

**Distribution, habitat associations and conservation
of the Eastern Cape rocky *Sandelia bainsii*
(Perciform: Anabantidae) in the Great Fish River
system**

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ABSTRACT

In South Africa, freshwater ecosystems are under pressure due to increasing anthropogenic factors degrading river systems. The Cape Fold, the lower Southern Temperate Highveld and the Amatolo – Winterberg Highlands freshwater ecoregions contain some of the heavily impacted river systems in the country. One such river system is the Great Fish River system, which has been affected by anthropogenic modification (hydrological modification, water pollution and invasion by non-native fish species). These modifications have raised conservation concerns on an imperilled anabantid fish, the Eastern Cape rocky *Sandelia bainsii* that is confined to the Kat and Koonap rivers, which are tributaries of the Great Fish River. Historically, the species was reportedly common and abundant, with a range spanning across seven river systems (Kowie, Great Fish, Keiskamma, Igoda, Gxulu, Buffalo and Nahoon rivers) in the Eastern Cape Province. However, surveys have indicated that the species has experienced decline in population size and distribution range, with localised extirpations being reported. This resulted in its listing on the IUCN Red List of threatened species as Endangered. The proposed shale gas exploration and potential infrastructure development in the Karoo basin, which encompasses the headwaters of the Kat River, represent a potential future threat to the remnant populations of this species. The aim of the present study was to review the past and present distribution range of *S. bainsii*, determine the habitat associations and assess the conservation of *S. bainsii* in the Great Fish River system. This information is essential for informing conservation and management decisions for this species in the Great Fish River system.

This study undertook a comprehensive survey of the Kat and the Koonap rivers in 2017, and augmented this information with data from more recent surveys that were conducted from 2009 - 2014 to map the distributions freshwater fishes in the Great Fish River system. Data from these recent surveys (2009 – 2017) were compared with historical records (1961 – 2005) obtained from the South African Institute for Aquatic Biodiversity to evaluate changes in distribution patterns of *S. bainsii*. Historical records indicated that *S. bainsii* occurred at 11 localities in the Kat River and three localities in the Koonap River. Data from recent surveys indicated that the species has persisted at these historical localities, except one locality in the Koonap River where no individuals of *S. bainsii* were captured, despite the use of multiple sampling approaches and gears. Although the species still persists at two of the three historical localities in the Koonap River, the population sizes have considerably

declined as the species was reportedly in high abundance at these localities in the 1980s. Factors threatening the continued existence of this species in the Koonap River likely include non-native species, extreme drought conditions and the associated excessive abstraction of water which has resulted in the drying of some of the refugia pools where the species was historically common and abundant.

In contrast to the Koonap River, the 2017 survey indicated that in the Kat River, *S. bainsii* had a similar distribution range compared to historical records. The species occurred at 11 of the 41 localities that were sampled in the Kat River, and the sampled populations consisted of all size classes. *Sandelia bainsii* occurred with other native fish species in the system, including *Labeo umbratus*, *Enteromius anoplus* and *Glossogobius callidus*. A comparison of historical and recent data indicates that non-native fishes have likely not expanded their ranges in the Kat River. This is likely due to the presence of weirs and dams that have prevented upstream movement of non-native fish species *L. aeneus*, *C. gariepinus* and *T. sparrmanii* which are now abundant in the lower Kat and mainstem Great Fish River.

Surveys from the present study indicated that although *S. bainsii* still persists within the Kat River, there are a number of factors that pose a considerable threat to the continued existence of this species in the Great Fish River system. Chapter 3 evaluated the habitat associations of *S. bainsii* based on 10 physical and chemical variables and distribution data collected from 30 localities in the Upper Kat River and seven of its tributaries. A non-metric multidimensional scaling model was used to assess the distribution patterns of *S. bainsii* in relation to environmental variables. Furthermore, a negative binomial generalised linear model (GLM) was used to assess the relationship between the abundance of *S. bainsii* and environmental variables.

Results from the present study indicated that *S. bainsii* was a habitat generalist as the distribution of this species showed no specific association with any of the habitat variables that were considered in this study. The species occurred in a wide range of habitats, including rocky sections in the upper Kat River and its tributaries as well as the mainstem sections. The GLM however indicated that the abundance of *S. bainsii* was negatively associated with increasing conductivity. In addition, the Kat River contains multiple weirs of variable sizes. Future studies should therefore evaluate the effects of these weirs on population fragmentation, gene flow and long-term evolutionary potential of *S. bainsii* through the application of techniques such as mitochondrial DNA and microsatellites.

“This thesis consists of chapters prepared as stand-alone chapters; as a result unavoidable repetitions may occur between chapters”

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CHAPTER 1

General Introduction

1. Background Information

The Amatolo – Winterberg Highlands, the lower Southern Temperate Highveld and the Cape Fold freshwater ecoregions (Abell et al. 2008) contain highly threatened narrow range endemic freshwater fishes in southern Africa (Skelton et al. 1995; Clark et al. 2009; Tweddle et al. 2009; Ellender et al. 2017). The freshwater fishes in these regions are threatened by hydrological modifications through construction of dams, weirs and inter-basin water transfers, water pollution from domestic sewage effluent and agricultural activities, as well as invasion by non-native species (Mayekiso and Hecht 1988a; Cambray 2000; Clark et al. 2009; Tweddle et al. 2009; Ellender and Weyl 2014; Ellender et al. 2017; Shelton et al. 2017a). The combined negative impacts caused by these factors include population declines and localised extinctions of freshwater fishes which have been reported in many river systems (Clark et al. 2009; Marr et al. 2010; Ellender and Weyl 2014; Van Der Walt et al. 2016; Shelton et al. 2017b). The recent discovery and potential exploration of shale gas and related infrastructure development in the Karoo basin adds to the existing threats on the unique biodiversity of the Amatolo – Winterberg Highlands, Southern Temperate Highveld, Cape Fold and Karoo ecoregions (De Wit 2011; van Tonder et al. 2013; Netshishivhe 2014; Holness et al. 2016). Therefore, information on the distribution, ecology of threatened species is a critical prerequisite for biodiversity management and for informing sustainable development. The present study is part of a broader multi-disciplinary program (BioGaps) that aims to provide foundational biodiversity data to support the Strategic Environmental Assessment (SEA) for shale gas exploration and infrastructure development in the Karoo basin. The present study focuses on the Eastern Cape rocky, *Sandelia bainsii* (Castelnau, 1861), a highly threatened freshwater fish which has been a long-standing flagship species for aquatic biodiversity conservation in the Eastern Cape Province of South Africa (Cambray 1997a, 2000).

Sandelia bainsii (Figure 1) is endemic to the Eastern Cape Province where it has been recorded from seven river systems, namely the Kowie, Great Fish, Keiskamma, Igoda, Gxulu,

Buffalo and Nahoon rivers (Mayekiso 1986; Mayekiso and Hecht 1988a; Cambray 2000, 2007; Skelton 2001). Historically, this species occurred in the mainstem sections and tributaries of these river systems (Mayekiso 1986; Cambray 1997a, 2000). The Eastern Cape rocky has, however, suffered a severe decline in both its historical distribution range and population sizes due to habitat alteration, pollution, non-native fishes, proliferation of floating aquatic weeds, and hydrological modifications (Mayekiso and Hecht 1988a; Cambray 2007). Conservation concerns for this species were highlighted almost three decades ago when it was classified as Vulnerable under the IUCN Redlist of threatened species (Skelton 1987). The continued decline in population sizes and distribution range led to the downgrading of the species to Endangered in 1994 (Baillie and Groombridge 1996) and it has remained in this category since then (Cambray 2007; Chakona et al. 2018a). The remnant populations are highly fragmented and mainly confined to a few tributaries (Mayekiso 1986; Mayekiso and Hecht 1990; Cambray 1997a, 2007; Chakona et al. 2018a). The Kat River, a tributary of the Great Fish River system and the Yellowwoods River, a tributary of the Buffalo River system were identified as critical sanctuaries as they were considered to harbour the remaining viable populations of the Eastern Cape rocky (Cambray 2007; Nel et al. 2011). However, the last dedicated and comprehensive surveys for *S. bainsii* were conducted in the 1980s, but these were limited in terms of geographic coverage, as they largely focussed on the Kowie River system and sections of the Keiskamma River system (Mayekiso 1986; Mayekiso and Hecht 1988a; Cambray 1997a, 2007). The current distribution ranges and population sizes of the remnant populations of this species across its distribution range are therefore uncertain.



Figure 1: A picture of a specimen of the Eastern Cape rocky, *Sandelia bainsii*, collected from the Lushington River showing live colour pattern.

A recent genetic study based on mitochondrial 16S sequences revealed the existence of two lineages within *S. bainsii*, which have been informally referred to as *Sandelia* sp. ‘Kowie’ and *Sandelia* sp. ‘Buffalo’ (Chakona unpublished). *Sandelia* sp. ‘Kowie’ is distributed in the Kowie, Great Fish and the Keiskamma River systems, while *Sandelia* sp. ‘Buffalo’ occurs in the Igoda and Buffalo river systems (Figure 2). As the specimens that were used for the original description of *S. bainsii* were collected from the Kowie River system, *Sandelia* sp. ‘Kowie’ therefore represents *S. bainsii* sensu stricto (s.s.). The splitting of *S. bainsii* sensu lato into two lineages highlights the need to re-evaluate the distribution and ecology of these lineages. This is important as the reduction in geographic range increases their vulnerability to extinction. A recent survey of the Kowie River system suggests that *S. bainsii* s.s. is almost extirpated from this system as only three individuals were found following extensive sampling of the system between 2014 and 2017 (A. Chakona & O. Weyl. personal communication). The proposed shale gas exploration and potential infrastructure development in the Karoo basin represents a potential future threat to the remnant populations of this species (Holness et al. 2016). This is particularly the case for the population in the Great Fish River system, because the proposed area for shale gas exploration encompasses the headwaters of the Kat River which is one of the designated sanctuaries for *S. bainsii*. However, information on the distribution and ecology of this species in this river system is not current. The lack of knowledge on the current distribution and ecology of this species is a major impediment to effective conservation and management to protect remnant populations of this species.

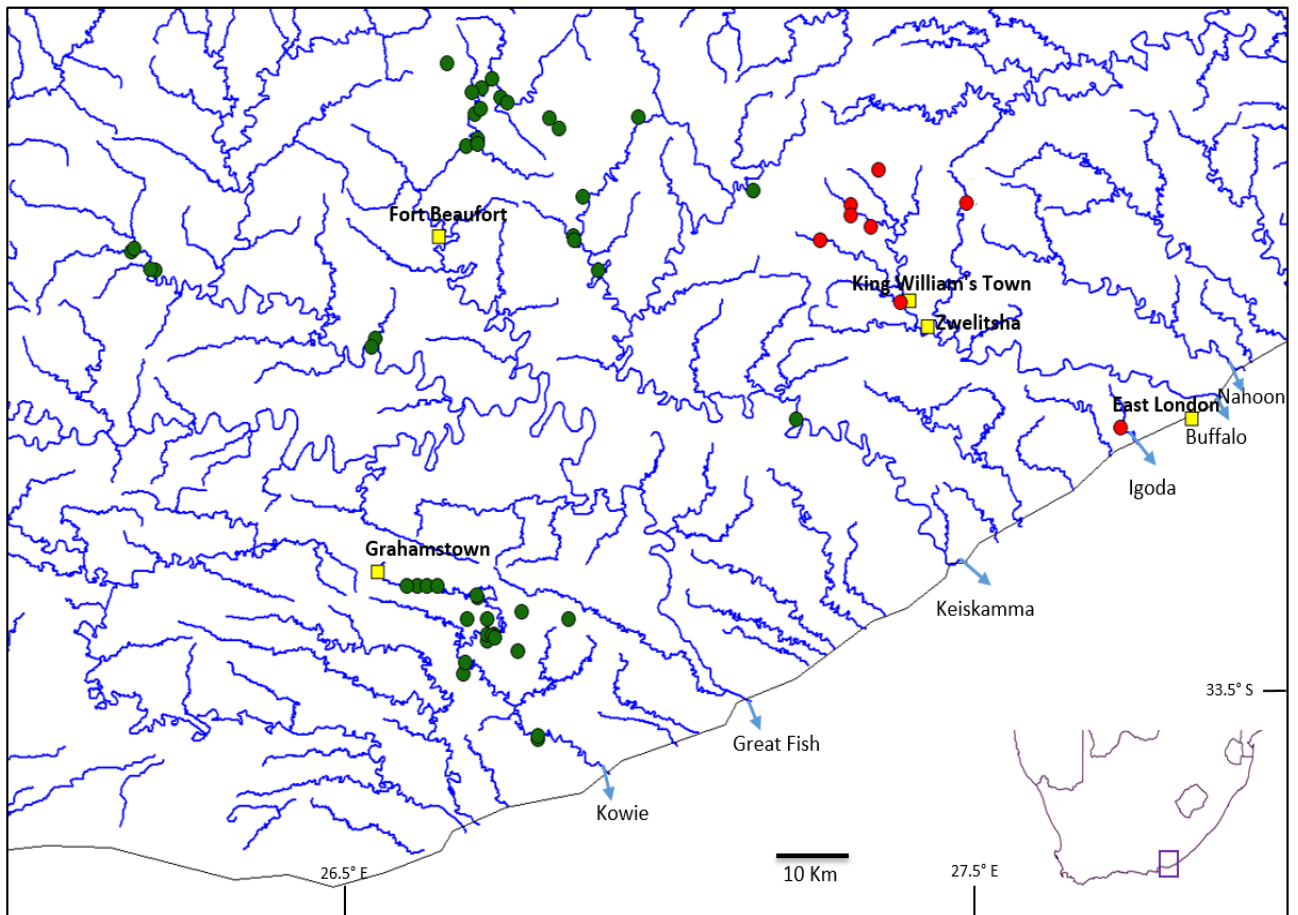


Figure 2: The distribution of the two lineages of *Sandelia bainsii* in the Eastern Cape Province. *Sandelia bainsii* sensu stricto (green points) occurs in the Kowie, Great Fish and Keiskamma River systems, while *Sandelia* sp. 'Buffalo' (red points) occurs in the Igoda, Buffalo river systems.

1.1 Causes of range reduction and population declines of *Sandelia bainsii* s.s.

Historical survey data and published reports indicate that *Sandelia bainsii* s.s. was fairly common and widespread in the Kowie River system where it occurred in both tributary and mainstem sections of this system (Gaigher et al. 1980; Skelton et al. 1995). However the species suffered severe decline in distribution range and population sizes over the past three decades (Skelton et al. 1995; Cambray 2007). Ongoing decline is likely as recent surveys have revealed only three specimens of *S. bainsii* indicating that the species is likely extirpated or in low numbers (A. Chakona & O. Weyl Personal communication). The status of the species in some of the river systems within its range, particularly the Great Fish River, is

however uncertain because the last dedicated surveys for this species in this system were done in the 1980s. This has necessitated the need to assess the current distribution and ecology for *S. bainsii* in the Great Fish River system. The major threats to the continued existence of this species include invasive species, pollution, habitat alteration and hydrological modification.

1.1.1 Non-native fish species

Non-native (a species that has been introduced outside of its biogeographic range (Essl et al. 2018)) invasive fish species have been identified as one of the most detrimental threats to the survival of native freshwater fishes worldwide (Cambray 2003; Richardson et al. 2003; Dudgeon et al. 2006; Clark et al. 2009; Maceda-Veiga 2012; Ribeiro and Leunda 2012; Simberloff et al. 2014; Ellender and Weyl 2014; Shelton et al. 2015; Phelps et al. 2017; Ruppert et al. 2017; Dwivedi 2018). They affect native species through direct predation, competition and habitat modification (Didham et al. 2007; Kloskowski 2011; van Kessel et al. 2011; Ellender and Weyl 2014; Shelton et al. 2015; van der Veer and Nentwig 2015; Gallardo et al. 2016; Pauchard et al. 2016; Van Der Walt et al. 2016; Weyl et al. 2016b). In South Africa, most non-native fish species have been introduced over the years for angling, aquaculture, as biological control and ornamental purposes (Cambray and Jubb 1977; de Moor and Bruton 1998; Ellender and Weyl 2014). Many of the species have become established and have dispersed to other systems in the region through inter-basin water transfers (IBWTs) and intentional introductions by anglers (Cambray and Jubb 1977; Laurenson and Hocutt 1985; Cambray 2003; Richardson et al. 2003; Ellender and Weyl 2014; Weyl et al. 2016b). Invasion of rivers particularly by non-native piscivores such as *Clarias gariepinus* (Figure 3) and *Micropterus salmoides* has been identified as one of the key reasons for the decline in distribution range and population sizes of *S. bainsii* (Mayekiso 1986; Mayekiso and Hecht 1988a; Cambray 2000, 2007).



Figure 3: *Clarias gariepinus* has established successful breeding populations in both the Kat and Koonap River catchments. This species was recorded in: (a) in the Kat River occurring with the native *Enteromius anoplus*, *Labeo umbratus* and *Glossogobius callidus*, (b) in the Koonap River, (c) in the Kat River occurring with the native *Enteromius anoplus* and *Labeo umbratus* and (d) in the Kat River below the town of Fort Beaufort.

A total of 7 non-native fish species (*Labeobarbus aeneus*, *Labeo capensis*, *Cyprinus carpio*, *C. gariepinus*, *Gambusia affinis*, *Lepomis macrochirus* and *Tilapia sparrmanii*) have been recorded from the Great Fish River system (Laurenson and Hocutt 1985; Skelton 2001; Kadye and Booth 2012a, 2013). Most of these were introduced through the Orange - Fish River IBWT and are now dominant in the mainstem sections of this system (Cambray and Jubb 1977; Laurenson and Hocutt 1985). Potential invasion of the upper tributary sections which represent the remaining refuges for *S. bainsii* remains a major threat to the persistence of this species (Cambray 2007).

