# Distribution and conservation status assessment of the freshwater fishes in the Krom River system in the Eastern Cape Province, South Africa

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By

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# ABSTRACT

The Cape Fold Ecoregion (CFE) contains the highest number of endemic freshwater fishes in Africa, south of the Zambezi River system. The CFE's unique fish fauna has, however, suffered severe decline in historical distribution ranges and population sizes due to multiple impacts, with the two main threats being introduced invasive piscivores and habitat degradation. Growing evidence shows that biodiversity is being lost at unprecedented levels due to increased human pressure and demand for limited resources. Future projections also indicate high extinction risk, particularly for freshwater ecosystems, as a result of global climate change. Yet for many regions, including the CFE, there is still limited biodiversity knowledge to guide decision making processes.

The Krom River system in the eastern Cape Fold Ecoregion (CFE) contains three native freshwater fish species. These are *Pseudobarbus senticeps*, which was recently revalidated, *Sandelia capensis* and a galaxiid lineage currently informally referred to as *Galaxias zebratus* sp. 'Joubertina'. Two of these taxa are listed under threatened categories of the International Union for Conservation of Nature (IUCN). These are *P. senticeps*, which is listed as Critically Endangered and *Galaxias zebratus* sp. 'Joubertina', which is listed as Endangered. Although *S. capensis* is currently listed as Data Deficient, the most recent information indicates that the species comprises three allopatric lineages, with the Krom River population belonging to a lineage that is distributed across a number of river systems on the south coast. Two non-native fishes, *Micropterus salmoides* and *Lepomis macrochirus*, have also been introduced into this river system.

Updated information on distribution patterns and habitat utilisation is required to guide authorities to develop effective management plans and conservation of the threatened fishes of the Krom River system. The aim of this study was to determine the current distribution of the fishes of the Krom River system and to provide recommendations for *in situ* conservation measures to protect remnant populations and promote recovery and range expansion of the native fishes. The primary objective of the study was to use historical and present data to map the distributions patterns. Data on the distribution of the fishes of the Krom River system were obtained from various sources, including the NRF-SAIAB database, studies published in peer reviewed literature and unpublished reports. Systematic sampling was conducted in the Krom River system and several of its tributaries to provide a snapshot of the current distribution of

both native and non-native fishes in this system at the time of this study. The presence of instream physical barriers was recorded, and habitat and water quality were visually assessed.

Only four species of freshwater fishes were recorded during the surveys. These were two native species, the Krom River redfin *P. senticeps* and the Cape kurper *Sandelia capensis*, and two introduced species, the black bass *M. salmoides* and bluegill sunfish *L. macrochirus*. *Pseudobarbus senticeps* was the most common and widely distributed species in the system, and was recorded at 20 localities (four mainstem and 16 tributary localities). *Sandelia capensis* was less common and was recorded at only eight localities (four mainstem and four tributary localities). These two native species co-occurred at all the eight localities where *S. capensis* was recorded, but *P. senticeps* was always more abundant than *Sandelia*. *Micropterus salmoides* was recorded at six localities (four mainstem and 2 tributary localities) while *L. macrochirus* was recorded at five localities (four mainstem and one off-stream dam site). Native fishes were not recorded at sites where non-native fishes were present except at two localities in the Wit Els River where juvenile *M. salmoides* were found amongst the native fish samples. However, unlike the other localities where all size classes (i.e., young of the year, juveniles, subadults and adults) were present, only adult redfins were found in the Wit Els River where juveniles of largemouth bass occurred.

A comparison of the past and present distribution patterns of the native fishes indicates a considerable decline in distribution range, and remnant populations are now fragmented compared to past observations. The major threats and impacts on the Krom River system are the presence of non-native piscivores, construction of instream physical barriers, and agricultural activities. The information from this study could form a basis for establishing long-term conservation measures that should focus on preventing the spread of non-native fishes, and rehabilitating critical habitats for the future persistence of remnant populations of native fishes.

