Evaluating the trophic ecology and feeding habits of three divergent lineages of *Sandelia bainsii* (Teleostei: Anabantidae), from the Eastern Cape Rivers using stable isotope analysis

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Abstract

Despite supporting a disproportionately large fraction of the global biodiversity, freshwater ecosystems are ranked as the most highly threatened habitats on the planet, ahead of both terrestrial and marine ecosystems. Many regions are still characterised by limited knowledge regarding the taxonomy and ecology of freshwater fish taxa. The need for ecological information is increasingly becoming important due to the discovery of new species and unique lineages, which require conservation management. The aim of this study was thus to evaluate the trophic ecology and feeding habits of the three recently described and divergent *Sandelia bainsii* lineages, namely *Sandelia* sp. '*bainsii* Kowie' from the Great Fish River, *Sandelia* sp. '*bainsii* Keiskamma' found in the Keiskamma River, and *Sandelia* sp. '*bainsii* Buffalo' confined to the Buffalo River system in the Amathole-Winterberg freshwater ecoregion in the Eastern Cape Province, South Africa. Based on the allopatric distribution and the generalist feeding habits of these lineages, this thesis postulated that these three lineages were likely to show similar trophic ecology patterns, different dietary composition and as a result would have variable trophic positioning in the different river systems. Therefore, the primary objectives were to use stable isotope analysis to (1) evaluate the food web patterns of the river systems where the three *S. bainsii* lineages occurred, and (2) determine dietary source contributions for the three lineages using isotope mixing models. The results revealed general variability and significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values for the different basal resources, macroinvertebrates and fish community across the different headwater streams. Within and across the different rivers, the *S. bainsii* lineages exhibited variable isotopic niche sizes, which appeared to coincide with the variation in the isotopic composition of the individual communities. Furthermore, these lineages did not exhibit any discernible patterns in their interspecific interactions in different habitats. This suggests that these lineages' isotopic niche patterns were largely influenced by spatial differences in both trophic resources and probable interactions with contraspecifics. Assessment of trophic positions of *S. bainsii* lineages showed that the three lineages had higher trophic positions than other co-occurring species at most sites, except in the Buffalo River. This suggest that the different lineages were generally top predators in the different river systems. Although *S. 'bainsii* Buffalo' had a lower trophic position compared to other co-occurring species, its trophic position was generally characterised by high uncertainty, indicating that this lineage was likely influenced by the occurrence of diet sources that had highly variable stable isotope values. Findings from stable isotope mixing models revealed that the diet sources varied from the dominance of either single diet source in the Fairburn and Tyume 1 River to the importance of multiple prey sources from the Lushington and Kat River system. This suggests that despite being a top predator at most sites, the diet sources for the different lineages were highly variable. The patterns observed in this study did not appear to be attributed to species divergence possibly caused by allopatric speciation, but rather differences in food web characteristics of the river systems, as well as the lineages interspecific relationships and their generalized feeding strategies. Understanding the trophic dynamics of these lineages will assist in implementing effective conservation strategies and policies dealing with narrowly distributed species that are threatened by habitat fragmentation and invasion of piscivorous fish.

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CHAPTER 1

General Introduction

Despite supporting a disproportionately large fraction of the global biodiversity, freshwater ecosystems are ranked as the most highly threatened habitats on the planet, ahead of both terrestrial and marine ecosystems (Collen *et al*. 2014; Reid *et al*. 2019). In many regions, there is ongoing destruction of freshwater ecosystems, their associated biodiversity, and the essential goods and services they provide. This is due to habitat degradation, hydrological modifications, overexploitation, the spread of non-native species and extreme changes in global climate conditions (Dugeon *et al*. 2006; Geist 2011; Carpenter *et al*. 2011; Kiruba-Sankar *et al*. 2018; Hassan *et al*. 2020; Shelton *et al*. 2018a). In addition to human-induced disturbances, unfortunately, for many regions, knowledge of taxonomic diversity, ecology, and distribution patterns of species remain rudimentary. This has been identified as the major impediment to effective conservation and management of freshwater ecosystems as it affects conservation status assessment and conservation prioritisation (Giangrande 2003; Revenga *et al*. 2005; Collen *et al*. 2014; Leung *et al*. 2015).

Endemic and native freshwater fishes in streams and rivers face the general risk of extirpation due to, among other factors, challenges associated with habitat-related disturbances and invasions by non-native species (Dextrase and Mandrak 2006; Tarkan *et al*. 2015). Therefore, understanding the processes that influence the distribution patterns of endemic and threatened fish species in relation to these impacts is essential in the design of conservation strategies (Fausch *et al*. 2006; Strecker *et al*. 2011). In general, fish species distribution patterns within freshwater ecosystems are broadly influenced by abiotic factors, which include physical (e.g. climate, geology, macro- and microhabitat) and chemical (e.g. nutrients and dissolved gases) attributes, and biotic factors (e.g. predation and competition), which reflect interspecific interactions within communities (Jackson *et al*. 2001). From an ecological niche perspective, these factors are largely defined by environmental and trophic niches (Holt 2001). Environmental niches explore the role of abiotic factors, with the species niches being assumed to be a consequence of species tolerances to the physiological limits imposed by the environmental factors (Bulleri *et al*. 2016). In comparison, trophic niches evaluate the role of biotic factors, which describe the extent to which co-occurring species utilise the available food resources (Liebold 1995). The ecological niche, thus, identifies communities as comprising species that are adapted to a suite of environmental and trophic resources, with coexistence being facilitated by minimum overlap along the different resource axes (*sensu* Hutchinson 1957b).

Ecological impacts on freshwater habitats, such as habitat loss and degradation have negative effects on the sustainment of viable populations for different species (Collares-Pereira *et al*. 2002). For example, in degraded freshwater habitats, fishes have been found to occur in small and isolated patches, which sustain a limited number of individuals, due to the loss of critical habitats for spawning, feeding and refuge (Aarts *et al*. 2004; Gido *et al*. 2016). Similarly, ecological impacts due to biological invasions in freshwater habitats are increasingly becoming a growing challenge in the conservation of native fishes (Hui *et al*. 2015; Kiruba-Sankar 2018). Due to niche opportunism, successful invaders have been reported to influence the distribution patterns and population dynamics of native fishes directly through predation, often leading to localised extirpation of populations (Ogutu-Ohwayo 1993; Dextrase and Mandrak 2006) and indirectly by competing for similar resources, often resulting in altered habits and trophic niche shifts (Blanchet *et al*. 2007). Several studies have evaluated the effects of these major threats on endemic and native fishes both globally (Ogutu-Ohwayo 1990; Marchetti and Moyle 2001; Taylor *et al*. 2001; He *et al*. 2010) and in southern Africa (Skelton, 1990). Globally, much of the research has centred on biodiversity hotspots, including

Madagascar (Benstead 2003), Western Ghats, India (Raghavan *et al.* 2016) and the Meditteranean Basin (Cuttelod *et al.* 2009). In southern Africa, much of the research on freshwater fishes have focussed extensively on the Cape Fold Ecoregion (CFE) (Lowe *et al.* 2010; Weyl *et al.* 2014; Ellender *et al.* 2017). Studies in this ecoregion have revealed large-scale declines in the historical ranges of many fish taxa that are characterised by fragmented contemporary distribution mainly due to habitat degradation and invasion impacts (Chakona *et al.* 2020a). Recently there has been renewed interest in other biodiversity hotspots in southern Africa where similar impacts are prevalent, such as the Amatola-Winterberg (Eastern Cape), the Drakensberg Escarpment (Kwazulu/ Natal-Mpumalanga) and the Eastern Zimbabwe Highlands (Darwall *et al.* 2009; Chakona *et al.* 2018b). These are regarded as areas of major conservation concern (Skelton and Cambray 1995; Rosenfield 2002).

In addition to the common impacts, many regions are still characterised by limited knowledge regarding the taxonomy and ecology of freshwater fish taxa. These include dryland rivers in Mediterranean regions as well as regions in sub-Saharan Africa such as west Africa and southern Africa (Skelton 2000; Thieme *et al.* 2005; Dudgeon 2011; Chakona *et al.* 2018b). Ironically, freshwater systems in these regions contain a high proportion of highly threatened narrow range endemic species (Smith *et al.* 2014). Recent studies have highlighted the need for generating information on the distribution and ecology of recently described species to better manage biodiversity hotspots (Chakona *et al.* 2017; Chakona *et al.* 2019a; Revenga *et al.* 2005). The lack of this information has been attributed to the lack of comprehensive biodiversity exploration (Revenga *et al.* 2005), as evidenced by recent discoveries of extreme levels hidden diversity in stream fishes from several poorly explored regions in Africa (Chakona *et al.* 2018c: Schmidt *et al.* 2014, 2016, 2011; Thomson and Page 2010), Asia (Farhana *et al.* 2018) and South America (Pereira *et al.* 2010). These findings have stimulated a resurgence in studies that are re-evaluating the distribution, ecology and conservation status of the newly identified genetic lineages or recently described species (e.g. Kadye *et al*. 2016; Chakona *et al*. 2017; Chakona *et al*. 2019b). An example is the CFE that is found along the southern fringe of the African continent. The CFE has received considerable research focus compared to other regions in southern Africa, largely due to the existence of a unique assemblage of endemic freshwater fishes (Chakona and Skelton 2017; Chakona and Swartz 2013; Chakona *et al*. 2013). The generation of new information on the diversity and accurate distribution ranges of the fishes of this region necessitated the re-evaluation of the ecology, biology and conservation status of newly described species (Kadye *et al*. 2016; Chakona *et al*. 2019b) and genetic lineages even before they became formally described to enhance their conservation (Chakona *et al*. 2017). A similar situation is unfolding in adjacent ecoregions, such as the Karoo and Amatolo-Winterberg freshwater ecoregions where recent studies have identified hidden diversity within a monotypic genus *Amatolacypris trevelyani* and a narrow ranged endemic anabantid, *Sandelia bainsii* (Roos 2006; Skelton *et al*. 2018; Chakona *et al*. 2021).

Sandelia bainsii Castelnau, 1861, commonly known as the Eastern Cape rocky, has been a long-standing flagship species for biodiversity conservation in the Amatolo-Winterberg Ecoregion (Cambray 1997a;b). This species is a member of the family Anabantidae (Osteichthyes; Perciformes) also known as the climbing perches (Mayekiso and Hecht, 1988a). The family Anabantidae has four genera with disjunct distributions. These include *Anabas*, which occurs in south-east Asia, and three African genera, *Cnetopoma* and *Microcnetopoma*, occurring in west-central, central and south-east Africa, and *Sandelia*, which is found in the coastal rivers of the Cape, South Africa (Mayekiso and Hecht 1988b). *Sandelia* comprises of two morphologically distinct species, and have disjunct geographical distributions (Norris 1996). *Sandelia capensis* occurs in the Cape Fold Ecoregion (CFE) (Cambray 2004), whereas *Sandelia bainsii* has a restricted distribution in the Eastern Cape coastal systems (Cambray

1996). In general, the family Anabantidae is characterised by having a labyrinth that acts as an accessory breathing organ located in the chamber above the gills (Günter 1880). It allows members of this family to live in poorly oxygenated habitats such as tropical swamps and streams. However, the genus *Sandelia* is an exception because the labyrinth is reduced possibly due to the Cape coastal drainages being well oxygenated (Bernard 1943).

The Eastern Cape rocky is the largest of all the species in the family Anabantidae, reaching up to 260 mm standard length (SL) (Cambray 1996). Until recently, much of the ecological information on this species was based on studies conducted more than 20 years ago. These studies indicate that this species inhabits rocky streams where it is associated with coarse substrates such as boulders in slow flowing pools for refuge and feeding. *Sandelia bainsi* is reported to be euryphagous and an opportunistic feeder (Mayekiso and Hecht 1990). Invertebrates (insects and crustaceans) and fish are the three important groups in the diet of *Sandelia bainsii*. This species has been reported to undergo ontogenetic diet shift, with fish < 60 mm SL being insectivores, those between 60 - 90 mm SL largely feeding on insects and crustaceans whilst occasionally feeding on fish, whereas size classes > 100 SL feeding predominantly on crustaceans and fish (Mayekiso and Hecht 1990). Cannibalism has also been documented for this species (Mayekiso and Hecht 1990).

The Eastern Cape rocky is endemic to the Eastern Cape Province of South Africa (Mayekiso and Hecht 1988a). This species is listed as endangered in the IUCN red list, with remnant populations being severely fragmented (Cambray 1996; Chakona *et al*. 2018a). Recent molecular studies have further revealed that the Eastern Cape rocky has three divergent lineages; *Sandelia 'bainsii* Kowie', *Sandelia 'bainsii* Keiskamma' and *Sandelia 'bainsii* Buffalo' lineages, each with a much narrower distribution than the species as currently described (Chakona *et al*. 2020). The 'Kowie' lineage occurs in the Kowie and Great Fish River systems, *Sandelia bainsii* 'Keiskamma' occurs in the Keiskamma River system, whereas the

’Buffalo’ lineage is located in the Igoda and Buffalo River systems (Roos 2006, Chakona *et al.* 2021) (Fig. 1.1).

Similar to other endemic species in the CFE, the Eastern Cape rocky is threatened by anthropogenic factors such as habitat degradation and deterioration in water quality through agricultural activities and pollution from urban settlements (Chakona *et al.* 2019a; Cambray 1996). It is also threatened by potential predation and competition from invasive piscivores, particularly the invasive large-mouth bass *Micropterus salmoides* in the Tyume River (Mayekiso and Hecht 1988b) and the extralimital sharptooth catfish *Clarias gariepinus* in the Great Fish, Buffalo, Kowie River systems (Kadye and Booth 2011; Cambray 2003). These invasive fish were introduced in river systems as angling species (*Micropterus salmoides*) and through inter-basin water transfer, particularly in the Great Fish River (Cambray and Jubb, 2010; Van Rensberg *et al.* 2011).

Understanding of the functional interactions and processes in freshwater ecosystems is crucial for the restoration of altered habitats (Geist 2011). *Sandelia bainsii* is a narrow ranged endemic species known to be confined in three river systems. The populations of *Sandelia* in the river systems is highly fragmented and the discovery of these new lineages in an already threatened species raises major conservation concern as the narrowly distributed lineages may even face a greater risk of extinction (Chakona *et al.* 2021). However, information on the distribution, habitat association, trophic and feeding ecology as well as the key threats for the lineages are not well documented. This therefore limits the ability to draw effective conservation strategies. The most recent detailed ecological research focussed on assessing the distribution and habitat association of *Sandelia ‘bainsii* Kowie’ in the Koonap and Kat rivers, tributaries of the Great Fish River systems. The major findings of this study were that the Kowie lineage is extirpated from one locality of the Koonap tributary (Sifundza 2019). The present study therefore aims to evaluate the trophic ecology and feeding habits of the three divergent *Sandelia bainsii* lineages, namely, *Sandelia* sp. '*bainsii* Kowie', *Sandelia* sp. '*bainsii* Keiskamma' and *Sandelia* sp. '*bainsii* Buffalo' to inform effective conservation management.

Based on the allopatric distribution of these lineages, this thesis addressed two key research questions:

1. To what extent do the food webs, isotopic niche patterns and interspecific interactions of the three *Sandelia bainsii* lineages differ across their distribution range?
2. What are the isotopic diet sources contributions and trophic positions for the different *S. bainsii* lineages in their respective river systems?

To address these questions, this thesis is structured into four chapters. Chapter 1 provides a general background of the problem statement, the study taxa and an overview of the research questions. Chapter 2 addresses the first research question by evaluating the food web patterns of the communities where the different *S. bainsii* lineages occur, together with their isotopic niche patterns and interspecific interactions. Chapter 3 focuses on the second research question by elucidating the dietary source contributions of the different lineages using isotopic mixing models. Lastly, Chapter 4 is a general discussion of the thesis.

Study area

The streams under investigation drain the area around the Amathole-Winterberg Highlands Ecoregion (AWHE) (Thieme *et al*. 2005). The headwater streams are typically narrow, fast flowing, turbulent with rocky substrata (Hughes and Hughes, 1992). The climate of the AWHE is characterised as warm temperate, with perennial rainfalls, cool winters and warm summers (Story, 1952). Most of the rainfall occurs in summer, approximately 650 mm (December to February) and autumn (March to May) (Thieme *et al*. 2005; Hoare *et al*. 2006). The AWHE area as a whole receives between 800 mm and 1,000 mm rain per annum (Hoare and

Bredenkamp, 1999; Clark *et al*. 2011). Air temperature ranges from 19 to 29°C in summer and – 5 to 19°C in winter. Snowfall may occur at high altitudes at the Winterberg Mountains, with frost mostly occurring regularly between April and September (van Zyl, 1994). Vegetation of the region comprises of a mosaic of several veld types, including thornveld and grassveld that are mixed with deciduous dwalf *Vachellia karroo* (Hayne) Banfi and Galasso, a veld type called sourveld, which is dominated by *Themeda triandra* Forssk and other grasses in the plateau slopes, and montane forest trees in valleys and escarpment slopes (Meadows and Meadows, 1988)

The Great Fish River system is characterised by a warm-temperate climate with air temperatures ranging between 20°C and 35°C in summer (October to March) and between 0°C to 20°C in winter (April to September) (Hoare *et al*. 2016). It is also regarded as sub-humid to semi-arid receiving annual rainfalls of approximately 75% between October/November and February/March (Hoare *et al.* 2016). The Great Fish River has been altered by the Orange-Great Fish Inter-Basin Water Transfer (IBWT) scheme from seasonal to perennial flow with little monthly fluctuations (O'Keeffe and De Moore, 1988). Most of the tributaries of the Great Fish River such as the Kat River are, however not influenced by the IBWT and maintain natural flow regimes. The Kat River catchment drains wet catchments and has perennial flow (Laurenson and Hocutt, 1984; Clark *et al*. 2014). The vegetation is semi-succulent thorny scrub ca. 2m high, comprising of dwarf shrubland, succulent bushclump savanna and grassland communities which parallel a topo-moisture gradient (Tanser and Palmer, 2000). The geology of the catchment includes erodible Beaufort and Ecca groups, resulting in considerable amounts of salts being leached from ancient marine sediments due to runoff (O'Keeffe and Moor, 1988). The native ichthyofauna sampled from the Great Fish River was comprised of *Sandelia bainsii*, *Enteromius mandelai*, *Amatolacypris trevelyani* and *Glossogobius callidus*. Sampling was conducted from the Kat River, a major tributary of the Great Fish River, and from two of its headwater tributaries, the Lushington and Fairburn rivers (Figure 2.1).

East of the Great Fish River, the Keiskamma River catchment's climate is similar to that of the Amathole sub area, but with less humidity and slightly lower temperatures down the coast and inland. The mean annual precipitation from this area varies from 600 mm along the coast to a low of 450 mm in parts of the dryer coastal plateaus to over 1200 mm on the mountain peaks. Rain falls predominantly in the summer months with June and July being the driest months of the year (Mbikwana and Bushula, 2008). The Keiskamma River has high perennial flow which is nonetheless characterised by highly seasonal discharge (Hughes and Hughes, 1992). The natural vegetation mainly consists of coastal grasslands, savannah (thornveld or sourveld) from the coastal areas up to the escarpment. The river valleys are characterised by areas of dense bush (valley thicket) and indigenous forests are found in mountain zone (Mbikwana and Bushula, 2008). The geological formation confined to this area are Beaufort Series, which are sedimentary rocks varying in character from sandstones to mudstone. The Karoo Dolerite which covers 25% of the area and form intrusion into the lower Beaufort sediments, and alluvium which is caused by the meandering of the Kieskamma River (Ramsbottom, 1958). Native fish sampled from this river were *Sandelia bainsii* and *Amatolacypris trevelyani*. Sampling in this region was conducted from two sites within the Tyume River, which is a tributary of the Keiskamma River (Figure 1.2.

The Buffalo River drains the Amathole Mountains at an altitude of 1963 m asl and flows for 140 km, reaching the sea at East London as a fourth-order stream (Ginkel *et al*. 1993). The major tributaries of this river include Cwengcwe, Izele, Mgqakwebe, Ngqokweni, and Yellowwoods rivers. The catchment has four man-made impoundments along the river (Ginkel *et al*. 1993), these include the Maden, Rooikrans, Laing and the Bridle Drift dams (Palmer and O'Keeffe, 1990). The Buffalo River Catchment has a warm and temperate climate.

Temperatures are moderate in the coastal zone (8 to 39 °C) with a warm mean annual of 21°C. Inland temperatures vary from -4 to 42 °C with an mean annual of 18 °C (River Health Programme RHP 2004). The catchment receives an annual summer rainfall ranging from 400 to 1000 mm per year, which is double that of the winter rainfall, it also receives an annual mean rainfall of 700 mm (River Health Programme RHP 2004). The geology of the river is characterised by erodible Beaufort Series mudstone, sandstones and also resistant dolerite intrusions. The Buffalo River hosts a diverse group of macroinvertebrates, native fish including the border barb *Amatolacypris trevelyani*, chubbyhead barb *Enteromius anoplus* and the moggel *Labeo umbratus* (Skelton *et al*. 2001; Ellender and Weyl, 2014*)*. Non-native species, such as the sharp-tooth catfish (*Clarias gariepinus),* large-mouth bass *(Micropterus salmoides),* smallmouth bass (*Micropterus dolomieu*), the rainbow trout (*Oncorhynchus mykiss*) and banded tilapia *Tilapia sparrmanii*, are known to occur in this river (Skelton *et al*. 2001).