1.1.2 Hydrological modification

Construction of dams, weirs and the IBWTs have had major impacts on rivers as they alter the natural flow regimes and connectivity of biodiversity in freshwater ecosystems (Laurenson and Hocutt 1985; Kingsford 2000; Rosenberg et al. 2000; Weerts and Cyrus 2008; Liermann et al. 2012; Chigor et al. 2013). Impacts of dams and weirs include fragmentation and habitat alteration and prevention of movement in freshwater fish species (Meldgaard et al. 2003; Liermann et al. 2012; Maceda-Veiga 2012). In the Eastern Cape Province, dams and weirs have been constructed over the years mainly for irrigation and domestic purposes. For example, along the Kat River, there are approximately three weirs (0.5 – 3 m) per km but that number varies within the catchment and one large dam, the Kat River Dam (52 m) (Farolfi et al. 2005; Birkholz 2009). These impoundments have various sizes, with some forming impassable barriers to upstream movement of fish as they did not have fish ladders (Figure 4). These instream physical barriers have likely fragmented the previously widely distributed *S. bainsii* into small isolated populations, with possibilities that genetic exchange between these populations may have been disrupted. Small isolated populations face a high risk of extinction because of possible loss of genetic diversity due to genetic drift and inbreeding (Frankham et al. 2014; Fitzpatrick et al. 2016; Jang et al. 2017; Coleman et al. 2018). This is likely to increase the risk of extinction of *S. bainsii* as a decrease in gene flow will have a long-term effect on the evolutionary potential of this species. Despite these potential impacts, construction of instream physical barriers also is likely to have played an important role in preventing invasion of the upper sections of the Kat River by non-native species which are present in the lower reaches of this river and the main-stem Great Fish River, as reported for other systems elsewhere (Jackson et al. 2004; Ellender et al. 2011; Shelton et al. 2015; Van Der Walt et al. 2016).

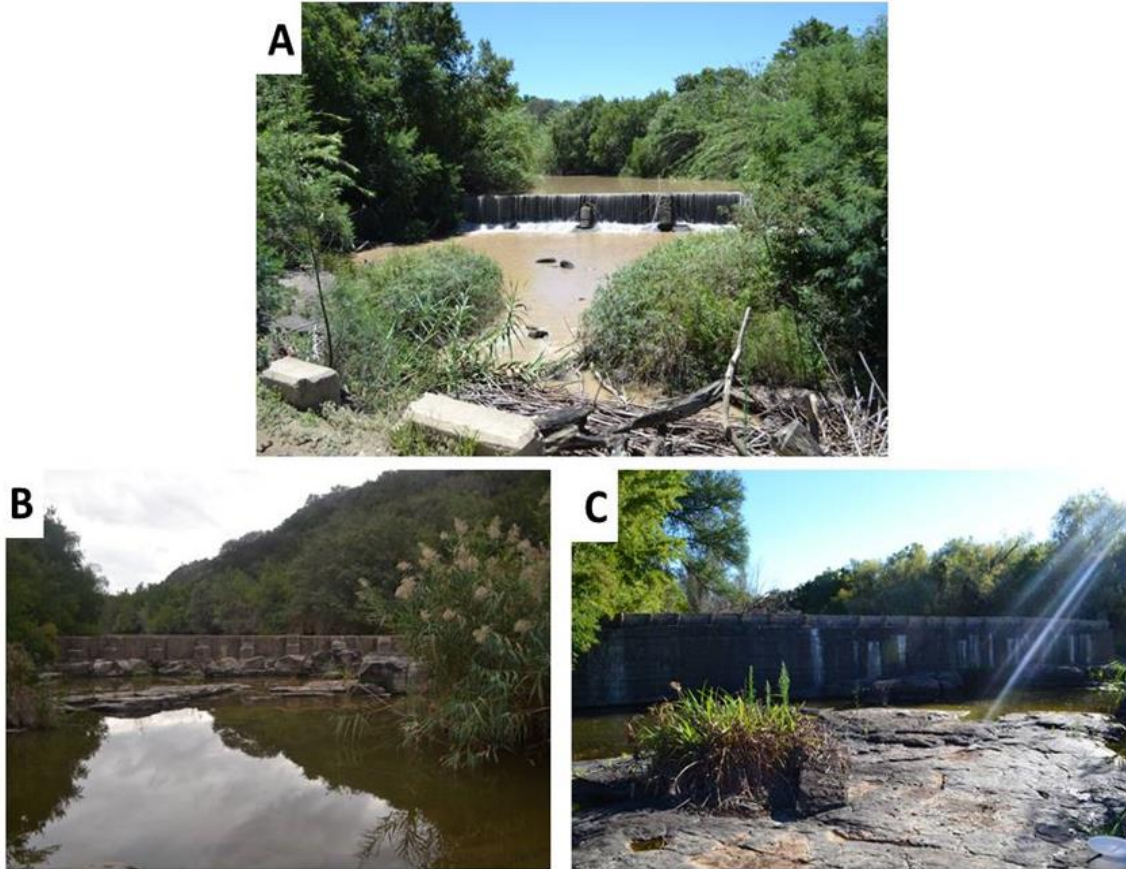


Figure 4: Some of the weirs that could be potential barriers for upstream movement of non-native fishes in the Kat, (a, b) and Koonap (c) rivers.

Southern Africa is a water scarce region, and this has prompted the construction of IBWTs to transfer water into some of the driest areas on the subcontinent (Laurenson and Hocutt 1985; Muller 1999; Turton et al. 2004; Stuart-Hill et al. 2012). For example, the Great Fish – Orange River IBWT was constructed in 1975 to transfer water from the Orange River system to the Great Fish River system from where it is further distributed to various parts of the Eastern Cape, which is one of the driest provinces in South Africa (Cambray and Jubb 1977; Laurenson and Hocutt 1985; Turton et al. 2004). Prior to the IBWT, the Great Fish was an intermittent system, with pronounced seasonality in flow regimes characterised by floods during the rainy season followed by rapid drawdown of water levels and recession of the river into a series of isolated pools during the dry season (Laurenson and Hocutt 1985; Rivers-Moore et al. 2007). The transfer of the Orange River water transformed the Great Fish from an intermittent system to a perennial system (Cambray and Jubb 1977; Laurenson and

Hocutt 1985). This is likely to have caused considerable ecological impacts through disruption of seasonal dynamics, change in the chemical and physical status of the water, accumulation of sediments in the river, as reported from other systems that have been similarly transformed (Snaddon et al. 1998; Kingsford 2000; Rosenberg et al. 2000; Weerts and Cyrus 2008; Grant et al. 2012). The construction of the Great Fish – Orange River IBWT has facilitated the spread of five fish species, *L. aeneus*, and *L. umbratus*, *L. capensis*, *C. gariepinus* and *T. sparrmanii* from the Orange to the Great Fish River system (Cambray and Jubb 1977; Laurenson and Hocutt 1985). These extralimital species now dominate the main-stem sections of the Great Fish River and represent a potential threat to the remnant populations of *S. bainsii* if the barriers to their upstream migration get breached.

The natural flow regime of rivers, defined as the magnitude, frequency, duration, timing and rate of change in flow, plays an important role in sustaining native biodiversity and ecosystem integrity because it regulates critical ecological and biological processes such as spawning and feeding migrations (Poff et al. 1997). Changes in the flow regime can thus be ecologically harmful (Poff and Zimmerman 2010; Arnell and Gosling 2013; McClain et al. 2014). Additionally, projected changes in global climate are expected to result in increase in temperatures, variability in rainfall and decrease in the total runoff for many regions in southern Africa, including the Cape Floristic Ecoregion (CFE) (Lumsden et al. 2009; Schulze 2011; Stuart-Hill et al. 2012; Arnell and Gosling 2013; Dallas and Rivers-Moore 2014). Flow modifications in rivers that currently harbour remnant populations of threatened species such as *S. bainsii* are likely to be intensified in the future due to the projected increase in human demand for water. Headwater streams are known to be vulnerable to small changes in temperature and run-off (Hrachowitz et al. 2010; Jones and Rinehart 2010; Ficklin et al. 2013; Luce et al. 2014). Thus, an increase in temperature and decrease in run-off represent real future threats to the continued survival of *S. bainsii*.

1.1.3 Water pollution

Water pollution is one of the major threats to freshwater biodiversity, and it has been implicated as one of the major causes for the extirpation of a number of freshwater fishes globally (Scott and Sloman 2004; Dudgeon et al. 2006; Gaber et al. 2013; Malaj et al. 2014). The main sources of pollution are domestic and industrial effluent discharge as well as diffuse sources from agricultural waste (Scott and Sloman 2004; Gaber et al. 2013; Malaj et al. 2014). This is likely to increase nutrient enrichment in receiving systems. While most of

these nutrients are not directly harmful to fish, excessive nutrient enrichment such as phosphorus is likely to lead to eutrophication, while industrial effluent may contain chemicals that are toxic to aquatic biota (Ibelings and Havens 2008; Malaj et al. 2014; Sukenik et al. 2015). Eutrophication is detrimental to the survival of freshwater fishes due to depletion of oxygen in the water column and production of toxins such as anatoxins, microcystins, nodularins, saxitoxins by blue-green algae (cyanobacteria) (Camargo and Alonso 2006; Ibelings and Havens 2008; Sukenik et al. 2015). These toxins have been found to affect fish behaviours, such as predator avoidance and reproduction due to disruption in sensory, endocrine, neurological and metabolic abilities (Scott and Sloman 2004; Malaj et al. 2014). Additionally, the bacteria that break down organic waste produces nitrogenous waste as a by-product which in high levels can damage fish gills (Lang et al. 1987; Levit 2010; Dong et al. 2013; Devaraj et al. 2014).

Within the Great Fish River catchment, sections of the rivers downstream of the towns of Adelaide, Fort Beaufort and Bedford have degraded water quality and show evidence of eutrophication likely due to discharge of domestic effluent (Figure 5). Deterioration in water quality was identified as one of the likely causes for the decline in the distribution and population sizes of *S. bairdii* in the Kowie River system (Skelton et al. 1995; Cambray 2007).

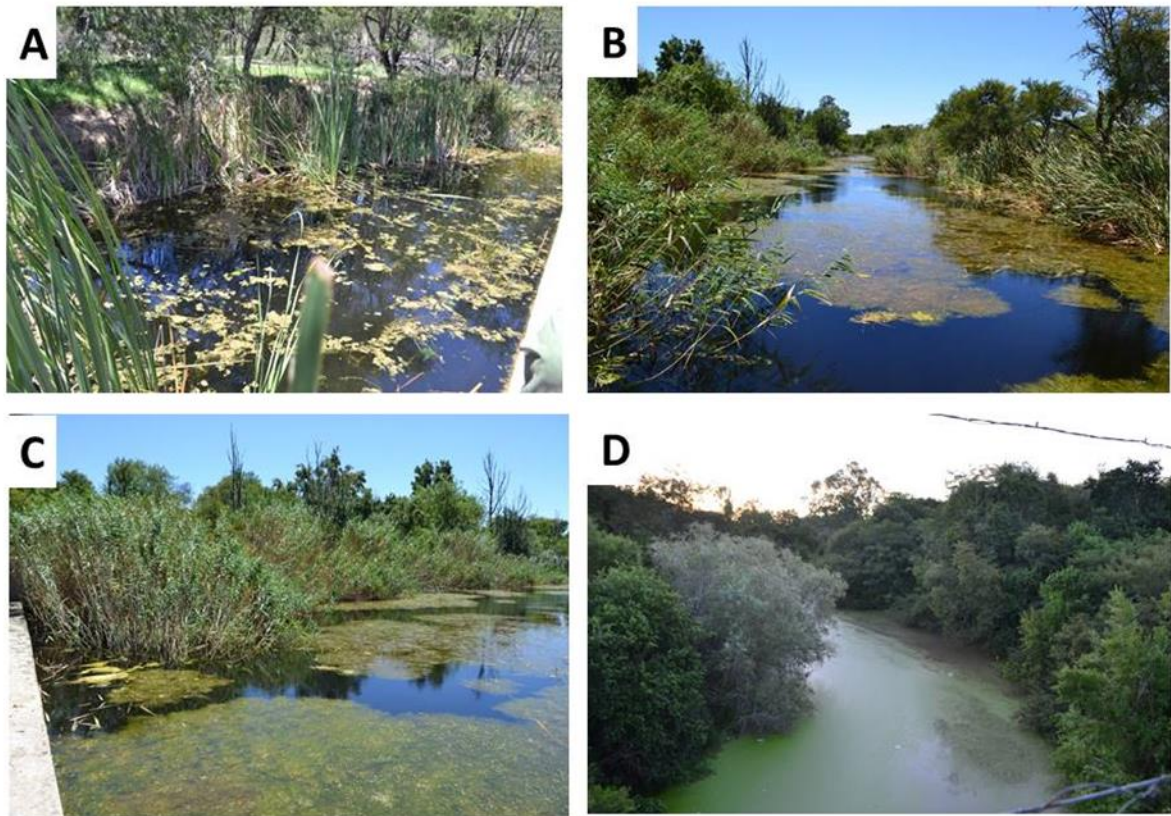


Figure 5: Sections of the Kat and Koonap rivers show sign of eutrophication, likely due to domestic effluent. Potential nutrient loading from the effluent has likely contributed to algal blooms in parts of the rivers (a, b and c) algal bloom at the tributary of the Koonap River next to Glen cliff, and (c) algal bloom along the Kat River below the town of Fort Beaufort.

1.1.4 Proposed shale gas exploration

An additional future threat to the remnant populations of *S. bainsii* is the proposed shale gas exploration and potential development through hydraulic fracturing commonly known as fracking in the Karoo basin (Figure 6). Fracking is a process of using liquid at high pressure to make fractures in rocks that allow extraction of natural gas or oil (Netshishivhe 2014; van Wyk 2014). The Karoo is an arid ecosystem characterised by low and unpredictable rainfall and episodic drought events (van Tonder et al. 2013; Netshishivhe 2014). Fracking uses a huge volume of water and this is likely to result in severe stress to the intermittent rivers in the Karoo basin (Vermeulen 2012; Netshishivhe 2014; Holness et al. 2016). Fracking can

also lead to contamination of freshwater due to ground and surface leakage during the extraction process (Vermeulen 2012; van Tonder et al. 2013; Warner et al. 2013; Netshishivhe 2014). This process has also been associated with negative impacts on freshwater fishes in other regions (Veil 2010; Gillen and Kiviat 2012; Maloney and Yoxtheimer 2012; Warner et al. 2013). For example, in the United States of America, fracking of the Marcellus-Utica region led to the deterioration in water quality, which in turn caused the restricted distribution of the bluebreast darter (*Etheostoma camurum*) and the tonguetied minnow (*Exoglossum laurae*) (Gillen and Kiviat 2012). These species have become vulnerable due to water pollution from spills of fracking wastewater (Gillen and Kiviat 2012). Fracking thus represents a severe threat to the remnant populations of *S. bainsii* in the Great Fish river system as the distribution of this species in the Kat River falls within the area that has been demarcated for exploration (Figure 6).

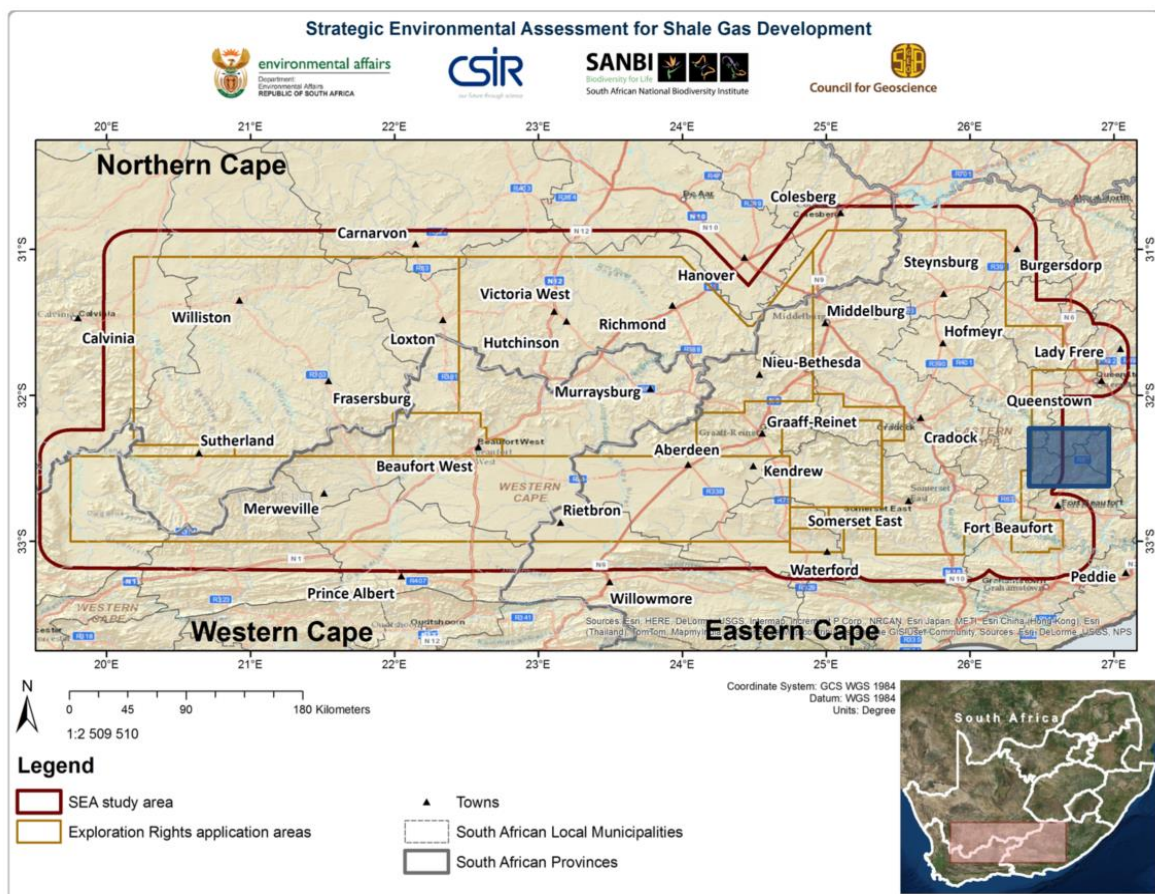


Figure 6: Map of the proposed area for shale gas exploration and potential infrastructure development in the Karoo basin. The distribution range of *Sandelia bainsii* in the Kat River falls within the area shown by the blue shaded shape (adapted from Holness et al. 2016).