The study also evaluated the implications of incomplete taxonomy on conservation status assessments and prioritisation, by evaluating case studies of species complexes of freshwater fishes whose taxonomy has been recently resolved, as well as two complexes with lineages that await formal recognition as distinct species. The aim was to demonstrate how incomplete knowledge of taxonomy affects the assessment of extinction risk and can potentially misdirect conservation prioritisation. Specifically, the study examined how change in taxonomy or recognition of undescribed genetic lineages in faunal listings affects range size and IUCN Red List risk category. The study taxa were *Pseudobarbus afer* sensu lato (sl),

*Enteromius anoplus* sl, *Amphilius natalensis* sl, *Sandelia capensis* and *S. bainsii*, and the study assessed the species complexes as Least Concern or Near Threatened. The majority of these taxonomically revised species and genetic lineages were determined to be Critically Endangered (CR), Endangered (EN) and Vulnerable (VU), as the extent of occurrence was estimated to be < 100 km$^2$; < 5000 km$^2$ and < 20 000 km$^2$ respectively. The species and lineages have been observed in fewer than ten locations, and the populations have been observed to be in decline. The results of this study indicate that the current taxonomic status of the native species in the Krom River system obscures the diversity of these fishes and affects conclusions for conservation assessments. Findings from this preliminary assessment highlight the need for advancing taxonomic knowledge through accurate delimitation of species boundaries, particularly within wide ranging taxa, in order to guide effective conservation and biodiversity management decisions.

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# ACKNOWLEDGEMENTS

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# CHAPTER 1

## General Introduction

Freshwater ecosystems host a large proportion of global biodiversity, and are among the most threatened habitats in the world, far greater than the declines experienced in marine and terrestrial ecosystems (Dudgeon et al., 2006; Lévêque et al., 2008; Collen et al., 2014; Reid et al., 2019). From an aquatic biodiversity perspective, freshwater ecosystems are important because they are critical habitats for fish refuge, feeding, spawning and for migration purposes (Roos et al., 2007; Welcomme et al., 2010; Davies & Stewart, 2013; Geist & Hawkins, 2016; McIntyre et al., 2016; Wantzen et al., 2016; Tickner et al., 2020). Despite supporting a greater proportion of diversity and distributions, freshwater ecosystems continue to face ongoing destruction in many regions. They are vulnerable to multiple stressors effects, ranging from the introduction of non-native species, habitat loss and degradation, hydrological modifications through damming and water diversions, overexploitation of species, unsustainable land use practices, and extreme changes in global climate conditions (Vörösmarty et al., 2000; Carpenter et al., 2011; Borgwardt et al., 2019; Albert et al., 2020). These factors contribute to the decline of aquatic biodiversity and their distribution.

In lotic habitats (rivers and streams), native and endemic freshwater fishes face the general risk of extirpation primarily due to habitat degradation and loss, and through invasions by non-native species (Dextrase & Mandrak, 2006). The loss of suitable habitats and ecological impacts of non-native freshwater fish due in freshwater ecosystems is a growing challenge to the survival and conservation of native fishes globally (Vitousek et al., 1997; Benstead et al., 2003; Cucherousset & Olden, 2011; Dwivedi, 2018; Kiruba- Sankar, 2018). Similarly, invasive non-native species that have managed to invade and establish in most systems have been reported to influence the distribution patterns of native freshwater fishes through direct predation, competition for resources, and hydrological modifications, resulting in population declines and extirpations (Ogutu-Ohwayo, 1993; Dextrase & Mandrak, 2006; Johnson et al., 2008; Gallardo et al., 2016; Pauchard et al., 2016; Golikov et al., 2020). Therefore, understanding the species-habitat relations and the processes that influence the distribution patterns of endemic and threatened fish species in relation to these impacts is essential in the design of conservation strategies (Fausch et al., 2006; Hayward, 2011; Strecker et al., 2011). Furthermore, understanding how species responds to the abiotic (physical) environment (e.g.

climate, geology, temperature, land formations, water quality), their biotic interactions (e.g. competition, predation, parasites, diseases) and the influence of global environment change has important implications for predicting future distributions of species (Ogutu-Ohwayo, 1993; Jackson et al. 2001; Huggett, 2004; Wisz et al., 2013).