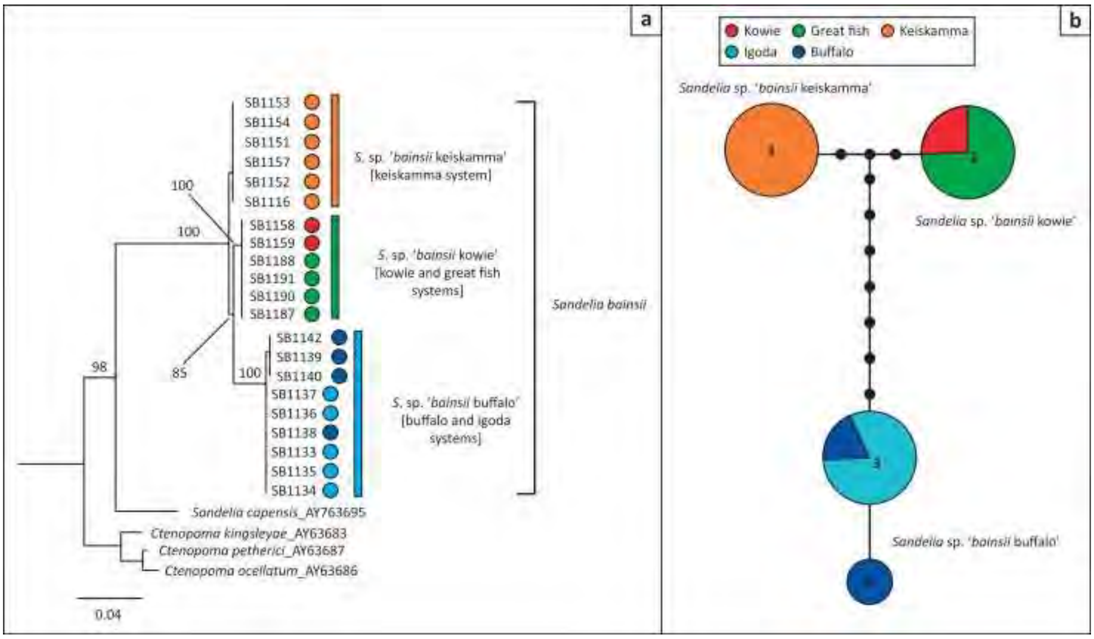

Figure 1.1. Maximum likelihood phylogeny (a) and TCS haplotype network (b) showing the existence of three lineages (*Sandelia* sp. '*bainsii* Kowie', *Sandelia* sp. '*bainsii* Keiskamma' and *Sandelia* sp. '*bainsii* Buffalo') within the Eastern Cape rocky, *Sandelia bainsii*. The river systems where each lineages were recorded are indicated (Chakona *et al*. 2021).

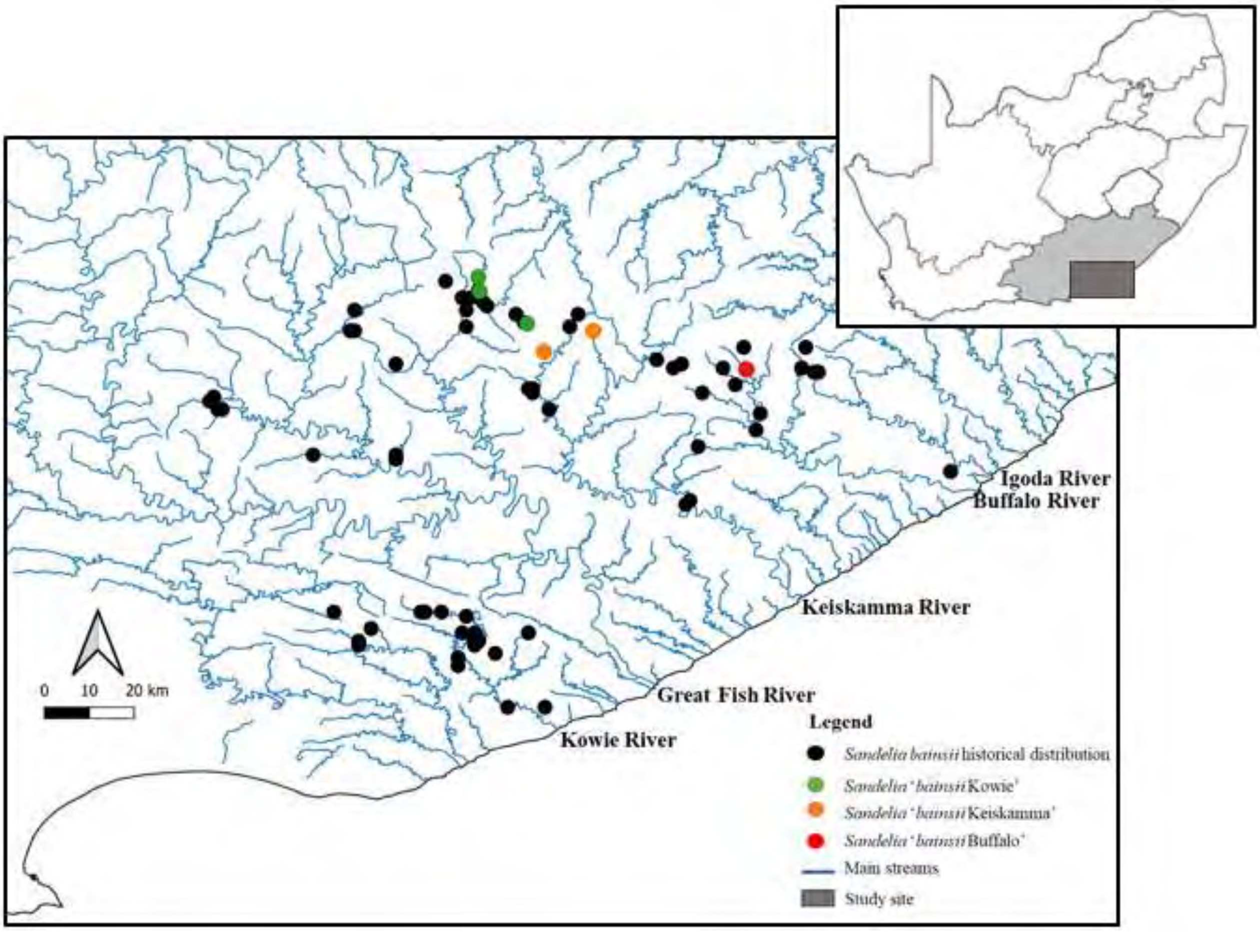

Figure 1.2. A map showing the historical distribution of *Sandelia bainsii* (black circles) and sampled sites of the *Sandelia bainsii* lineages from the Eastern Cape Rivers.

CHAPTER 2

Comparative assessment of isotopic food webs for *Sandelia bainsii* (Teleostei: Anabantidae), lineages within Eastern Cape river systems

Introduction

Ecological information provides insight on, among other factors, species distribution (McNyset 2005), trophic dynamics (Moore *et al.* 2004) and niche diversification of species (Wellenreuther *et al.* 2007; Winemiller 2014). In freshwater environments, the need for ecological information is increasingly becoming important due to the discovery of new species and unique lineages, which require conservation management (Chakona *et al.* 2017; Moore *et al.* 2017; Chakona *et al.* 2019a). This information broadly encompasses abiotic interactions, which relate to species-environment relationships (Eros *et al.* 2009) and biotic interactions, which describe intra- and inter-specific interactions in relation to food resources (Bernardi 2013; Gebrekiros *et al.* 2016). In general, studies on biotic interactions provide information on trophic ecology, which broadly describes aspects ranging from evaluating the feeding habits, behaviour and dietary preferences of individual species, lineages or populations (Braga *et al.* 2012) to exploring entire food webs that comprise multiple species within communities and ecosystems (Gelwick and McIntyre 2017). By exploring both intra- and inter-specific food resource patterns, studies on trophic ecology are important in understanding mechanisms that facilitate resource use patterns (Nunn *et al.* 2020) and species coexistence in different environments (Braga *et al.* 2012).

Studies on trophic ecology are particularly useful when evaluating resource use patterns and food web dynamics among closely related species and lineages that occur either in sympatry or in allopatry (Ornelas-García *et al.* 2018; Tran 2015; Carbia *et al.* 2020). Ecological niche theory posits that the functional role of individual species is constrained by a suite of abiotic and biotic factors, and these constraints facilitate the coexistence of different species (Hutchinson 1957a; Chase and Leibold 2003). From a trophic ecology perspective, closely related species and lineages are likely to exhibit trophic niche diversification when they occur in sympatry in order to minimise competitive exclusion (Tran *et al*. 2015; Kadye *et al*. 2016; Carbia *et al*. 2020). Resource partitioning for such species may trigger morphological differences linked to resource exploitation, and may lead to interspecific differences in prey selection and general feeding habits (Lu and Bernatchez, 2017; Ornelas-García *et al*. 2018). Other examples of mechanism that facilitate coexistence of species in sympatry include differences in diel activity patterns, ontogenetic diet shift and segregation of microhabitats (Alanärä *et al*. 2001; Sánchez-Hernández and Amundsen 2015; Polačik *et al*. 2014). In comparison, for closely related species that occur in allopatry, geographical barriers may facilitate incipient species occupying contrasting ecological environments to utilise diverse resources, due to differences in habitat structure, climate and a suite of competitors and predators (Schluter 2000; Nosil 2006). Vicariance processes and new ecological opportunities are likely to facilitate adaptive diversification in the trophic roles of such species through acting on ecologically important traits such as mouth morphology (Funk 1998; Schulter 1998; 2001). Alternatively, however, allopatric species may exhibit niche conservatism (Wiens 2004). Niche conservatism is the tendency of lineages to maintain their ancestral ecological niche when they are isolated and begin the process speciation. Niche conservatism may be an important aspect of allopatric speciation, although it is largely unstudied (Wiens 2004).

Stable isotope analysis (SIA) is increasingly becoming a preferred alternative tool in trophic ecology studies. The most commonly used stable isotopes are carbon ($\delta^{13}C$), which is used to infer energy sources for consumers (Post 2002) and nitrogen ($\delta^{15}N$), which is used to elucidate trophic positions of consumers in food webs (Vanderklift and Ponsard 2003).

Applications of SIA in trophic ecology studies include characterising resource partitioning within species assemblages and nutrient dynamics within food chains (Crawford *et al*. 2008), resolving spatial and temporal patterns of individual species (Dalerum and Angerbjörn 2005; Eurich *et al*. 2019), evaluating intra- and inter-specific resource use (Golikov *et al*. 2020) and reconstructing food webs within communities (Boecklen *et al*. 2011).

The utility of SIA has been shown when evaluating the trophic ecology of species that occur either in sympatry (Genner *et al*. 1999; Kadye *et al*. 2016) or in allopatry (Cucherousset *et al*. 2020; Carbia *et al*. 2020). For sympatric species, stable isotope-based studies have shown trophic niche separation among closely related species, which are similar in size, morphology, and environmental requirements with limited opportunity of habitat segregation (Polačik, 2014; Eurick, 2019). Species occurring in sympatry are generally subject to interspecific competition, this can lead to the development or differing trophic strategies to maximise energy requirements (Páez-Rosas, 2012; Alonso *et al*. 2019). For example, Kadye *et al*. (2016) showed that coexistence between the giant redfin *Pseudobarbus skeltoni* and its congener *Pseudobarbus burchelli* was promoted by trophic niche partitioning, largely due to differences in the mouth structure of the two species. Similar findings were shown among three sympatric Arctic cephalopods in a paper by Golikov *et al*. (2020). This study showed that the co-occurring three species of the genus *Rossia* shared no traits related to resource and habitat utilisation. In comparison, for allopatrically distributed species, SIA-based studies have shown that species niche differentiation is facilitated by differences in environmental characteristics of the ecosystems they inhabit, which facilitate new dietary niche opportunities. For example, Carbia *et al*. (2020) found changes in the dietary composition of the allopatric populations of *Bathygobius cocosensis*, which was likely associated with a shift in micro-habitat and intraspecific competition. Furthermore, in the absence of interspecific competition, species occurring in allopatry generally utilise wider ranges in food sources (Jackson *et al*. 2012;

Musseau *et al*. 2017).

This study evaluated the trophic patterns and food web ecology of *Sandelia bainsii* using stable isotope analysis. Recent molecular studies have revealed the occurrence of three divergent lineages within *Sandelia bainsii* (Chakona *et al*. 2020a). These are *Sandelia 'bainsii* Kowie' occurring in the Great Fish River system, *Sandelia 'bainsii* Keiskamma' occurring in the Keiskamma River, and *Sandelia 'bainsii* Buffalo' confined to the Buffalo River system (Chakona *et al*. 2020a). These lineages therefore represent closely related taxa that occur in allopatry. Studies have shown that congeneric allopatric species occurring in similar environments are likely to exhibit similar trophic niche patterns due to niche conservatism (Jackson *et al*. 2014; Larson *et al*. 2016). The niche conservatism hypothesis posits that closely related taxa may retain similar ancestral ecological characteristics (Kozak and Wiens 2006). From an ecological perspective, when such species occur in allopatry, they may exhibit similar habits in their distribution and resource utilisation patterns (Wiens and Graham 2005). In this study, the three *S. bainsii* lineages occurred in headwater habitats within the same climatic zone in the Amathole-Winterberg Afromontane ecoregion (Vlok *et al*. 2016; Mbikwana and Bushula, 2008; van Zyl, 1994). Previous studies on *S. bainsii sensu lato* have shown that it is stenotopic, occurring in habitats that are dominated by coarse substrates such as boulders and slow flowing pools (Mayekiso 1986). Nevertheless, the most recent detailed ecological research on one lineage in the Great Fish River system revealed that it had a propensity towards eurytopic behaviour likely due to the influence of habitat alteration (Sifundza *et al*. 2021). Historical trophic ecology-based research on this species, based on stomach content analysis, showed that it is a generalist predator (Mayekiso 1986). With the recent research showing occurrence of three allopatric lineages that have recently diverged (Chakona *et al*. 2020a), this study postulates that these lineages are likely to exhibit limited morphological differentiation and likely to show similar trophic ecology patterns that are consistent with niche conservatism.

Thus, the objectives of this study were to (1) examine the broad patterns of the food web dynamics in habitats where the three lineages of *Sandelia bainsii* occur, (2) determine the isotopic niche patterns of each *Sandelia bainsii* lineage from the three river systems, and (3) evaluate the interspecific interactions of the *Sandelia bainsii* lineages with other co-occurring species.

Materials and methods

Study area

Sampling was conducted in the headwater streams of the Kat River, a major tributary of the Great Fish River, the Keiskamma River and the Buffalo River systems (Figure 2.1). These rivers drain the area around the Amathole-Winterberg Highlands Ecoregion (AWHE) (Abelle *et al*. 2008). Headwater streams of these rivers are typically narrow, fast flowing, turbulent with rocky substrata (Hughes and Hughes, 1992). Site selection was done to cover the representative distribution patterns of the three *Sandelia bainsii* lineages based on pilot surveys. In the Kat River, sampling was done at three sites; two in the headwater streams, the Lushington and Fairburn River, and one in the mainstem (Figure 2.1). The natural vegetation in this catchment was dominated by deciduous broad-leafed forests and grasslands (Vlok *et al*. 2006). Other important vegetation components of this catchment include, *Vachellia karroo* (Hayne) Banfi and Galasso and succulent thicket, comprising *Euphorbia* spp. and *Diospyros dichrophylla* (Gand.) De Winter and *Olea europaea*. The geology of this catchment includes erodible Beaufort and Ecca groups resulting in considerable amounts of salts being leached to ancient marine sediments due to runoff (O'Keeffe and De Moor 1988). The rainfall in the catchment varies widely, with the upper catchment receiving the highest rainfall of approximately 1000 mm per annum (Jari and Fraser 2009). The mean annual rainfall is approximately 500 mm, with the rainfall peaking around March and the lowest rainfall being recorded in winter months (June/July). The maximum daily temperature varies from 30 ºC in summer (November to February) to 21 ºC in winter (June to August), whereas the minimum mean daily temperature varies from 17 ºC in summer to 8 ºC in winter.

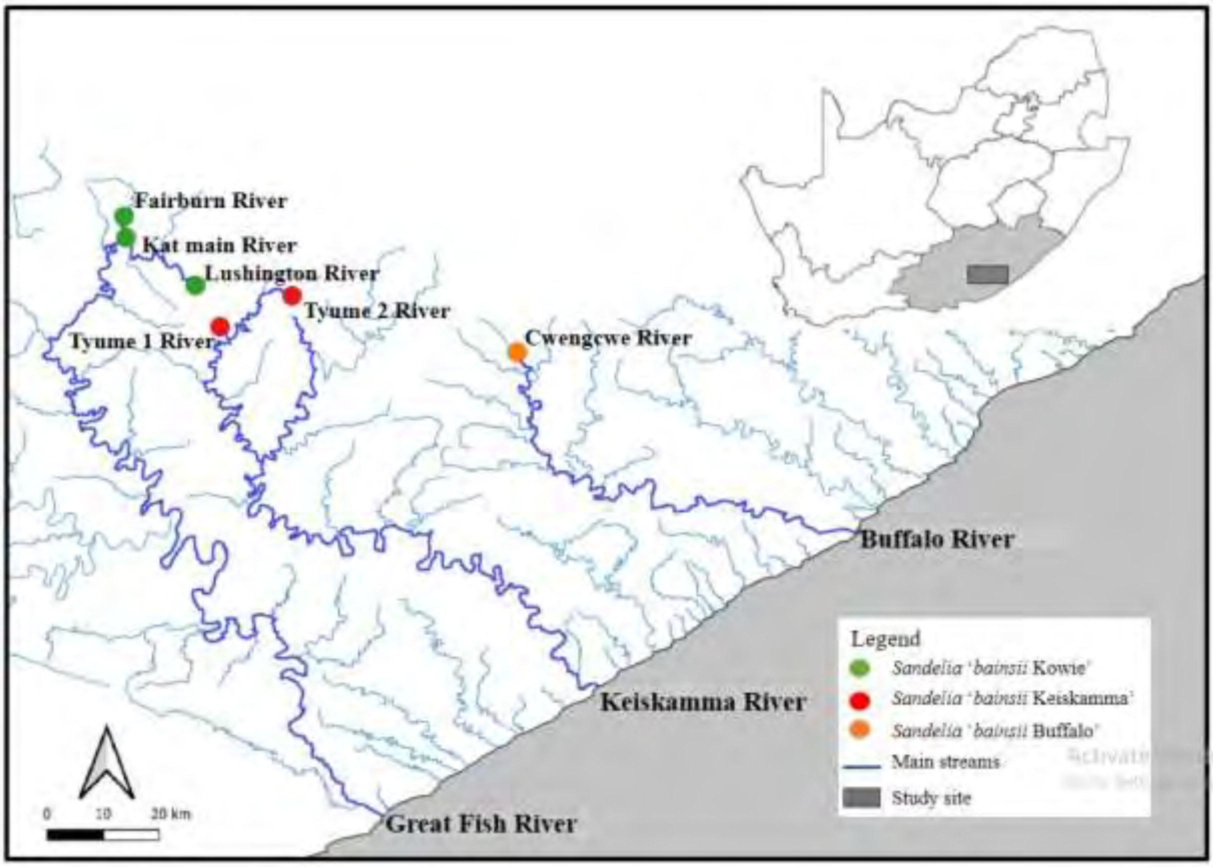

Figure 2.1. A map showing sampled locations of the three different *Sandelia bainsii* lineages in the rivers of the Eastern Cape Province.

In the Keiskamma River catchment, sampling was conducted from two sites within the Tyume River, its major tributary (Figure 2.1). This catchment has a natural vegetation mainly consisting of coastal grasslands, savannah (thornveld or sourveld) from the coastal areas up to the escarpment. The river valleys were characterised by areas of dense bush (valley thicket) and indigenous forests are found in mountain zone (Mbikwana and Bushula 2008). The geological formation confined to this area are Beaufort Series, which are sedimentary rocks varying in character from sandstones to mudstone. The Karoo Dolerite which covers 25% of the area and form intrusion into the lower Beaufort sediments, and alluvium which is caused by the meandering of the Kieskamma River (Ramsbottom 1958). The catchment's climate is humid and slightly lower temperatures down the coast and inland. The mean annual precipitation from this area varies from 600 mm along the coast to a low of 450 mm in parts of the dryer coastal plateaus to over 1200 mm on the mountain peaks. Rain falls predominantly in the summer months with June and July being the driest months of the year (Mbikwana and Bushula 2008). The Keiskamma River has high perennial flow which is characterised by high seasonal discharge (Hughes and Hughes 1992). Native fish sampled from this river were *Sandelia bainsii* and *Amatolacypris trevelyani*.

In the Buffalo River catchment, sampling was conducted at one site of its major tributary, the Cwengcwe River. The geology of the river includes erodible Beaufort Series mudstone, sandstones and also resistant dolerite intrusions. The Buffalo River catchment is characterised by a warm and temperate climate with temperatures that are moderate in the coastal zone (8 to 39 °C) with a warm mean annual of 21°C. Inland temperatures vary from -4 to 42 °C with a mean annual of 18 °C (River Health Programme 2004). Furthermore, this system receives summer rainfall, ranging between 400 to 1000 mm per year which is double that of the winter season. It also receives a mean annual rainfall of 700 mm (River Health Programme 2004). Similar to the Keiskamma system, the Buffalo River has a perennial flow which is characterised by high seasonal discharge (Hughes and Hughes 1992).

Sample collection

Sampling was conducted in the summer months between October 2018 and May 2019. The six representative sites for *Sandelia bainsii* lineages were selected because they comprised habitats that were uninvaded by non-native fishes. Three sites were sampled from the Kat River system, a major tributary of the Great Fish River. These were the Lushington and Fairburn rivers where *Sandelia 'bainsii* Kowie' co-occurred with *Enteromius mandelai*, and the Kat River mainstem where this lineage co-occurred with *E. mandelai* and *Glossogobius callidus*. In the Keiskamma River system, *Sandelia 'bainsii* Keiskamma' occurred as the only fish taxa at the Tyume River site 1, and co-occurred with *Amatolacypris trevelyani* at the Tyume River site 2. In the Buffalo River system, *Sandelia 'bainsii* Buffalo' was sampled in the Cwengcwe River where it co-occurred with *E. mandelai* and *A. trevelyani*. To examine food web patterns and trophic niche relationships, the following were collected from each site; potential basal food sources, aquatic invertebrates and fish fin tissue for stable isotope analysis. Potential basal food sources included coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), epilithic algae, filamentous algae, and macrophytes. Coarse particulate organic matter is particulate organic matter > 1000 µm in size (Cummins, 1974). It is mostly of allochthonous origin, such as leaves, wood, bark, flowers, seeds and fruits, or of autochthonous origin such as macrophytes and filamentous algae. The CPOM of the studied river systems was primarily comprised of C_3 (leaves and plant debris) and C_4 plant matter (grasses) of terrestrial origin. It was collected by dislodging organic matter within an area of 1 m^2 and washing it through a 250 µm net. Organic particles that were considered as FPOM were in the size range > 0.45 to <1000 mm that were either suspended in the water column or deposited within lotic habitats. The FPOM was collected by filtering 25 litres of stream water through a 100 µm net. Three replicate samples were collected for both CPOM and FPOM at each site. Epilithic algae were scraped from the substratum using a scalpel blade, washed and stored in Eppendorf tubes. Filamentous algae and small bits of aquatic macrophytes were hand-picked and stored in Eppendorf tubes.

