species within the lower Great Fish River (LGFR) based on isotopic diversity indices.



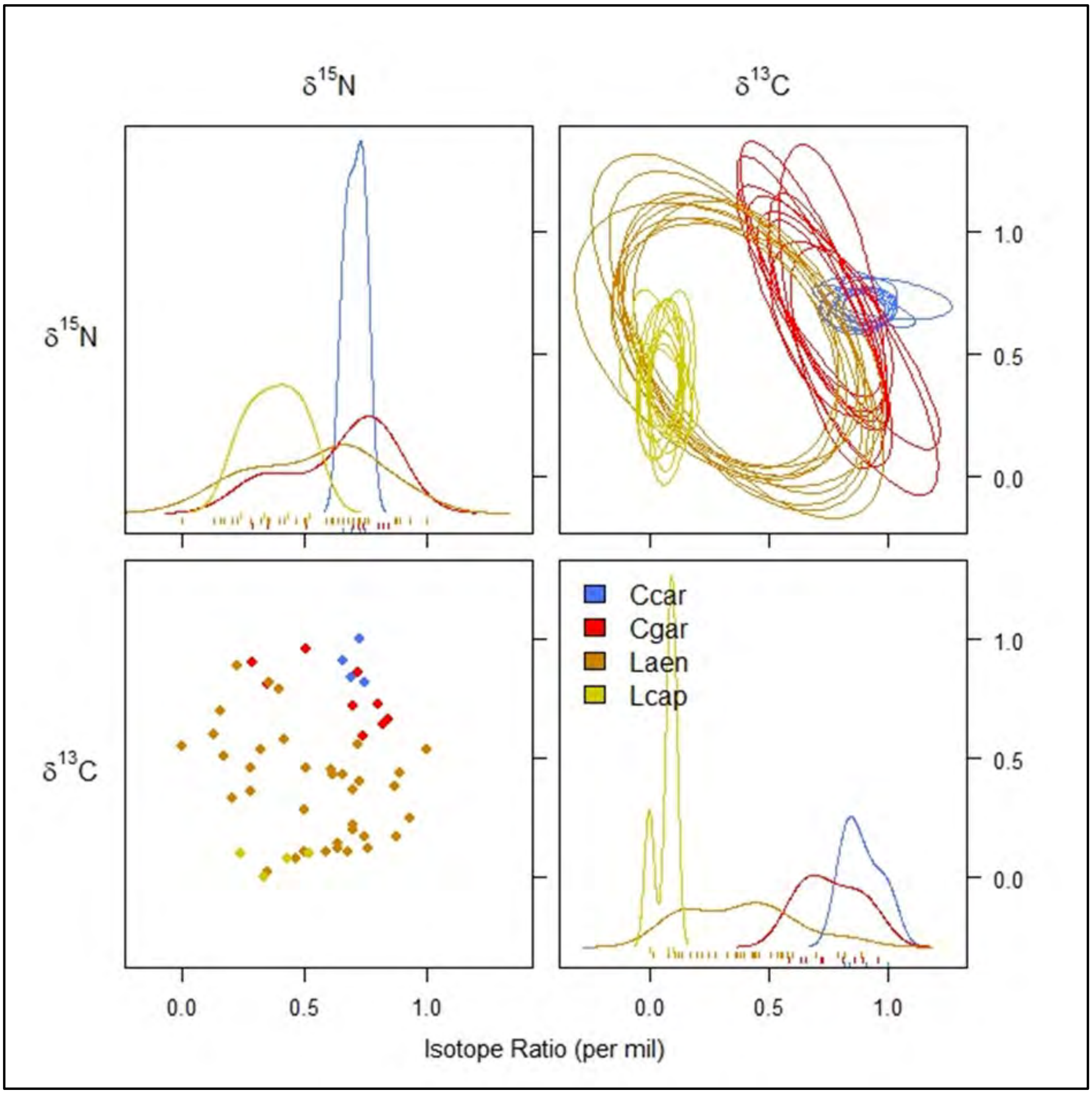
Supplementary Figure 8. Isotopic niche variability of fish assemblage (native and non-native species) within the Koonap River based on isotopic diversity indices.