Unfortunately, in addition to the anthropogenic disturbances, many regions worldwide are still characterised by limited information on the species distribution patterns and knowledge on the ecology and taxonomy of freshwater fish taxa. Cryptic diversity, which comprises two or more distinct lineages within a species that are classified as one due to their morphological similarity, is more common than previously thought worldwide (Bickford et al., 2007; Struck et al., 2018). This is the case for several freshwater fish species in southern Africa where molecular studies have uncovered genetically distinct lineages or candidate species, but many of these remain undescribed (Swartz et al., 2007, 2009; Chakona et al., 2018, 2020b; Mutizwa et al., 2021). The lack of this information has been identified as the major impediment to effective conservation and management of freshwater ecosystems (Scotland et al., 2003; Arthington et al., 2016). Identifying discrete biological units within threatened species has important implications for conservation assessments (Giangrande 2003; Collen et al., 2014; Leung et al., 2015). Most traditional taxonomic approaches dominate conservation efforts for defining geographic boundaries and identifying biological units, which often result in cryptic diversity to go undetected and likely become extinct. These findings have stimulated a resurgence in studies on re-evaluating the distribution, ecology, taxonomy and conservation status of the newly identified genetic lineages or recently described species (e.g. Kadye et al., 2016; Chakona & Skelton, 2017; Chakona et al., 2019).

An example is the Cape Fold Ecoregion (CFE), located at the southern tip of Africa, which is a hotspot for unique and highly threatened freshwater fish fauna (Skelton, 2001; Tweddle et al., 2009; Weyl et al., 2014; Ellender et al., 2017; Chakona et al., 2022). The CFE has larger river systems that include the Olifants, Berg, Breede, Gourits, Gamtoos and Sundays River systems (Fig.1.1). These systems span the Western Cape and Eastern Cape provinces (Swartz et al., 2009). The CFE is one of the most extensively studied regions in southern Africa, and there is evidence from recent International Union for Conservation of Nature (IUCN) Red List assessments that the unique biodiversity of this region is under severe threat and faces a high risk of extinction (Darwall et al., 2009; Tweddle et al., 2009; Chakona et al., 2022). The region supports various taxonomic groups and narrow-range endemic fish species. The primary threat to the aquatic biodiversity of this region is the spread of non-native fish species, particularly the salmonids, *Salmo trutta* (brown trout) and *Oncorhynchus mykiss* (rainbow trout), the centrarchids *Micropterus* spp. (bass) and *Lepomis macrochirus* (bluegill sunfish) and the extralimital *Clarias gariepinus* (sharptooth catfish), which have been associated with declines in historical ranges and localised extinctions of native fishes in the region (Skelton, 2001; Ellender et al., 2011; Shelton et al., 2014; Weyl et al., 2014; Ellender et al., 2017; Shelton et al., 2017, 2018; Chakona et al., 2020a).

In general, non-native fish species have been shown to impact native fish populations through direct predation, competition for food and habitat, habitat alteration and sometimes through the introduction of parasites (Skelton, 2001; Tweddle et al., 2009; David et al., 2017; Shelton et al., 2017; Chakona et al., 2018; Chakona et al., 2019b; Kadye & Booth, 2020). These impacts can, in turn, have cascading effects on ecosystem functioning (Cucherousset & Olden, 2011; Kadye & Booth, 2013; Koel et al., 2019; Cerrilla, 2020; Kadye & Booth, 2020). Within the CFE, additional threats on freshwater fishes include habitat loss (Tweddle et al. 2009; Chakona et al., 2022), as well as growing concerns regarding projected global climate change, which is anticipated to have devastating impacts on sensitive ecosystems, in particular on the Mediterranean climate regions (Thieme et al., 2010; Vörösmarty et al., 2010; Rivers-Moore et al., 2012; Shelton et al., 2017, 2018; Chakona et al, 2019). The projected impacts of global climate change (Beaumont et al., 2011; Cooke et al., 2012) are likely to influence river flow patterns, with a shift from perennial to intermittent flow regimes due to reduced precipitation and increases in demand for water, negatively affecting most of the ecological processes (Grant et al., 2007) and the unique endemic stream fish fauna of the CFE (Dallas & Rivers-Moore, 2014; Shelton et al., 2018; Chakona et al., 2019). As a result of these multiple impacts, most native freshwater fishes of the CFE have experienced severe reduction in both population size and distribution ranges, with some species, such as *Cheilobarbus capensis*, having been extirpated from the Berg River system (Clark et al., 2009), whereas remnant populations in the Breede River system are highly fragmented (Chakona et al., 2020a).