Macroinvertebrates were collected from instream and vegetated habitats in the headwater streams. Instream, macroinvertebrates were collected using a 250 µm mesh size net by disturbing the substrate through kicking for one minute. Vegetated sections were sampled by repeatedly sweeping a hand net through and over the vegetation for approximately one minute. The samples were sorted in the field using trays to isolate the macroinvertebrates from the detritus. The macroinvertebrates were sorted and identified to family level and stored on ice in the field. A reference sample of the macroinvertebrates was preserved in 70% ethanol to verify the identification using a stereomicroscope upon returning to the laboratory. Fish were sampled using a SAMUS backpack electrofisher, which was employed for 10 minutes, or by seine (3 m length × 3 mm mesh size net) netting. Current strength and setting for electrofishing gear were set to target species and conditions in the study area, such as conductivity, water depth and survey area. After multiple electrofishing passes were carried out, all fish captured were immediately removed from the net and placed in aerated buckets filled with ambient water to avoid prolonged exposure. The fish were kept in a bucket containing stream water and then euthanised using clove oil. The caudal fin was excised and placed in Eppendorf tubes for stable isotope analysis. Only a small piece of fin tissue was collected from *Sandelia bainsii* and the fish was returned to the stream alive. The basal food sources, aquatic invertebrates and fish were all sampled on the same day. All samples were kept on ice in the field, and thereafter transported to the Freshwater Ecology Laboratory at the Department of Ichthyology and Fisheries Science at Rhodes University, Makhanda, South Africa.

Stable isotope processing and analysis

In the laboratory, the macroinvertebrates were identified to the lowest taxonomic unit possible using a light microscope. For stable isotope analysis, macroinvertebrates of the same taxonomic group were pooled to achieve sufficient mass for analysis. The samples of fish, macroinvertebrates and basal food sources were oven-dried at 60 °C for 48 hours, after which they were ground into a fine powder with a mortar and pestle. The ground samples were packaged in Eppendorf tubes and sent for stable isotope analysis at the Stable Isotope Laboratory, Mammal Research Institute at University of Pretoria in South Africa. Aliquots of approximately 1-12 mg of fin tissue or plant materials were weighed into tin capsules that had been pre-cleaned with toluene.

The ratios of the stable isotopes were measured against the reference standards, Pee Dee belemnite for $\delta^{13}C$ (Craig, 1957) and the atmospheric nitrogen gas for $\delta^{15}N$ (Ehleringer and Rundel, 1989) (Equation 1). A laboratory running standard (Merck Gel: $\delta^{13}C$ = -20.26‰, $\delta^{15}N$ = 7.89‰ and DL-Valine: $\delta^{13}C$ = -10.57‰, $\delta^{15}N$ = 6.15‰) and a blank sample were run after every 11 samples. The delta notation (δ), the difference (‰, parts per thousand) between the isotopic ratio of the sample and the standard was calculated as:

$$\delta X(‰) = [(Rsample - Rstandard)/Rstandard - 1] \qquad (1)$$

where X = $\delta^{15}N$ or $\delta^{13}C$ and R represents $^{15}N/^{14}N$ or $^{12}C/^{13}C$, respectively. Analytical precision was < 0.08‰ for $\delta^{13}C$ and <0.13‰ for $\delta^{15}N$. 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), housed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria, South Africa.

Data analysis

To discern the food web patterns of the river systems where *S. bainsii* lineages occurred, stable isotope biplots were used based on the $\delta^{13}C$ and $\delta^{15}N$ values of the basal sources, macroinvertebrates and fish species across the river systems. Single factor analysis of variance (ANOVA) was used to examine whether there was a variation for both $\delta^{13}C$ and $\delta^{15}N$ values of the groups (basal sources, macroinvertebrates and fish) between the different river systems. The ANOVA assumptions were tested as follows; normality of residuals was tested and met using the Shapiro-Wilk test at $\alpha = 0.05$, whereas homogeneity of variance was tested using the Levene's test at $\alpha = 0.05$. Where significant ANOVA variations were found ($\alpha < 0.05$), Tukey HSD test was used for pairwise comparison of isotopic values between rivers.

In order to compare isotopic niche sizes of the different *S. bainsii* lineages among the river systems, sample-size corrected standard ellipse area (SEAc) were calculated using fin tissue isotopic data (Jackson *et al.* 2011). Standard ellipse area (SEA) is expressed as ‰^{2}, as a measure of trophic niche structure. In addition, community-wide metrics (Layman *et al.* 2007) were used to evaluate isotopic niche patterns of individual communities. The community-wide metrics included; δ^{13}C range (CR) which is the distance between the species with the most enriched and depleted δ^{13}C range, providing for niche diversification at the base of the food web; δ^{15}N range (NR) provides information of the trophic length; distance to centroid (CD), which is the average Euclidean distance of all components to the centroid indicating the average degree of trophic diversity within the food web; mean nearest neighbour distance (NND) provides information on trophic redundancy in an isotopic space and standard deviation of nearest neighbor distance (SDNND) measures the evenness of species packing in bi-plot space that is less influenced than NND by sample size. Low SDNND values suggest more even distribution of trophic niches (Layman *et al.* 2007; Jackson *et al.* 2011).

To evaluate the interspecific interactions of *Sandelia bainsii* lineages with other species, a Bayesian isotope niche overlap analysis was conducted following Swanson *et al.* (2015). This followed a two-step approach. Firstly, the isotope niche size for each species was inferred based on the joint probability distributions of δ^{13}C and δ^{15}N values of fin tissues. These probability distributions (α = 95%), indicated the most plausible isotopic trophic niche sizes from a multi-dimensional isotopic-space. This was denoted 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 were determined as the extent of overlap between two species. This was expressed as $O_B^A = P(Y_A \in N_R(B))$, where O_B^A is the probability of species A onto the isotopic niche of species B (Swanson *et al.* 2015). Isotope niche overlaps were based on Bayesian posterior distributions using means and 95% credibility intervals. Bayesian distributions were based on Monte Carlo simulations with 1,000 iterations.

All these analyses were performed using the R statistical program (R Core Team 2021). The following packages were used; *SIBER* (Jackson *et al.* 2011) for stable isotope ellipses and Layman metrics and *nicheROVER* (Lysy *et al.* 2014) for stable isotope

Results

Isotopic food web patterns in habitats with Sandelia bainsii lineages

Within the Great Fish River system, the three sites where *Sandelia 'bainsii* Kowie' was captured exhibited a wide variation in the stable isotope values for the different trophic groups (Figure 2.2). The Lushington River isotope food web was mostly distinguished by having a narrow δ^{13}C breadth for basal sources, ranging from -30.22 ‰ for C_4 to -26.04 ‰ for FPOM (Figure 2.2, Table 2.1). In comparison, the Fairburn River had a wide δ^{13}C breadth for basal sources, ranging from -24.82 ‰ for C_3 to -12.56 ‰ for C_4. In the Kat River mainstem, the basal sources had wide breadth for both δ^{13}C (from -12.01 ‰ for C4, to -29.22 ‰ for C3) and δ^{15}N (5.57 ‰ to 12.92 ‰) (Figure 2.2, Table 2.1). Despite these wide variations, the basal sources exhibited no significant variation in δ^{13}C (ANOVA, df= 2, F = 3.46, P = 0.07) among these three sites. For the macroinvertebrates, the Lushington and Kat rivers were characterised by both narrower breadths in δ^{13}C values and wider breadths in δ^{15}N values than the Fairburn River (Figure 2.2, Table 2.1). The macroinvertebrate groups did not significantly differ for δ^{13}C

(ANOVA, F = 2.48, P = 0.10), but differed significantly for $\delta^{15}N$ (ANOVA, F = 24.49, P < 0.001). Post-hoc tests showed pairwise significant difference between Fairburn and Kat rivers (Tukey HSD, P < 0.001), and between Lushington and Kat rivers (Tukey HSD P < 0.001). Specifically, the Kat River mainstem was characterised by higher $\delta^{15}N$ values for macroinvertebrates (10.64±1.37 ‰) than the Lushington (7.26±1.25 ‰) and Fairburn (5.39±1.15 ‰) rivers (Table 2.1). In addition, these three sites were characterised by a variation in the composition of macroinvertebrate taxa. In particular, the Lushington River site was characterised by the presence of Ephemeroptera taxa such as *Acanthiops*, *Castanophlebid* and *Dicentroptelum*, whereas the Fairburn River had Odanata taxa such as *Aeshna*, *Anax* and *Lestes*, which were absent at the Kat River mainstem site (Table 2.2). At these three sites, *Sandelia 'bainsii* Kowie' was most distinguished by having higher $\delta^{15}N$ (Lushington River, $\delta^{15}N$ = 10.19 ‰; Fairburn River, $\delta^{15}N$ = 9.46 ‰; Kat River, $\delta^{15}N$ = 12.91 ‰) than other co-occurring native fishes (Figure 2.2, Table 2.3). This indicated that *Sandelia 'bainsii* Kowie' was generally a top predator in these rivers.

In the Keiskamma River, the Tyume River site 1's isotope food web was distinguished by having a wide $\delta^{13}C$ breadth for basal sources, ranging from -31.00 ‰ for C_4 to -20.59 ‰ for FPOM, whereas the Tyume River site 2's isotope food web had both enriched and narrow $\delta^{13}C$ breadth ranging from -18.07 ‰ for CPOM to -12.09 ‰ for C_4. In comparison, the Buffalo River was mostly distinguished by having a wide $\delta^{13}C$ breadth, ranging from -29.78 ‰ for C_4 to -21.29 ‰ for FPOM (Table 2.1, Figure 2.3). Overall, the basal sources exhibited a significant variation in $\delta^{13}C$ (ANOVA, F = 5.42, P = 0.03), with the Tyume River site 1 having the lowest values (-31.00±0.03) and the Tyume River site 2 having the highest values (-12.09±0.05). Post-hoc tests showed pairwise significant difference between Tyume River sites (Tukey HSD, P = 0.05), and between Tyume River site 2 and the Buffalo River (Tukey HSD P = 0.03). Specifically, the Tyume River site 2 had higher $\delta^{13}C$ values, ranging from ~ -18 to ~ -12 ‰, than those for Tyume River site 2 and Buffalo River (Table 2.1). For macroinvertebrates, the Tyume River site 1 had narrow δ^{13}C breadth ranging from -24.85 to -20.98 ‰. However, the Tyume River site 2 was mostly characterised by having a wide δ^{13}C breadth, ranging from -24.74 ‰ to 17.99 ‰. Similarly, the Buffalo River isotope food web was distinguished by having a wide δ^{13}C breadth ranging from -31.33 ‰ to -24.63 ‰ (Figure 2.3, Table 2.2). All three river sites were characterised narrow δ^{15}N ranges. The macroinvertebrates differed significantly for both δ^{13}C (ANOVA, F= 31.08, P < 0.001), and δ^{15}N (ANOVA, F= 31.43, P < 0.001). Post-hoc tests showed pairwise significant difference between all three rivers (Tukey HSD, P < 0.001). The Tyume River site 2 had the highest δ^{13}C (-19.80±1.99 ‰) compared to the Tyume River site 1 (-23.75±1.38 ‰) and Buffalo River site (-24.43±1.69 ‰) (Table 2.1). Conversely, the δ^{15}N for macroinvertebrates was lowest at the Tyume River site 2 (7.99±1.06 ‰), intermediate at the Buffalo River site (10.64±1.37 ‰) and highest at the Tyume River site 1 (12.03±1.28±1.38 ‰). In addition, the Tyume River site 2 was distinguished by the presence of Odonata taxa, *Lestes* and *Microgomphus*, and Diptera taxa, *Tipula* and *Simulium*, whereas the Buffalo River site was characterised by the presence of Hemiptera taxa, *Appasus* and *Plea* (Table 2.2). In the Keiskamma River, *Sandelia* '*bainsii* Keiskamma' was most distinguished by having higher δ^{15}N (Tyume 1 = 14.15±0.45, Tyume 2 = 10.93±0.63) than other co-occurring fishes (Figure 2.2. Table 2.3), whereas in the Buffalo River, *Sandelia 'bainsii* Buffalo' had lower δ^{15}N (8.99±2.86) than other co-occurring fishes (Figure 2.3, Table 2.3).

to -21.29 ‰ for FPOM (Table 2.1, Figure 2.3). Overall, the basal sources exhibited a significant variation in δ^{13}C (ANOVA, F = 5.42, P = 0.03), with the Tyume River site 1 having the lowest values (-31.00±0.03) and the Tyume River site 2 having the highest values (-12.09±0.05). Post-hoc tests showed pairwise significant difference between Tyume River sites (Tukey HSD, P = 0.05), and between Tyume River site 2 and the Buffalo River (Tukey HSD P = 0.03). Specifically, the Tyume River site 2 had higher δ^{13}C values, ranging from ~ -18 to ~ -12 ‰, than those for Tyume River site 2 and Buffalo River (Table 2.1). For macroinvertebrates, the Tyume River site 1 had narrow $\delta^{13}C$ breadth ranging from -24.85 to -20.98 ‰. However, the Tyume River site 2 was mostly characterised by having a wide $\delta^{13}C$ breadth, ranging from -24.74 ‰ to 17.99 ‰. Similarly, the Buffalo River isotope food web was distinguished by having a wide $\delta^{13}C$ breadth ranging from -31.33 ‰ to -24.63 ‰ (Figure 2.3, Table 2.2). All three river sites were characterised narrow $\delta^{15}N$ ranges. The macroinvertebrates differed significantly for both $\delta^{13}C$ (ANOVA, F= 31.08, $P < 0.001$), and $\delta^{15}N$ (ANOVA, F= 31.43, $P < 0.001$). Post-hoc tests showed pairwise significant difference between all three rivers (Tukey HSD, $P < 0.001$). The Tyume River site 2 had the highest $\delta^{13}C$ (-19.80±1.99 ‰) compared to the Tyume River site 1 (-23.75±1.38 ‰) and Buffalo River site (-24.43±1.69 ‰) (Table 2.1). Conversely, the $\delta^{15}N$ for macroinvertebrates was lowest at the Tyume River site 2 (7.99±1.06 ‰), intermediate at the Buffalo River site (10.64±1.37 ‰) and highest at the Tyume River site 1 (12.03±1.28±1.38 ‰). In addition, the Tyume River site 2 was distinguished by the presence of Odonata taxa, *Lestes* and *Microgomphus*, and Diptera taxa, *Tipula* and *Simulium*, whereas the Buffalo River site was characterised by the presence of Hemiptera taxa, *Appasus* and *Plea* (Table 2.2). In the Keiskamma River, *Sandelia* '*bainsii* Keiskamma' was most distinguished by having higher $\delta^{15}N$ (Tyume 1 = 14.15±0.45, Tyume 2 = 10.93±0.63) than other co-occurring fishes (Figure 2.2. Table 2.3), whereas in the Buffalo River, *Sandelia 'bainsii* Buffalo' had lower $\delta^{15}N$ (8.99±2.86) than other co-occurring fishes (Figure 2.3, Table 2.3).

Table 2.1. Mean values of carbon ($\delta^{13}C$ ‰) and nitrogen ($\delta^{15}N$ ‰) stable isotopes (±standard deviation) for basal sources and macroinvertebrates collected from the Lushington, Fairburn, Kat River of the Great Fish River and Tyume River sites 1 and 2 (Tyume 1 and Tyume 2) of the Keiskamma River system and the Buffalo River system.

Group	Lushington River		Fairburn River		Kat River		Tyume 1		Tyume 2		Buffalo River	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Algae	-	-	-9.69±0.53	4.61±0.68	-14.36±0.17	10.46±0.24	-	-	-	-	-	-
C_3	-29.49±0.35	1.92±0.19	-24.82±0.09	9.09±0.35	-29.22±0.05	12.92±0.42	-27.53±2.72	4.02±1.71	-	-	-29.68±0.19	1.34±0.24
C_4	-30.22±0.52	2.38±0.58	-12.56±0.12	1.73±0.11	-12.01±0.12	5.57±0.17	-31.00±0.03	7.97±0.32	-12.09±0.05	4.73±0.42	-29.78±0.03	7.78±0.03
CPOM	-26.46±0.79	1.84±0.89	-	-	-25.74±0.17	5.89±0.46	-25.30±0.78	9.26±0.38	-18.07±0.86	4.45±0.25	-27.22±0.25	2.92±0.58
FPOM	-26.04±0.26	7.98±1.98	-	-	-21.79±0.47	3.02±0.73	-20.59±3.71	5.59±0.71	-	-	-21.29±1.39	.34±0.96
Invertebrates	-24.28±1.9 5	7.26±1.25	-23.58±2.65	5.39±1.15	-22.43±1.69	10.64±1.37	-23.75±1.38	12.03±1.28	19.80±1.99	7.99±1.06	-24.43±1.69	10.64±1.37

Table 2.2. Mean values of carbon (δ^{13}C ‰) and nitrogen (δ^{15}N ‰) stable isotopes (±standard deviation) for macroinvertebrates collected from the Lushington, Fairburn, Kat rivers of the Great Fish River and Tyume 1 and Tyume 2 of the Keiskamma River and the Buffalo River system

		Lushington		Fairburn		Kat mainstem		Tyume 1		Tyume 2		Buffalo	
Order	Taxa	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
Ephemeroptera	Baetidae	-	-	-25.97±0.92	5.27±0.14	-21.48±0.00	11.81±0.00	-24.84±0.21	11.89±0.04	-20.22±0.44	7.36±0.04	-	-
	Caenis	-	-	-	-	-	-	-23.28±0.10	10.20±0.09	-	-	-28.05±0.23	7.43±0.73
	Acanthiops	-25.65±2.54	6.35±1.43	-	-	-	-	-	-	-	-	-	-
	Castanophlebid	-24.43±0.37	6.94±0.78	-	-	-	-	-	-	-	-	-	-
	Dicentroptelum	-24.46±0.12	6.11±0.00	-	-	-	-	-	-	-	-	-	-
	Leptophlebiidae	-26.64±0.00	7.16±0.00	-25.55±0.00	7.27±0.00	-24.63±0.20	12.18±0.49	-25.08±0.00	11.76±0.00	-21.27±0.00	6.84±0.00	-30.95±0.00	8.54±0.00
	Tricorythus	-	-	-21.50±0.36	5.20±0.22	-23.60±0.03	9.69±0.26	-24.74±0.00	7.13±0.00	-20.74±0.11	6.97±0.12	-	-
	Afronurus		-	-28.43±0.00	5.14±0.00	-	-	-	-	-24.74±0.00	7.13±0.00	-	-
Trichoptera	*Cheumatopsyche*	-24.26±0.00	7.35±0.00	-18.13±0.00	6.08±0.00	-22.08±0.08	11.94±0.10	-	-	-		-	-
	Hydropsyche	-	-	-	-	-21.79±0.01	11.67±0.22	-23.81±0.18	13.81±0.14	-19.24±0.11	9.17±0.03	-	-
Odonata	*Aeshna*	-	-	-24.27±0.00	6.51±0.00	-	-	-	-	-	-	-	-
	Anax	-	-	-23.00±0.00	6.58±0.00	-	-	-	-	-	-	-	
	Lestes	-	-	-22.48±0.00	6.66±0.00	-	-	-	-	-18.82±0.00	8.59±0.03	-27.39±0.17	8.95±0.65
	Microgomphus	-	-	-	-	-	-	-	-	-17.99±0.08	8.96±0.07	-	-
Coleoptera	Scirtidae	-24.62±0.00	6.97±0.00	-	-	-	-	-	-	-	-	-	-
	Psephenidae	-	-	-25.00±0.32	3.38±0.13	-22.07±0.00	7.50±0.00	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
Diptera	Unidentified *diptera*	-24.47±0.00	8.75±0.00	-	-	-	-	-	-	-	-	-	-

	Tipula	-23.04±0.00	0.21±0.00	-	-	-	-	-	-	-20.39±0.00	10.31±0.03	-	-
	Simulium	-	-	-21.72±0.00	4.80±0.07	-22.07±0.00	7.50±0.00	-	-	-20.91±0.01	7.10±0.13	-26.95±0.00	8.12±0.00
	Athericidae	-	-	-	-	-21.48±0.00	11.82±0.00	-24.55±0.18	12.70±0.36	-	-	-31.34±0.00	8.44±0.00
Hemiptera	Notonectidae	-	-	-	-	-21.37±0.25	9.21±0.15	-	-	-	-		-
	Appasus	-	-	-	-	-	-	-	-	-	-	-25.69±0.00	9.74±0.00
	Plea	-	-	-	-	-	-	-	-	-	-	-24.63±0.37	8.85±1.01
Araneae	*Tetragnatha*	-	-	-	-	-21.75±0.00	11.90±0.00	-	-	-	-	-	-
Decapoda	*Potamonautes*	-20.44±0.00	7.12±0.00	-	-	-19.07±0.29	9.43±0.22	-20.98±0.09	10.97±0.07	-16.68±0.58	7.96±0.25	-	-
Anura	Tadpole	-	-	-	-	-	-			-	-	-27.84±0.00	9.16±0.00

Table 2.3. Mean values of carbon ($\delta^{13}C$ ‰) and nitrogen ($\delta^{15}N$ ‰) stable isotopes (±standard deviation) for consumer species including *Sandelia bainsii, Enteromius mandelai, Amatolacypris trevelyani, Glossogobius callidus*, samples from Lushington River, Fairburn River, Kat River, Tyume River sites 1 and 2 (Tyume 1 and Tyume 2) and the Buffalo River system.