The sustainable exploitation of shale gas requires scientific information in order to reduce the impact and loss of biodiversity in the proposed region. While conservation of freshwater fishes is important, there is a need to strike a balance between socio-economic development and natural resource utilisation. Given the multiple threats faced by *S. bainsii*, there is a need for dedicated surveys to establish the full range of the remnant populations of this species and generate data that will inform effective conservation and management strategies. The present study was part of a Foundational Biodiversity Information Programme (FBIP) (a joint initiative of the Department of Science of Technology (DST), the National Research Foundation (NRF) and the South African National Biodiversity Institute (SANBI)), multi-disciplinary project, referred to as the BioGaps Project, whose aim was to identify areas of biodiversity importance in the Karoo basin and adjacent systems.

1.2 Description of study area

The Great Fish River is the third largest systems in the Eastern Cape Province of South Africa, with a catchment area of approximately 30 243 km². The climate of the Great Fish River system is warm-temperate with an unevenly distributed rainfall within the catchment and ranges from 400 to 800 mm (O’Keeffe and Moor 1988; Jari and Fraser 2009; Cochet et al. 2015). The Great Fish River system is regarded as sub-humid to semi-arid and receives 75% of the annual rainfall between October/November and February/March (Jari and Fraser 2009; Cochet et al. 2015). The annual air temperatures recorded for summer ranges between 20 – 35° C and winter temperatures range from 0 – 20°C (Lerotholi et al. 2004; Cochet et al. 2015). Frost is common in winter at high altitude, especially during the months of July and August (O’Keeffe and Moor 1988; Cochet et al. 2015). The geology of the system consists of the Karoo super group, sandstones and shales (O’Keeffe and Moor 1988; Hoare and Bredenkamp 1999; Cochet et al. 2015). At high elevation, the catchments are dominated by the strong non-eroded dolerite rocks (O’Keeffe and Moor 1988).

The major tributaries of the Great Fish river system are the Little Fish (Klein), the Kat and the Koonap rivers (Figure 7). The Little Fish is an intermittent river as it drains the Karoo basin which receives approximately 350 – 550 mm rainfall per annum (O’Keeffe and Moor 1988; Rivers-Moore et al. 2007). The Kat River is approximately 147 km long from its headwaters to the confluence with the Great Fish River and has a total catchment area of

approximately 1 715 km² (Lerotholi et al. 2004; Birkholz 2009). The Koonap River has a total catchment area of 3360 km² and is approximately 220 km long from its headwaters to the confluence with the Great Fish River. In the Great Fish River system, *S. bainsii* has only been recorded from the Kat and Koonap rivers (Gaigher et al. 1980; Laurenson and Hocutt 1985) which both drain the wetter southern slopes of the Winterberg Mountains (Jari and Fraser 2009).

The upper section of the Kat River includes the Elands, Eyre and Lushington rivers which flow into the Katriver Dam (Figure 7) (Mitchell 2012). The Katriver Dam (32°34'8" S; 26°45'2" E), which was constructed in 1969 at Seymour (Cochet et al. 2015), covers a surface area of 212.9 ha and has a depth of 52 m at maximum capacity. The middle section of the Kat River (the section between the Katriver Dam and Fort Beaufort town), which is moderately modified, includes the following tributaries: Readsdales, Balfour, Buxton, Mankazana and Blinkwater rivers (Figure 7) (Mitchell 2012). The lower section of the Kat River, mostly modified, comprises the stretch of the river from the town of Fort Beaufort down to the confluence with the Great Fish River (Mitchell 2012). Both the river and the dam supply water for irrigation and domestic use to the surrounding areas (Farolfi et al. 2005).

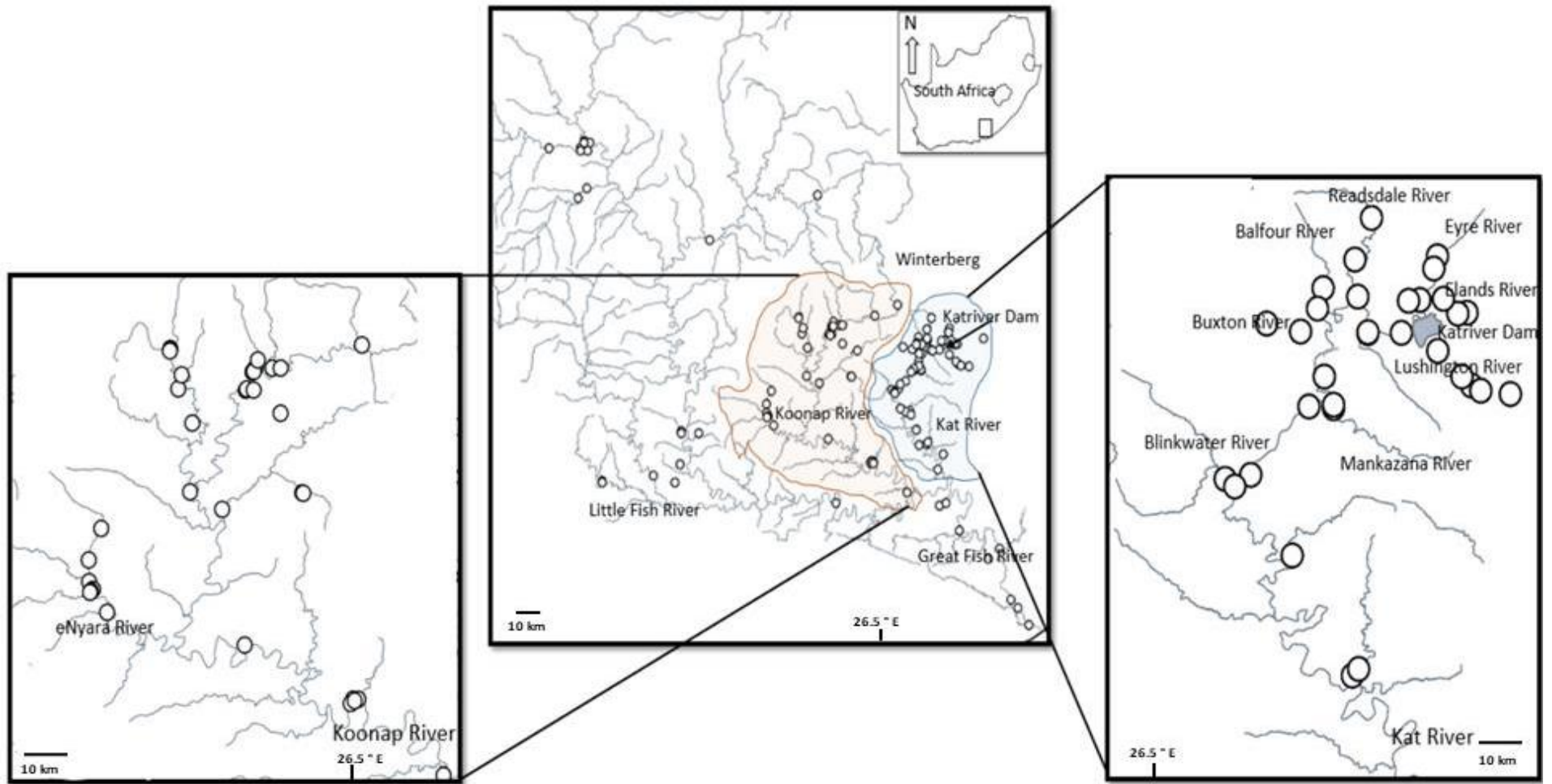


Figure 7: Map of the Great Fish River system showing all sampled localities (represented by open circle) (centre). Right and left pane showing the two major tributaries of the system, the Kat and Koonap river catchments and their tributaries.

1.3 Thesis outline

The present study provides updated information based on a comprehensive ichthyofaunal survey of the Great fish River system, with specific focus of the Kat and Koonap river catchments, to assess the current distribution, conservation and habitat association of *Sandelia bainsii* in this river system. This study was largely based on data generated from fine-scale surveys of the Kat and Koonap rivers that were conducted in 2017, and augmented with data from previous surveys that were conducted between 2009 and 2015, extensive literature review and collection records obtained from the National Research Foundation - South African Institute for Aquatic Biodiversity (from 1961 to 2005).

1.3.1 Objectives and questions

The aim of this study was to contribute to the conservation and management decisions for *S. bainsii* in the Great Fish River system. The first objective of this study was to determine whether there have been any changes in the distribution of *S. bainsii* in the Great Fish River system based on historical collections and recent data from field surveys. The second objective was to conduct comprehensive surveys in the Kat River to map the distribution of *S. bainsii* and determine the factors that are associated with the species' distribution patterns. This information is important for informing the development of effective conservation and habitat management strategies to prevent loss of biodiversity.

The following questions will be addressed:

1. Have there been any changes in the historical and recent distribution of *S. bainsii* in the Great Fish River system?
2. What are the factors that are associated with the distribution of *S. bainsii* in the Kat River catchment?

1.3.2 Thesis structure

To address the above objectives and questions the thesis is structured into four chapters. Firstly, chapter 1 provides an introduction on *S. bainsii*, threats to the species, description of study area and the thesis aims and objectives. Chapter 2 reviewed the past and present distribution patterns of *S. bainsii* in the Great Fish River system. Chapter 3 determined the habitat association of *S. bainsii* in the Kat River catchment. Lastly, Chapter 4 summarised the thesis findings, detailed management options for the conservation of *S. bainsii* in the Great

Fish River system and future research. This information is important for the long-term survival of *S. bairdii* in the system.

CHAPTER 2

Comparison of the past and present distribution patterns of *Sandelia bainsii* in the Great Fish River system

Introduction

Freshwater ecosystems around the world have been affected by multiple human-induced impacts, with profound implications on the persistence of native species (Clark et al. 2009; Habit et al. 2010; Venter et al. 2010; Ellis et al. 2013; Kadye and Booth 2013; Maceda-Veiga et al. 2017). There is strong evidence that many species have experienced severe range reductions and declines in population sizes (Clark et al. 2009; Tweddle et al. 2009; Habit et al. 2010; Maceda-Veiga 2012; Ellis et al. 2013; Gallardo et al. 2016; Shelton et al. 2017a, 2017b), or suffered localised extirpations (Clark et al. 2009; Hammer et al. 2015; Ellender et al. 2017; Liu et al. 2017) due to these impacts. As anthropogenic impacts continue to increase, there is a need for accurate spatial distribution data to facilitate monitoring of the trends and evaluate the ecological consequences of these human-induced changes in order to identify and protect species facing the risk of extinction (Dudgeon et al. 2006; Strayer and Dudgeon 2010).

In South Africa, there is growing concern about the conservation of native freshwater fishes, particularly the endemic species of the Cape Floristic, Amatolo – Winterberg Highlands and lower Southern Temperate Highveld ecoregions (Clark et al. 2009; Tweddle et al. 2009; Russell 2011; Ellender et al. 2017; Shelton et al. 2017b, 2018). Although it is widely reported that many of these endemic fishes have declined (Tweddle et al. 2009), much of the evidence is circumstantial as it is based on expert opinion, because the available distribution data is either outdated or comprehensive surveys have not been done. Furthermore, the discovery of hidden diversity within species that were previously thought to have wide distribution ranges also necessitates the re-evaluation of distribution ranges and conservation to include recently identified lineages even before they are formally described in order to facilitate conservation action (Chakona et al. 2018b).

The Eastern Cape rocky *Sandelia bainsii*, occurs in the coastal rivers of the Eastern Cape Province where its distribution spans seven river systems from the Kowie River to the Nahoon River (Mayekiso 1986; Mayekiso and Hecht 1988a; Cambray 2000). Recent genetic studies have revealed two lineages within *S. bainsii* (Roos 2004; Chakona et al. in prep) which highlights the need to re-evaluate its distribution and conservation. In the Great Fish River, the population of *S. bainsii* is distributed in two major tributaries, the Koonap and Kat rivers (Mayekiso 1986; Skelton 2001). The species has been severely affected by anthropogenic impacts over the years which contributed to its listing on the IUCN Redlist as Endangered (Mayekiso 1986; Mayekiso and Hecht 1988a; Cambray 1997a, 2000). An additional future threat to the species is the proposed shale gas exploration and potential infrastructure development in the Karoo (Holness et al. 2016), as the proposed area encompasses the remnant population of *S. bainsii* in the Great Fish River system.

The Kat River catchment was recently designated as one of the sanctuaries for *S. bainsii* (Nel et al. 2011), but there has been no recent published information on the recent distribution of this species in the Great Fish river system. Therefore, the objective of this chapter was to determine whether there have been any changes in the distribution of *S. bainsii* in the Great Fish River system based on historical collections made between 1961 and 2005 and data from recent field surveys conducted between 2009 and 2017. Through this process, the second objective was to further identify management options that will contribute towards conserving the population of *S. bainsii* within the Great Fish River system.

Materials and Methods

Historical and current distribution data

The study used historical data (1961 – 2005) obtained from specify database at the South African Institute for Aquatic Biodiversity (SAIAB) and published literature and current data from recent field surveys (2009 - 2017) (Appendix 1). Extensive sampling of river catchments (Kat and Koonap rivers) within the Great Fish River system was conducted from 2009 to 2017. A total of

118 localities were surveyed during that period to map the distribution of *Sandelia bainsii* in the Koonap and Kat rivers (Appendix 2). Of the localities sampled, 53 sites were surveyed from January to May 2017 and 3 sites were obtained from specify. Seven of the 53 sites were dry at the time of sampling. Supplementary data (62 sites) was obtained from Kadye & Booth (2012), based on surveys that were conducted in 2009 – 2011.

Multiple sampling gears (Appendix 2), including seine nets (3 m length × 3mm mesh size and a 30 m length × 8mm mesh size), fyke nets (8 m long net × ring diameter of 55 cm × a 10 mm mesh size) and electrofishing (SAMUS-725MP) were used to cover all available habitats at each locality. The sampling gears varied across sites depending on the stream size and depth. Electrofishing was the mostly used sampling with 80% of sites sampled using it. Seine net and fyke net were used on sites with deep pools (0.5 m). Two fyke nets were set in the evening and collected the next morning from two localities. Fish sampled were identified using keys in Skelton (2001) and fishes were returned to the collection localities alive. The presence and absence of fish species was noted. Additionally, at each site, GPS coordinates were recorded using a Global Positioning System (Garmin etrex Ltd) unit to an accuracy of 10 m.

Results

A total of 68 localities were sampled in the Great Fish River system between 1961 and 2005 (Appendix 1, Figure 8). Of these localities, *S. bainsii* was only recorded from 14 localities, three localities (Locality A-C) in the Koonap River catchment and 11 localities (Locality 1-11) in the Kat River catchment (Appendix 1, Figure 8). *Sandelia bainsii* occurred with the native *Labeo umbratus*, *Enteromius anoplus* and *Anguilla mossambica*. These historical surveys indicated that *E. anoplus* was the most widespread species. In addition, the non-native species *Labeobarbus aeneus*, *Cyprinus carpio*, *Micropterus salmoides*, *Clarias gariepinus* and *Tilapia sparrmanii* were also recorded.

During the recent surveys a total of 118 localities were sampled in the Great Fish River. *Sandelia bainsii* was present at 13 of these localities, two localities in the Koonap River and 11 localities in the Kat River (Figure 8). Data from recent surveys indicate that the species has persisted at the historical localities, with the exception of one locality (Locality C) in the Koonap River where no specimens of *S. bainsii* were sampled, despite the use of multiple sampling approaches and gears (Figure 8, Table 1). The species was only present at two historical localities (Locality A and B) in the eNyara River, a tributary of the Koonap River (Figure 8). *Sandelia bainsii* occurred at 11 (Locality 1 to 11) of the 41 localities that were sampled in the Kat River, and it was represented by all size classes. It was present in two tributaries, the Lushington and Readsdales rivers and the mainstem section of the river (Figure 8). In the Lushington River, *S. bainsii* was recorded at two localities (Locality 1 and 2) and in the Readsdales River, it was only recorded at one locality (Locality 5). In the mainstem section of the Kat River, *S. bainsii* was recorded just below the Katriver Dam (Locality 3 and 4) extending to the middle section of the river (Locality 6 to 11), after the confluence with Mankazana River (Figure 8).