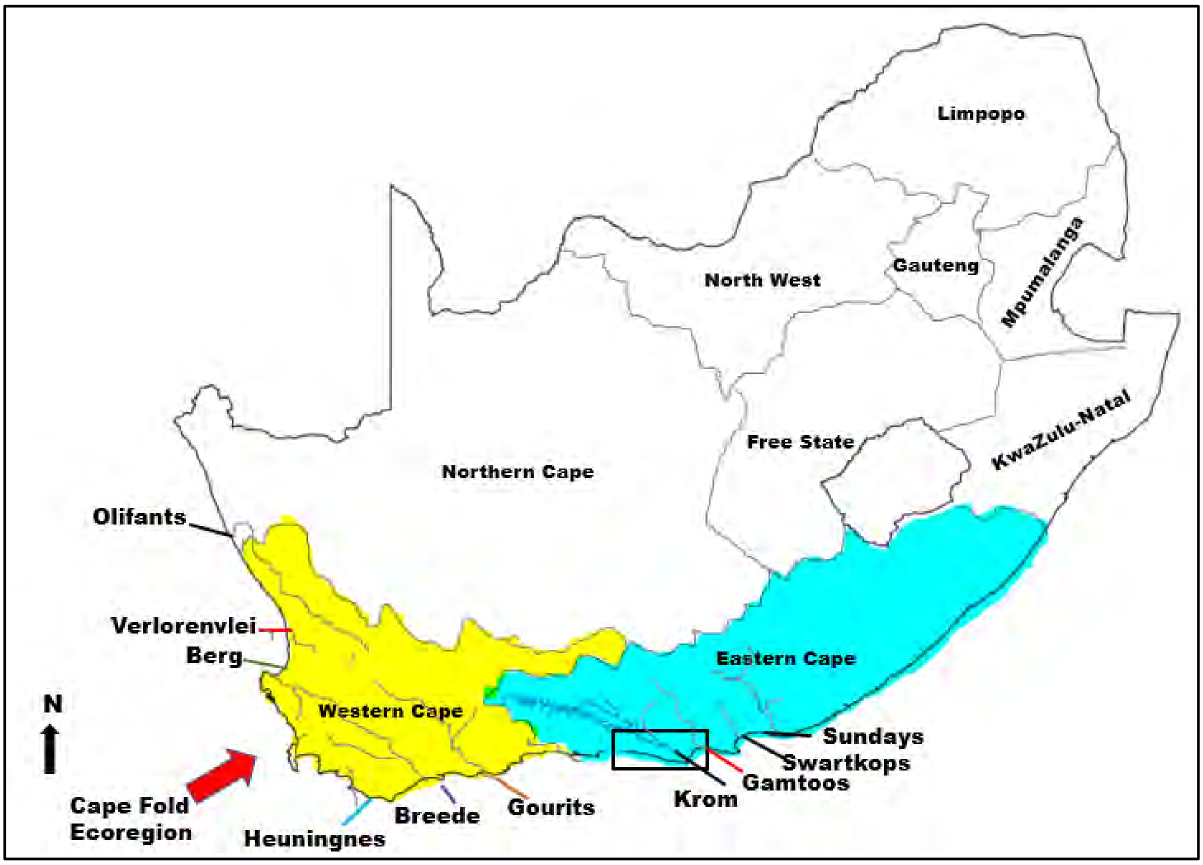


Figure 1.1: The Cape Fold Ecoregion (CFE) and river systems associated with the region referred to in this study, which indicate the distribution of historically isolated lineages of *Pseudobarbus*, *Sandelia* and *Galaxias* in the region.