Species	Lushington River		Fairburn River		Kat River		Tyume 1		Tyume 2		Buffalo River	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
S. bainsii	-23.03±0.73	10.19±1.23	-18.07±2.14	9.46±0.18	-20.39±0.80	12.91±0.44	-24.49±0.34	14.15±0.45	-19.47±1.12	10.93±0.63	-24.57±1.84	8.99±2.86
E. mandelai	-21.93±0.47	9.30±0.21	-19.39±1.81	9.09±0.35	-	-	-	-	-	-	-24.16±0.79	11.65±0.17
A. trevelyani	-	-	-	-	-	-	-	-	-	10.75±0.23	-23.00±1.30	11.09±0.34
G. callidus	-	-	-	-	-19.66±0.89	12.63±0.99	-	-	-18.39±1.31	4.45±0.25	-27.22±0.25	-

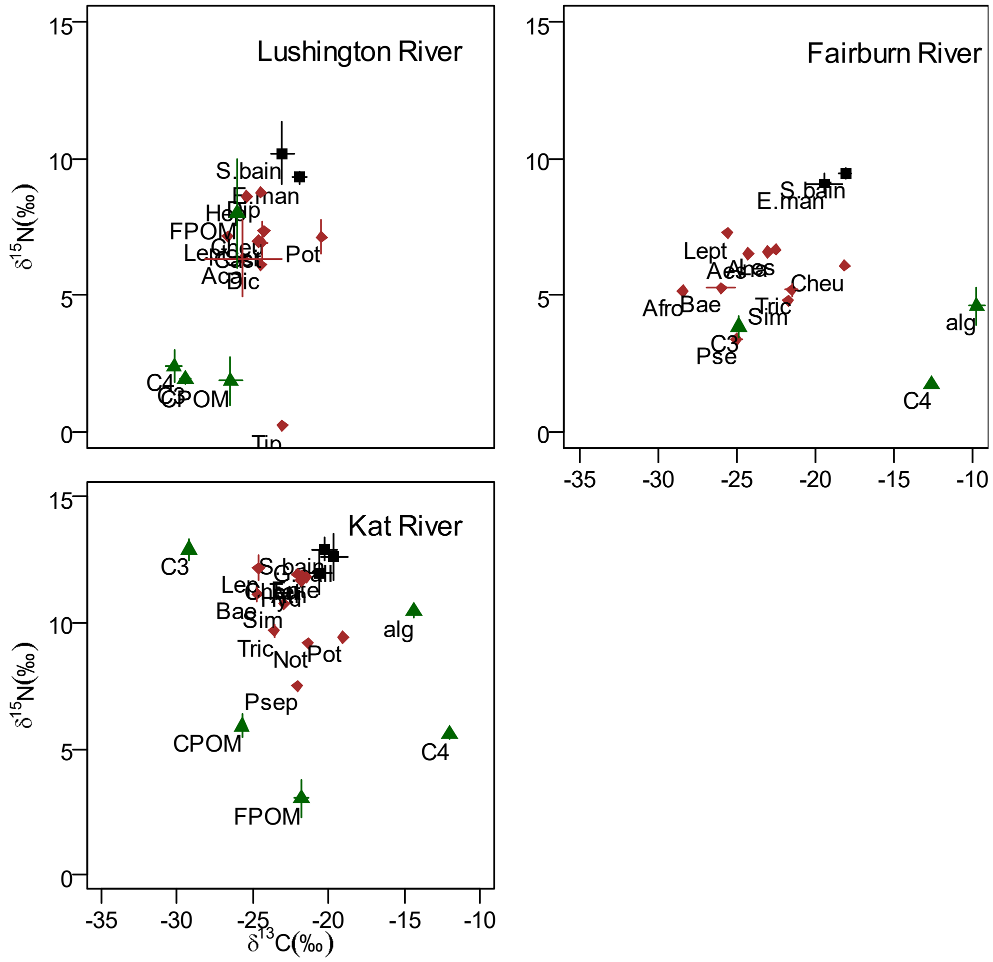

Figure 2.2. Stable isotope biplots for basal sources, aquatic invertebrates and fishes collected in habitats with *Sandelia 'bainsii Kowie'* in the Lushington, Fairburn and Kat rivers, which are tributaries of the Great Fish River system. The dfferent consumer and basal sources are abbreviated as S.bain = *Sandelia 'bainsii Kowie',* E. mand = *Enteromius mandelai,* G.cal = *Glossogobius callidus,* Bae = *Baetidae,* Aca = *Acanthiops,* Cas = *Castanophlebid,* Dic = *Dicentroptelum,* Lep = Leptophlebiidae, Tri = *Tricorythus,* Afro = *Afronurus,* Cheu = *Cheumatopsyche,* Hyd = *Hydropsyche,* Aes = *Aeshna,* Ana = *Anax,* Les = *Lestses,* Sci = Scirtidae, Pse = Psephenidae, *Dip* = *Diptera, Tip* = *Tipula, Sim* = *Similium, Ath* = Athericidae, *Not* = Notonectidae, Tet = *Tetragnatha, Pot* = *Potamonautes.*

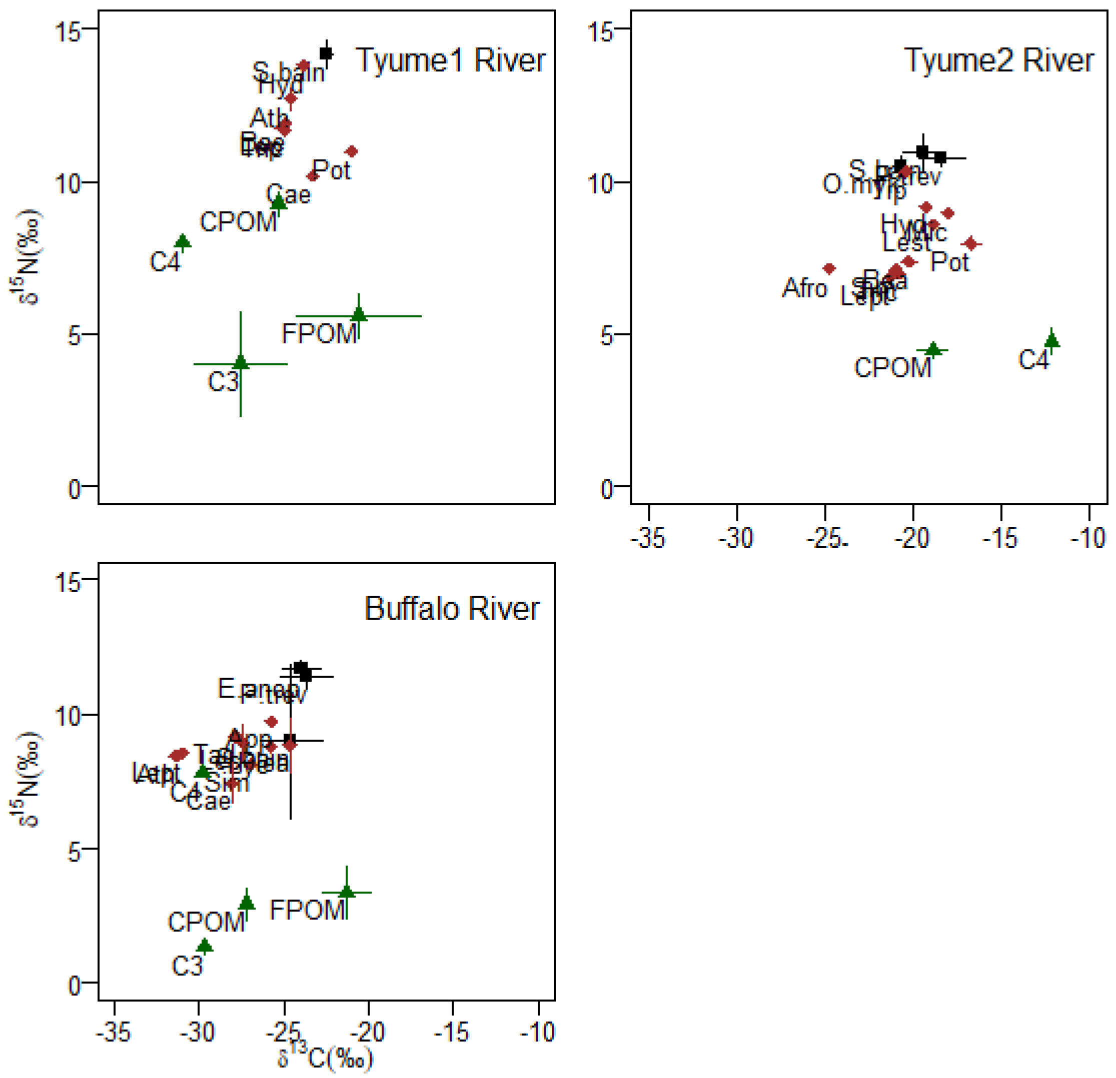

Figure 2.3. Stable isotope biplots for basal sources, aquatic invertebrates and fishes collected in habitats with *Sandelia ‘bainsii* Keiskamma’ from the Keiskamma River and *Sandelia ‘bainsii* Buffalo’ within the Buffalo River system. The different consumer and basal sources are abbreviated as S.bain = *Sandelia ‘bainsii* Kowie’, E. mand = *Enteromius mandelai*, G.cal = *Glossogobius callidus*, Sim = Simuliidae, Bae = Baetidae, Lep = Leptophlebiidae, Tri = *Tricorythus*, Afro= *Afronurus*, Hyd = *Hydropsyche*, Aes = *Aeshna*, Les = *Lestes*, Mic = *Microgomphus*, Tip = *Tipula*, Sim = *Simulium*, Ath = Athericidae, App = *Appasus*, Ple = *Plea*, Pot = *Potamonautes*, Tad = Tadpole.

Isotopic niche patterns and trophic interrelationships of Sandelia bainsii lineages

Within the Great Fish River, *Sandelia 'bainsii* Kowie' exhibited variable isotopic niche sizes (Figure 4). This lineage's isotope niche was largest in the Lushington River (SEAc = 1.71 ‰^{2}), intermediate in the Kat River (SEAc = 1.23 ‰^{2}) and smallest in the Fairburn River (SEAc = 0.26 ‰^{2}) (Table 2.4). Comparison of the headwater tributary sites, where *Sandelia 'bainsii* Kowie' co-occurred with *E. mandelai*, revealed that the Lushington River community was most distinguished by high nitrogen range (NR = 0.89 ‰), whereas the Fairburn River community was most distinguished by high carbon range (CR = 1.51 ‰). The high NR value for the Lushington River community indicated high trophic diversity, with corresponding low trophic redundancy due to high mean distance to centroid (CD = 0.71 ‰) and mean nearest neighbour distance (NND = 1.42 ‰) (Table 2.5). By comparison, the high CR values for the Fairburn River community indicated a wide range in basal sources, with corresponding low trophic redundancy due to high CD (0.82 ‰) and NND (1.63 ‰). The isotope niche for *Sandelia 'bainsii* Kowie' exhibited low and comparable probabilities of interspecific overlap onto the isotope niche of *E. mandelai* in the Lushington River (mean = 10.59 %, 95 % credibility interval (CI) = 1.0 – 26.0 %) (Figure 2.5), and Fairburn River, albeit with high uncertainty (mean = 11.97 %, 95 % CI = 0.0 – 63.0 %) (Figure 2.6). Conversely, the probability of *E. mandelai*'s isotopic niche overlap onto that of *Sandelia 'bainsii* Kowie' was high in the Lushington River (mean = 65.50 %, 95 % CI = 14.0 – 100.0 %) and low in the Fairburn River (mean = 2.42 %, 95 % CI = 0.0 - 14.0 %) (Figures 5 and 6).

The Kat River mainstem community, where *Sandelia 'bainsii* Kowie' co-occurred with *E. mandelai* and *G. callidus,* was characterised by intermediate values for CR (1.06 ‰) and NR (0.78 ‰) compared to the headwater tributary sites (Table 2.5). The intermediate NR and CR values for the Kat River community indicated intermediate trophic diversity, with high trophic redundancy due to low CD (0.54 ‰) and NND (0.82 ‰) (Table 2.5). The isotope niche for *Sandelia 'bainsii* Kowie' exhibited high and comparable probabilities of interspecific overlap onto the isotope niche of both *E. mandelai* (mean = 88.84 %, 95 % CI = 58.0 – 100.0 %) and *G. callidus* (mean = 77.41 %, 95 % CI = 44.0 – 96.0 %) (Figure 2.7). In contrast, the probability of *E. mandelai's* isotopic niche overlap onto that of *Sandelia 'bainsii* Kowie' was low (mean = 47.94 %, 95 % CI = 18.0 – 69.0 %), whereas the probability of interspecific overlap onto the isotope niche of *G. callidus* was high (mean = 87.53 %, 95 % CI = 46.0 - 97.0 %) (Figure 2.3). *Glossogobius callidus* exhibited low probability of interspecific overlap into *Sandelia 'bainsii* Kowie' (mean = 56.69 %, 95 % CI = 20.0 – 77.0 %) and high probabilities with *E. mandelai* (mean = 88.91 %, 95 % CI = 49.0 – 78.0 %).

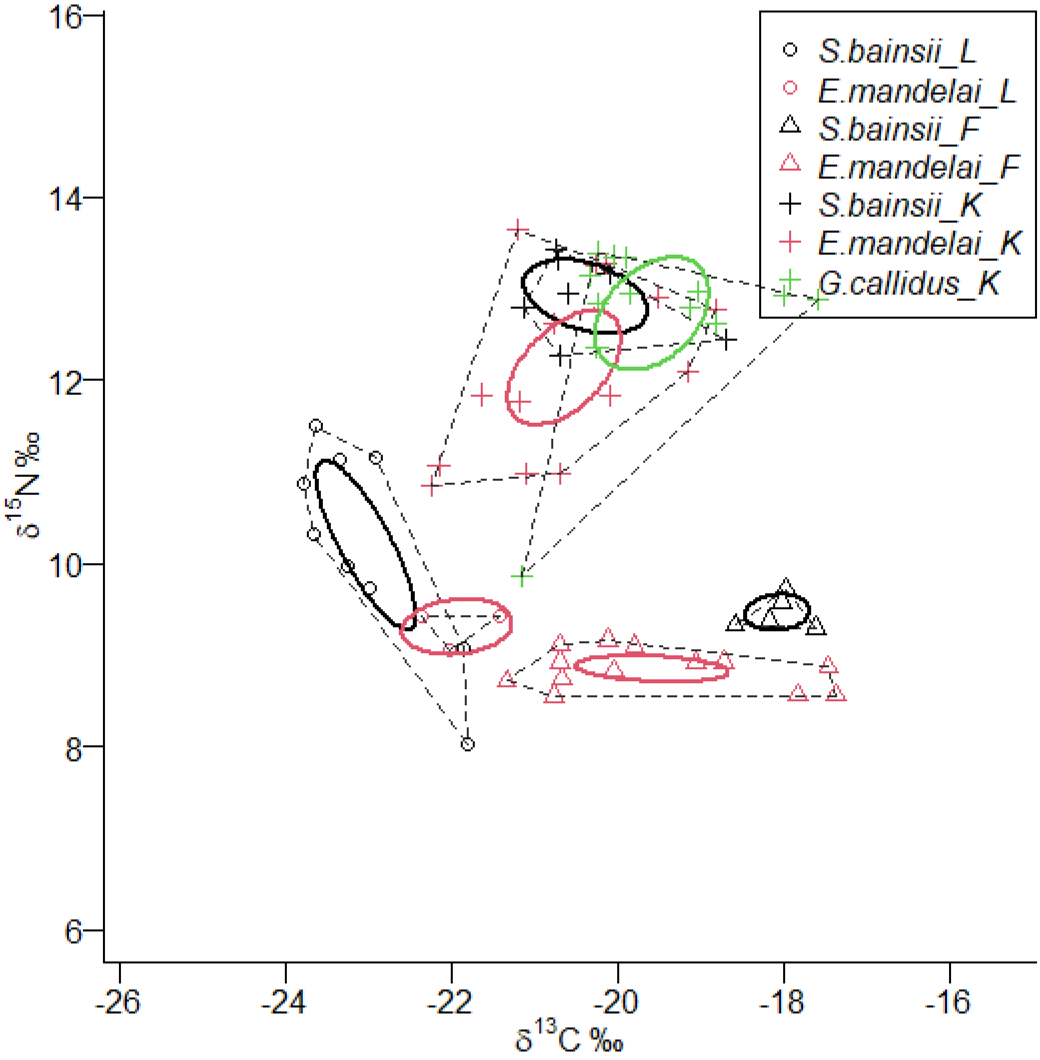

Figure 2.4. Standard Ellipse Area (SEAc; solid lines) and dashed convex hulls (dashed polygon) of *Sandelia ‘bainsii* Kowie’ and its co-occurring species, *Enteromius mandelai* and *Glossogobius callidus* collected from the Lushington River (circles), Fairburn River (triangles) and the Kat River mainstem (cross).

Table 2.4. Summary statistics of the standard ellipse areas (SEAc) of the *Sandelia bainsii* lineages and its co-occurring species, *Enteromius mandelai*, *Glossogobius callidus* and *Amatolacypris trevelyani* lineages in the different river system.

Sites	*S. bainsii*	*E. mandelai*	*G. callidus*	*A. trevelyani*
Lushington River	1.71	0.62	-	-
Fairburn River	0.26	0.95	-	-
Kat River	1.23	2.91	2.93	-
Tyume 1	0.61	-	-	-
Tyume 2	1.90	0.95	-	-
Buffalo River	5.44	1.04	-	1.89

Table 2.5. Community Layman metrics values of *Sandelia bainsii* and its co-occurring species from the different river systems.

Rivers	NR	CR	CD	NND
Lushington River	0.89	1.09	0.71	1.41
Fairburn River	1.51	0.82	1.63	0.62
Kat River	0.78	1.06	0.54	0.82
Keiskamma River	3.41	4.09	2.17	2.20
Buffalo River	2.66	0.88	1.18	1.09[1]

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[1] Nitrogen range (NR), carbon range (CR), distance to centroid (CD), mean nearest neighbour distance (NND)

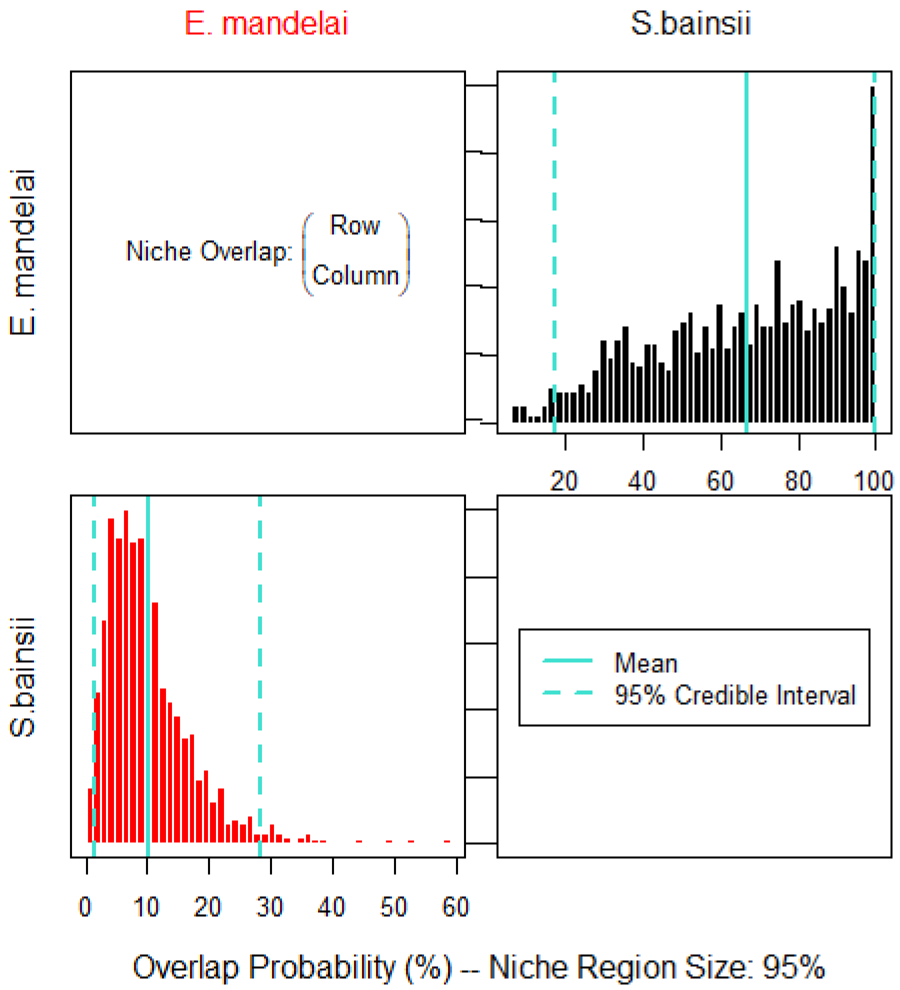

Figure 2.5. Bayesian posterior probabilistic isotopic trophic niche overlap for *Sandelia bainsii* and its co-occurring species *Enteromius mandelai* in the Lushington River. Probabilities of niche overlap (mean and 95% credibility intervals) are specified as the overlap of the *Sandelia ‘bainsii* Kowie’ into the isotopic niche of *Enteromius mandelai* (column) and *Enteromius mandelai* into *Sandelia ‘bainsii* Kowie’ (rows).