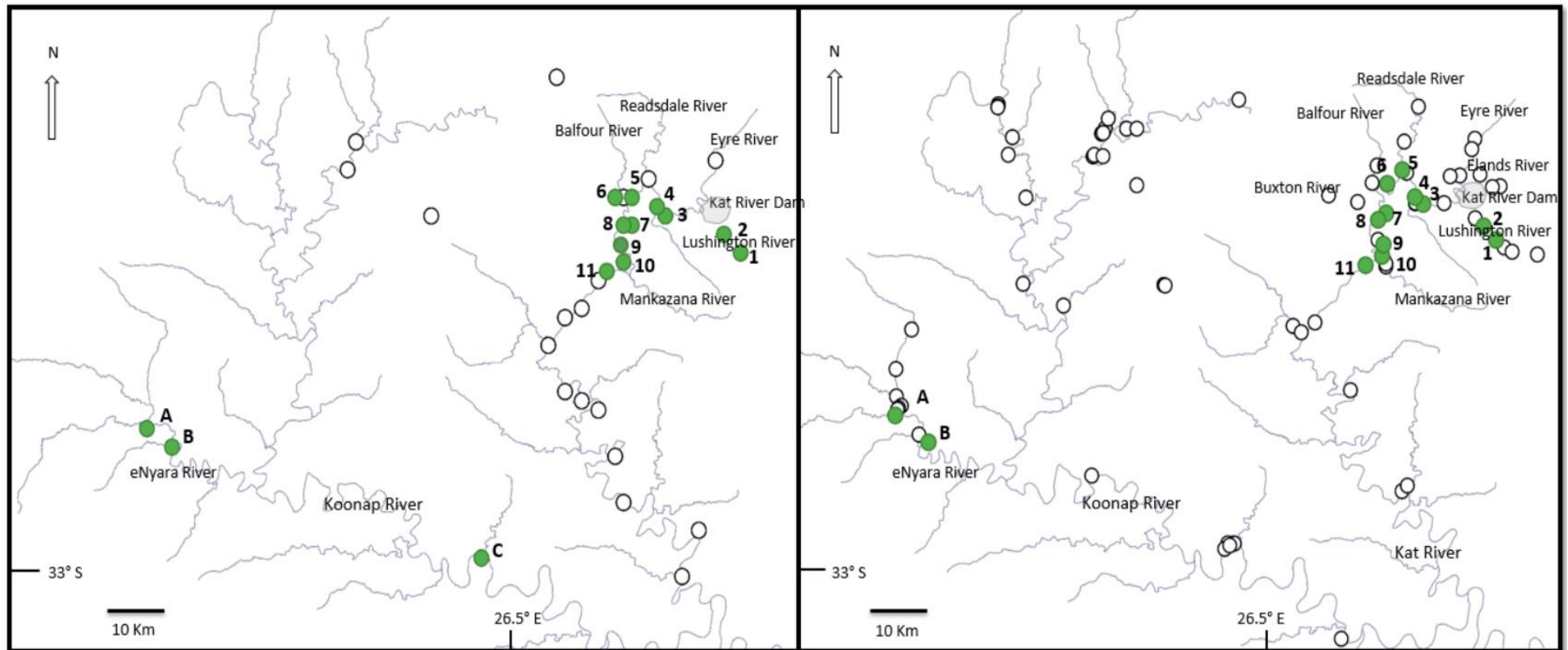


Figure 8: Map of all sampled sites (open circles) and sites where *Sandelia bainsii* was present (green circles) in the Kat and Koonap river catchments in the Great Fish River system. Left pane shows historical records (1961-2005), and the right pane shows the current records from the recent surveys (2009-2017).

Table 1: Comparison of the past and present distribution of *Sandelia bainsii* at 14 localities in the Great Fish River system.

River	Locality	Latitude	Longitude	Historical	Current
Kat	1	-32.60	26.80	✓	✓
Kat	2	-32.62	26.81	✓	✓
Kat	3	-32.58	26.73	✓	✓
Kat	4	-32.57	26.72	✓	✓
Kat	5	-32.54	26.72	✓	✓
Kat	6	-32.56	26.69	✓	✓
Kat	7	-32.59	26.69	✓	✓
Kat	8	-32.60	26.68	✓	✓
Kat	9	-32.62	26.69	✓	✓
Kat	10	-32.63	26.68	✓	✓
Kat	11	-32.64	26.66	✓	✓
Koonap	A	-32.81	26.11	✓	✓
Koonap	B	-32.83	26.15	✓	✓
Koonap	C	-32.95	26.51	✓	×

In addition to *S. bainsii*, four native fish species, *A. mossambica*, *E. anoplus*, *L. umbratus* and *Glossogobius callidus* were sampled in the Kat and Koonap river catchments (Table 2). *Sandelia bainsii* was not found at localities that were invaded by non-native species in the Kat River catchment. Whereas, in the Koonap *S. bainsii* occurred with the non-native *T. sparrmanii*. In the Koonap River catchment, the non-native *L. aeneus* and *C. gariepinus* occurred at a locality where *S. bainsii* previously occurred (Table 2).

Table 2: Fish species collected at localities where *Sandelia bainsii* historically occurred in the Great Fish River system as part of surveys from 2009 to 2017. The numbers 1 to 11 and letters A to B represent localities where *S. bainsii* occurred in the Kat and Koonap river catchments respectively. ✓ represents presence and × represents absence.

		Kat River											Koonap River		
		1	2	3	4	5	6	7	8	9	10	11	A	B	C
<i>Anguilla mossambica</i>	Longfin eel	×	×	×	×	×	×	×	✓	×	×	×	×	✓	×
<i>Enteromius anoplus</i>	Chubbyhead barb	×	×	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓
<i>Labeobarbus aeneus</i> *	Smallmouth yellowfish	×	×	×	×	×	×	×	×	×	×	×	×	×	✓
<i>Labeo umbratus</i>	Moggel	×	×	×	×	×	×	×	×	✓	✓	×	×	✓	✓
<i>Clarias gariepinus</i> *	Sharptooth catfish	×	×	×	×	×	×	×	×	×	×	×	×	×	✓
<i>Tilapia sparrmanii</i> *	Banded tilapia	×	×	×	×	×	×	×	×	×	×	×	✓	✓	×
<i>Sandelia bainsii</i>	Eastern cape rocky	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
<i>Glossogobius</i>	River goby	×	×	×	✓	×	×	×	✓	✓	✓	✓	×	×	×
<i>callidus</i>															

* Non-native fish species

Based on the distribution of *S. bainsii*, the Kat River can be divided into a native and non-native fish section (Figure 9). The lower range of the native fish section is demarcated by a weir upstream of the confluence of Kat and the Blinkwater. This weir which is approximately 3 m high is likely to be an effective barrier to non-native fishes, as all non-native fishes that were collected in the Lower Kat River were not found above this weir. The only species that were found in the native fish section were *E. anoplus*, *L. umbratus*, *S. bainsii* and *G. callidus*. Non-native fish species, *L. aeneus*, *C. gariepinus*, and *T. sparrmanii*, occurred in the middle and lower sections of the river.

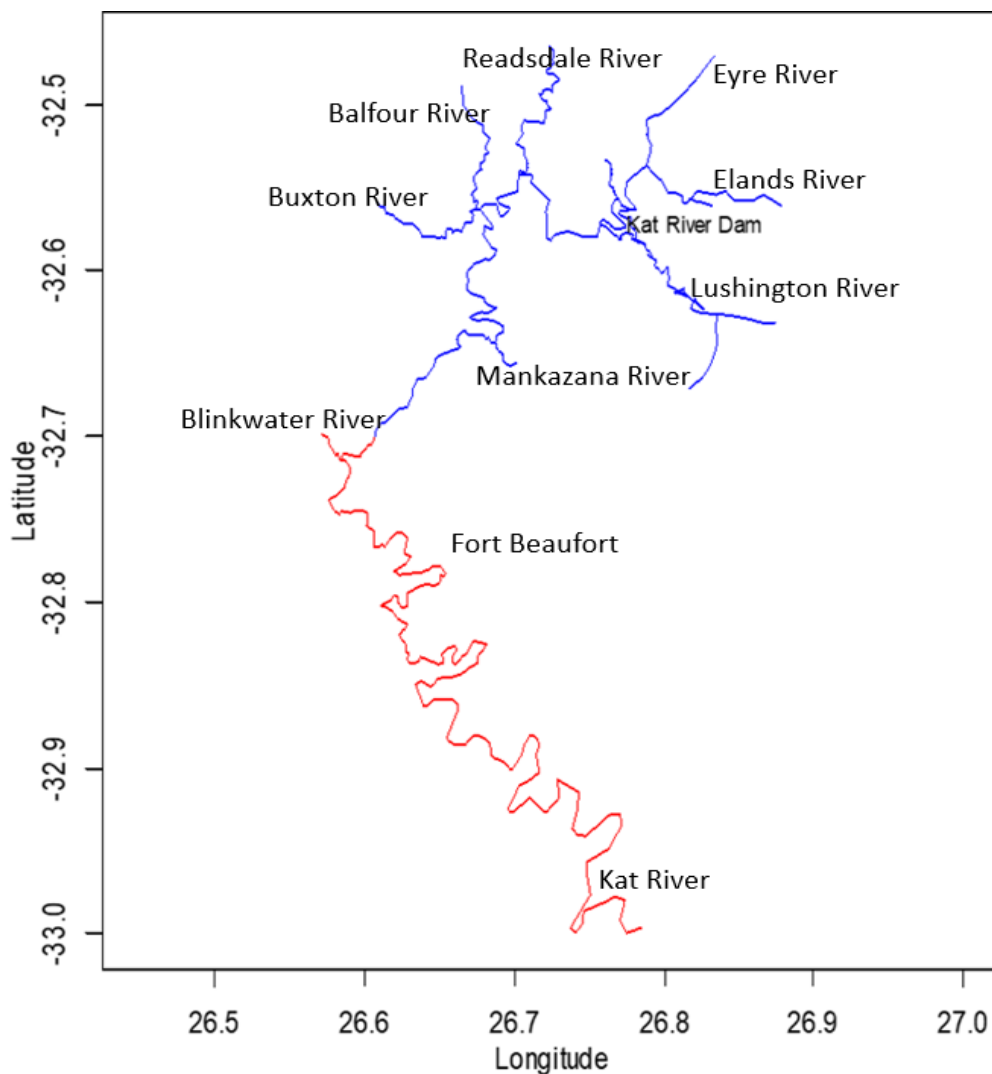


Figure 9: Map of the Kat River catchment showing section (blue) with native fish species, *Sandelia bainsii*, *Labeo umbratus*, *Glossogobius callidus*, *Enteromius anoplus* were distributed, and the section (red) invaded by non-native fish species, *Labeobarbus aeneus*, *Clarias gariepinus* and *Tilapia sparrmanii*.

Discussion

Based on the historical and current records of *S. bainsii* in the Great Fish River system, the results from the present study have shown that the species has a patchy distribution pattern. Historical records indicate that *S. bainsii* occurred at 11 localities in the Kat River and three localities in the Koonap River. Data from recent surveys indicate that the species has persisted at these historical localities, with the exception of one locality in the Koonap River where no specimens of *S. bainsii* were sampled, despite the use of multiple sampling gears. Although the species still persists at two of the three historical localities in the Koonap River, the population sizes have considerably declined as the species was reportedly common at these localities in the 1980s (Gaigher et al. 1980; Laurenson and Hocutt 1985; Cambray 1997a). Factors that were observed that likely threaten the continued existence of this species include the combination of non-native species, hydrological modification and pollution. This was also observed from the southern-most distribution of the species, where agricultural activities and the invasive species were identified as one of the likely causes for the decline in the distribution and population sizes of *S. bainsii* in the Bloukrans and Kowie rivers (Skelton et al. 1995; Cambray 2007). The combined anthropogenic impacts on the population of *S. bainsii* in the Koonap River catchment is of concern as the species is currently confined to only two pools in the eNyara River. Studies have shown that fish species with small isolated populations face a high risk of due to possible loss of genetic diversity due to genetic drift and inbreeding (Frankham et al. 2014; Fitzpatrick et al. 2016; Jang et al. 2017; Coleman et al. 2018).

In contrast to the Koonap River, the 2017 survey indicated that *S. bainsii* in the Kat River had a similar distribution range compared to historical records. The species occurred at 11 of the 41 localities that were sampled in the Kat River, and it was represented by all size classes. These results indicate that *S. bainsii* maintains viable populations in the Kat River catchment, confirming and supporting the designation of this section of the Great Fish River system as a sanctuary (Nel et al. 2011) for this species. Sanctuaries have been designated in freshwater conservation as they act as breeding and feeding grounds for fish species (Hermoso et al. 2016). Although the historical distribution of *S. bainsii* in the Kat River catchment has not changed and maintained a viable population, there are potential future threats in the catchment likely to influence the species. These include hydrological modification, pollution and introduction and potential expansion of non-native fish species

from the Katriver Dam into the streams where the species occurs. Additionally, the species will likely experience further pressure from the proposed shale gas exploration and development in the Karoo basin (Holness et al. 2016).

Historical records from the SAIAB database indicate that specimens of *S. bainsii* were collected from pools at Edgehill farm in the Koonap River where two non-native species, *T. sparrmanii* and *C. gariepinus* were also found. Two native species, *L. umbratus* and *E. anoplus* also occurred at that locality. However, the recent surveys indicated that only *L. umbratus*, *L. aeneus*, *E. anoplus* and *C. gariepinus* occurred in the pools and weir at Edgehill farm. Remnant populations of *S. bainsii* in the Koonap River were confined to small sections of the eNyara River at Herbert's Hope Farm. In the Kat River, *S. bainsii* occurred with only native fish species, including *A. mossambica*, *L. umbratus*, *E. anoplus* and *G. callidus* which is similar to historical records (Cambray and Jubb 1977; Gaigher et al. 1980; Laurenson and Hocutt 1985; SAIAB database 2018). Furthermore, *S. bainsii* in the Kat River never occurred in polluted sections of the catchment. This likely contributes to the species maintaining viable reproducing populations in the catchment.

Comparisons of historical and recent data indicate that non-native fishes have not expanded their ranges, particularly in the Kat River. In the Kat River, there was a clear separation in the distribution ranges of fish species, native occurring in the upper sections and non-native in the lower sections of the catchment. This is likely due to the presence of weirs in the catchment that have likely prevented upstream movement of the non-native fish species *L. aeneus*, *C. gariepinus* and *T. sparrmanii* which are now abundant in the lower Kat and mainstem Great Fish River. In addition, surveys of the Kat River Dam have indicated that the non-native *C. gariepinus*, *L. aeneus* and *M. salmoides* were also present (Potts 2003; Potts et al. 2008). These non-native fish species likely have the potential to invade the sections of Kat River where *S. bainsii* occurs. Invasion of rivers particularly by non-native piscivores such as *C. gariepinus* and *M. salmoides* has been identified as one of the key reasons for the decline in distribution range and population size of *S. bainsii* (Mayekiso 1986; Mayekiso and Hecht 1988a; Cambray 2000, 2007). These species have been widely implicated in the decline of native fish species through predation, competition and habitat degradation (Didham et al. 2007; Kloskowski 2011; van Kessel et al. 2011; Shelton et al. 2015; van der Veer and Nentwig 2015; Gallardo et al. 2016; Pauchard et al. 2016; Van Der Walt et al. 2016). Therefore, if these non-native fish species manage to invade the streams that harbour *S. bainsii* they will likely have a negative influence on the species.

An additional threat to *S. bainsii* population in the Great Fish River system is the presence of major weirs along the profile of the river and the Kat River Dam. *Sandelia bainsii*' distribution range is currently fragmented into several subpopulations due to these impoundments. This fragmentation will likely have a negative impact on the distribution and abundance of *S. bainsii* in the Kat River in the long-term. Studies have shown that small isolated populations are at a much greater extinction rate because of possible loss of genetic diversity due to genetic drift and inbreeding (Frankham et al. 2014; Fitzpatrick et al. 2016; Jang et al. 2017; Coleman et al. 2018). This is likely to increase the risk of extinction of *S. bainsii* as decreasing gene flow will have a long-term effect on the evolutionary potential of this species. Future research in the Great Fish River should focus on the effect fragmentation due to the constructions of weirs have on the genetic diversity and structuring of the population of *S. bainsii*.

Conclusion

The results, therefore, have important implications for the conservation and management of *S. bainsii* in the Great Fish River system. Foremost, because the stronghold population of *S. bainsii* in the system is likely that of the Kat River catchment due to the consistency in its distribution range over the years, it is important to focus conservation prioritise in this catchment. In addition to the catchment being classified a sanctuary; conservation efforts should also include surveys and monitoring programmes. Conserving this catchment will protect other native fish species that are found in this area. Furthermore, in addition to understanding *S. bainsii*' distribution patterns, research is necessary in order to determine the species critical habitat (see Chapter 3) so as to better manage the population in the Great Fish River system.

CHAPTER 3

Distribution and habitat associations of an imperilled narrow range endemic anabantid fish, *Sandelia bainsii*, in the Kat River a tributary of the Great Fish River system, South Africa

Introduction

Understanding species-environmental relationships is important in identifying effective conservation measures in order to minimise the loss of biodiversity (Buisson et al. 2008; Senay et al. 2015; Maceda-Veiga et al. 2017; Sehr and Keckeis 2017; Finch et al. 2018; Gray et al. 2018). In streams and rivers, the distribution limits of freshwater fishes are determined by both abiotic and biotic factors that act simultaneously over spatial and temporal scales (Kadye et al. 2008; Chakona and Swartz 2012; Senay et al. 2015; Kadye et al. 2016; Maceda-Veiga et al. 2017). Abiotic factors include habitat characteristics, both at micro - (e.g. substratum type, depth and flow) and macro-scale (e.g. stream size, order and landscape characteristics) and the physicochemical factors (e.g. dissolved oxygen, pH and temperature) (Kadye et al. 2008; Chakona and Swartz 2012; Schnurr et al. 2014; Holt and Jorgensen 2015), which directly or indirectly impose physiological limits on fish survival (Torresdowdall et al. 2013; Tesfay 2017; de Magalhaes Lopes et al. 2018). Biotic factors include interspecific and intraspecific interactions that influence the persistence of both individual species and that of their community (Crow et al. 2010; Kadye and Booth 2012b; Santos et al. 2018). This basic ecological information is essential for identifying areas that need to be targeted for intervention and protection, managing threats and monitoring population trends of threatened species (Clavero *et al.* 2010; Dubey *et al.* 2012; Ellis *et al.* 2013; Maceda-Veiga 2013).