Within the CFE, the Cyprinidae is the dominant fish family with 18 species in six genera (Skelton, 2001; Chakona & Swartz, 2013; Chakona et al., 2014; Chakona & Skelton, 2017; Skelton et al., 2018), and 50% (9) of these species are listed under highly threatened categories of the IUCN (Chakona et al., 2022). The families Austroglanidae, Anabantidae and Galaxiidae are represented by a single genus each, with only one or two species in each genus (Skelton, 2001). With a total of ten formally described species and six genetic lineages, *Pseudobarbus* is the most speciose cyprinid genus in the CFE (Skelton, 2001; Chakona & Swartz, 2013; Chakona et al., 2014; Chakona & Skelton, 2017). However, 60% (six species) of these taxa are listed as either Critically Endangered (CR) or Endangered (EN) (Tweddle et al., 2009; Ellender et al., 2017; Chakona et al., 2022), indicating that they face an elevated risk of extinction if intervention measures are not taken. Previous and ongoing studies in the CFE have also uncovered several new species and genetic lineages, particularly in the genera *Sandelia* and *Galaxias* (Chakona et al., 2013; Bronaugh et al., 2020; Chakona et al., 2020a).

The Krom River is part of the south costal river systems in the eastern part of the CFE. Three indigenous freshwater fish species inhabit the Krom River system, namely *Pseudobarbus senticeps*, *Sandelia capensis* and a unique lineage of the Cape Galaxias, *Galaxias zebratus* sp. 'Joubertina' (Chakona & Skelton, 2017; Chakona et al., 2018). *Galaxias zebratus* sp. 'Joubertina' also occurs in the adjacent Gamtoos River system (Chakona et al., 2018). The Krom River redfin, *P. senticeps* (Smith, 1936), which was recently revalidated, was previously considered a synonym of the Eastern Cape redfin, *P. afer* (Skelton, 1988, 2001; Chakona & Skelton, 2017). This species is among the fishes listed as Critically Endangered within the genus *Pseudobarbus* in the CFE. Previous ecological studies focused on the Gamtoos River system population of the *P. afer sensu lato* (which represents the recently described *P. swartzi*) as well as the populations in the Baakens, Swartkops and Sundays River systems, which represent *P. afer sensu stricto* (Cambray, 1996). The inclusion of *P. senticeps*, a narrow-range endemic species confined to the Krom River system, under a single widespread species, *P. afer*, has clearly obscured the magnitude of the threats faced by this species (Skelton, 2001; Tweddle, 2009; Chakona et al., 2022).

A recent study revealed that *S. capensis* (Cuvier 1829) of the family Anabantidae, comprises three allopatric lineages, namely, the west coast lineage, the Klein lineage and the southern lineage (Bronaugh et al., 2020). The southern lineage is distributed from the Swartkops River system to the Breede River system, and thus its range encompasses the Krom River system.  In comparison, molecular studies have identified that *G. zebratus* is a species complex consisting at least 10 distinct lineages, including *Galaxias zebratus* sp. 'Joubertina' (Waters & Cambray, 1997; Wishart et al., 2006; Chakona et al., 2013; Chakona et al., 2018). *Galaxias zebratus* sp. 'Joubertina' is a narrow range endemic taxon confined to few localities in the upper Krom and tributaries of the Gamtoos River system in the eastern CFE. This lineage has potentially smaller population sizes and is highly fragmented (Cambray et al., 1995; Chakona et al., 2013; Chakona et al., 2015; Chakona et al., 2018). *Galaxias zebratus* sp. 'Joubertina' is listed as Endangered due to its restricted geographic range and the increasing threats to the few remnant populations (Chakona et al., 2018). The potential threats to this lineage include the invasion by non-native fish species, water abstraction and habitat degradation. Due to the increasing anthropogenic impacts in this lineage, the future persistence of its remnant populations is uncertain.