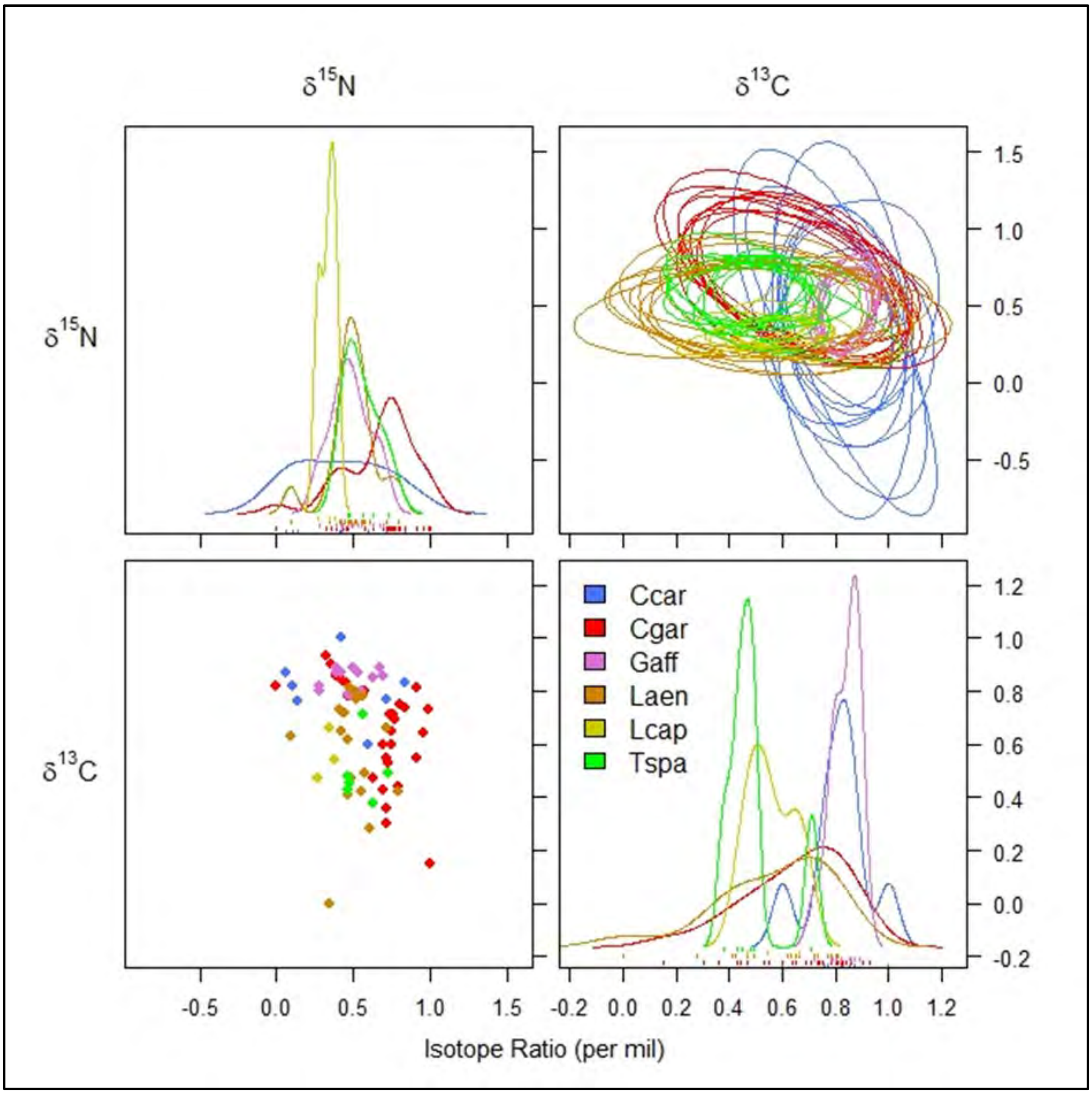
Supplementary Figure 9. Isotopic niche variability of native fish species within the Koonap River based on isotopic diversity indices.