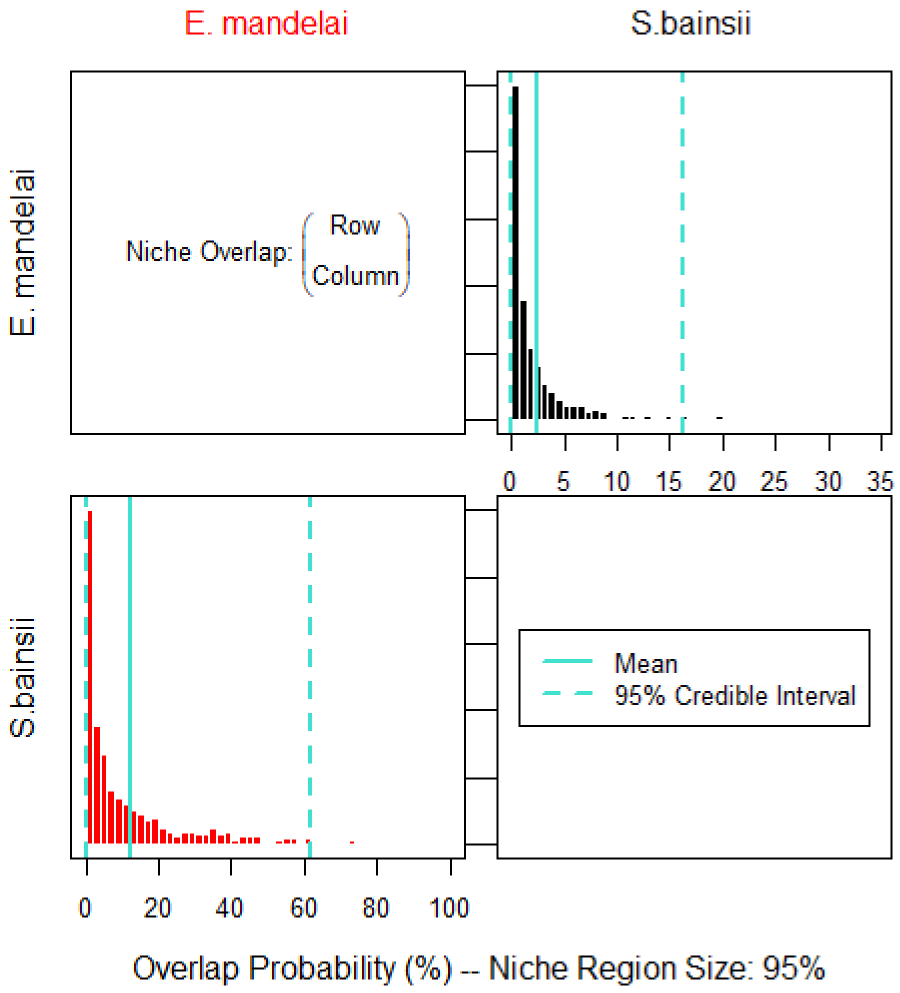

Figure 2.6. Bayesian posterior probabilistic isotopic trophic niche overlap for *Sandelia 'bainsii* Kowie' and its co-occurring species *Enteromius mandelai* from the Fairburn River. Probabilities of niche overlap (mean and 95% credibility intervals) are specified as the overlap of the *Sandelia 'bainsii* Kowie' into the isotopic niche of *Enteromius mandelai* (column) and *Enteromius mandelai* into *Sandelia 'bainsii* Kowie' (rows).

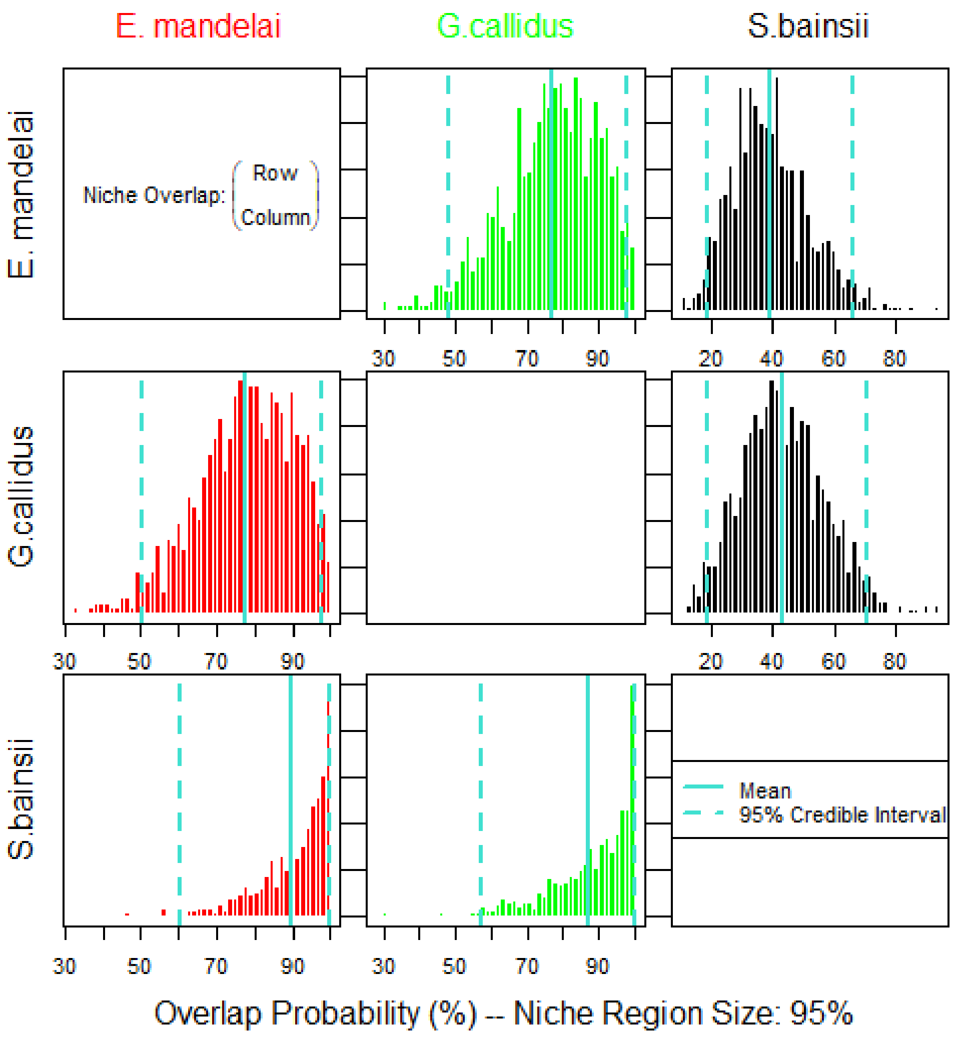

Figure 2.7. Bayesian posterior probabilistic isotopic trophic niche overlap for *Sandelia 'bainsii* Kowie' and its co-occurring species *Enteromius mandelai* and *Glossogonius callidus* from the Kat River. Probabilities of niche overlap (mean and 95% credibility intervals) are specified as the overlap of the *Sandelia 'bainsii* Kowie' into the isotopic niche of *Enteromius mandelai* (column) and *Glossogobius callidus* (column). *Enteromius mandelai* and *Glossogobius callidus* into *Sandelia 'bainsii* Kowie' (rows).

Within the Keiskamma River, *Sandelia 'bainsii* Keiskamma' exhibited variable isotopic niche sizes (Figure 2.8). This lineage's isotope niche was larger in the Tyume River site 2 (SEAc = 1.90 ‰2) than in the Tyume 1 River (SEAc = 0.61 ‰2) (Table 2.4). In the

Buffalo River, *Sandelia 'bainsii* Buffalo' had a large isotopic niche (SEAc = 5.44 ‰2) compared to that of the Kat River lineage (SEAc = 1.23 ‰2) where both species co-occurred with two other species (Table 2.4, Figure 2.9). The Keiskamma River community, where *Sandelia 'bainsii* Keiskamma' co-occurred with *A. trevelyani*, was characterised by high values for CR (4.09 ‰) and low NR (3.41 ‰). This is compared to the Buffalo River community where *S. 'bainsii* Buffalo' co-occurred with *E. mandelai* and *P. trevelyani* was characterised by low CR values (0.88 ‰) and intermediate NR values (2.66 ‰) (Table 2.5). The high NR and CR values for the Keiskamma River community indicated high trophic diversity, with corresponding low trophic redundancy due to high CD (2.17 ‰) and NND (2.20 ‰) (Table 2.5). By comparison, the Buffalo River had low CR values indicating a low range of basal sources, and an intermediate NR value suggesting an intermediate trophic diversity, with corresponding high trophic redundancy due to low CD (1.18 ‰) and NND (1.09 ‰).

Sandelia 'bainsii Keiskamma's isotopic niche exhibited low probability of interspecific overlap onto the isotope niche of *A. trevelyanii* in the Keiskamma River (mean = 3.06 %, 95 % credibility interval (CI = 0.0 – 45.0 %) (Figure 2.10). However, the probability of *A. trevelyani*'s isotopic niche overlap onto that of *Sandelia 'bainsii* Keiskamma' was high (mean = 13.71 %, 95 % CI = 0.0 – 50.0 %) (Figure 2.10). In the Buffalo River, isotope niche for *Sandelia 'bainsii* Buffalo' exhibited low probabilities of interspecific overlap onto the isotopic niches of both *E. mandelaii* (mean = 9.78 %, 95 % CI = 1.0 – 20.0 %) and *A. trevelyani* (mean = 17.20 %, 95 % CI = 3.0 – 30.0 %) (Figure 2.11). By comparison, the probability of *E. mandelai*'s isotopic niche overlapping onto that of *Sandelia 'bainsii* Buffalo' was intermediate (mean = 39.89 %, 95 % CI = 18 – 75.0 %) (Figure 2.11), and high for *A. trevelyani* (mean = 91.36 %, 95 % CI = 75.0 – 100.0 %). Additionally, *A. trevelyani* showed high probabilities of interspecific overlap onto the isotopic niche of *Sandelia 'bainsii* Buffalo' (mean = 44.52 %, 95 % CI = 21.0 – 75.0 %), and the isotopic niche of *E. mandelai* (mean = 63.46 %, 95 % CI = 40.0 – 83.0 %).

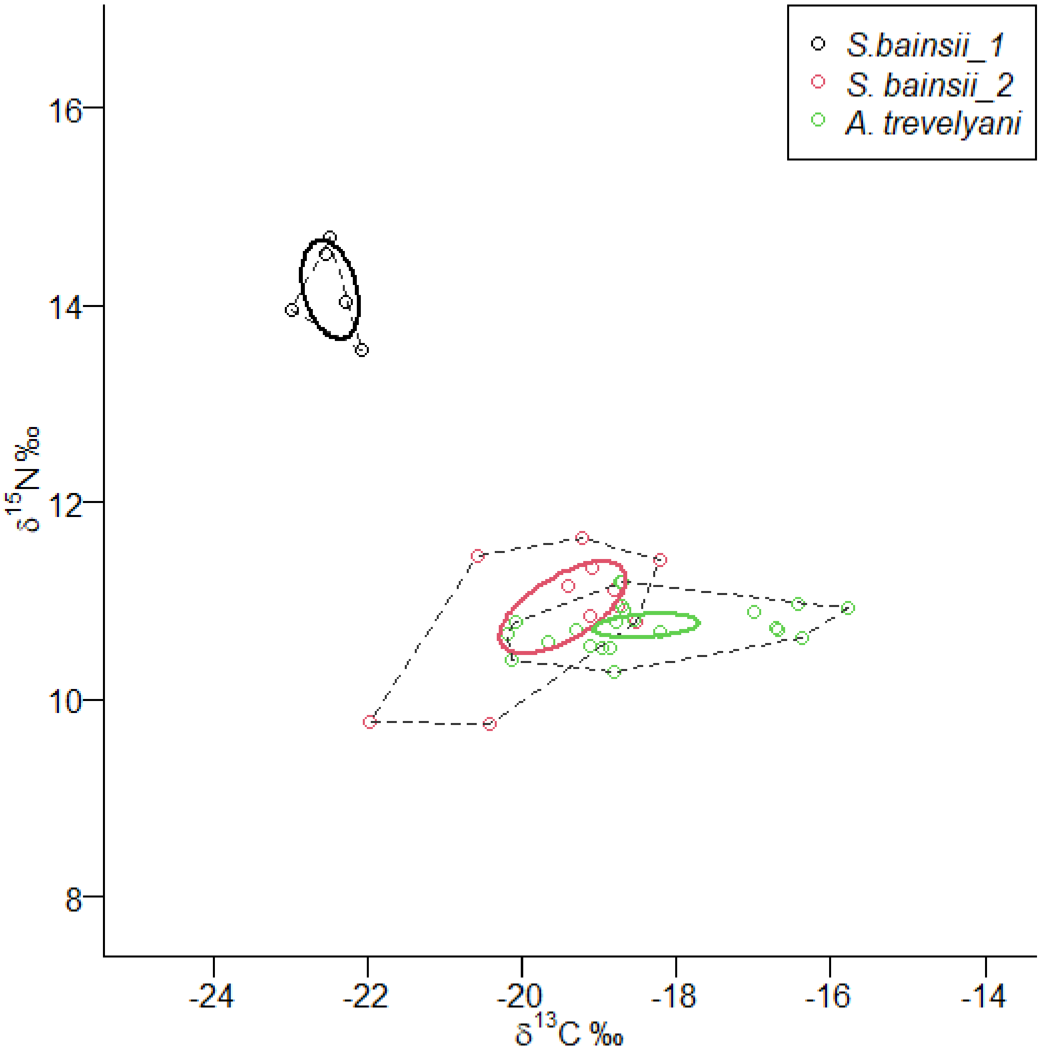

Figure 2.8. Standard Ellipse Area (SEAc; solid lines) and dashed convex hulls (dashed polygon) of two populations of *Sandelia 'bainsii* Keiskamma' and its co-occurring species, *Amatolacypris trevelyani* collected from the Tyume 1 River (black circles), Tyume 2 River (green circles).

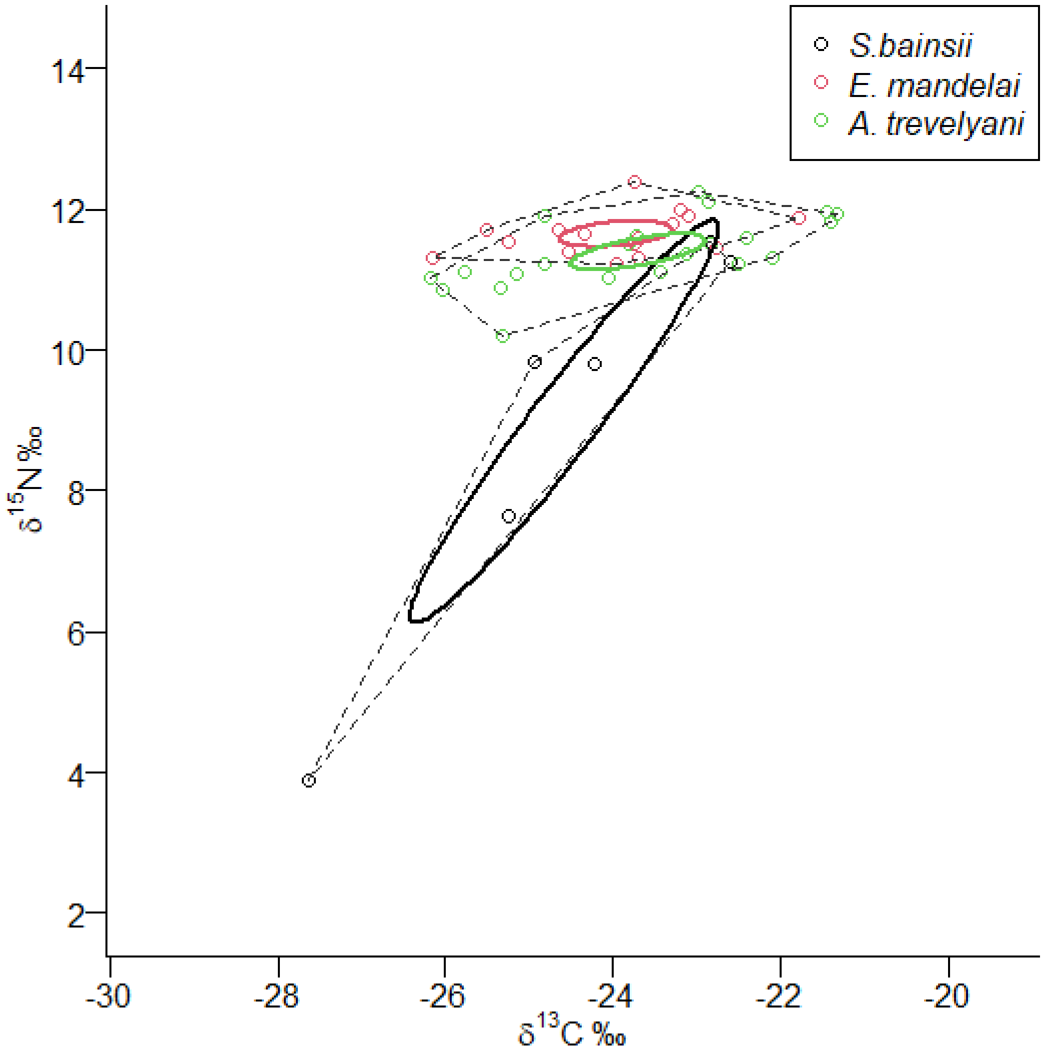

Figure 2.9. Standard Ellipse Area (SEAc; solid lines) and dashed convex hulls (dashed polygon) of *Sandelia 'bainsii* Buffalo' and its co-occurring species, *Enteromius mandelai* and *Pseudobarbus trevelyani* collected from the Buffalo River system.

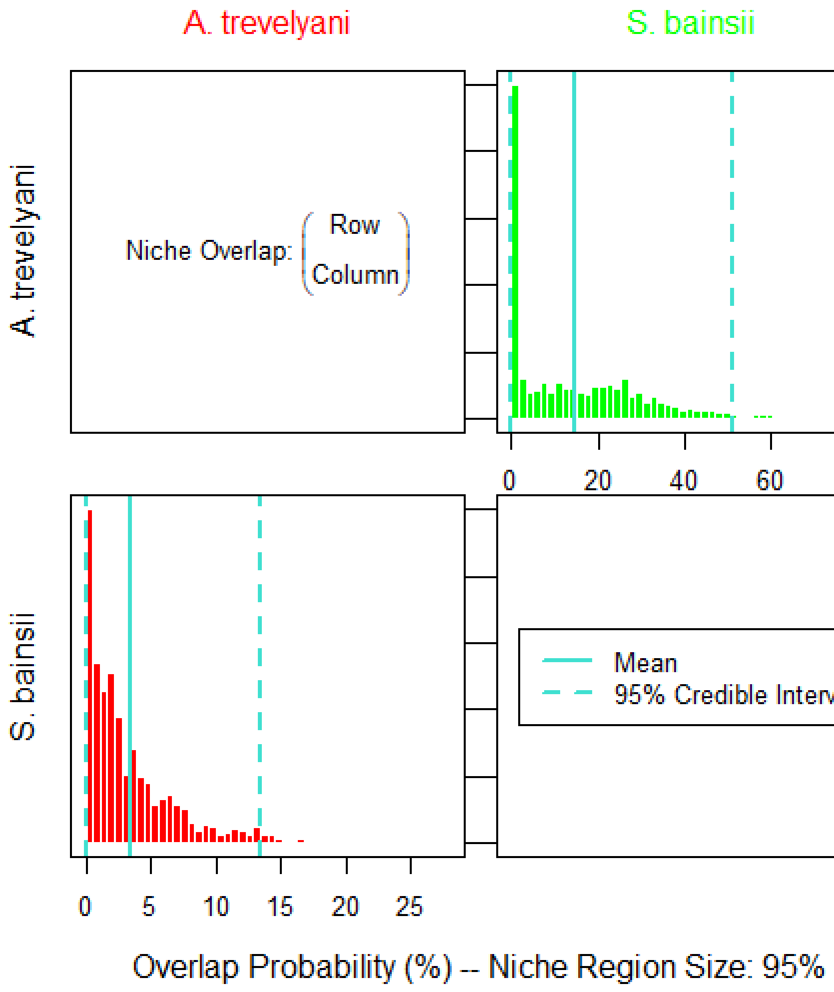

Figure 2.10. Bayesian posterior probabilistic isotopic trophic niche overlap for *Sandelia ‘bainsii* Keiskamma’ and its co-occurring species *Amatolacypris trevelyani* from the Keiskamma River. Probabilities of niche overlap (mean and 95% credibility intervals) are specified as the overlap of the *Sandelia ‘bainsii* Keiskamma’ into the isotopic niche of *Amatolacypris trevelyani* (column) and *Amatolacypris trevelyani* into *Sandelia ‘bainsii* Keiskamma’ (rows).

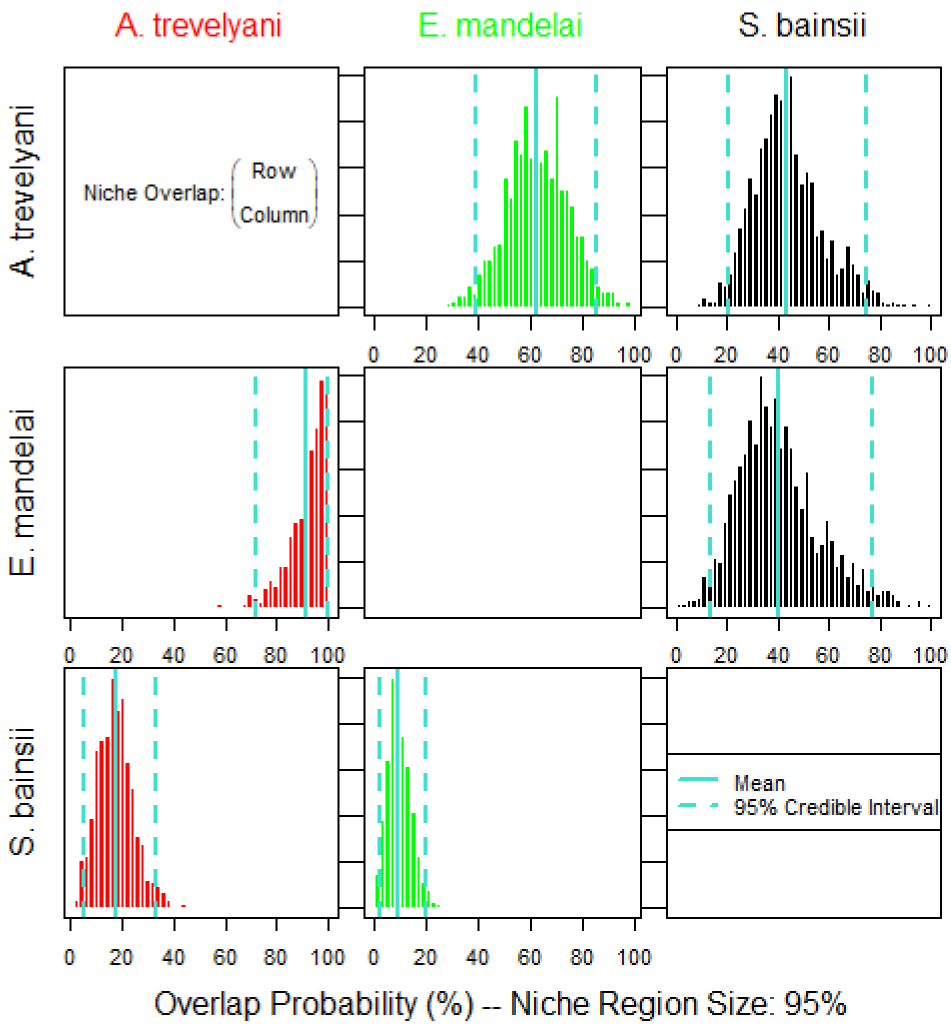

Figure 2.11. Bayesian posterior probabilistic isotopic trophic niche overlap for *Sandelia 'bainsii* Buffalo' and its co-occurring species *Enteromius mandelai* and *Amatolacypris trevelyani f*rom the Buffalo River. Probabilities of niche overlap (mean and 95% credibility intervals) are specified as the overlap of the *Sandelia 'bainsii* Buffalo' into the isotopic niche of *Enteromius mandelai* (column) and *Amatolacypris trevelyani* (column). *Enteromius mandelai* and *Amatolacypris trevelyani* into *Sandelia 'bainsii* Buffalo' (rows).