Sandelia bainsii, commonly known as the Eastern Cape rocky, has been a long-standing flagship species for conservation of aquatic biodiversity in the Eastern Cape Province of South Africa (Cambray 1997a, 2000; Skelton 2001). The species is endemic to the Eastern Cape Province where it has been recorded from seven river systems, namely the Kowie, Great Fish, Keiskamma, Igoda, Buffalo, Gxulu and Nahoon rivers (Mayekiso 1986; Mayekiso & Hecht 1988a; Skelton 2001). Historically, the species was common and

abundant in both the main-stem and tributary sections of these rivers. However, similar to many other regions across southern Africa, river systems in the Eastern Cape Province have been severely modified by multiple anthropogenic impacts that include pollution, non-native species and hydrological modification (Tweddle et al. 2009; Kadye and Booth 2012a, 2013; Ellender and Weyl 2015). As a result, the historical range of *S. bainsii* has declined and is almost extirpated from some river systems such as the Kowie River system (Chakona et al. unpublished) where it was historically common and abundant. The species' IUCN conservation threat status has since increased from Vulnerable in 1988 to Endangered in 1994 and has remained in this highly threatened category thus far (Cambray 2007; Chakona et al. 2018a). Recent and ongoing surveys have shown that the Kowie and Koonap rivers populations of *S. bainsii* are likely at the verge of extinction because they are represented by only a few remaining individuals (Chakona et al. unpublished).

Past ecological studies on *S. bainsii* have indicated that both abiotic and biotic factors are important determinants of the distribution and abundance patterns of this species (Mayekiso 1986; Mayekiso and Hecht 1988a, 1990). Previous studies and historical collection records indicate that *S. bainsii* had a patchy distribution pattern within the river systems where it occurred, and it was largely associated with boulders in slow-flowing pools (Mayekiso 1986; Mayekiso and Hecht 1988b, 1990). These habitats are considered to be important for both refuge and feeding of this species (Mayekiso 1986; Mayekiso and Hecht 1988a, 1990; Cambray 2007). While its interspecific interactions with other native fishes have largely been unexplored, studies seem to infer that interactions with non-native fishes, particularly piscivores such as sharptooth catfish *Clarias gariepinus*, largemouth bass *Micropterus dolomieu* and smallmouth bass *M. salmoides*, have likely had a negative impact that has led to the decline in both its distribution ranges and population sizes (Mayekiso and Hecht 1988a; Cambray 2007). Furthermore, several anthropogenic factors, which have altered habitat integrity, flow regimes and water quality, such as water abstraction, weirs and impoundments, and pollution, have been inferred to have additional restrictions on the distribution of *S. bainsii* (Mayekiso and Hecht 1988a; Cambray 2007).

Recent molecular studies have shown that this species, as it is currently known, contains two divergent lineages that are allopatrically distributed (Chakona et al. unpublished; Roos 2004). One of these newly identified lineages is distributed in the Kowie, Great Fish and the Keiskamma River systems. As the specimens that were used for the original description of *S. bainsii* were collected from the Kowie River system, this lineage

represents *S. bainsii* sensu stricto. The second lineage, *Sandelia* sp. 'bainsii Buffalo' is distributed in the Buffalo and Igoda river systems. The narrow ranges of these lineages increase their risk of extinction. This has implications on the conservation of this species and raises the need to examine ecological aspects that influence the distribution and abundance of this species.

The recent discovery and proposed exploration of shale gas and associated infrastructure development in the Karoo basin adds to the existing threats on the unique biodiversity of the Karoo basin (De Wit 2011; van Tonder et al. 2013; Netshishivhe 2014; Holness et al. 2016). The proposed area encompasses the Kat River which is one of the National Freshwater Ecosystem Priority Areas because it contains the remnant strong-hold population of *S. bainsii* in the Great Fish River (Nel et al. 2011). Studies from elsewhere have shown that shale gas development has profound negative impacts of several aspects of stream function, such as habitat integrity, flow dynamics and water quality (Veil 2010; Gillen and Kiviat 2012; Maloney and Yoxtheimer 2012; Warner et al. 2013).

Persistence of *S. bainsii* in the Kat River is therefore uncertain due to the multiple anthropogenic stressors described above as they have been found to have adverse impacts on freshwater fishes elsewhere. Therefore the aim of this study was to conduct comprehensive surveys in the Kat River to map the distribution of *S. bainsii* and determine the factors that are associated with the species' distribution patterns. This information is important for informing the development of effective conservation and habitat management strategies to prevent loss of biodiversity.

Materials and Methods

Data collection

Extensive sampling of Kat River catchment within the Great Fish River system was conducted from Jan to May 2017. A total of 33 localities (three sites were dry) in the Kat River catchment were surveyed to determine fish distribution, abundance and collect environmental data. Fish were sampled using multiple sampling gears, including two seine nets (3 m length × 3mm mesh size and a 30 m length × 8mm mesh size) and an electrofisher

(SAMUS-725MP). Electrofishing was the mostly used sampling with 80% of sites sampled using it. Seine nets were used on sites with deep pools (0.5 m). The presence and absence of fish species and their abundances was noted. Sampled fishes were identified to species based on Skelton (2001) and counted. For *S. bairdii*, the standard length was measured. All sampled fishes were returned to their localities alive.

Additionally, at each site, GPS coordinates and elevation were recorded using a Global Positioning System (Garmin etrex Ltd) unit to an accuracy of 10 m. Water temperature (°C), conductivity ($\mu\text{S}/\text{cm}$), total dissolved solids (TDS) (ppm), pH and dissolved oxygen (DO) (mg/l) were measured once using a HANNA HI 98129 Combo meter, whereas turbidity (NTU) was measured using a HANNA HI 98703 turbidity meter.

In order to evaluate microhabitat characteristics for each site, 7 to 10 transects were set across the sampled area, perpendicular to the direction of flow to measure the width, depth, length and determine substratum type. Three depth measurements (two the near banks and one at the centre of transect) were taken along each transect using a graduated pole. The substratum type was also characterised at these three points of each transect. Substratum types were qualitatively assessed and classified following a modified Wentworth scale (Cummins 1962; Hynes 1970) as silt (<0.05 cm), sand (0.05–2 cm), gravel (2–10 cm), cobble (10–30 cm), boulder (30–50 cm) and bedrock (>50 cm). Substratum types were expressed as proportions (%) of the total sampled points. The presence or absence of vegetation was also noted along the banks of each transect.

Data analyses

Firstly, each of the physical environmental variables was standardised using a z-scores, calculated as $x_i = (x_i - \bar{x}) / s$, where x_i was the individual measurement for each variable, \bar{x} was the mean, s was the standard deviation and $X = (x_1, x_2, \dots, x_n)$ was the vector for each x_i variable. Therefore, each variable was standardised to normal distribution such that $X \sim N(0,1)$. Predictor variables were standardised in order to allow comparability for predictor variables that were measured in different units (Quinn and Keough 2002). Secondly, environmental and habitat variables were pre-screened for multicollinearity. Multicollinearity occurs when a regression model includes multiple predictor variables that

are correlated (Quinn and Keough 2002). If redundant predictor variables are not removed, they may increase standard errors of the coefficients, which would influence the significant independent predictor variables (Quinn and Keough 2002). Multicollinearity was evaluated by comparing the variance inflation factors (VIF) that were based on a generalised linear regression model. The VIF evaluates how much variance is associated with correlated predictor variables. If none of the predictor variables are correlated, then their VIF will be close to one (Quinn and Keough 2002). For this study, VIF values less than 5 were retained. Six possible explanatory variables used include altitude, conductivity, depth, width, area and dissolved oxygen as they had a VIF of less than 5.

In order to assess the distribution patterns of *S. bairdii* in relation to other species, both native and non-native fishes and environmental variables, a non-metric multidimensional scaling (NMDS) ordination was performed on a binomial distance matrix for the fish data. NMDS is specifically designed to graphically represent relationship between variables (species and environmental parameters) in a multidimensional space (Quinn and Keough 2002). Species data were presented as $n \times p$ (sample \times species) data matrix, where species data were based on presence/absence. Environmental data were presented as $n \times q$ (sample \times environmental variable) data matrix. The model with the smallest stress value was calculated using the meta-MDS function in the MASS and vegan packages of statistical software R. The NMDS stress values that was obtained at the third run was 0.065. The two-dimensional solution of the ordination indicated a sufficient estimate of the spatial distribution matrix as it was less than 0.2.

In order to assess the relationship between the abundance of *S. bairdii* and possible environmental variables, a general linear model (GLM) was used. Electrofishing data were used to estimate abundance for the study species. The standard null model for the distribution of organisms in many ecological studies is that individuals are distributed at random. Therefore, the Poisson model was firstly fitted as it assumes that the mean is equal to variance. If the variance is greater than the mean, or the dispersal parameter (variance/mean) is greater than 1.5 then the model is considered over-dispersed (Zuur et al. 2009). The Poisson model fitted had a dispersal parameter of 13.85, which indicated over-dispersion. Therefore, a negative binomial model was fitted as it accounts for over-dispersion (dispersal parameter = 0.95). All analyses were performed using the statistical software R (version 3.5.1).

Results

Spatial patterns in physicochemical and physical variables

Physico-chemical parameters ranges varied across all sampled sites (Appendix 3). The sampled sites were characterised by a wide range of conductivity (33 to 1618 $\mu\text{S}/\text{cm}$) and total dissolved oxygen (19 ppm to a high 990 ppm). Water pH ranged from neutral (7.83) to basic (10.71) and the water temperature ranged from 20.1 to 30.7 °C. Dissolved oxygen levels ranged from 3.2 to 14.6 mg/l. Water clarity ranged from clear (8.36 NTU) to turbid (720 NTU).

The elevation of the sampled sites ranged from 303 to 1022 m asl (Appendix 3). The sampled sites had variable depth that ranged from 13.7 to 86.3 cm. Similarly, average stream width varied from 2.51 to 21.2 m. Bedrocks and boulders were the most common substrate types in the upper and middle sections of the sampled sites (Figure 10). Silt and gravel were the predominant substrates in the middle and lower sections of the Kat River catchment.

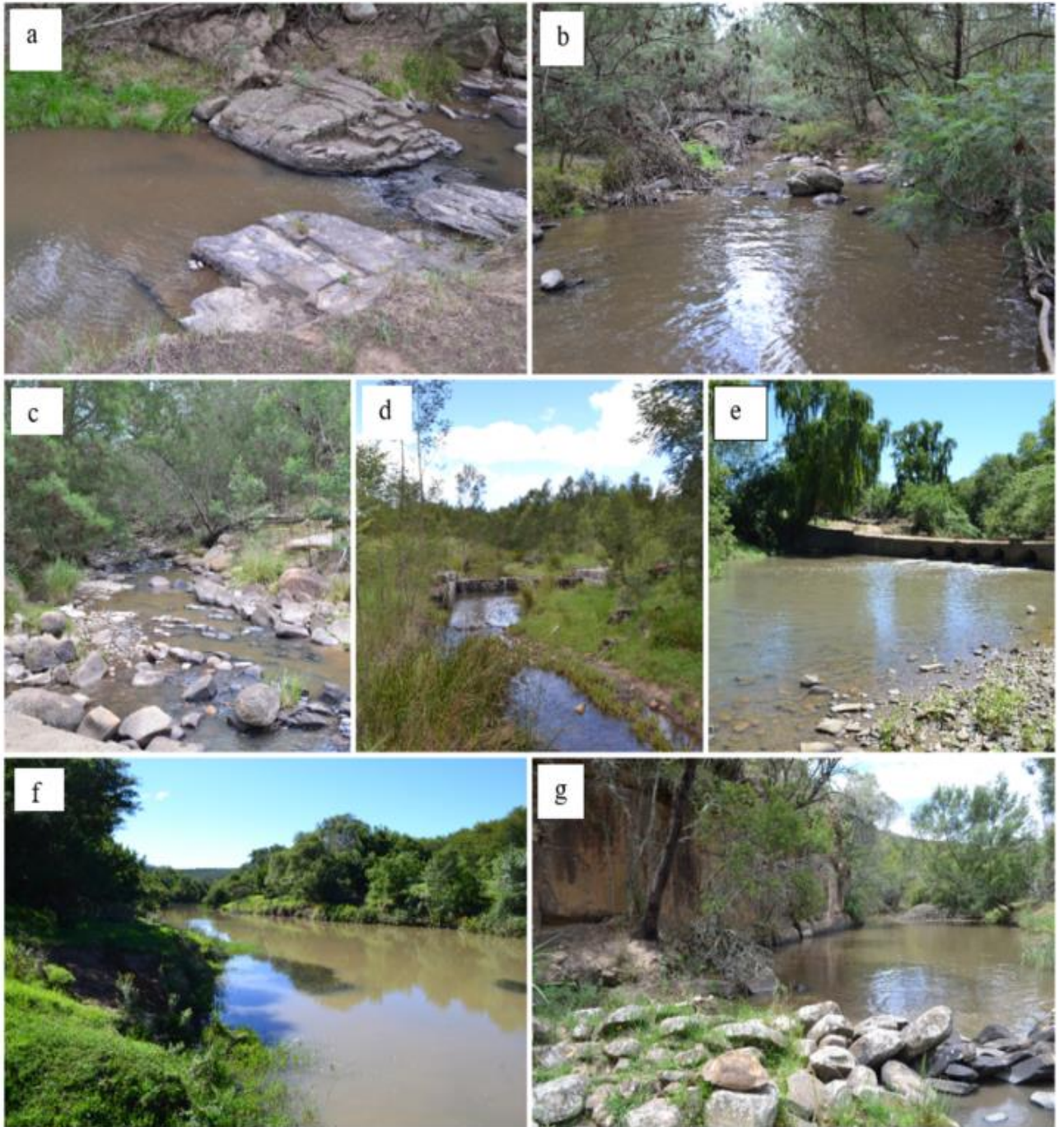


Figure 10: The substrate types at some of the sites where *Sandelia bainsii* occurred. These ranged from boulders to cobbles in the headwaters (a, b, c, d, e and g) and sand to silt (f) in the mainstem of the Kat River catchment.

Fish distribution and population size structure

Sandelia bainsii occurred at 13 out of the 30 sampled localities in the Kat River catchment during the surveys (Table 3). *Sandelia bainsii* only occurred at localities within the middle and upper sections of the Kat River catchment. Within the upper section, the species occurred in only one (Lushington River) out of the three tributaries. In the middle section, it was only found in the Readsdales River, a tributary of the Kat River and the mainstem of the river. Although *S. bainsii* occurred in the upper section of Kat River, it was absent in the other tributaries (Eyre and Elands rivers) that had similar physicochemical and microhabitat. Where it was captured, *S. bainsii*'s numbers ranged from one to 65 individuals at each locality. In addition to *S. bainsii*, four native and three non-native fish species were also sampled. These included *Anguilla mossambica*, *Enteromius anoplus*, *Labeobarbus aeneus*, *Labeo umbratus*, *Clarias gariepinus*, *Tilapia sparrmanii* and *Glossogobius callidus* (Table 3). The Chubbyhead barb *Enteromius anoplus* was the most widespread species, occurring in 80% of sampled sites (Table 3).

The length frequencies showed that *S. bainsii* collected in the Kat River had sizes ranging from 20 to 170 mm (SL) (Figure 11). Different length classes represented the upper and middle sections. The upper section, Lushington River, was represented by the small length classes. Similar to the upper section of the river, the Readsdales River was mostly represented by the small length classes. In comparison to the Lushington and Readsdales rivers, the mainstem of the Kat River was represented by all length classes.

Table 3: Sites sampled in the Kat River catchment and the different fish species caught during the 2017 surveys with their abundances. Non-native fish species are indicated by *. Elec = Electrofishing and Seine = Seine net.