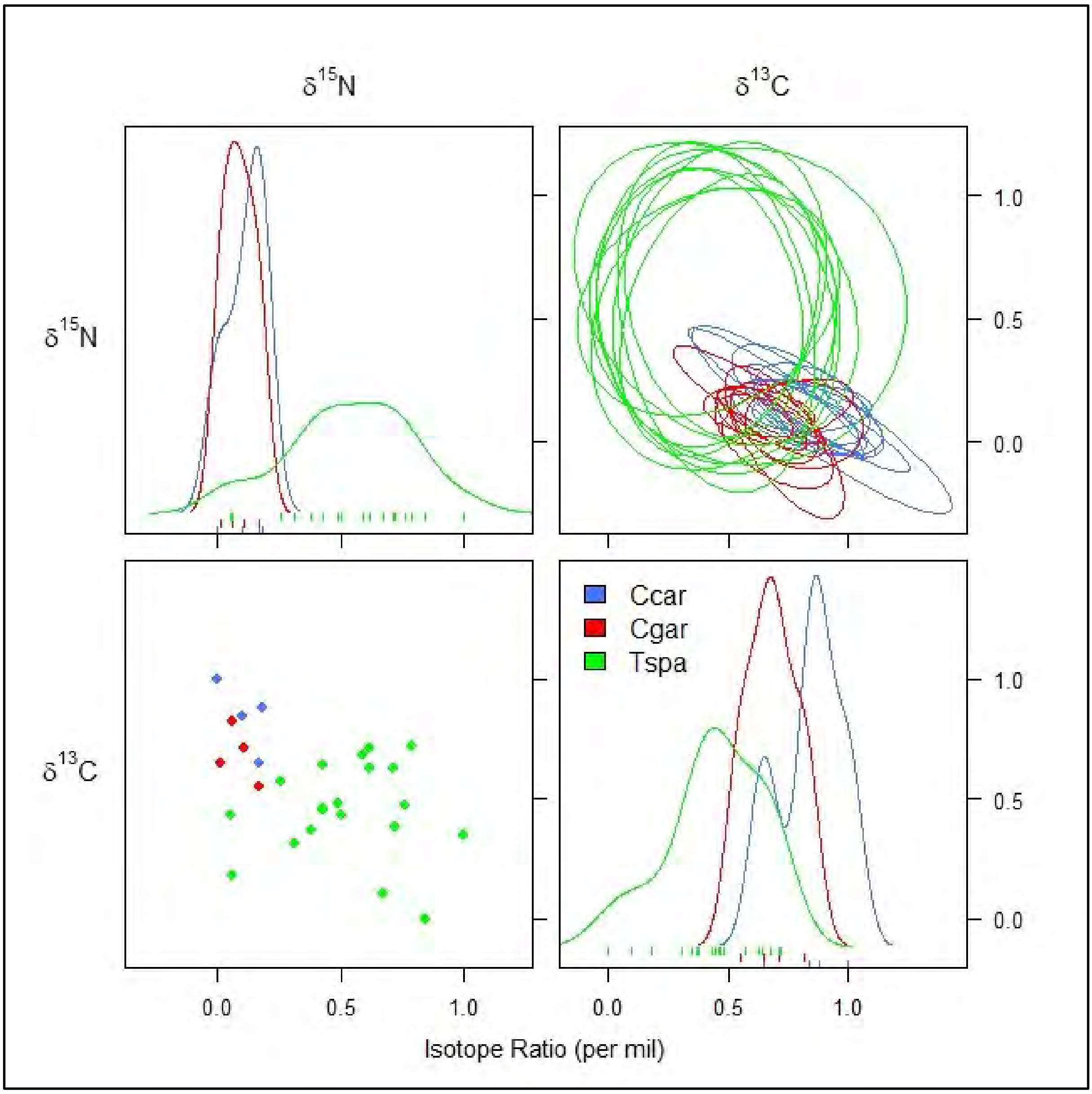
Supplementary Figure 10. Isotopic niche variability of non-native fish species within the Koonap River based on isotopic diversity indices.



Supplementary Figure 11. Random elliptical projections of trophic niche region (NR) for each non-native species within the upper Great Fish River. Also displayed are one-dimensional density plots (lines) and two-dimensional scatterplots. Ccar = *Cyprinus carpio*, Cgar = *Clarias gariepinus*, Laen = *Labeobarbus aeneus*, Lcap = *Labeo capensis*.



Supplementary Figure 12. Random elliptical projections of trophic niche region (NR) for each non-native species within the lower Great Fish River. Also displayed are one-dimensional density plots (lines) and two-dimensional scatterplots. Ccar = *Cyprinus carpio*, Cgar = *Clarias gariepinus*, Gaff = *Gambusia affinis*, Laen = *Labeobarbus aeneus*, Lcap = *Labeo capensis*, Tspa = *Tilapia sparrmanii*.



Supplementary Figure 13. Random elliptical projections of trophic niche region (NR) for each non-native species within the Koonap River. Also displayed are one-dimensional density plots (lines) and two-dimensional scatterplots. Ccar = *Cyprinus carpio*, Cgar = *Clarias gariepinus*, Tspa = *Tilapia sparrmanii.*

Supplementary Tables

Supplementary Table 1. Physico-chemical variables and habitat conditions (unit of measurement) recorded from 67 sites within the Great fish River system. Abbreviations are: Lat. = latitude, Long. = longitude, Alt = altitude, Temp = temperature, Con = conductivity, TDS = total dissolved solids, Turb = turbidity, DO = dissolved oxygen, Dep. = average depth, A.W = average width, Tot. L = sampled site length, bed. = bedrocks, bou. = boulders, sand = sand, silt = silt, cob = cobbles, gra = gravel and veg = vegetation.