Discussion

This study set out to examine the trophic patterns and food web dynamics in habitats where *Sandelia bainsii* lineages occurred using stable isotope analysis. The results showed variability in the $\delta^{13}C$ and $\delta^{15}N$ values for the different basal resources, macroinvertebrates and fish community across the different headwater streams. Within and across the different rivers, the *Sandelia bainsii* lineages exhibited variable isotopic niche sizes, which appeared to coincide with the variation in the isotopic composition of the individual communities. Furthermore, these lineages did not exhibit any discernible patterns in their interspecific interactions in different habitats. This suggest that these lineages' isotopic niche patterns were largely influenced by spatial differences in both trophic resources and probable interactions with other native fishes. This appeared to be consistent with the generalist feeding behaviour that was previously reported for the species (Mayekiso 1986; Mayekiso and Hecht 1990).

The Kat River represented a mosaic of habitats within which the *Sandelia 'bainsii* Kowie' lineage persisted. The most recent ecological research on species-habitat relationships for this lineage revealed that it occurred in varying densities with no clear habitat affinities and co-occurred with different native fishes within its sampled range (Sifundza *et al.* 2021). The current study thus sheds more light on the broad trophic ecology of this lineage, which likely explain its persistence and probable coexistence mechanisms. Specifically, at two of the sites where this lineage co-occurred with *E. mandelai*, it was characterised by variable but distinct isotopic niches, and generally exhibited low interspecific isotopic niche overlap. This suggests the likely importance of trophic niche differentiation in these headwater stream habitats, which could facilitate coexistence between the natives (Ross 1986; Shirantha *et al.* 2005). By comparison, in the Kat River mainstem, *Sandelia 'bainsii* Kowie' had an isotopic niche that generally overlapped with that of other species. This lineage showed higher isotopic niche overlap onto the niches of its co-occurring species than the reverse. This could suggest that resources were limiting in its environment and *S. bainsii* is able to switch diets as in their generalist feeding behaviour. Previous studies have also shown that having fish with generalist feeding strategies are favoured in stressful or variable environments (Laske *et al.* 2018).

Overall, in addition to trophic niche differentiation, these observations suggest the relative importance of interspecific interactions in habitats where this lineage co-occurred with multiple native species.

Similarly, the Keiskamma and Buffalo River were found to show spatial variability in food web structure. Carbon food resource dynamics in streams can be highly patchy, and factors such as canopy cover and stream reach morphology may influence carbon assimilation pathways (Sullivan 2013). Communities that can assimilate both allochthonous and autochthonous carbon in their system are expected to have a higher diversity at the base of the food web (high CR value) which could subsequently lead to more trophic levels (high NR value), and thus more food resources for consumers (Layman 2007). This pattern can also be observed in both the Keiskamma and Buffalo River systems, where there is wide resource use by *S. bainsii*, resulting in a low interspecific overlap onto the isotopic niches of other species. These findings are in support of the results found by Kadye *et al*. (2016) which showed *Sandelia capensis* a member of the family Anabantidae to have low probabilities of overlapping onto those of redfins. The study further suggested that this lineage of *S. capensis* was perhaps influenced by niche partitioning mechanisms and through trophic niche segregation that it enabled it to co-occur with redfins. The three *S. bainsii* lineages seem to exhibit a similar pattern in terms of interspecific interactions, which could be assumed to be characteristic of members of the family Anabantidae. Previous studies have also suggested that *S. capensis* tolerates a wide range of physical and chemical water conditions (Harrison 1952). Therefore as much as these lineages are adaptable to a wide range of environmental factors, they are not highly competitive and tend to resort to resource partitioning.

To conclude, this study suggests that the variable isotopic niche exhibited by the three *Sandelia bainsii* lineages result from varied food web characteristics possibly due to differences in resource availability, spatial heterogeneity and land use patterns. *S. bainsii* is a generalist feeder, but is not a highly competitive predator, where it co-exists with other native predators, it tends resort to resource partitioning to reduce competition. This could possibly explain why it persists in headwater streams, as the middle and lower sections are invaded by *Clarias gariepinus*, *Micropterus salmoides* and *Micropterus domomieu* which are known to be top predators and generalist feeders (Cambray 2003; Wasserman 2011; Shelton *et al.* 2014). In addition, these lineages have only recently been separated by paleodrainage connections, river captures or intermittent connection of low drainage divides (Chakona *et al.* 2020), therefore they could still retain ancestral ecological characteristics, hence the similar trophic ecology patterns in terms of niche partitioning through trophic niche segregation (Kadye *et al.* 2016).

These findings have important implications on the conservation of *Sandelia bainsii* as a threatened endemic species with a fragmented distribution. The study by Sifundza *et al.* 2020 found that *Sandelia* '*bainsii* Kowie' was extirpated from one locality from the Koonap tributary of the Kat River, possibly due to the presence of a non-native species *Clarias gariepinus*. The results of this study thus shed more light to those findings, showing that the *Sandelia bainsii* lineages are not rigorous competitors when it comes to resource utilisation and as a result are generalist feeders, a possible adaptation to avoid competition. Moreover, in cases where native species such as *Sandelia bainsii* have more restricted distribution ranges, the presence of invasive fishes may have more significant effects. Therefore, it can be assumed that when occurring in systems with top predators such as *C. gariepinus* and *M. salmoides S. bainsii* will be outcompeted and driven to localised extirpation as demonstrated in other studies where the presence of non-native fishes resulted in localised extirpation of native fish in the Cape Fold Ecoregion (Van der Walt *et al.* 2016; Chakona *et al.* 2018c; Ellender *et al.* 2018). It is therefore important to protect not only headwater stream, but the middle and lower reaches from continued threats posed by invasive fishes which have been shown to have deleterious impacts on native biota in many parts of its introduced ranges (Shelton *et al*. 2014; van der Walt *et al*. 2016).

CHAPTER 3

The application of stable isotope analysis to evaluate the feeding habits of three divergent lineages of *Sandelia bainsii* (Teleostei: Anabantidae), from rivers of the Eastern Cape Province

Introduction

Evaluating the feeding habits of an animal is important because this aspect is closely related to the ecological niche of the species (Rodriguez-Silva *et al*. 2021). Knowledge on feeding ecology contributes to the broad understanding of aspects such as resource partitioning, habitat preference, prey selection, competition and predation, amongst others (Domingo *et al*. 2016; Yazıcıoğlu *et al*. 2016; Jensen, 2017). Such ecological information is of value when developing conservation strategies, and is therefore a key element in protecting species and ecosystems (Braga *et al*. 2012). Studies on feeding ecology have since shifted from basic descriptions of food consumption to understanding community structure (Jennings, 2002), interspecific relationships such as competition and predation (Aroujo *et al*. 2008), niche overlap (Bellwood *et al*. 2006), food web structure (Zanden and Vadeboncoeur, 2002) and the trophic positions of different species (Stergiou *et al*. 2002). Despite the importance of studying fish feeding ecology, few studies have focused on threatened species, particularly those cited on the IUCN red list. Therefore, there is a knowledge gap on the basic biology of species that are endangered (Braga *et al*. 2011). In addition, studying endangered fish is challenging due to ethical and other practical considerations. This is because most studies on fish diets and general trophic ecology rely on invasive methods, such as examinations of the gut content, which often require euthanasia (Froese and Pauly, 2011). However, recently, several methods have been developed to facilitate research on endangered species, such as non-lethal methods of extraction of gut content (Castro *et al.* 2008), direct observation (Cole 2010), fatty acid analysis and the use of stable isotope analysis (Litz *et al.* 2017).

The application of stable isotope analysis (SIA) in trophic ecology studies is increasing (Boecklen *et al.* 2011). Stable isotope of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are the most applied in ecological studies (Boecklen *et al.* 2011; Perkins *et al.* 2014). Because the $\delta^{13}C$ and $\delta^{15}N$ values of a consumer is related to the ratios in their prey, these markers can be used to reconstruct the resource use of an organism, predator–prey interactions and that of the entire food webs (Post 2000; 2002; McKechnie 2004). This is because the prey items for animals often exhibit characteristic isotopic signatures (Gannes *et al.* 1997). For example, plant-based diet sources are derived from different photosynthetic pathways (e.g. C_3 versus C_4) and will consequently exhibit contrasting $^{13}C/^{12}C$ ratios (Marshal *et al.* 2007; Cernusak *et al.* 2014). Similarly, food sources from marine systems will have different isotopic composition than food sources derived from terrestrial and freshwater sources due to differences in the environmental composition of carbon and nitrogen sources (Smith and Epstein, 1971; Larsen *et al.* 2013). Specifically, these differences have been used to identify the source contribution to an animal's diet consisting of basal sources with varying photosynthetic pathways from different environments (Gannes *et al.* 1997).

Due to $\delta^{13}C$ values being conserved up the food chain, but vary at the base of the food web, the $\delta^{13}C$ of consumers can only provide information about the sources of energy to higher consumers. However, consumers become enriched in $\delta^{15}N$ relative to their food by 3-4‰ (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Cabana and Rasmussen, 1994). Consequently, due to the stepwise trophic enrichment of $\delta^{15}N$ by consumers, $\delta^{15}N$ signatures serve as a time-integrated indicator of trophic position based on pathways and energy flows (Fry 1988). Moreover, macroinvertebrates play a fundamental role in aquatic ecosystems as primary consumers at an intermediate level, thus serving as channels by which bottom-up and top-down forces are transmitted (Wallace *et al.* 1999; Díaz Villanueva 2012). Different food sources of macroinvertebrates include epithilic layer that grows on the surfaces of substrates (consumed by scrapers), the coarse detritus, composed mainly of leaves falling down from riparian vegetation (consumed by shredders), the fine detrituts that is rather deposited on the substrate (consumed by gatherers) or suspeneded in the water column (consumed by filterers), and live animals (consumed by predators) (Uwadiae 2010). Therefore, functional feeding groups are a useful way to categorise prey items in a food web system. Moreover, Cabana and Rasmussen (1996) used primary consumers, rather than primary producers as baseline indicators, because their larger body size and greater longevity result in less seasonality.

The use of stable isotopic composition of consumers and their foods to make inferences about the composition of the animal's diet can be accomplished by the use of stable isotope mixing models (Phillips, 2012; Phillips *et al.* 2014). Stable isotope mixing models convert isotopic data into estimates of food source contributions from the various components of an animal's diet (Fry, 2006; Hopkins and Ferguson, 2012). Mixing models are used to quantify the links between consumers and their dietary sources based on their stable isotopic values and to provide probabilistic estimates of source contribution into dietary mixtures (Jackson *et al.* 2011; Layman, 2012; Kadye *et al.* 2020). The choice in the stable isotope mixing model approach is dependent on multiple factors, such as the consumer species of choice, its food sources, sample size, the familiarity a user has with a particular framework analysis and the desired level of inference (e.g. population-level or individual level) (Hopkins and Ferguson, 2012). A critical aspect when applying isotope mixing models is using diet-tissue discrimination factors (DTDFs) to address the systematic differences in isotope values between food sources and consumer tissues (Kadye *et al.* 2020). Ideally, these DTDFs can be considered as correction factors that are essential in linking sources to consumer isotope signatures in order to provide the correct estimates for their dietary composition. However, the use of appropriate

DTDFs remains a key challenge, and this challenge is critical as DTDFs are a major source of uncertainty in mixing models and stable isotope mixing models (Phillip, 2012; Kadye *et al.* 2020).

Although stable isotope analysis has become a relevant tool in trophic ecology, one of its disadvantage in the study of fishes is that it is often destructive (Hayden *et al.* 2015). This is because muscle tissue is the most commonly used tissue in these studies (Chen *et al.* 2012; Durante *et al.* 2020). However, in most of the cases, obtaining a sufficient sample often requires the animal to be sacrificed (Fredriksen, 2003; Vizzini and Mozzola, 2009; Vander Zanden *et al.* 2015). This has negative ethical implications, especially when studying threatened species (Parris *et al.* 2010). In recent years, studies employing the use of non-lethal sampling has increased (Valladares and Planas, 2012). In fish biology, the use of scales and fin clips provide an alternative non-lethal method to commonly sampled muscle and liver tissue (Jardine *et al.* 2005; Sanderson *et al.* 2009; Busst *et al.* 2015; Hayden *et al.* 2015). However, fish scales are known to be challenging due to their heterogenous structure, which characterises the lifetime of fish (Hutchinson and Trueman, 2006). Alternatively, fin clips are routinely used because their isotopic turnover rate is generally comparable to that of muscle tissue (Jardine *et al.* 2011). For example, Hanisch *et al.* (2009) demonstrated that due to the strong relationships between the isotopic values of fin and muscle tissues, the former could be considered good surrogates for the latter in stable isotope studies of salmonids. Other studies have also reported similar findings (e.g. Willis *et al.* 2013). This is particularly important when studying an endangered species that is known to have fragmented distributions (Sanderson *et al.* 2009).

Sandelia bainsii is endemic to the Eastern Cape Province of South Africa, occurring in short sections of the Great Fish, Kowie, Keiskamma, Igoda, Buffalo and Nahoon river systems (Skelton 2001). It comprises three allopatric lineages (Chakona *et al.* 2020). Chapter 2 focused on the broader trophic ecology patterns of these three lineages. Specifically, the previous chapter explored the food web characteristics of habitats with the different lineages, their isotopic niche patterns and their interspecific interactions. This chapter aims to evaluate whether there are any distinguishable differences in the feeding habits and trophic positions of the three *S. bainsii* lineages through the use of stable isotope analysis. Previous studies based on stomach content analysis have shown *S. bainsii sensu latto* to be a generalist feeder, feeding on insects, crustaceans, fish and molluscs (Mayekiso 1986). However, this traditional method would be inappropriate to use for this threatened species. Therefore, this study was based on the application of stable isotope analysis using fin tissue, which has been considered to be less invasive (Sanderson *et al*. 2009). The three lineages of *S. bainsii* are allopatrically distributed and occur in river systems that have varying food web structures and subsequently exhibiting different trophic ecology patterns (see chapter 2). This study postulates that because this species is known to be a dietary generalist (e.g. Mayekiso 1986), these lineages likely have different dietary composition and as a result would have variable trophic positioning in the different river systems. Thus, the objectives of this study were to (1) estimate the trophic positions of the different *S. bainsii* lineages and compare with other co-occurring species using stable isotopes and (2) assess if there is a difference in source contribution to dietary mixtures of the *S. bainsii* lineages using stable isotope mixing models.

Materials and methods

Data collection, stable isotope processing and analysis

Methods of data collection, stable isotope processing and analysis of data are from the same sites as described in Chapter 2.

Data analysis

To estimate the trophic positions of the three *S. bainsii* lineages and other co-occurring fish species, the ratios of stable isotope nitrogen ($\delta^{15}N$) were used because $\delta^{15}N$ of a consumer is typically enriched by 3-4 ‰ relative to its diet. Trophic position was calculated using the following formula: $\lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}})/\Delta n$, where λ was the trophic position of the organism used to estimate $\delta^{15}N_{\text{base}}$ (e.g. $\lambda = 1$ in primary producers), and $\delta^{15}N_{\text{secondary consumer}}$ was the stable isotope value of any higher consumer measured directly. It is important to note that some studies use unionid mussels as a baseline indicator for estimating trophic positions of fish, thereby correcting the among system differences in $\delta^{15}N_{\text{base}}$ (Cabana and Rasmussen 1996; Vander Zanden *et al.* 1997). However, because unionid mussels were not present in the sampled systems, this study used the taxa Baetidae from the order Ephemeroptera as an alternative baseline. This is because it feeds on fine detritus from submerged rocks, woods and macrophytes which form the base of the food webs. However, in systems where this taxon was absent, such as the Buffalo River the taxon *Caenis* was used as a baseline, and the taxon *Acanthiops* from the order Ephemeroptera in was used in the Lushington River. Finally, the Δn was the enrichment in $\delta^{15}N$ per trophic level, which is generally assumed to be between 3‰ and 4‰ (Peterson & Fry, 1987).

A Bayesian mixing model, using the R package MixSIAR (Stock *et al.* 2018), was used to determine the proportion of source contribution to the diets of the different *S. bainsii* lineages. The sources were different aquatic invertebrate groups, and they were categorised into functional feeding groups following Palmer *et al.* (1993a; b; 1996) and Kambikambi *et al.* (2019) (see Table 3.1). The groups were as follows: brushers (use setae to remove loosely deposited and lightly attached organic matter), gatherers (use structures other than setae to remove loosely deposited and lightly accreted organic material from surfaces), filterers (suspension feeders of FPOM and detrital matter), omnivores (feed on a wide prey range, in this case *Potamonautes*), and predator 1 (feed on small insects using their hinged lower jaw to impale their prey), predator 2 (insert their stylets into their selected prey), predator 3 (piercing sucking form of predation) (see table 3.1). As a result of the predators having different feeding strategies, they were categorised as predator 1, 2 and 3. Predator 1 were the order Odonota, the order Hemiptera were grouped under predator 2 and the order Diptera grouped under predator 3. The diet-tissue discrimination factors (DTDF) were derived from literature following Post (2002). These were 1‰ for carbon and 3‰ for nitrogen. The mixing model was based on Monte Carlo Markov Chain (MCMC) simulation method, with 10 000 iterations, and model convergence was evaluated using the Gelman-Rubin diagnotics whereby the potential scale reduction factors were approximately 1 (Gelman and Rubin, 1992). Subsequently, the simulation process was developed to produce a posteriori densities of the potential source contributions. The source contributions were expressed using means and 95% credibility intervals (CI). All these analyses were performed using the R statistical program (R Core Team 2021).

Table 3 1. Mean values of carbon δ^{13}C ‰) and nitrogen (δ15N ‰) stable isotopes (±standard deviation) for macroinvertebrates and feeding functional groups (FFG) collected from the Lushington, Fairburn, Kat rivers of the Great Fish River and Tyume River sites 1 and 2 (Tyume 1 and Tyume 2) of the Keiskamma River as well as the Buffalo River systems.

		Lushington		Fairburn		Kat mainstem		Tyume 1		Tyume 2		Buffalo		FFG
Order	Taxa	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	
Ephemeroptera	Baetidae	-	-	-25.97±0.92	5.27±0.14	-21.48±0.00	11.81±0.00	-24.84±0.21	11.89±0.04	-20.22±0.44	7.36±0.04	-	-	Gatherer
	Caenis	-	-	-	-	-	-	-23.28±0.10	10.20±0.09	-	-	-28.05±0.23	7.43±0.73	Gatherer
	Acanthiops	-25.65±2.54	6.35±1.43	-	-	-	-	-	-	-	-	-	-	Gatherer
	Castanophlebid	-24.43±0.37	6.94±0.78	-	-	-	-	-	-	-	-	-	-	Gatherer
	Dicentroptelum	-24.46±0.12	6.11±0.00	-	-	-	-	-	-	-	-	-	-	Gatherer
	Leptophlebiidae	-26.64±0.00	7.16±0.00	-25.55±0.00	7.27±0.00	-24.63±0.20	12.18±0.49	-25.08±0.00	11.76±0.00	-21.27±0.00	6.84±0.00	-30.95±0.00	8.54±0.00	Brusher
	Tricorythus	-	-	-21.50±0.36	5.20±0.22	-23.60±0.03	9.69±0.26	-24.74±0.00	7.13±0.00	-20.74±0.11	6.97±0.12	-	-	Gatherer
	Afronurus		-	-28.43±0.00	5.14±0.00	-	-	-	-	-24.74±0.00	7.13±0.00	-	-	Scraper
	Heptageniidae	-25.40±0.00	8.61±0.00											Scraper
Trichoptera	*Cheumatopsyche*	-24.26±0.00	7.35±0.00	-18.13±0.00	6.08±0.00	-22.08±0.08	11.94±0.10	-	-	-		-	-	Gatherer
	Hydropsyche	-	-	-	-	-21.79±0.01	11.67±0.22	-23.81±0.18	13.81±0.14	-19.24±0.11	9.17±0.03	-	-	Filterer
Odonata	*Aeshna*	-	-	-24.27±0.00	6.51±0.00	-	-	-	-	-	-	-	-	Predator 1
	Anax	-	-	-23.00±0.00	6.58±0.00	-	-	-	-	-	-	-		Predator 1
	Lestes	-	-	-22.48±0.00	6.66±0.00	-	-	-	-	-18.82±0.00	8.59±0.03	-27.39±0.17	8.95±0.65	Predator 1
	Microgomphus	-	-	-	-	-	-	-	-	-17.99±0.08	8.96±0.07	-	-	Predator 1
Coleoptera	Scirtidae	-24.62±0.00	6.97±0.00	-	-	-	-	-	-	-	-	-	-	Gatherer
	Psephenidae	-	-	-25.00±0.32	3.38±0.13	-22.07±0.00	7.50±0.00	-	-	-	-	-	-	Scraper
Diptera	Diptera	-24.47±0.00	8.75±0.00	-	-	-	-	-	-	-	-	-	-	Gatherer

	Tipula	-23.04±0.00	0.21±0.00	-	-	-	-	-	-	-20.39±0.00	10.31±0.03	-	-	Predator 3
	Simulium	-	-	-21.72±0.00	4.80±0.07	-22.07±0.00	7.50±0.00	-	-	-20.91±0.01	7.10±0.13	-26.95±0.00	8.12±0.00	Filterer
	Athericidae	-	-	-	-	-21.48±0.00	11.82±0.00	-24.55±0.18	12.70±0.36	-	-	-31.34±0.00	8.44±0.00	Predator 3
Hemiptera	Notonectidae	-	-	-	-	-21.37±0.25	9.21±0.15	-	-	-	-		-	Predator 2
	Appasus	-	-	-	-	-	-	-	-	-	-	-25.69±0.00	9.74±0.00	Predator 2
	Plea	-	-	-	-	-	-	-	-	-	-	-24.63±0.37	8.85±1.01	Predator 2
Decapoda	*Potamonautes*	-20.44±0.00	7.12±0.00	-	-	-19.07±0.29	9.43±0.22	-20.98±0.09	10.97±0.07	-16.68±0.58	7.96±0.25	-	-	Omnivore

[2] The functional feeding groups of invertebrates were based Palmer *et al*. (1993a; b; 1996) and Kambikambi *et al*. (2019).