River	Co-ordinates	Altitude	<i>S.</i> <i>bainsii</i>	<i>E.</i> <i>Anoplus</i>	<i>L.</i> <i>umbratus</i>	<i>G.</i> <i>callidus</i>	<i>A.</i> <i>mossambica</i>	* <i>C.</i> <i>gariepinus</i>	* <i>T.</i> <i>sparrmanii</i>	* <i>L.</i> <i>aeneus</i>	<i>Fishing</i> <i>method</i>
Lushington	32°37'54.7"S 26°51'57.6"E	948	1	0	0	0	0	0	0	0	Elec
Lushington	32°37'43.9"S 26°50'0.5"E	892	6	6	0	0	0	0	0	0	Elec
Lushington	32°37'27"S 26°49'36.8"E	872	28	6	0	0	0	0	0	0	Elec
Eyre	32°30'26.2"S 26°47'33"E	850	0	125	0	0	0	0	0	0	Elec
Lushington	32°36'58"S 26°49'01.9"E	846	17	0	0	0	0	0	0	0	Elec
Readsdale	32°28'21.5"S 26°43'33.8"E	841	0	0	0	0	2	0	0	0	Elec
Elands	32°33'33.3"S 26°48'47.1"E	820	0	111	0	0	0	0	0	0	Elec
Eyre	32°31'6.1"S 26°47'19.9"E	807	0	42	0	0	0	0	0	0	Elec

Table 3 continued

River	Co-ordinates	Altitude	<i>S.</i> <i>bainsii</i>	<i>E.</i> <i>Anoplus</i>	<i>L.</i> <i>umbratus</i>	<i>G.</i> <i>callidus</i>	<i>A.</i> <i>mossambica</i>	* <i>C.</i> <i>gariepinus</i>	* <i>T.</i> <i>sparrmanii</i>	* <i>L.</i> <i>aeneus</i>	<i>Fishing</i> <i>method</i>
Kat	32°33'51.3"S 26°45'48.4"E	777	0	51	0	0	0	0	0	0	Elec
Lushington	32°35'34.2"S 26°47'34.2"E	773	47	0	0	0	0	0	0	0	Elec
Eyre	32°32'48.4"S 26°46'29.3"E	762	0	60	0	0	1	0	0	0	Elec
Buxton	32°34'5.4"S 26°37'13.4"E	754	0	12	0	0	0	0	0	0	Elec
Readsdale	32°30'36.9"S 26°44'33.7"E	688	65	33	0	0	0	0	0	0	Elec
Buxton	32°34'31.2"S 26°39'16.3"E	663	0	13	0	0	0	0	0	0	Elec
Kat	32°34'35.5"S 26°45'22.2"E	659	0	55	1	3	1	0	0	0	Elec
Kat	32°34'36.3"S 26°43'20.5"E	639	10	39	1	0	0	0	0	0	Elec

Table 3 continued

River	Co-ordinates	Altitude	<i>S.</i> <i>bainsii</i>	<i>E.</i> <i>Anoplus</i>	<i>L.</i> <i>umbratus</i>	<i>G.</i> <i>callidus</i>	<i>A.</i> <i>mossambica</i>	* <i>C.</i> <i>gariepinus</i>	* <i>T.</i> <i>sparrmanii</i>	* <i>L.</i> <i>aeneus</i>	<i>Fishing</i> <i>method</i>
Kat	32°34'33.5"S 26°43'20.9"E	637	14	10	9	1	0	0	0	0	Elec
Balfour	32°32'10.1"S 26°40'38.3"E	631	0	161	0	0	0	0	0	0	Elec
Kat	32°32'37.8"S 26°42'44"E	609	7	8	0	32	0	0	0	0	Elec
Buxton	32°33'16.6"S 26°40'17.8"E	598	0	41	0	0	2	0	0	0	Elec
Kat	32°33'49.8"S 26°40'38.2"E	577	17	42	0	60	2	0	0	0	Elec & seine
Kat	32°36'57.9"S 26°40'42.4"E	546	37	109	188	97	0	0	0	0	Seine
Kat	32°38'29.7"S 26°41'16.1"E	540	1	0	10	5	0	0	0	0	Elec & seine
Kat	32°38'35.2"S 26°39'46.6"E	512	1	2	0	31	0	0	0	0	Elec

Table 3 continued

River	Co-ordinates	Altitude	<i>S.</i> <i>bainsii</i>	<i>E.</i> <i>Anoplus</i>	<i>L.</i> <i>umbratus</i>	<i>G.</i> <i>callidus</i>	<i>A.</i> <i>mossambica</i>	<i>*C.</i> <i>gariepinus</i>	<i>*T.</i> <i>sparrmanii</i>	<i>*L.</i> <i>aeneus</i>	<i>Fishing</i> <i>method</i>
Kat	32°42'17.8"S 26°36'15.5"E	473	0	0	4	26	0	0	0	0	Elec
Kat	32°42'55.9"S 26°35'17.6"E	464	0	12	8	430	7	2	1	0	Elec & seine
Kat	32°53'11.5"S 26°42'24.2"E	314	0	0	0	10	0	2	1	2	Elec
Kat	32°46'40.3"S 26°38'45.9"E	312	0	0	0	0	0	10	0	0	Elec
Kat	32°52'49.9"S 26°42'46.9"E	303	0	0	0	0	0	1	0	0	Elec

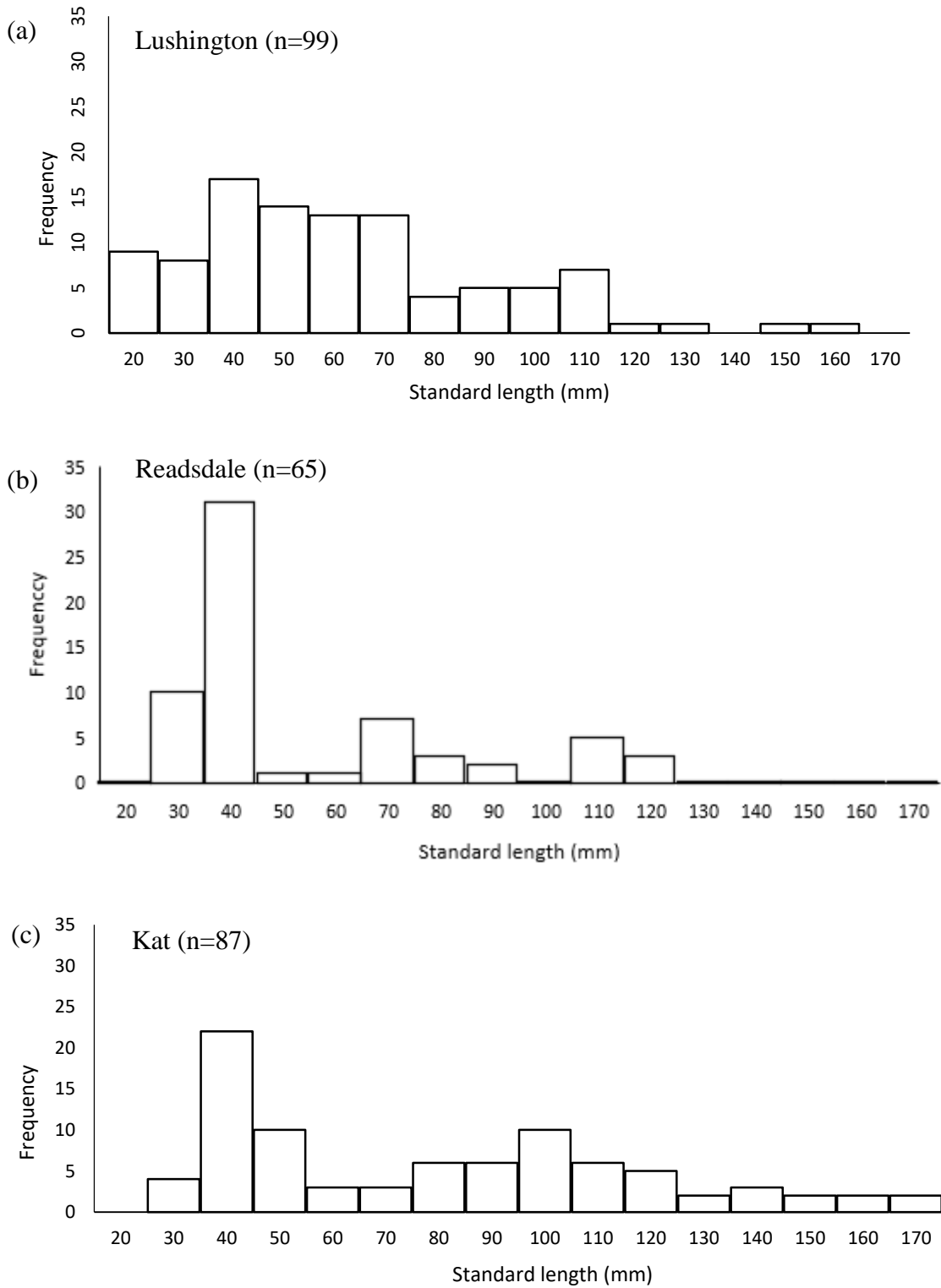


Figure 11: Length frequency graphs for *Sandelia bainsii* based on individuals captured using all sampling methods in the (a) Lushington River, (b) Readsdale River and (c) mainstem section of the Kat Rivers.

Exploratory distribution pattern between fish species and environmental variables

The non-metric multidimensional scaling ordination had a small residual value (stress value = 0.065), indicating a good consistency between the spatial distribution and distance among variables. The ordination showed that *S. bainsii* co-occurred with *Enteromius anoplus* in the upper section and with *Labeo umbratus* and *Glossogobius callidus* in the middle section of the Kat River catchment (Figure 12 & 13). In the upper section, *S. bainsii* was associated with low conductivity, shallow and smaller streams. At the middle section, *S. bainsii* was associated with habitats that were much deeper and wider (Figure 12). *Sandelia bainsii* was not found in association with the non-native species, *Labeobarbus aeneus*, *Clarias gariepinus* and *Tilapia sparrmanii* (Figure 14). Sites with these non-native fish species were characterised by high conductivity, and these were at the lower sections of the Kat River catchment (Figure 12).

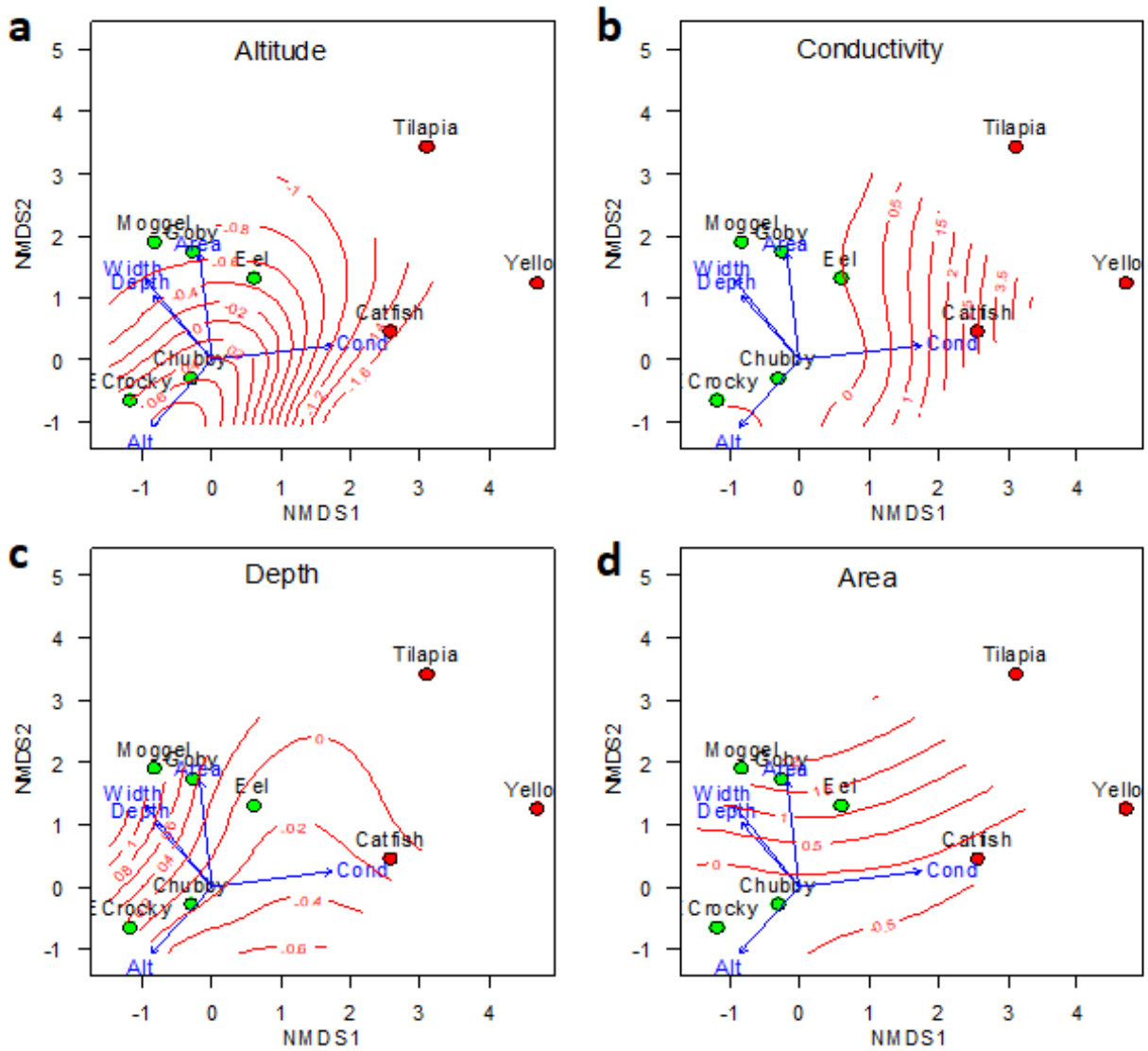


Figure 12: Non-metric multidimensional scaling (NMDS) ordinations of the spatial distribution of fish species in relation to altitude (a), conductivity (b), depth (c) and area (d) in the Kat River catchment. The green circles represent native and red circles represent non-native fish species. The surfaces fitting (red lines) represent the different environmental parameters with values indicating a decline or increase in the parameters. The blue lines (vectors) represent the different environmental parameters. EC rocky *Sandelia bainsii*, chubby *Enteromius anoplus*, moggel *Labeo umbratus*, goby *Glossogobius callidus*, yellowfish *Labeobarbus aeneus*, catfish *Clarias gariepinus*, and tilapia *sparrmanii*.

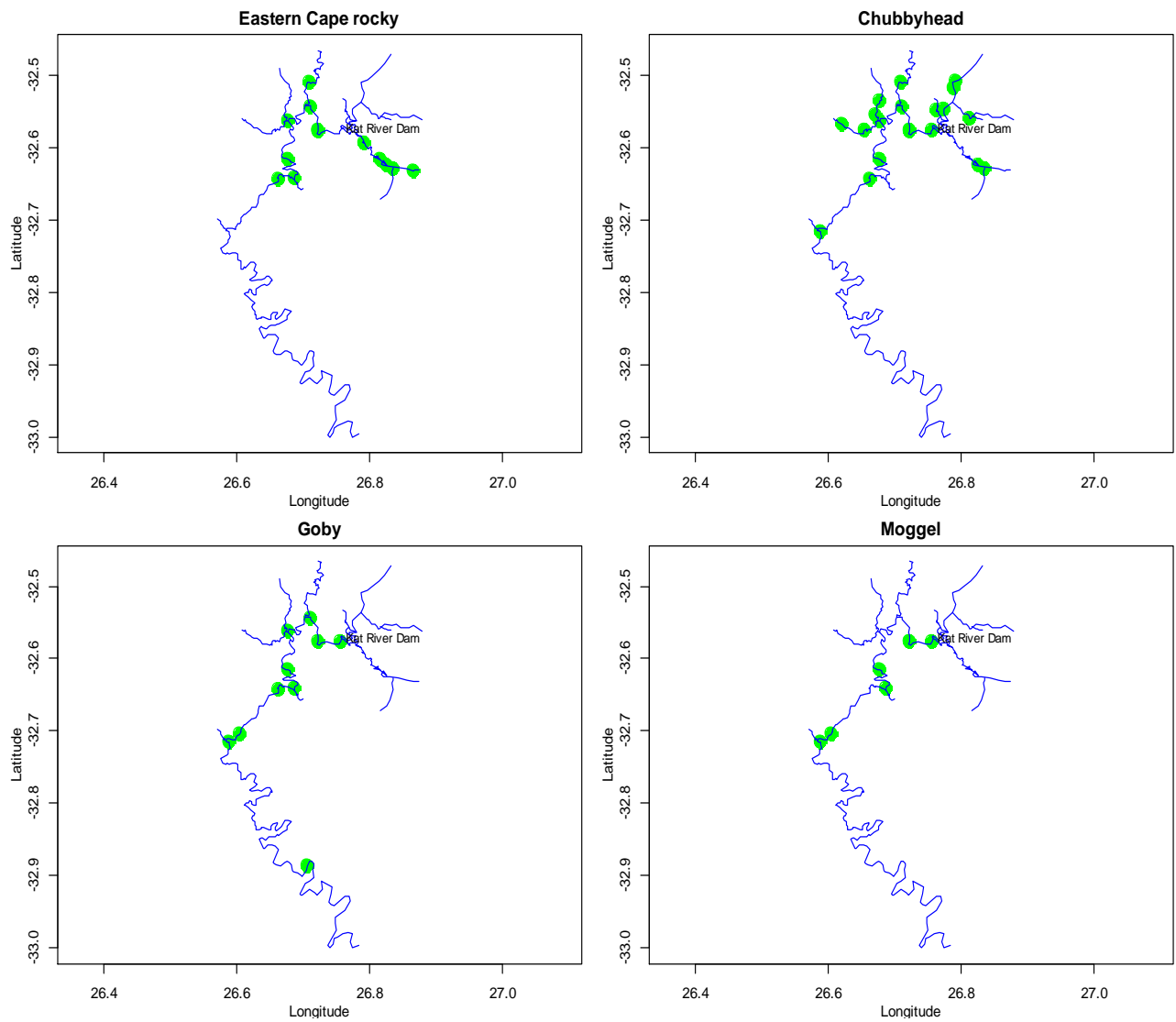


Figure 13: *Sandelia bainsii* co-occurred with three native fish species, chubbyhead barb *Enteromius anoplus*, moggel *Labeo umbratus* and river goby *Glossogobius callidus* in the upper and middle sections of the Kat River catchment. Bubbles represent where the different fish species occurred.