Site	Lat.	Long.	Alt (m)	Temp (°C)	Cond. (μs)	pH	TDS (ppm)	Turb. (ntu)	DO (mg/L)	Dep. (cm)	A. Wid. (m)	Tot. L (m)	bed. (%)	bou. (%)	sand (%)	silt (%)	cob (%)	gra (%)	veg (%)
1	-32.624	26.827	872	30.2	86.0	8.06	41.00	55.80	7.00	24.05	2.97	46.83	15.00	51.67	0.00	6.67	10.00	16.67	28.33
2	-32.626	26.834	892	24.1	33.0	7.83	19.00	19.20	6.40	22.33	2.51	26.70	40.00	16.67	0.00	43.33	0.00	0.00	46.67
3	-32.616	26.817	846	24.1	65.0	8.08	39.00	25.90	6.80	27.80	4.22	35.40	13.33	76.67	0.00	0.00	3.33	6.67	93.33
4	-32.559	26.813	820	24.2	161.0	8.66	94.00	40.40	7.80	41.90	6.70	43.80	0.00	36.67	6.67	36.67	20.00	0.00	23.33
5	-32.546	26.799	793	28.5	216.0	8.42	127.00	28.90	5.50	33.23	6.79	36.10	3.33	6.67	10.00	40.00	23.33	16.67	6.67
6	-32.547	26.775	762	26.3	202.0	9.24	123.00	22.90	10.50	29.20	3.28	20.40	6.67	20.00	3.33	0.00	53.33	16.67	3.33
7	-32.518	26.789	807	26.0	186.0	9.19	109.00	43.30	7.60	18.41	2.97	50.00	3.33	53.33	10.00	26.67	0.00	6.67	6.67
8	-32.507	26.793	850	23.5	150.0	8.19	86.00	26.90	7.40	29.77	6.92	29.63	40.00	10.00	3.33	46.67	0.00	0.00	36.67
9	-32.564	26.763	777	27.4	380.0	8.38	223.00	8.35	6.50	37.00	2.67	10.40	10.00	0.00	0.00	0.00	0.00	90.00	76.67
10	-32.577	26.722	639	22.9	129.0	9.67	76.00	70.30	8.00	30.33	8.13	12.00	100.00	0.00	0.00	0.00	0.00	0.00	41.67
11	-32.576	26.722	637	24.2	130.0	8.70	76.00	69.90	8.00	41.73	5.71	38.60	93.33	6.67	0.00	0.00	0.00	0.00	20.00
12	-32.564	26.677	577	25.0	110.0	8.35	64.00	272.00	8.20	24.16	6.75	41.50	0.00	70.00	0.00	6.67	0.00	23.33	53.33
13	-32.544	26.712	609	25.1	132.0	8.68	77.00	64.10	8.30	32.57	8.70	29.60	0.00	66.67	0.00	6.67	0.00	26.67	36.67
14	-32.510	26.743	688	26.3	97.0	8.92	57.00	18.70	7.80	28.60	7.63	44.10	56.67	10.00	0.00	26.67	0.00	6.67	23.33
15	-32.473	26.726	841	24.3	82.0	9.13	48.00	33.20	7.50	19.17	3.68	15.00	30.00	36.67	0.00	26.67	6.67	0.00	50.00
16	-32.616	26.678	546	23.4	118.0	9.22	69.00	275.00	7.20	83.23	21.50	23.50	0.00	0.00	30.00	33.33	0.00	36.67	23.33
17	-32.642	26.688	540	25.6	121.0	9.10	71.00	31.80	8.10	86.25	12.33	22.00	58.33	8.33	0.00	0.00	33.33	12.50	0.00
18	-32.643	26.663	512	27.3	130.0	8.12	74.00	518.00	7.20	44.17	3.95	22.60	6.67	56.67	0.00	0.00	0.00	36.67	16.67