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[2]

Results

Trophic positions of Sandelia bainsii lineages and its co-occurring species

In general, the three *S. bainsi*i lineages had the highest trophic positions across all river systems, except in the Buffalo River (Figure 3.1). This indicated that this species was generally a top predator. Within the Great Fish River, in the headwater streams where *Sandelia ‘bainsii* Kowie’ co-occurred with *Enteromius mandelai* revealed that the former occupied the highest trophic position in both the Fairburn (TP = 3.40) and Lushington River (TP = 3.36) compared to the latter, which occupied a lower trophic position in both the Fairburn (TP = 3.19) and Lushington River (TP = 3.06). Similarly, in the Kat River, *S. ‘bainsii* Kowie’ occupied a higher trophic position (TP = 3.07), relative to other co-occurring fishes *E. mandelai* (TP = 2.81) and *Glossogobius callidus* (TP = 3.01) which occupied lower trophic positions. A similar pattern was observed in the Keiskamma River where *S. ‘bainsii* Keiskamma’ co-occurred with *Amatolacypris trevelyani* in the Tyume River site 2. At this site, *S. ‘bainsii* Keiskamma’ occupied a higher trophic position (TP = 3.19) than *A. trevelyani* (TP = 3.12). In contrast to other sites, within the Buffalo River, *S. ‘bainsii* Buffalo’ occupied the lowest trophic position (TP = 2.52), with *E. mandelai* being the top predator (TP = 3.40) followed by *A. trevelyani* (TP = 3.32) (Figure 3.1).

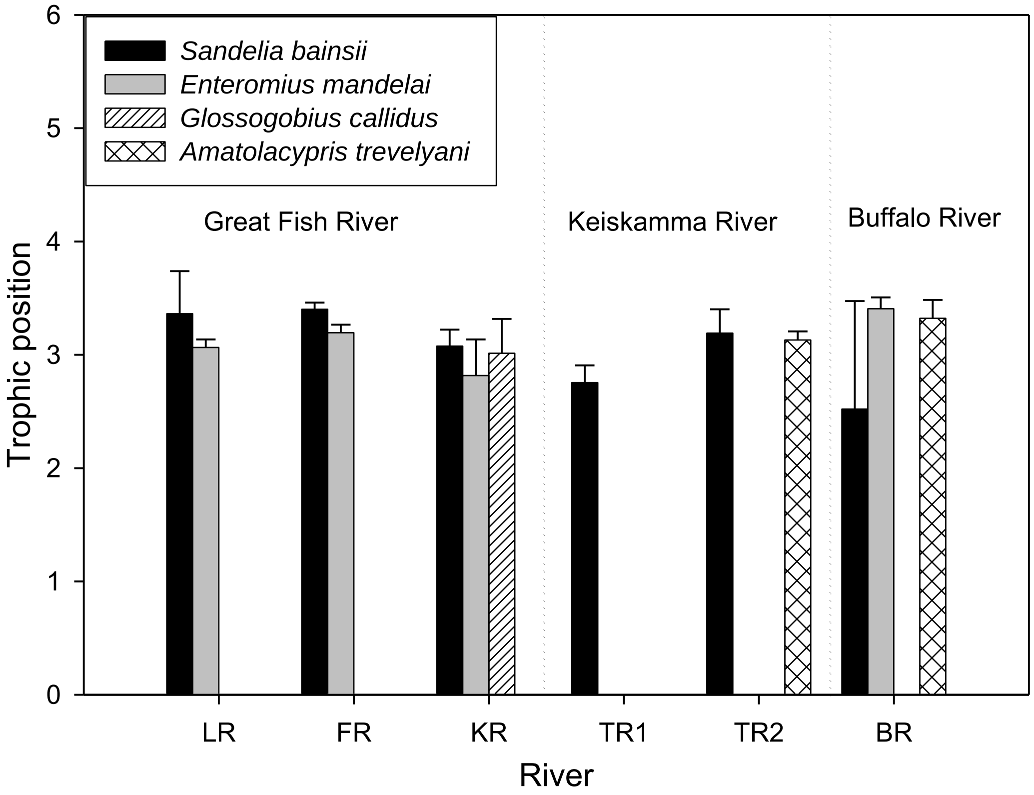

Figure 3.1. The average trophic position of *Sandelia bainsii* lineages, *Enteromius mandelai*, *Glossogobius callidus* and *Amatolacypris trevelyani* in the Lushington River (LR), Fairburn River (FR) and Kat River (KR) within the Great Fish River system, the Tyume River the site 1 (TR1) and Tyume River site 2 (TR2) within the Keiskamma River system and Buffalo River system (BR).

Source contribution to dietary mixtures of the Sandelia bainsii lineages

The application of isotope mixing models showed that the contributions of respective diet sources within the *S. bainsii* lineages were variable. Within the Great Fish River system, the Lushington River population of *S.* '*bainsii* Kowie' showed that brushers (mean = 28.1% %, 95% CR = 0.0-65.4%), gatherers (mean = 42.3 %, 95% CR 95% CR = 0.0-100%), and omnivores (mean = 21.1 %, 95% CR = 0.0-42.6%) as most important sources than predator 3 group (order Diptera) (mean= 2.9 %, 95% CR= 0.0-15.7%) and scrapers (mean = 5.6 %, 95%

CR = 0.0-30.1%) (Figure 3.2). In contrast, in the Fairburn River, *S. 'bainsii* Kowie' was characterised by higher source contributions of only gatherers (mean = 91.8 %, 95% CR = 60.6-100%) to their diet than any other sources (Figure 3.3). By comparison, in the Kat River *S.* '*bainsii* Kowie' was characterised by the relatively high importance of scrapers (mean = 24.3%, 95% CR = 0.0-45.9%) and omnivores (mean = 22.1%, 95% CR = 0.0-38.6%) as most important sources compared to brushers (mean = 0.9%, 95% CR = 0.0-22.1%), filterers (mean = 0.8 %, 95% CR = 0.0-27.4%), gatherers (mean = 0.8 %, 95% CR = 0.0-27.0%), predator 2 (mean = 14.0 %, 95% CR = 0.0-48.4%) and predator 3 group (order Diptera) (mean = 13.5%, 95% CR = 0.0-100%) (Figure 3.4).

Within the Keiskamma River, at Tyume River site 1, the mixing model revealed that gatherers were the most important diet source (mean = 53.2%, 95% CR = 0.4-100%), whereas brushers (mean = 11.9 %, 95%, CR= 0.0-45.5%), filterers (mean= 10.0%, 95%, CR=0.0-36.6%), omnivores (mean= 15.0%, 95%, CR=0.0-37.2%) and predator 3 (mean= 9.9%, 95%, CR=0.0-38.5%) showed lower contribution to the diet of *S. 'bainsii* Keiskamma' (Figure 3.5). By comparison, at the Tyume River site 2, *S. 'bainsii* Keiskamma' had a relatively higher source contribution of gatherers (mean = 44.8%, 95% CR=0.0-99.5%) and filterers (mean = 33.2%, 95% CR = 0.0-99.0%), compared to brushers (mean= 10.9%, 95% CR = 0.0-63.2%), omnivores (mean = 3.0%, 95% CR = 0.0-14.9%), predator 1 (mean = 2.2%, 95% CR = 0.0-11.7%) and predator 3 (mean = 3.6%, 95% CR = 0.0-14.8%) (Figure 3.6). Similarly, *Sandelia* '*bainsii* Buffalo' from the Buffalo River system had high source contributions of gatherers (mean = 80.4%, 95% CR = 0.0-100%) than any other sources. Moreover, predator 2 (mean = 16.6%, 95% CR = 0.0-69.2%) was also an important source in the diet of this lineage (Figure 3.7).

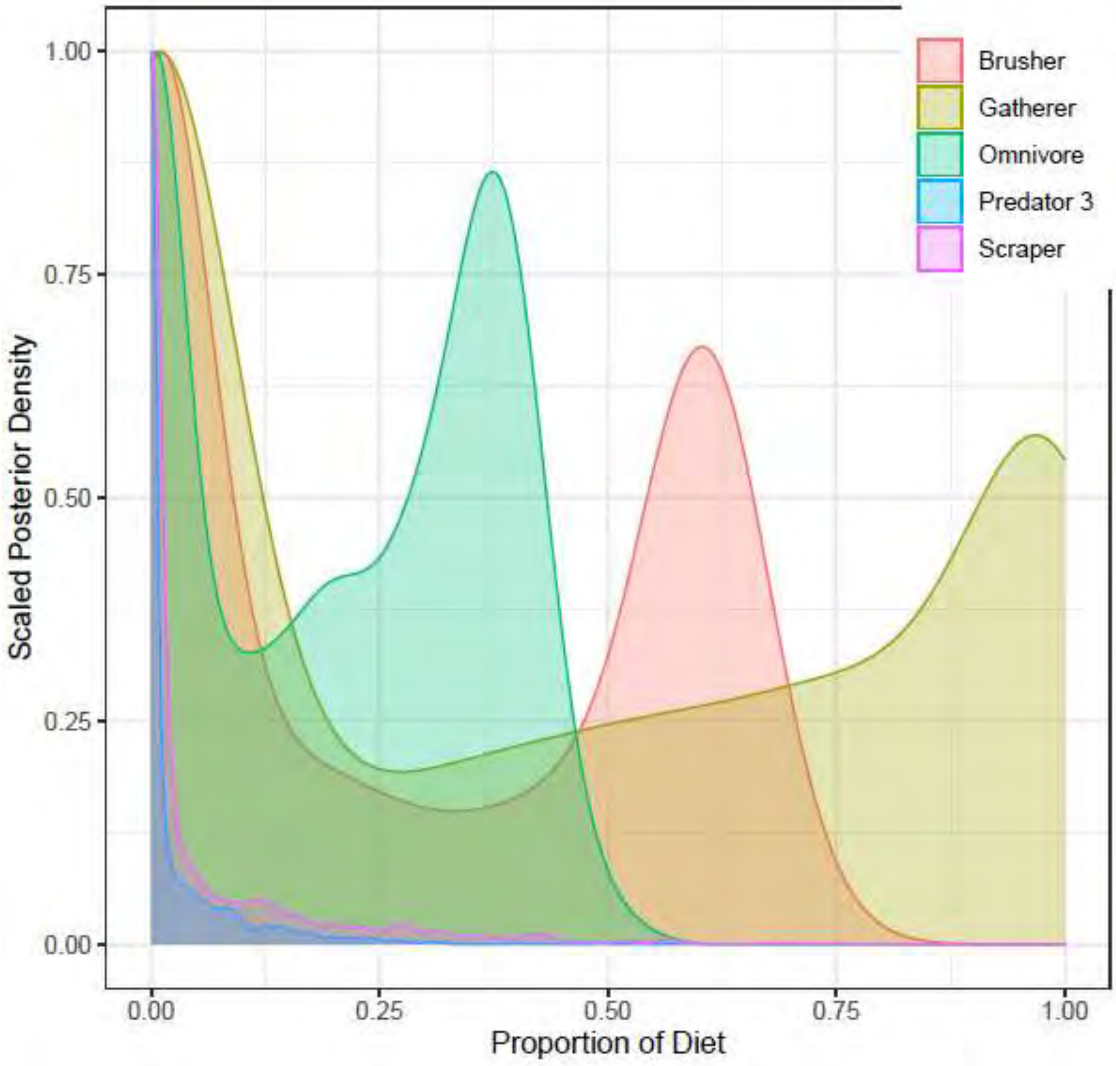

Figure 3.2. MixSIAR estimated source contributions for consumer group, *Sandelia 'bainsii* Kowie' showing Bayesian credibility intervals and posterior densities of sources from the Lushington River system.

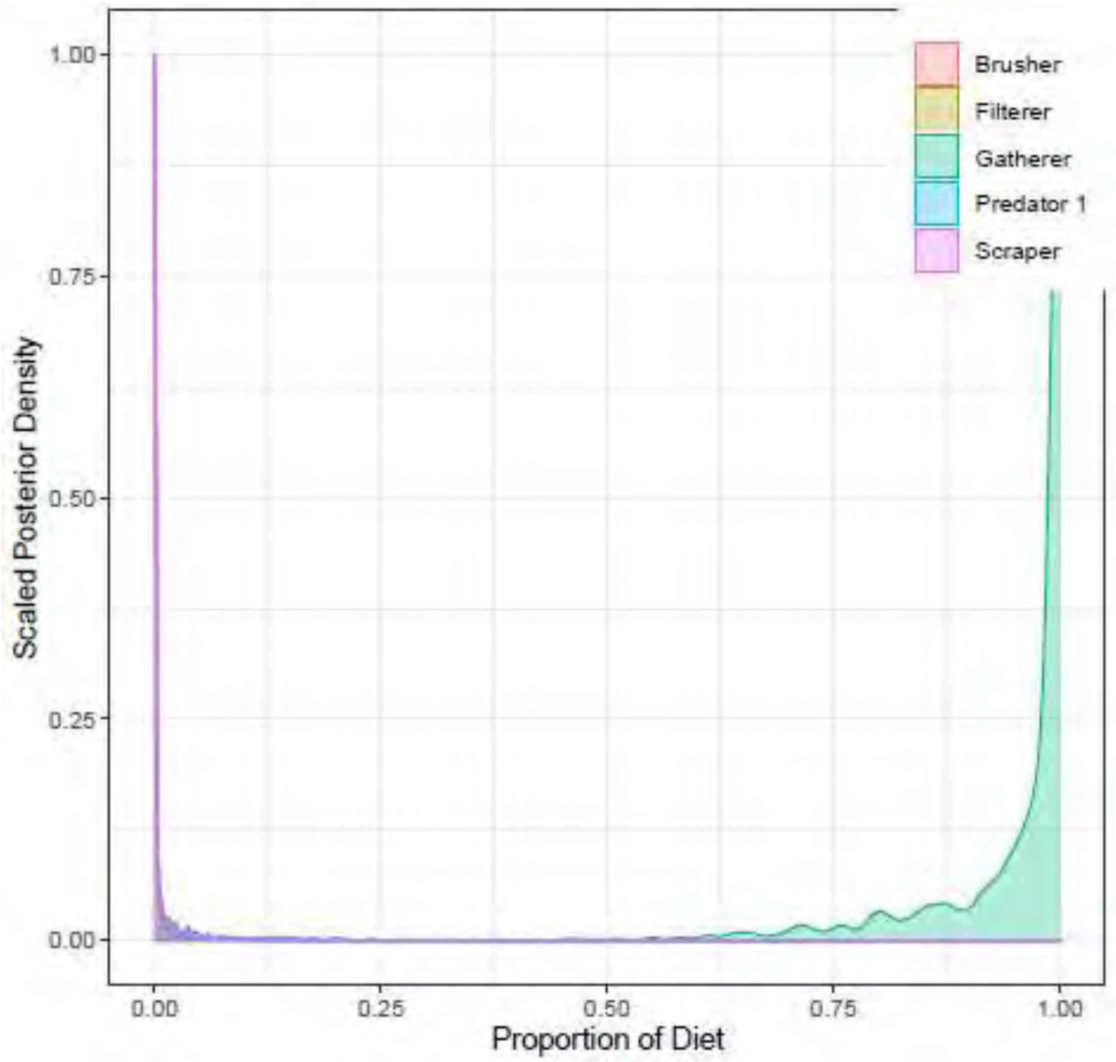

Figure 3.3. MixSIAR estimated source contributions for consumer group, *Sandelia 'bainsii* Kowie' showing Bayesian credibility intervals and posterior densities of sources from the Fairburn River.

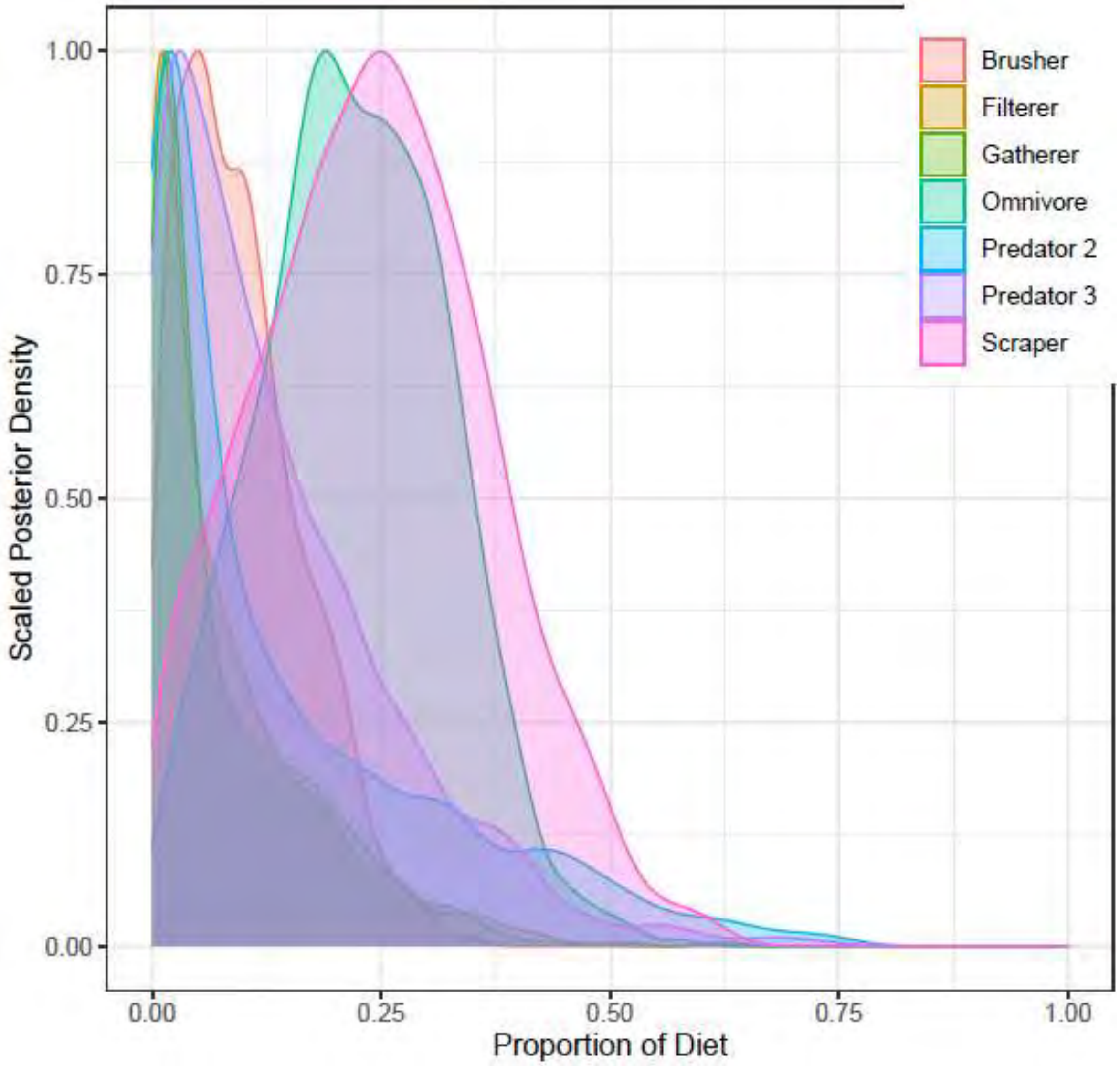

Figure 3.4. MixSIAR estimated source contributions for consumer group, *Sandela 'bainsii* Kowie' showing Bayesian credibility intervals and posterior densities of sources from the Kat main River.