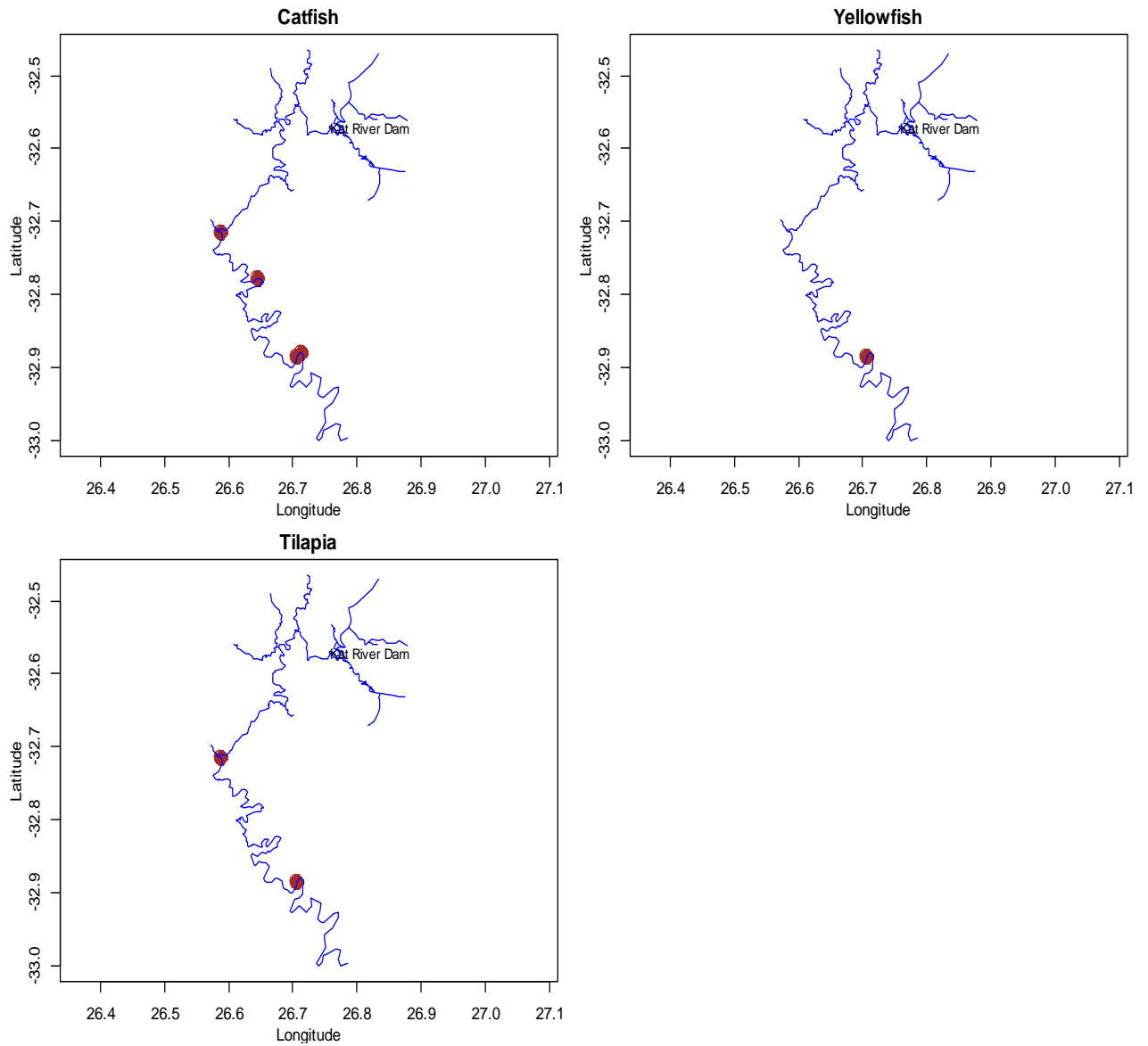


Figure 14: Non-native fish species, smallmouth yellowfish *Labeobarbus aeneus*, sharptooth catfish *Clarias gariepinus*, and banded tilapia *Tilapia sparrmanii*, occurred in the middle and lower sections of the Kat River catchment. Bubbles represent where the different fish species occurred.

Relationship between Sandelia bainsii's abundance and environmental variables

The negative binomial GLM demonstrated that out of the environmental variables that were sampled, *S. bainsii's* abundance was negatively associated with conductivity (Table 6). Other environmental variables (altitude, depth, width, area and dissolved oxygen) used in the regression did not have a significant association with *S. bainsii's* abundance.

Table 4: Summary of negative binomial GLM outcome of *S. bainsii's* abundance in relation to altitude, conductivity, depth, width, area, and dissolved oxygen at the Kat River catchment. Standard error is represented by Std error.

	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-3.59	1.49	-2.42	0.02*
Altitude	-0.85	0.79	-1.08	0.28
Conductivity	-16.46	4.33	-3.80	<0.05**
Depth	-0.10	0.71	-0.15	0.88
Width	0.77	0.83	0.92	0.36
Area	-0.44	0.66	-0.65	0.52
Dissolved Oxygen	-0.01	1.11	-0.01	1.00

Significant codes: 0 '***' 0.05 '**'

Discussion

Results from the present study indicated that *Sandelia bainsii* was confined to the middle and upper sections of the Kat River and two of its tributaries, the Lushington and Readsdales rivers. The middle and upper sections of the catchment were less influenced by anthropogenic factors when compared to the lower sections. This pattern is consistent with findings from other studies that have shown that remnant populations of threatened native fishes now only persist in the least impacted upper rivers in the CFE (Clark et al. 2009; Ellender et al. 2011; Chakona and Swartz 2012; Kadye and Booth 2012b; Weyl et al. 2014; Ellender and Weyl

2015; Sterling and Warren 2017). The headwater sections of the Kat River should therefore be prioritised for protection to prevent non-native fish and habitat degradation as it represents an important sanctuary for the Eastern Cape rocky (Nel et al., 2011).

In the Kat River, the habitat associations of *S. bainsii* was characterised by a broad range of physicochemical and microhabitat variables. Across its distribution range (middle and upper mainstem and headwater tributaries), *S. bainsii* appeared to show no specific microhabitat preferences. In contrast to this study, previous studies have shown that *S. bainsii* was associated with rocky and shallow habitats in other systems (Mayekiso 1986; Mayekiso and Hecht 1988a, 1990, Cambray 1997a, 2000; Skelton 2001). The lack of association between *S. bainsii* with the recorded variables observed in the Kat River likely indicates that *S. bainsii* is a habitat generalist. This is because it was able to utilise a wide range of habitats, from shallow and rocky to deeper and sandy streams. Studies elsewhere have shown that habitat generalist fish species are able to utilise different habitats in order to survive (Hamilton et al. 2017; Chen and Olden 2018). Additionally, studies have inferred that habitat generalist fish species found in headwater streams are able to utilise a wide range of habitat as part of their life cycle stages (Meyer et al. 2007; Hamilton et al. 2017; Turschwell et al. 2017), this is probably the case with *S. bainsii* in the Kat River catchment.

Although *S. bainsii* was distributed across a broad range of physicochemical variables, the negative binomial GLM model indicated that the abundance of the species was negatively associated with increasing conductivity. In the present study, conductivity is likely to be a surrogate or an indicator of increasing pollution in the lower sections of the Kat River due to domestic effluent and other diffuse sources from settlements along the course of the river. Pollution in riverine ecosystems is known to alter dissolved oxygen, conductivity, dissolved organic and inorganic matter of the water and further increase ions on the system (Malaj et al. 2014; Moore et al. 2017; Diamantini et al. 2018). Additionally, pollution affects aquatic biodiversity through increased mortality due to acute toxicity and bioaccumulation, degraded water quality (e.g. eutrophication and sedimentation) and reduced fitness (developmental and reproductive irregularities) (Mulk et al. 2016; Karaouzas et al. 2018; Schmeller et al. 2018). This is likely to cause local extirpation on vulnerable fish species. An example, in the southern Murray-Darling Basin of southern Australia, pollution and other anthropogenic impacts led to the deterioration in water quality, which in turn caused local extirpation of the southern purple-spotted gudgeon *Mogurnda adspersa*. Therefore, the

increasing pollution in the parts of the Kat River catchment has likely contributed to the decline in the abundance of *S. bainsii*.

While *S. bainsii* occurred in some parts of the upper sections of the Kat River, its absence from the upper tributaries, Eyre and Elands rivers was unexpected because these tributaries appeared to have similar habitats as those where the study species was present. In addition, the historical distribution records of *S. bainsii* dating back to 1961 (see chapter 3) indicated that the species was not caught in those tributaries. The absence of this species in some tributaries is likely due to possible micro-climatic and hydrological differences due to the unevenly distributed rainfall (Jari and Fraser 2009; Cochet et al. 2015) in the Kat River catchment. Therefore, further studies in the catchment need to investigate the micro-climatic and hydrological differences in order to better understand the absence of the species in those tributaries.

These results indicate that anthropogenic factors may have potentially had an impact on the distribution and abundance of *S. bainsii* and may be responsible for the lower limit of the current distribution in the Kat River catchment. Below the lower limit of *S. bainsii*, a number of potential impacts were observed during the surveys. These include habitat degradation due to weirs, invasion by non-native fish species, banded tilapia *T. sparrmanii* and sharptooth catfish *C. gariepinus*, and pollution due to agricultural activities and the town of Fort Beaufort. These multiple impacts are likely limiting the lower distribution of *S. bainsii* in the catchment by creating unsuitable habitats for the species. Accumulating evidence from other studies have shown similar patterns for many river systems across southern Africa (Clark et al. 2009; Tweddle et al. 2009; Chakona and Swartz 2012) and other regions (Habit et al. 2010; Strayer and Dudgeon 2010; Ellis et al. 2013).

Conclusion

Relatively few studies have examined the habitat associations of *S. bainsii*, a necessary approach needed when the objective is to advise on management action in order to protect and restore the remaining population. The habitat association of *S. bainsii* provides new findings for the species. The species is a habitat generalist and was able to utilise a wide range of habitats. This is in contrary to previous studies in other systems that have indicated that *S. bainsii* was associated with shallow and rocky streams. The study further showed that

S. bainsii in the Kat River was confined to the middle and headwater streams of the catchment, an important area for the conservation of this species. Therefore, conservation measures in the Kat River should focus on protecting and maintaining the catchment where the species occurs.

CHAPTER 4

General Discussion

Overview

The present thesis has identified the distribution of *Sandelia bainsii* in the Great Fish River system to be confined in the Koonap and Kat rivers. This distribution is similar to historical records from the SAIAB database except for one locality in the Koonap River, where the species was not present (see Chapter 2). In the Koonap River catchment, *S. bainsii* persist only at two localities in the eNyara River. The Koonap River catchment appears to be affected by a combination of anthropogenic factors, including pollution, hydrological modification and invasive species. In the Kat River, all size classes of *S. bainsii* were collected in the upper mainstem and tributaries of the catchment. These results indicate that *S. bainsii* maintained a viable population in the catchment. Additionally, *S. bainsii* was able to utilise a wide range of habitats in the Kat River catchment (see Chapter 3). Therefore, in the Great Fish River, it is important to focus conservation prioritise in the Kat River catchment as it has the stronghold population of *S. bainsii*.

In the Kat River, *S. bainsii* was confined to the upper sections and tributaries of the catchment. As a result, these sections are critically important in conserving the remaining population of *S. bainsii* in the Great Fish River system. The Kat River catchment, above Fort Beaufort, has been classified as a sanctuary for endangered species as part of the freshwater ecosystem priority areas in South Africa (Nel et al. 2011). Therefore, the stronghold population of *S. bainsii* confirms and supports the importance of designation the upper sections of the Kat River as a sanctuary. Additionally, in the Kat River catchment, the native *L. umbratus* likely represents the genetic integrity of the population at the Great Fish River as it was isolated before the Sundays-Fish inter-basin water transfer scheme (Ramoejane 2011). Therefore, *S. bainsii* and *L. umbratus* are primarily restricted to the upper sections of the Kat River catchment, which warrants their conservation and management.

Conservation measures needed for persistence of Sandelia bainsii in the Kat River catchment

Appropriate conservation and management strategies for *S. bainsii* in the Kat River catchment is the continuous protection of the species habitats. The population of *S. bainsii* in the Kat River seem to be protected from major pollution and expansion of non-native fish species by weirs constructed in the catchment. However, these weirs need to be monitored as they will likely contribute to further fragmentation of native fish species in the catchment, including *S. bainsii*.

Additionally, non-native fish species in the Katriver dam need to be controlled, as they have the potential to expand into the upper section of the river where *S. bainsii* occurs. The eradication of established fish species can be costly and at times impractical. Many projects in South Africa and elsewhere have used rotenone, a piscicide, to successfully eradicate non-native fish species (Weyl et al. 2014, 2016a; Donaldson and Cooke 2016; Sandodden et al. 2018). While the advantage of using rotenone is complete eradication, it can also kill native fish species. Therefore, in the case of the Katriver dam, eradication using rotenone is not feasible due to the native fish species, including *S. bainsii*, downstream of the dam. An ideal solution is the construction of weirs that will act as a barrier to reduce potential invasion upstream and downstream where *S. bainsii* occurs. But such barriers will need to be scientifically tested and monitored so as to not further fragment the population of *S. bainsii* and other native species in the catchment. In addition to these barrier weirs, eradication using physical methods (e.g. fishing) and management against further invasion and impacts should also be part of the conservation strategies.

Continuous monitoring of *S. bainsii* in the Kat River catchment should include certain objectives: firstly, evaluating the size structures and population numbers of the species in order to detect changes early. Secondly, assessing and protecting the habitat which the species occupies from pollution and habitat degradation. Lastly, seasonal surveys are needed in order to evaluate the influence of microclimate on the distribution and abundance of the species.

Education and awareness of *S. bainsii* through social media, public engagement, posters along the species distribution ranges and details pertaining to the anthropogenic impacts on the species are important for the conservation strategies of this species. These should be tailored in such a way that they include the impacts to the species and what are the likely consequences if not conserved. Furthermore, the public should be made aware of the

consequences of pollution, introduction of non-native fish species and the illegal construction of weirs on the population *S. bainsii* and other native fish species.

Future research on Sandelia bainsii in the Great Fish River system

Sandelia bainsii' distribution range in the Kat River catchment is currently separated into several small subpopulations due to the constructions of impoundments such as weirs and the Katriver dam. Surveys of the Kat River catchment have suggested that there are approximately three weirs constructed per km and that the lower catchment had approximately +/- 25 weirs (Farolfi et al. 2005; Birkholz 2009). Studies have shown that species with small isolated distribution ranges that are fragmented have led to the decline in population and local extinction in other regions (Frankham et al. 2014; Fitzpatrick et al. 2016; Jang et al. 2017; Coleman et al. 2018). Therefore, future studies need to first assess the genetic diversity within *S. bainsii* in the Great Fish River system. Secondly, assess the consequences of fragmentation on the genetic diversity and structure of *S. bainsii*. This is important as: (i) effective strategies can be implemented to stop or at least slow the process of extinction resulting from these causes, (ii) information on relationships of currently fragmented subpopulations are useful for evaluating the population's status, and identifying the most endangered subpopulation in order to allocate resources. Therefore, research using sensitive molecular techniques such as microsatellites is required to determine the effects of fragmentation on the genetic diversity and structuring of the population of *S. bainsii* in order to effectively manage the species.

In the Karoo basin, the planned development for shale gas exploration adds a potential future threat to the remaining population of *S. bainsii* at the Kat River catchment. Holness et al. (2016) mentioned the impacts the planned developments would likely have on the Karoo basin. These include habitat loss, water pollution/contamination, spread of invasive alien species, fragmentation of natural habitat and changes in water flow patterns. Therefore, the developments in the Karoo basin needs to take into consideration the population of *S. bainsii* and its habitat in the Kat River. Future studies should focus on how the planned development will influence the catchment so that effective prevention measures can be put in place.

Furthermore, a dietary analysis of *S. bainsii* using stable isotopes is also needed as it would provide insight on how the species is able to utilise a wide range of habitats in terms of food sources. This is an important component in the conservation of *S. bainsii*. Efforts should

be made to protect this species, through enforcing the recommendations from this study and previous studies (Cambray 1997b, 2000; Nel et al. 2011). In order for all the mentioned management strategies to be effective, they need to be enforced by both conservation managers and the public sector.

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Appendix

Appendix 1: Historical data retrieved from the SAIAB database (Specify) showing sampled localities in the Great Fish River system from 1961 to 2005. *Sandelia bainsii* was recorded from only 14 localities (✓) and was absent (×) from 50 localities.