Site	Lat.	Long.	Alt (m)	Temp (°C)	Cond. (μs)	pH	TDS (ppm)	Turb. (ntu)	DO (mg/L)	Dep. (cm)	A. Wid. (m)	Tot. L (m)	bed. (%)	bou. (%)	sand (%)	silt (%)	cob (%)	gra (%)	veg (%)
19	-32.887	26.707	314	30.7	1678.0	9.84	990.00	8.72	14.60	35.20	4.00	28.80	100.00	0.00	0.00	0.00	0.00	0.00	100.00
20	-32.778	26.646	312	24.5	383.0	9.40	227.00	51.20	3.20	36.23	4.18	73.34	83.33	0.00	0.00	3.33	13.33	0.00	60.00
21	-32.716	26.588	464	29.0	185.0	9.32	109.00	699.00	7.00	36.40	10.31	69.38	0.00	26.67	0.00	63.33	0.00	10.00	60.00
22	-32.555	26.672	598	28.3	98.0	9.72	57.00	58.50	8.00	24.97	4.61	53.60	56.67	0.00	3.33	10.00	6.67	23.33	56.67
23	-32.575	26.655	663	23.7	156.0	8.44	90.00	118.00	8.10	15.27	3.07	25.22	6.67	53.33	3.33	16.67	0.00	20.00	26.67
24	-32.568	26.620	754	26.3	106.0	8.50	62.00	29.30	7.40	16.37	3.29	45.04	10.00	50.00	0.00	26.67	6.67	6.67	30.00
25	-32.536	26.677	631	26.2	94.0	8.57	55.00	146.00	7.80	27.17	5.26	40.36	0.00	0.00	30.00	33.33	0.00	36.67	70.00
26	-32.705	26.604	473	23.2	185.0	9.37	109.00	720.00	7.50	31.37	8.73	65.78	0.00	23.33	0.00	16.67	60.00	0.00	23.33
27	-32.593	26.793	773	23.4	60.0	9.74	34.00	29.10	7.80	15.87	5.72	43.20	3.33	96.67	0.00	0.00	0.00	0.00	16.67
28	-32.632	26.866	948	22.3	43.0	9.31	24.00	17.20	8.20	13.73	2.89	45.50	0.00	6.67	0.00	0.00	93.33	0.00	36.67
29	-32.687	26.308	654	20.3	225.0	9.92	132.00	1000.00	5.50	48.23	6.37	30.40	3.33	10.00	6.67	43.33	10.00	26.67	20.00
30	-32.526	26.355	821	25.5	478.0	9.72	283.00	54.50	10.40	28.90	8.70	23.44	20.00	16.67	0.00	53.33	10.00	0.00	50.00
31	-32.496	26.395	873	28.0	525.0	9.48	310.00	31.70	8.00	49.53	8.46	51.50	3.33	6.67	26.67	3.33	50.00	10.00	30.00
32	-32.465	26.515	1022	27.5	335.0	9.02	196.00	4.57	9.30	23.60	3.35	27.58	0.00	86.67	0.00	3.33	6.67	3.33	13.33
33	-32.486	26.361	916	29.5	285.0	8.59	166.00	384.00	3.70	59.41	5.34	13.20	6.67	6.67	3.33	70.00	0.00	13.33	0.00
34	-32.571	26.264	786	25.9	633.0	8.81	371.00	10.60	8.00	54.03	3.65	41.30	30.00	70.00	0.00	0.00	0.00	0.00	0.00
35	-32.577	26.756	659	20.1	134.0	10.71	77.00	10.60	9.90	29.73	7.44	82.40	86.67	3.33	0.00	0.00	10.00	0.00	16.67
36	-32.502	26.364	845	21.0	310.0	7.95	182.00	92.20	7.10	54.68	9.83	19.00	90.30	9.70	0.00	0.00	0.00	0.00	0.00
37	-32.564	26.677	577	16.8	120.0	8.16	71.00	37.20	9.03	49.77	8.43	43.10	0.00	30.00	3.30	3.30	43.30	20.00	0.00
38	-32.544	26.712	615	17.6	129.0	8.21	77.00	98.60	8.84	44.40	8.63	35.40	0.00	50.00	6.70	0.00	26.70	16.70	0.00
39	-32.510	26.709	699	19.0	103.0	8.40	59.00	59.00	9.20	31.30	5.77	16.60	70.00	30.00	0.00	0.00	0.00	0.00	0.00
40	-32.465	26.515	990	14.9	329.0	7.74	192.00	17.20	4.76	44.41	5.60	10.50	8.60	11.40	8.60	40.00	20.00	11.40	0.00
41	-32.395	26.512	1139	16.5	97.0	8.53	54.00	4.30	7.56	40.29	5.47	12.00	43.30	26.70	3.30	0.00	23.30	3.30	0.00