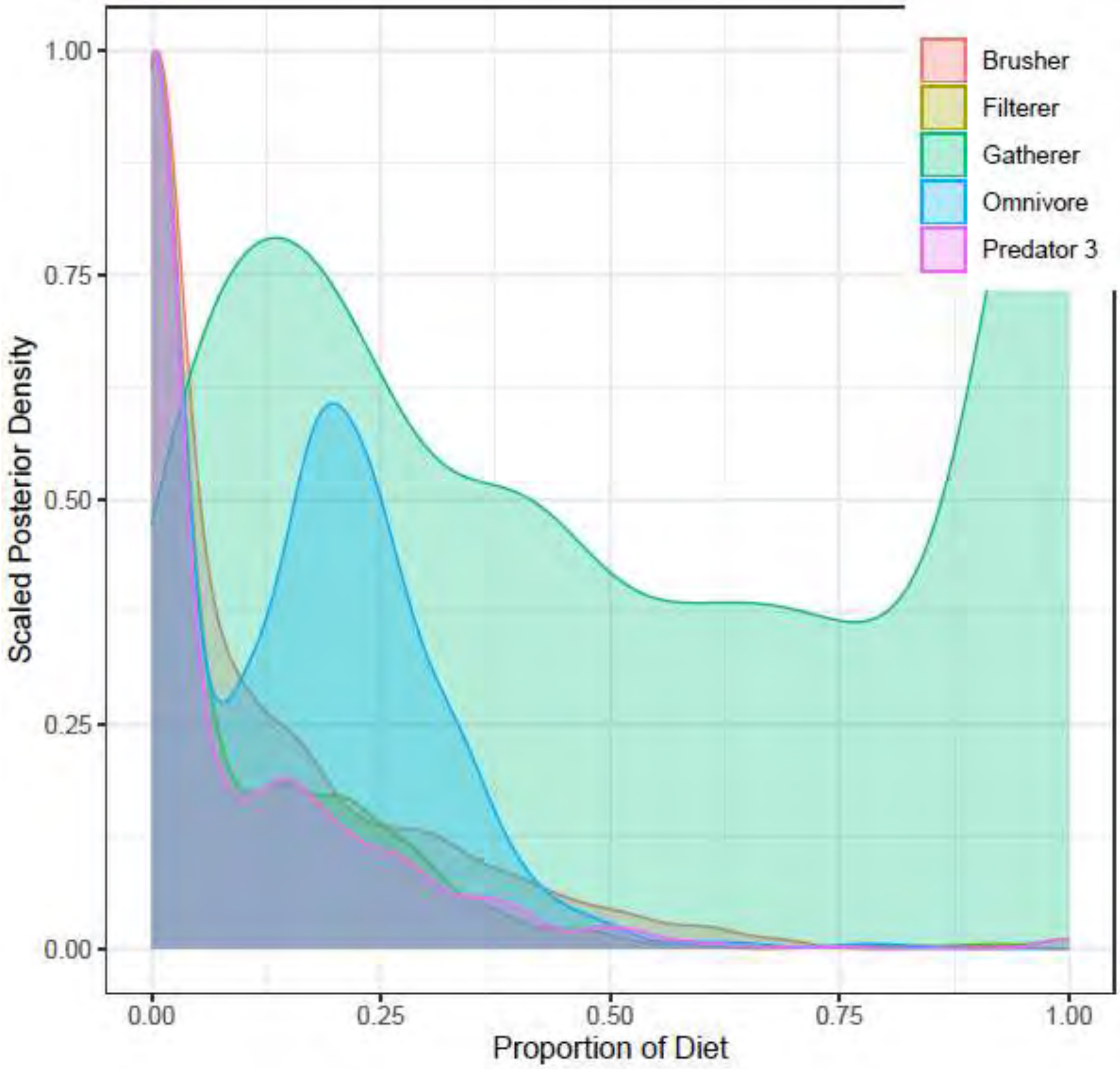

Figure 3.5. MixSIAR estimated source contributions for consumer group, *Sandelia ‘bainsii* Keiskamma’ showing Bayesian credibility intervals and posterior densities of sources from Tyume River site 1.

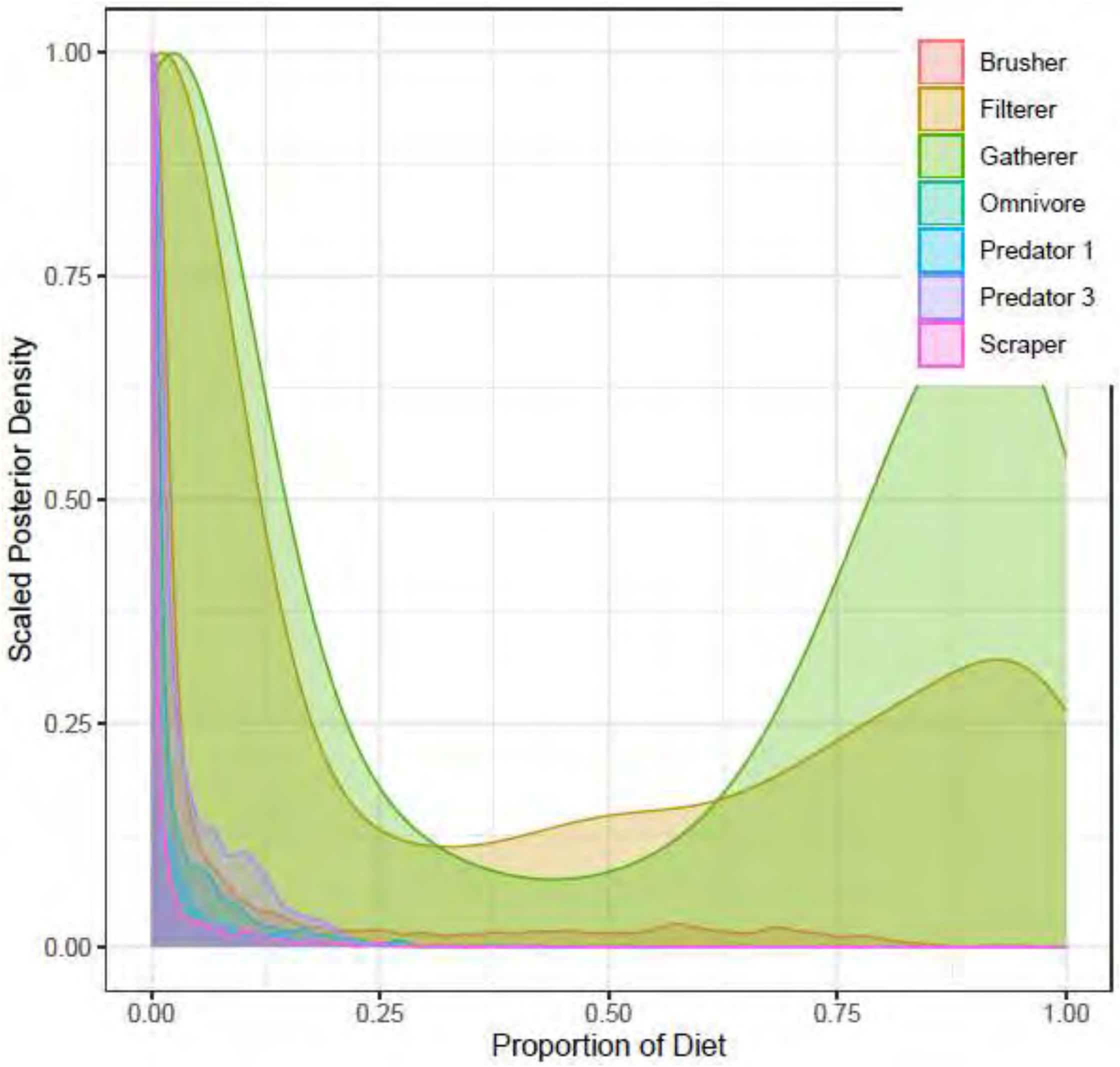

Figure 3.6. MixSIAR estimated source contributions for consumer group, Sandelia 'bainsii Keiskamma' showing Bayesian credibility intervals and posterior densities of sources from Tyume River site 2.

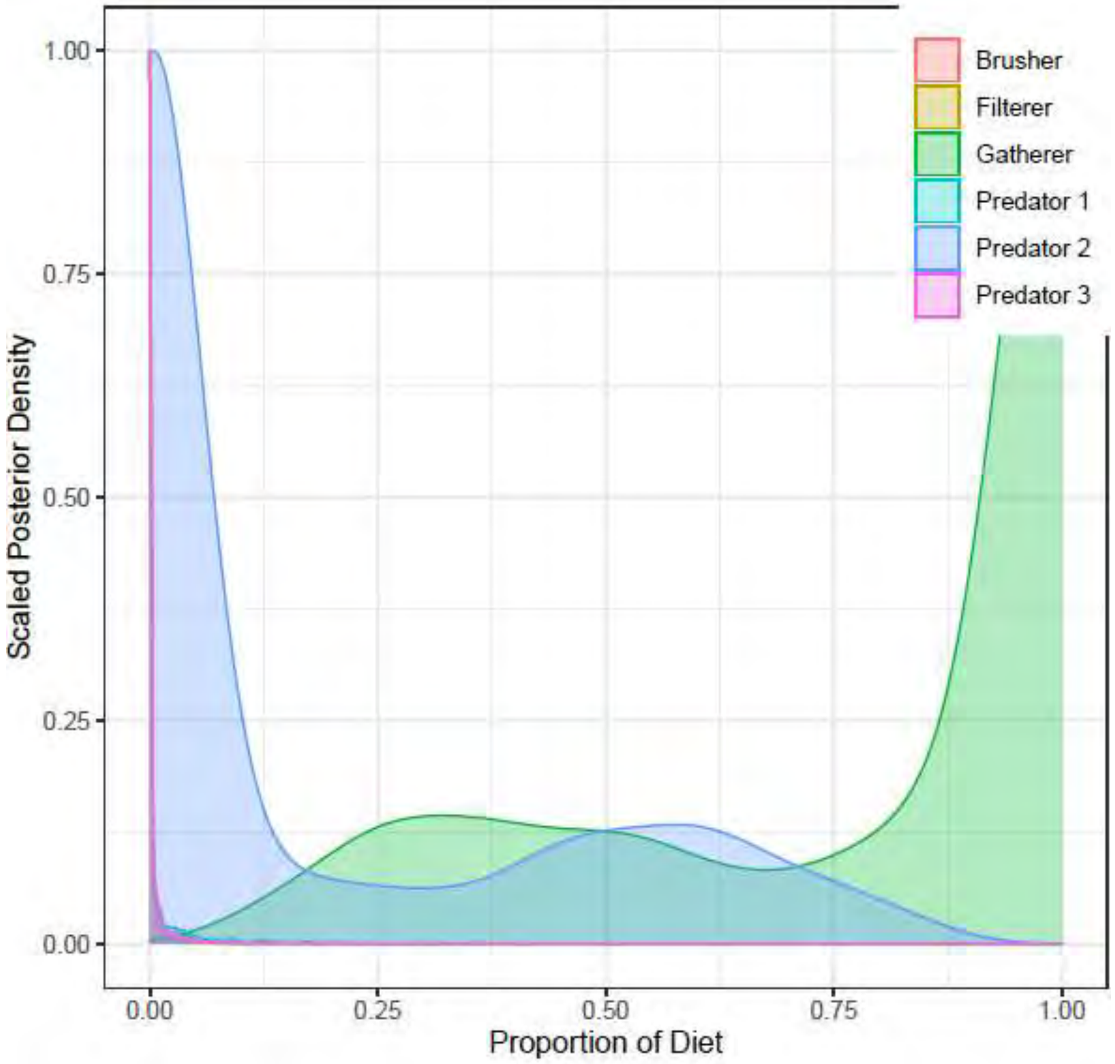

Figure 3.7. MixSIAR estimated source contributions for consumer group, Sandelia ‘bainsii Buffalo’ showing Bayesian credibility intervals and posterior densities of sources from the Buffalo River.

Discussion

The primary objectives of this study were to examine the feeding habits and trophic positions of the three *S. bainsii* lineages through the use of stable isotope analysis. This study showed that the three *S. bainsii* lineages had higher trophic positions than other co-occurring species at most sites, except in the Buffalo River. This suggest that the different lineages were generally top predators in the different river systems. Although *S. 'bainsii* Buffalo' had a lower trophic position compared to other co-occurring species, its trophic position was generally characterised by high uncertainty, possibly due to the use of the taxa *Caenis* as a basal taxon in the Buffalo system. Vander Zanden and Rasmussen (1999) showed it is important to note that disparate baseline values may lead to two populations that may exhibit the same $\delta^{15}N$ values to occupy different trophic positions. The lower trophic position could also indicate that the Buffalo lineage was likely influenced by the occurrence of diet sources that had highly variable stable isotope values. Findings from stable isotope mixing models revealed that the diet sources varied from the dominance of either single diet source, such as in the Fairburn River and Tyume River site 1, or two prey sources, such as in the Tyume River site 2 and Buffalo River, to the importance of multiple prey sources, such as in the Lushington and Kat rivers. This suggest that despite being a top predator at most sites, the diet sources for the different lineages were highly variable, i.e. exhibiting a typical generalist feeding pattern (Wang *et al*. 2019b)

In general, the results from this study were consistent with Mayekiso (1986) who found *Sandelia bainsii sensu latto* to be an opportunistic predator whose diet varied according to food availability. Mayekiso (1986) also showed that *S. bainsii* did not have preferred prey but exploited abundant and available food sources. This could be attributed to *Sandelia bainsii* still retaining its ancestral predatory feeding mode from the family Anabantidae (Mayekiso 1986). In the past, the diet and feeding habits of *S. bainsii* were reported to be similar to those found in other anabantid groups (Siegfried 1963; Bruton 1979; Singh and Samuel, 1981). Mayekiso and Hecht (1990) also revealed that there was a greater overlap in the diet of *Sandelia bainsii* and predators such as, *Oncorhychus mykiss* and *Micropterus salmoides* in the Tyume River, compared to the indigenous lineages which could have facilitated co-existence of the *Sandelia bainsii* lineages with other natives. A study by Shelton *et al*. (2018a) showed that the closely related *Sandelia capensis* consumed vertebrate prey (fish and amphibians) and was an opportunistic predator on vertebrates and invertebrates, which could somewhat explain the elevated trophic position of *S. capensis* relative to two other species. Similar results were found by Kadye *et al*. (2016) who revealed that this species is able to co-exist with other natives due to them occupying different trophic niches. Additionally, Shelton *et al*. (2018a) revealed that dietary differences of *S. capensis* were not consistent among sites and were probably influenced by site-specific factors such as resource availability, which could be used to explain the variability in the proportion of source contribution to the diets of *Sandelia bainsii* lineages.

Studies on other streams also revealed that generalist feeding techniques are closely related to food web dynamic of a stream (López van Oosterom *et al*. 2013; Melo *et al*. 2014; Choi *et al*. 2020). The variation in the isotopic niches of *S. bainsii* lineages coincided with the variation in the isotopic composition of the individual communities (see chapter 2). This further supports that the patterns observed in the feeding ecology of *S. bainsii* are likely influenced by resource availability rather than morphological differences that may have developed due to allopatric speciation, as Chakona *et al*. (2020) mentioned that these lineages could have experienced a recent range expansion. However, studies that look into the morphological aspect of these lineages are currently ongoing to determine whether they constitute separate entities (Chakona *et al*. 2020). Furthermore, the feeding morphology of these *Sandelia bainsii* lineages is an aspect worth exploring going forward, as it could offer complementary insights into the functional ecology of the lineages explored in this study.

To conclude, the results demonstrated that the *Sandelia bainsii* lineages occupied high trophic positions in their respective systems with the exception of the Buffalo lineage. These findings could be reflective of the opportunistic feeding habits and behavior exhibited by these lineages. Through the application of isotope mixing models, it was revealed that the proportion source contribution to the diets of these lineages varied across river systems. It is important to note that Bayesian isotopic mixing models should be interpreted with caution due to the uncertainty in the mean and variance of sources (Ward *et al*. 2010). In systems where there is source isotopic signatures that overlap, this prevents source discrimination. Moreover, some consumers may feed on a diversity of prey that stable isotope mixing models may only provide a blurry picture of (Phillips *et al*. 2014). However, the results from the model support previous studies which considered *Sandelia bainsii* to be a generalist feeder (Mayekiso 1986; 1990). Therefore, this study further suggests that the differences in source contribution in the diets of *S. bainsii* are likely due to the variability in the resource availability in their respective environments as seen in Chapter 2 of the thesis. Although these lineages are allopatrically distributed, they exhibit similar trophic positions as well as feeding patterns, suggesting that they share similar ecological functions. As mentioned by Wiens and Graham (2005), that from an ecological perspective, when closely related species occur in allopatry, they may exhibit similar habits in their distribution and resource utilisation patterns. Although the *Sandelia bainsii* lineages exhibit different habits in terms of food web characteristics (Chapter 2), they share similar resource utilisation patterns by being generalist predators.

From an ecological perspective, understanding the feeding patterns and trophic positions of the three *Sandelia bainsii* lineages are important with informing conservation strategies and policies in dealing with this narrowly distributed species that is threatened by habitat fragmentation and invasion of piscivorous fish. In the case of dietary competition with non-native species such as sharptooth catfish *Clarias gariepinus,* largemouth bass *Micropterus* *salmoides* and smallmouth bass *Micropterus dolomieu,* conservation measures should target restricting population of the invasives from further invasion into habitats where these native persist. In the case where no such competition exists, other measures should become investigated such as habitat degradation and deterioration in water quality due to agricultural activities as potential reasons for decline of this species.

CHAPTER 4

General discussion

Knowledge of the trophic and feeding ecology of *Sandelia bainsii* is not well documented and is only limited to a few studies (Mayekiso 1986; 1990). This therefore limits the ability to draw effective conservation strategies, which is necessary because this species is considered endangered by the IUCN Red List of endangered species. A recent study by Chakona *et al.* 2020 found three divergent lineages within this species, raising further concern as the populations of evolutionary significant units might be smaller than previously thought. This study therefore evaluated the trophic ecology and feeding patterns of the three allopatrically distributed *Sandelia bainsii* lineages using stable isotope analysis in order to increase ecological data on these lineages.

Stable isotope analysis (SIA) in trophic ecology studies provides insight on resource partitioning among species assemblages, evaluation of intra- and interspecific resource use as well as reconstructing food web patterns amongst other things (Crawford *et al.* 2008; Boecklen *et al.* 2011; Golikov *et al.* 2020). The key research question for the first data chapter was, given that these newly discovered lineages are allopatrically distributed, how different are the river systems they occupy in terms of food web patterns, and from a trophic ecology perspective, are there any variations in the isotopic niche patterns and interspecific interactions of these lineages with other co-occurring species?. Based on the results, the variable isotopic niche sizes exhibited by these lineages resulted from varied food web isotopic compositions of the rivers they occur in. Wang *et al.* (2019), showed that variations in stable isotope composition in food webs plays a vital role in trophic niche sizes exhibited by species, as well as their trophic niche overlap patterns. Moreover, there were no discernible interspecific interactions in the different habitats of *S. bainsii* and other co-occurring fish. Trophic niche differentiation appeared to be one of the major drivers of low interspecific interaction in the different habitats, as there was generally low interspecific trophic overlap of *S. bainsii* onto other species. Resource competition is thought to play a major role in driving evolutionary diversification, for instance, coexisting species evolve to use different resources, reducing the effects of interspecific competition (Svanbäck *et al.* 2007). Individuals may mitigate competition by switching to use alternative resources not used by competitors. These finding were in line with studies looking into the feeding habits of *Sandelia bainsii* using stomach content analysis (Mayekiso, 1986; Mayekiso and Hecht, 1990) as well as studies looking at the closely related *Sandelia capensis* using stable isotope analysis from other streams (Kadye *et al.* 2019), which revealed that resource partitioning facilitates co-existence amongst species.

From the results of this study it is evident that the isotopic niche patterns of the three *Sandelia bainsii* lineages are influenced by food web patterns in their respective river systems and that the co-existence of *S. bainsii* with other natives is facilitated by trophic niche partitioning which is revealed through the interspecific interactions of these lineages in their habitats. This is supported by other studies that show that food web dynamics influence the trophic niche patterns of species (Trans 2015), and trophic niche partitioning facilitates co-existence among native fish occurring in the same habitats (Neves *et al.* 2018). *Sandelia bainsii* lineages are not rigorous competitors when it comes to resource utilisation and as a result are generalist feeders. Therefore in systems where it occurs with invasive fish it is outcompeted and driven to localised extirpation leading to conservation concerns around the viability of the population sizes of this species.

The ratios of stable isotope nitrogen ($\delta^{15}N$) can be used estimate the trophic position of fish species, and the stable isotope mixing models assists in using stable isotopic composition of consumer and their foods to make inferences about the composition of an animals diet (Post, 2002; Philips, 2012). The research question for the second data chapter, was to probe which sources contributed to the diet composition of *Sandelia bainsii* lineages. The findings in this study suggested that the *Sandelia bainsii* lineages were top predators because of the high trophic positions they occupy in most sites, except for the Buffalo River, where its trophic position was generally characterised by high uncertainty. Furthermore, *S. bainsii* lineages where characterized by variable source contribution to their diets which reflected a generalist feeding behavior. These findings are in line with some studies (Thomas *et al*. 2017; Laske *et al*. 2018), which revealed that generalist feeding strategies are favored in stressful and highly variable environments. In general, the results from this study were consistent with Mayekiso (1986) who found *Sandelia bainsii sensu latto* to be an opportunistic predator whose diet varied according to food availability. Shelton *et al*. (2018a) also revealed that dietary differences amongst species were not consistent between sites and were probably influenced by site-specific factors such as resource availability, which could be used to explain the variability in the proportion of source contribution to the diets of *Sandelia bainsii* lineages. The use of the functional feeding groups (FFG's) to categorise the available resources, revealed the high taxonomic patchiness of the prey community resulting in extremely variable diet profiles which the Bayesian model attempted to define. This showed the rivers to be extremely variable from a food web structure perspective as well as taxonomic perspective of the FFG's.

In conclusion, from the results it can be inferred that the variation in the isotopic niches, trophic interrelationships, and source contribution in the diets of these lineages could be attributed to factors such as spatial differences in trophic resources and interactions of *Sandelia bainsii* with other co-occurring fishes. The patterns observed in this study do not appear to be attributed to species divergence possibly caused by allopatric speciation, but rather variability in the food web and taxonomic perspective of the river systems and interspecific relationships. This study suggests that the different *Sandelia bainsii* lineages are not highly competitive predators, and have adapted by being general feeders to avoid possible competition from other predators. Therefore, it can be assumed that when occurring in systems with top predators such as *C. gariepinus* and *M. salmoides*, *S. bainsii* will be outcompeted and driven to localised extirpation. Therefore, therefore important to protect streams where these lineages occur due to the continued threats posed by invasive fishes which have been shown to have deleterious impacts on native biota in many parts of its introduced ranges (Shelton *et al*. 2014; van der Walt *et al*. 2016). Moreover, findings from this study are important in possibly assisting to implement effective conservation strategies and policies which are dedicated to dealing with narrowly distributed species that are threatened by habitat fragmentation and invasion of piscivorous fishes.

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