River	Locality Description	Latitude	Longitude	Present/Absent	Date
Kat	Katberg	-32.52	26.63	✓	1961
Kat	Katberg	-32.52	26.63	×	1961
Kat	Fort Beaufort	-32.63	26.68	✓	1972
Koonap	Adelaide; crosses Post Retief	-32.53	26.36	×	1972
Great	Hunts Drift	-33.23	26.98	×	1976
Koonap	Fort Beaufort District; Edge Hill	-32.93	26.52	×	1980
Koonap	Fort Beaufort District; Edge Hill	-32.933	26.85	×	1980
Little Fish	Pump Station, pool above weir	-32.99	25.69	×	1981
Koonap	Edgehill Farm	-32.95	26.51	✓	1981
Great	National Road bridge	-33.23	26.98	×	1981
Blinkwater	Heraldtown	-32.60	26.55	×	1981
Koonap	Driefontein; Headwaters	-32.43	26.60	×	1981
Koonap	Spioenkop; Below road bridge	-32.48	26.47	×	1981
Blinkwater	Upstream Fairview Heraldtown	-32.52	26.53	×	1981
Koonap	Elizabeth Moorcroft bridge	-32.50	26.35	×	1981

Appendix 1 continued

River	Locality Description	Latitude1	Longitude1	Present/Absent	Date
Kat	Orangefarm; Seymour	-32.59	26.69	✓	1983
Kat	Thorny Croft, Outside Seymour;	-32.60	26.80	✓	1983
Kat	Causeway on Gonzana Farm	-32.69	26.61	×	1983
Kat	Next to river on Milbank Farm	-32.74	26.59	×	1983
Kat	Causeway on Gonzana Farm	-32.69	26.61	×	1983
Kat	Next to river on Milbank Farm	-32.74	26.59	×	1983
Kat	FarmLorraine; F.Beaufort	-32.64	26.66	✓	1983
Kat	Katberg Pass/Fairbairn road	-32.53	26.71	×	1983
Mankazana	Farm Amherst; Seymour/Fort	-32.64	26.68	✓	1983
Koonap	Thornhill Farm	-32.58	26.45	×	1983
Koonap	weir- Adelaide/Balfour	-32.50	26.36	×	1983
Koonap	Langdraai Balfour/Adelaide	-32.53	26.35	×	1983
Elands	Alice/Hogsback road	-32.54	26.92	×	1983
GFR	Bedford/Cradock road bridge	-32.59	25.89	×	1983
Nyara	Herberts Hope Farm	-32.81	26.11	✓	1983
GFR	Cradock/Graaff-Reinet bridge	-32.08	25.41	×	1983
GFR	Great Fish River mouth	-33.53	27.10	×	1983

Appendix 1 continued

River	Locality Description	Latitude1	Longitude1	Present/Absent	Date
Koonap	Adelaide/Balfour road bridge	-32.49	26.47	×	1983
GFR	Orange/Great Fish tunnel outlet	-31.42	25.64	×	1983
GFR	Double Drift	-33.08	26.78	×	1985
GFR	Double Drift	-33.08	27.78	×	1986
GFR	Mayfair Farm	-33.08	26.37	×	1991
GFR	Stream in Kudu Reserve	-33.10	26.66	×	1995
Kat	Sheshego dam	-32.92	26.77	×	1997
Kat	Kat river	-32.58	26.74	×	1997
Kat	Site 2 - Kat river	-32.54	26.71	×	1997
GFR	Committees drift	-33.17	26.83	×	1998
Kat	Site 1: Kat River	-32.55	26.72	×	1999
Kat	Below weir	-32.57	26.68	×	1999
Kat	Kat River	-32.54	26.71	×	1999
Kat	Kat River	-32.51	26.79	×	1999
Kat	Fort Beaufort	-32.78	26.63	×	1999
Kat	Kat River	-32.72	26.58	×	1999
Kat	Gonzana Bridge	-32.68	26.63	×	1999

Appendix 1 continued

River	Locality Description	Latitude1	Longitude1	Occurrence	Date
Nyara	Eye White's Farm	-32.83	26.15	✓	2001
Kat	Eyre Stream	-32.52	26.79	×	2003
Kat	Kat River	-32.58	26.73	×	2003
Kat	Kat River	-32.56	26.69	✓	2003
Kat	Lushington	-32.62	26.82	✓	2003
Kat	Kat River	-32.60	26.68	✓	2003
Kat	Kat River	-32.65	26.65	×	2003
Kat	Kat River	-32.69	26.61	×	2003
Kat	Kat River	-32.77	26.61	×	2003
Kat	Kat River	-32.79	26.65	×	2003
Kat	Kat River	-32.84	26.67	×	2003
Kat	Kat River	-32.89	26.68	×	2003
Kat	Kat River	-32.97	26.75	×	2003
Kat	Kat River at Amhurst Farm	-32.62	26.69	×	2005
Kat	Kat River at Charlesgrove Farm	-32.89	26.72	×	2005
Kat	Kat River at Hertzog Farm	-32.57	26.72	✓	2005
Kat	Balfour River	-32.56	26.67	✓	2005
Kat	Kat River at Hertzog Farm	-32.57	26.72	×	2005

Appendix 2: Localities sampled in the Great Fish River system as part of surveys from 2009 to 2017 showing localities that were positive and negative for *Sandelia bainsii*.

River	Locality description	Latitude	Longitude	Present/Absent	Year
Great Fish	Kap	-33.48	27.09	×	2009
Great Fish	Double Drift	-33.089	26.78	×	2009
Great Fish	Koonap	-33.05	26.66	×	2009
Great Fish	Middleton1	-32.95	25.82	×	2009
Great Fish	Middleton2	-32.84	25.82	×	2009
Great Fish	Holmes	-33.01	25.52	×	2009
Great Fish	Brak	-31.89	25.44	×	2009
Great Fish	Fish	-31.92	25.42	×	2009
Great Fish	Tarka	-32.22	25.93	×	2009
Great Fish	Pauls	-32.05	25.46	×	2009
Great Fish	Fish	-31.92	25.31	×	2009
Great Fish	LF1	-33.42	27.05	×	2009
Great Fish	LF2	-33.40	27.02	×	2009
Great Fish	Hanshoek	-33.26	26.97	×	2010
Great Fish	Double Drift	-33.09	26.78	×	2010

✓ Present, × Absent

Appendix 2 continued

River	Locality description	Latitude	Longitude	Present/Absent	Year
Great Fish	Double Drift	-33.09	26.78	×	2010
Great Fish	Middleton1	-32.95	25.82	×	2010
Great Fish	Middleton2	-32.84	25.82	×	2010
Great Fish	Holmes	-33.01	25.52	×	2010
Great Fish	Brak	-31.89	25.44	×	2010
Great Fish	Fish	-31.92	25.42	×	2010
Great Fish	KE4	-32.67	26.44	×	2010
Great Fish	KE5	-33.66	26.44	×	2010
Great Fish	KE6	-32.67	26.44	×	2010
Great Fish	KE7	-32.53	26.36	×	2010
Great Fish	KE8	-32.53	26.35589	×	2010
Great Fish	KE9	-32.50	26.37	×	2010
Great Fish	KE10	-32.50	26.36	×	2010
Great Fish	KE11	-32.50	26.37	×	2010
Great Fish	KE12	-32.50	26.40	×	2010
Great Fish	KE13	-32.47	26.24	×	2010
Great Fish	KE14	-32.47	26.24	×	2010
Great Fish	KE15	-32.47	26.24	×	2010

✓ Present, × Absent

Appendix 2 continued

River	Locality description	Latitude	Longitude	Present/Absent	Year
Great Fish	KE16	-32.47	26.24	×	2010
Great Fish	KE17	-32.47	26.24	×	2010
Great Fish	KE18	-32.47	26.24	×	2010
Great Fish	KE19	-32.47	26.24	×	2010
Great Fish	KE20	-32.47	26.24	×	2010
Great Fish	KE21	-32.51	26.26	×	2010
Great Fish	KE22	-32.52	26.25	×	2010
Great Fish	Koonap	-32.66	26.27	×	2014
Great Fish	Koonap	-32.70	26.31	×	2014
Great Fish	Koonap	-32.87	26.35	×	2014
Kat	Lushington River	-32.62	26.83	✓	2017
Kat	Low bridge on your way to Hogsback	-32.63	26.83	✓	2017
Kat	1.28 km away from bridge to Hogsback	-32.62	26.82	✓	2017
Kat	Drift on the road to Michelle's pass	-32.56	26.82	×	2017
Kat	871 m away from dry site on Eland River	-32.56	26.81	×	2017
Kat	Drift at the pump house	-32.55	26.80	×	2017
Kat	On transect under the bridge	-32.55	26.78	×	2017
Kat	Under bridge on road to Nicomaline	-32.52	26.79	×	2017

✓ Present, × Absent

Appendix 2 continued

River	Locality description	Latitude	Longitude	Present/Absent	Year
Kat	1.28 up road from broken bridge	-32.51	26.79	×	2017
Kat	Bridge on road to Queenstown/Seymour	-32.56	26.76	×	2017
Kat	Bridge on road to Seymour	-32.58	26.72	✓	2017
Kat	Bridge on road to Seymour 2	-32.58	26.722	✓	2017
Kat	Bridge on road to Balfour	-32.56	26.68	✓	2017
Kat	Bridge at Fairbairn	-32.54	26.71	✓	2017
Kat	Readsdale River at Fairbairn on road to Katberg	-32.51	26.74	✓	2017
Kat	Low water bridge to Katberg next to farmers	-32.47	26.73	×	2017
Kat	Kat River next to small village on road to Seymour	-32.62	26.68	✓	2017
Kat	Low water bridge dirty road to Alice (Venture area)	-32.64	26.69	✓	2017
Kat	Bridge on road to Fort Beaufort	-32.65	26.69	×	2017
Kat	Low water bridge dirty road to Alice (Venture area)	-32.64	26.66	✓	2017
Kat	Blinkwater on road to Fort Fordyce	-32.71	26.58	×	2017
Kat	Mcelo tributary on Jannie's farm	-32.88	26.71	×	2017
Kat	Below weir on Jannie's farm	-32.89	26.71	×	2017
Kat	Low water Bridge on dirty road from Beaufort/Alice	-32.78	26.65	×	2017
Kat	Low water bridge on confluence of the Blinkwater	-32.72	26.59	×	2017
Kat	Road to Maqoma's place below the weir	-32.56	26.67	×	2017

✓ Present, × Absent

Appendix 2 continued

River	Locality description	Latitude	Longitude	Present/Absent	Year
Kat	Low water bridge after Maqoma's place	-32.58	26.66	×	2017
Kat	Drift on dirty road in Barkstine	-32.57	26.62	×	2017
Kat	Next to central police station	-32.54	26.68	×	2017
Kat	Next to school around blink water area	-32.71	26.60	×	2017
Kat	Bridge above Katriver dam	-32.59	26.79	✓	2017
Kat	After Mzimkhulu secondary school	-32.63	26.87	✓	2017
Koonap	Below Kartriver dam	-32.58	26.76	×	2017
Koonap	Bridge upstream of Vulindlela School	-32.69	26.308	×	2017
Koonap	Low bridge on Benskraal	-32.56	26.40	×	2017
Koonap	Low water bridge at Waterfalls	-32.53	26.36	×	2017
Koonap	Low water bridge on road to Post Retief	-32.50	26.40	×	2017
Koonap	Low water bridge in Prieshoek	-32.47	26.52	×	2017
Koonap	Pool on way to Tarkastad	-32.49	26.36	×	2017
Koonap	Pools below weir- Makhandzana on road to Tarkastad	-32.57	26.26	×	2017
Koonap	Nyaga by the weir at Elizabeth's place	-32.71	26.13	×	2017
Koonap	Drift below the dam next to Kingsvale	-32.76	26.11	×	2017
Koonap	Malanskraal bridge on Grahamstown/ Bedford road	-32.79	26.11	×	2017
Koonap	Above weir at Herbet's farm	-32.80	26.12	×	2017

✓ Present, × Absent

Appendix 2 continued

River	Locality description	Latitude	Longitude	Present/Absent	Year
Koonap	Big pool downstream at edgefarm	-32.94	26.51	×	2017
Koonap	Small pool next to small bridge at edge hill	-32.94	26.51	×	2017
Koonap	Big pool next to the bridge at edge hill	-32.94	26.50	×	2017
Koonap	Clifton next to small bridge	-32.95	26.50	×	2017
Koonap	Clifton next to small bridge	-32.95	26.50	×	2017
Koonap	Clifton next to small bridge	-32.83	26.14	✓	2017
Koonap	Herbets hope next to bridge	-32.80	26.12	×	2017
Koonap	Further downstream of herberts hope	-32.80	26.11	✓	2017

✓ Present, × Absent

Appendix 3: Physico-chemical and substrate types of sampled sites in the Kat River catchment. Physico-chemical: Alt = Altitude, T = Temperature, Con = Conductivity, TDS = Total dissolved solids, Tur = Turbidity, DO = Dissolved oxygen, A. D = Average depth and A. W = Average width. Substrate types: BD = Bedrocks, BL = Boulders, CL= Cobbles, GR= Gravel, SN = Sand, SL = Silt, V = Vegetation. Fishing method: Elec = Electrofishing, Seine = Seine net and - = dry sites.

River	Co-ordinates	Alt- (m)	T (°C)	Con (µs)	pH	TDS ppm	Tur (ntu)	DO mg/L	A. D (cm)	A.W (m)	Area	% BD	% BL	% CL	% GR	% SN	% SL	%V	Fishing method
Lushington	32°37'54.7"S 26°51'57.6"E	948	22	43	9	24	17	8	14	3	131	0	7	0	0	93	0	37	Elec
Lushington	32°37'43.9"S 26°50'0.5"E	892	24	33	8	19	19	6	22	3	67	40	17	0	43	0	0	47	Elec
Lushington	32°37'27"S 26°49'36.8"E	872	30	86	8	41	56	7	24	3	139	15	52	0	7	10	17	28	Elec
Eyre	32°30'26.2"S 26°47'33"E	850	24	150	8	86	27	7	30	7	205	40	10	3	47	0	0	37	Elec
Lushington	32°36'58"S 26°49'01.9"E	846	24	65	8	39	26	7	28	4	149	13	77	0	0	3	7	93	Elec
Readsdale	32°28'21.5"S 26°43'33.8"E	841	24	82	9	48	33	8	19	4	55	30	37	0	27	7	0	50	Elec
Elands	32°33'33.3"S 26°48'47.1"E	820	24	161	9	94	40	8	42	7	294	0	37	7	37	20	0	23	Elec

Appendix 3 continued

River	Co-ordinates	Alt- (m)	T (°C)	Con (µs)	pH	TDS ppm	Tur (ntu)	DO mg/L	A. D (cm)	A.W (m)	Area	% BD	% BL	% CL	% GR	% SN	% SL	%V	Fishing method
Eyre	32°31'6.1"S 26°47'19.9"E	807	26	186	9	109	43	8	18	3	149	3	53	10	27	0	7	7	Elec
Kat	32°33'51.3"S 26°45'48.4"E	777	29	216	8	127	29	6	33	7	245	3	7	10	40	23	17	7	Elec
Lushington	32°35'34.2"S 26°47'34.2"E	773	27	380	8	223	8	7	37	3	28	10	0	0	0	0	90	77	Elec
Eyre	32°32'48.4"S 26°46'29.3"E	762	23	60	10	34	29	8	16	6	247	3	97	0	0	0	0	17	Elec
Buxton	32°34'5.4"S 26°37'13.4"E	754	26	202	9	123	23	11	29	3	67	7	20	3	0	53	17	3	Elec
Readsdale	32°30'36.9"S 26°44'33.7"E	688	26	106	9	62	29	7	16	3	148	10	50	0	27	7	7	30	Elec
Buxton	32°34'31.2"S 26°39'16.3"E	663	26	97	9	57	19	8	29	8	336	57	10	0	27	0	7	23	Elec

Appendix 3 continued

River	Co-ordinates	Alt- (m)	T (°C)	Con (µs)	pH	TDS ppm	Tur (ntu)	DO mg/L	A. D (cm)	A.W (m)	Area	% BD	% BL	% CL	% GR	% SN	% SL	%V	Fishing method
Kat	32°34'35.5"S 26°45'22.2"E	659	20	134	11	77	11	10	30	7	613	87	3	0	0	10	0	17	Elec
Kat	32°34'36.3"S 26°43'20.5"E	639	23	129	10	76	70	8	30	8	98	10	0	0	0	0	0	42	Elec
Kat	32°34'33.5"S 26°43'20.9"E	637	24	130	9	76	70	8	42	6	220	93	7	0	0	0	0	20	Elec
Balfour	32°32'10.1"S 26°40'38.3"E	631	26	94	9	55	146	8	27	5	212	0	0	30	33	0	37	70	Elec
Kat	32°32'37.8"S 26°42'44"E	609	25	132	9	77	64	8	33	9	258	0	67	0	7	0	27	37	Elec
Buxton	32°33'16.6"S 26°40'17.8"E	598	28	98	10	57	59	8	25	5	247	57	0	3	10	7	23	57	Elec
Kat	32°33'49.8"S 26°40'38.2"E	577	25	110	8	64	272	8	24	7	280	0	70	0	7	0	23	53	Elec & Seine

Appendix 3 continued

River	Co-ordinates	Alt- (m)	T (°C)	Con (µs)	pH	TDS ppm	Tur (ntu)	DO mg/L	A. D (cm)	A.W (m)	Area	% BD	% BL	% CL	% GR	% SN	% SL	%V	Fishing method
Kat	32°36'57.9"S 26°40'42.4"E	546	23	118	9	69	275	7	83	22	505	0	0	30	33	0	37	23	Seine
Kat	32°38'29.7"S 26°41'16.1"E	540	26	121	9	71	32	8	86	12	271	54	3	0	0	30	13	0	Seine & Elec
Kat	32°38'35.2"S 26°39'46.6"E	512	27	130	8	74	518	7	44	4	89	7	57	0	0	0	37	17	Elec
Kat	32°42'17.8"S 26°36'15.5"E	473	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Kat	32°42'55.9"S 26°35'17.6"E	464	23	185	9	109	720	8	31	9	575	0	23	0	17	60	0	23	Elec
Kat	32°53'11.5"S 26°42'24.2"E	314	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Kat	32°46'40.3"S 26°38'45.9"E	312	29	185	9	109	699	7	36	10	715	0	27	0	63	0	10	60	Elec & Seine
Kat	32°52'49.9"S 26°42'46.9"E	303	31	167	10	990	9	15	35	4	115	10	0	0	0	0	0	100	Elec
				8								0							