Site	Lat.	Long.	Alt (m)	Temp (°C)	Cond. (µs)	pH	TDS (ppm)	Turb. (ntu)	DO (mg/L)	Dep. (cm)	A. Wid. (m)	Tot. L (m)	bed. (%)	bou. (%)	sand (%)	silt (%)	cob (%)	gra (%)	veg (%)
42	-32.869	26.354	413	18.9	205.0	8.31	120.00	719.00	5.98	60.73	12.10	19.80	60.00	3.30	0.00	0.00	13.30	23.33	0.00
43	-32.546	26.797	827	17.9	87.0	8.01	51.00	61.50	10.04	55.43	4.40	13.00	0.00	33.30	6.70	36.70	23.30	0.00	0.00
44	-32.632	26.866	980	9.8	39.0	7.63	24.00	24.20	13.36	31.00	2.67	23.70	80.00	16.70	3.30	0.00	0.00	0.00	0.00
45	-32.624	26.827	902	12.1	56.0	7.68	33.00	42.50	12.43	35.10	5.26	38.00	46.70	13.30	16.70	0.00	23.30	0.00	0.00
46	-32.616	26.817	897	13.8	54.0	8.08	32.00	46.10	13.90	65.23	6.09	34.00	6.67	63.30	30.00	0.00	0.00	0.00	0.00
47	-32.518	26.789	861	12.8	64.0	7.33	36.00	77.30	15.98	49.73	3.96	26.00	13.30	26.70	20.00	3.30	36.70	0.00	0.00
48	-32.632	26.866	865	15.5	63.0	7.75	36.00	80.00	11.50	31.27	4.09	21.00	20.00	43.30	16.70	0.00	20.00	0.00	0.00
49	-32.559	26.813	870	16.1	101.0	8.10	60.00	22.00	9.30	37.53	4.54	29.00	17.60	41.10	29.40	2.90	8.80	0.00	0.00
50	-32.553	26.810	852	18.3	105.0	8.27	63.00	23.60	14.20	52.94	5.74	25.00	18.80	12.50	12.50	31.30	25.00	0.00	0.00
51	-32.553	26.671	646	19.9	117.0	8.22	68.00	18.70	7.83	43.52	3.95	38.00	66.70	6.70	0.00	0.00	26.70	0.00	25.00
52	-32.575	26.655	731	26.6	428.0	7.21	192.00	82.30	4.42	23.47	2.30	13.00	3.30	63.30	13.30	20.00	0.00	0.00	40.00
53	-32.567	26.620	800	19.3	137.0	7.93	78.00	35.70	6.71	34.93	5.35	20.60	0.00	0.00	16.70	80.00	0.00	3.30	70.00
54	-32.536	26.677	680	24.0	106.0	8.67	62.00	13.50	7.05	33.10	3.52	30.00	0.00	73.30	6.70	0.00	23.30	0.00	5.00
55	-32.510	26.709	699	25.8	109.0	8.54	63.00	19.30	7.82	30.97	5.14	14.00	66.70	36.70	0.00	0.00	0.00	0.00	1.00
56	-32.473	26.726	880	27.6	118.0	8.34	70.00	30.01	5.67	51.70	5.93	45.00	30.00	50.00	3.30	3.30	0.00	10.00	5.00
57	-33.261	26.970	22	22.2	974.0	8.74	851.00	43.50	8.48	78.07	32.79	46.33	0.00	10.00	26.67	6.67	16.67	40.00	13.33
58	-33.259	26.971	29	21.2	1437.0	8.71	852.00	95.80	9.70	65.84	33.77	48.63	26.67	20.00	20.00	6.67	13.33	13.33	0.00
59	-33.238	26.995	41	21.3	1517.0	8.64	897.00	58.20	8.76	67.03	22.54	38.50	0.00	10.00	13.33	26.67	3.33	46.67	6.67
60	-33.238	26.995	43	21.2	1482.0	8.72	677.00	48.70	9.32	68.75	34.49	45.00	0.00	23.33	13.33	6.67	16.67	40.00	23.33
61	-33.233	26.980	44	21.2	1474.0	8.79	867.00	63.00	8.71	56.18	43.59	32.35	13.33	70.00	0.00	16.67	0.00	0.00	16.67
62	-33.233	26.979	44	21.7	1474.0	8.08	867.00	68.20	8.53	76.30	36.90	27.50	6.67	63.33	0.00	30.00	0.00	0.00	0.00
63	-33.160	26.838	86	25.6	469.0	8.25	798.63	1074.00	7.93	74.14	24.80	43.40	0.00	10.00	0.00	0.00	13.33	76.67	36.67
64	-33.161	26.839	86	25.6	469.0	8.25	798.63	1074.00	7.93	71.39	26.02	39.20	0.00	10.00	0.00	0.00	13.33	76.67	36.67

Site	**Lat.**	**Long.**	**Alt (m)**	**Temp (°C)**	**Cond. (µs)**	**pH**	**TDS (ppm)**	**Turb. (ntu)**	**DO (mg/L)**	**Dep. (cm)**	**A. Wid. (m)**	**Tot. L (m)**	**bed. (%)**	**bou. (%)**	**sand (%)**	**silt (%)**	**cob (%)**	**gra (%)**	**veg (%)**
65	-33.048	26.636	259	23.8	3952.0	8.05	2000.00	191.00	10.95	36.13	5.39	31.33	0.00	20.00	3.33	3.33	73.33	0.00	53.33
66	-32.953	25.815	505	22.6	1176.0	8.32	689.00	304.00	8.01	76.94	14.61	69.70	0.00	13.33	73.33	0.00	13.33	0.00	13.33
67	-32.953	25.814	505	21.3	1153.0	8.37	663.00	315.00	7.89	64.65	13.39	69.70	0.00	0.00	53.33	23.33	16.67	6.67	13.33

Supplementary Table 2. Eleven functional ecomorphological traits (SML) used in the present study, modified from Santos et al. (2019).