Although the recent IUCN Red List assessment indicated that most native fishes of the Krom River system are highly threatened (Chakona et al., 2022), *S. capensis* is currently listed as Data Deficient due to the taxonomic uncertainties on the species, particularly due to poor information on the geographic ranges of its lineages (Bronaugh et al., 2020; Chakona et al., 2013). As has been the case in other river systems in the CFE, the Krom River system is impacted by the introduction of non-native species, as well as agricultural activities that have transformed the riparian zone and increased instream sedimentation, whilst the construction of weirs and water abstraction have altered the hydrological regime of the system (Lee et al., 2014; Rebelo, 2012). As a result of these impacts, most of the remnant native fish populations precariously persist in short stretches of least impacted river sections, or uninvaded catchments above barriers that prevent upstream migration of non-native fishes. Similar trends have been observed elsewhere where freshwater fish species have been found to occur in small and isolated patches sustaining a limited number of individuals due to degradation and loss of suitable habitats (Collares-Pereira et al., 2002; Aarts et al., 2004; Franssen & Tobler, 2013; Gido et al., 2016). The pattern is further supported by Gilliam and Fraser (2001), who observed low abundance of small fishes in mainstem sections likely due to local extirpation compared to populations in small tributaries.

Effective management of threatened species requires knowledge of distribution patterns and ecological attributes as this information is integral to conservation status assessments following the IUCN criteria (IUCN, 2003). However, as with all newly described or recently resurrected species, conservation efforts are often be hampered by limited or complete lack of knowledge on a species' ecological requirements (Chakona et al., 2019a). This is indeed the case for the freshwater fishes of the Krom River system. There is, therefore, a need to address this knowledge gap and to identify appropriate measures to guide *in situ* conservation measures to protect remnant populations and promote the recovery and range expansion of the native fishes.

The aim of this study was to evaluate the distribution patterns of the freshwater fish taxa in the Krom River system and the impacts of incomplete taxonomy, in order to contribute to the conservation and management decisions for these fishes. The first objective of the study was to map the historical distribution of the fishes in the Krom River system and then to determine whether there have been any changes in the distribution based on the historical records and recently collected data from field surveys. The second objective was to highlight the implications of incomplete taxonomy on conservation status assessments and prioritisation. The data obtained from this study is anticipated to contribute towards the body of knowledge for establishing long-term monitoring of both native and non-native species and determination of the effectiveness of any future management intervention measures.

The study aimed to address the following questions:

1. Have there been any changes between the past and present distribution of the freshwater fishes in the Krom River system?
2. What are the implications of cryptic diversity on conservation status assessments?

***Study area***

The Krom River system, located in the eastern section of the Cape Fold Ecoregion (CFE), is about 109 km in length and drains a catchment area of approximately 1152 $km^2$. The region has a Mediterranean climate, with an annual rainfall of between 200 and 600 mm (Mucina & Rutherford, 2006; Nsor & Gambiza, 2013; Rebelo et al., 2015). The geology of this region is characterised by quartzite sandstones and shales (Rebelo, 2012). The main natural vegetation types in the catchment are grasslands and mountain fynbos. The riparian zone is dominated by the native Palmiet (*Prionium serratum*) and invaded by non-native black wattle (*Acacia mearnsii*) (Sieben, 2012; Nsor & Gambiza, 2013; Rebelo et al., 2015). The instream habitat is characterised by rocky substratum (boulders and cobble), with small pools less than 1 m deep, bounded with riffles upstream and downstream (Chakona et al., 2018).