Trait	Interpretation	Trait Functional category
Compression body index (Maximum body height / Maximum body width)	Indicate position of fish in water column. High values indicate laterally compressed body, expected in fishes exploring habitats with low flow (Watson & Balon, 1984)	Habitat use
Caudal peduncle compression index (Caudal peduncle height / Caudal peduncle width)	High values indicate less active/slow swimmers (Gatz, 1979; Watson & Balon, 1984)	Locomotion
Relative body height (Maximum body height / Standard length)	Low values indicate elongated fish (Gatz, 1979)	Locomotion, habitat use
Relative head length (Head length / Standard length)	Directly related to prey size. High values are associated to fishes which feed of large preys (Gatz, 1979)	Feeding
Relative eye diameter (Eye diameter/ Head height)	Related to feeding and foraging	Feeding
Relative eye position (Eye height / Head height)	Related to preferable position of the fish in the water column. High values indicate dorsal eyes, generally related to benthic fishes (Pouilly et al., 2003)	Habitat use
Relative mouth width (Mouth width / Maximum body width)	Directly related to prey size. High values are associated to fishes which feed of large preys (Gatz, 1979)	Feeding
Relative mouth height (Mouth height/maximum body height)	High values are associated to fishes which feed of large preys (Gatz, 1979)	Feeding
Mouth aspect ratio (Mouth height/Mouth width)	High values indicate narrow mouths (Pessanha et al. 2015)	Feeding
Relative caudal peduncle length (Caudal peduncle length / Standard length)	Fishes with long caudal peduncle are good swimmers. (Watson & Balon, 1984)	Locomotion
Fineness coefficient (Standard length / √Maximum body height *Maximum body width)	Lower values are generally associated with high swimming efficiency (Ohlberger et al., 2006)	Locomotion

Supplementary Table 3. Trait data for the 13 species used in the trait-environment association analyses. [a] and [b] denote categorial and numerical variables respectively. Also included is the number of levels for each trait (factor). For categorial variables, each species is assigned a value of between 1 and its respective maximum level. Values for SL traits are given in averages. Amos = *Anguilla mossambica,* Cgar = *Clarias gariepinus,* Ccar = *Cyprinus carpio,* Eman = *Enteromius mandelai,* Gaff = *Gambusia affinis,* Gcal = *Glossogobius callidus,* Lumb = *Labeo umbratus,* Laen = *Labeobarbus aeneus,* Mfal = *Monodactylus falciformis,* Omos = *Oreochromis mossambicus,* Pcap = *Pseudomyxus capensis,* Sbai = *Sandelia bainsii, and* Tspa = *Tilapia sparrmanii.*

Trait source	**Trait**	**Levels**	**Amos**	**Cgar**	**Ccar**	**Eman**	**Gaff**	**Gcal**	**Lumb**	**Laen**	**Mfal**	**Omos**	**Pcap**	**Sbai**	**Tspa**
Regional literature (PRL)	Body size[a]	3	BS3	BS3	BS3	BS1	BS1	BS1	BS3	BS3	BS2	BS2	BS2	BS1	BS1
	Diet[a]	4	DT3	DT2	DT2	DT2	DT3	DT3	DT4	DT5	DT3	DT2	DT5	DT3	DT2
	Feeding strata[a]	5	FS3	FS5	FS5	FS5	FS4	FS5	FS3	FS3	FS2	FS3	FS5	FS5	FS5
	Body morphology[a]	7	BM4	BM6	BM2	BM5	BM7	BM6	BM5	BM5	BM2	BM1	BM2	BM2	BM2
	Barbels[a]	2	BB2	BB1	BB1	BB1	BB2	BB2	BB1	BB1	BB2	BB2	BB2	BB2	BB2
	Migration[a]	2	SM1	SM1	SM1	SM2	SM1	SM2	SM1	SM1	SM1	SM1	SM2	SM1	SM1
	Mouth position[a]	3	MP2	MP2	MP2	MP2	MP1	MP2	MP3	MP3	MP2	MP2	MP2	MP2	MP2
	Temperature preference[a]	3	TP2	TP3	TP2	TP1	TP2	TP2	TP2	TP2	TP2	TP1	TP2	TP1	TP1

Trait source	Trait	Levels	Amos	Cgar	Ccar	Eman	Gaff	Gcal	Lumb	Laen	Mfal	Omos	Pcap	Sbai	Tspa
Standard linear measurements (SML)	Compression body index (CI)[b]	1	1.33	0.77	1.96	1.74	1.58	1.04	1.61	1.46	4.39	2.36	1.71	2.12	2.09
	Relative body height (RBH)[b]	1	0.06	0.14	0.37	0.26	0.26	0.17	0.26	0.23	0.59	0.43	0.24	0.31	0.43
	Relative caudal peduncle length (RCPL)[b]	1	0.00	0.04	0.19	0.26	0.36	0.26	0.16	0.19	0.11	0.12	0.20	0.12	0.15
	Caudal peduncle compression index (CPCI)[b]	1	0.00	7.52	2.18	1.59	2.88	1.45	1.68	1.48	3.39	3.41	2.31	3.11	2.31
	Relative head length (RHL)[b]	1	0.13	0.28	0.31	0.27	0.24	0.32	0.26	0.25	0.35	0.34	0.25	0.40	0.34
	Relative eye position (REP)[b]	1	0.70	0.56	0.64	0.51	0.31	0.80	0.56	0.59	0.54	0.64	0.60	0.65	0.62
	Relative mouth width (RMW)[b]	1	0.88	0.66	0.46	0.55	0.57	0.69	0.50	0.50	0.61	0.59	0.57	0.82	0.48
	Relative mouth height (RMH)[b]	1	0.40	0.53	0.22	0.28	0.10	0.60	0.23	0.29	0.10	0.18	0.22	0.28	0.23
	Mouth aspect ratio (MAR)[b]	1	0.60	0.61	0.92	0.89	0.28	0.89	0.74	0.87	0.73	0.72	0.66	0.72	1.02
	Fineness coefficient (FC)[b]	1	21.61	6.38	3.75	5.11	4.78	6.01	4.84	5.32	3.54	3.54	5.35	1.28	3.35

Supplementary Table 4. Basal resources, macroinvertebrates and fish samples used for stable isotope analyses in the present study. Abbreviations were used in the analyses. Macroinvertebrates were identified to either the family or genus level whereas fish species were identified to species level.

	Sample	**Abbreviation**
Basal resources	Algae	alga
	C3 plants	C3
	C4 plants	C4
	Coarse particulate organic matter	CPOM
	Fine particulate organic matter	FPOM
Macroinvertebrates	Acanthiops	Acan
	Aeshnidae	Aesh
	Afronurus	Afro
	Anax	Anax
	Ancylidae	Ancy
	Athericidae	Athe
	Atyiidae	Atyi
	Baetidae	Baet
	Belostomatidae	Belo
	Caenis	Caen
	Cheumatopsyche	Cheu
	Chimarra	Chim
	Coenagrionidae	Coen
	Cordullidae	Cord
	Corixidae	Cori
	Dytiscidae	Dyti
	Aeshna	Eash
	Heptagynidae	Hept
	Hirudinae	Hiru
	Hydropsyche	Hydr
	Leptophlebiidae	Lept
	Lestidae	Lest
	Libellulidae	Libe
	Lymnaidae	Lymn
	Muscidae	Musc
	Notonectidae	Noto
	Oligochaeta	Olig
	Orectogyrus	Orec
	Physidae	Phys
	Pleidae	Plei
	Potamonautes	Pota
	Psephenidae	Psep
	Scirtidae	Scir
	Simuliidae	Simu
	Tabanidae	Taba
	Tetragnatha	Tetr
	Tricorythidae	Tric
Native fish species	*Anguilla marmorata*	Am
	Anguilla mossambica	Ams

	Sample	**Abbreviation**
	Enteromius mandelai	Em
	Gilchristella aestuaria	Ga
	Planiliza macrolepis	Lm
	Labeo umbratus	Lu
	Pseudomyxus capensis	Pc
	Mugil cephalus	Mc
	Monodactylus falciformis	Mf
	Oreochromis mossambicus	Om
	Pomadasys commersonnii	Pco
	Psammogobious knysnaensis	Pk
	Redigobius dewaalii	Rd
	Rhabdosargus holubi	Rh
	Sandelia bainsii	Sb
Non-native fish species	*Cyprinus carpio*	Cc
	Clarias gariepinus	Cg
	Gambusia affinis	Gaf
	Labeobarbus aeneus	La
	Labeo capensis	Lc
	Tilapia sparrmanii	Ts