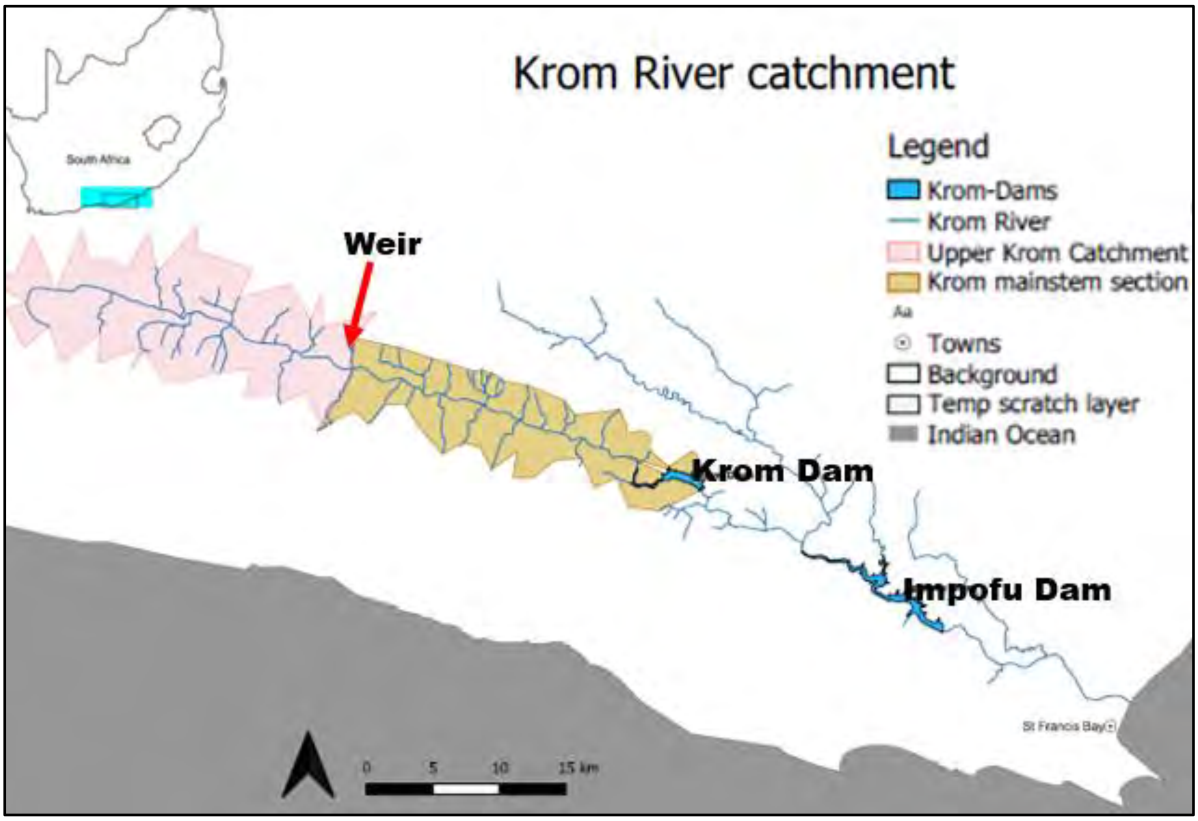


Figure 1.2: Map of the Krom River catchment, showing the upper, middle and lower Krom reaches, the weir forming an upper limit for non-native fishes, including the Krom (previously Churchill Dam) and Impofu Dam.

The mainstem Krom River system is impounded by two large dams, the Impofu and Krom dams, which both act as physical barriers for upstream fish movement. The construction of these dams has led to increased levels of sedimentation (Lee et al., 2014). The dams are used for providing potable water to the Nelson Mandela Bay Metropolitan Municipality and for irrigation (Haigh et al., 2004; Rebelo et al., 2015). The Krom River catchment is heavily degraded by human activity, including intensive agricultural activities (fruit, vegetables) and irrigated grazing land for livestock farming (Rebelo et al., 2015). Recent surveys have also recorded two non-native centrarchid species, the black bass *M. salmoides* and the bluegill sunfish *L. macrochirus*, in this system (Chakona & Skelton, 2017; this study).

***Thesis outline***

To address the above objectives and questions of the present study, the thesis is structured into four chapters. Chapter 1 serves as a general introduction by providing background on the research study, the fishes of the Krom River system, the threats to these fish species, and the issues around incomplete taxonomic knowledge. The chapter further outlines the thesis aims and objectives, research questions and gives a description of the study area. The first data chapter (Chapter 2) provides a description of the general methods used in this research study, to gather information in order to achieve the research aims and objectives. Furthermore, this chapter reviews the past and present distribution patterns of the fishes in the Krom River system. The second data chapter (Chapter 3) evaluates case studies of species complexes of freshwater fishes whose taxonomy has been resolved recently, as well as two complexes with lineages that await formal recognition as distinct species and reassessment of their conservation status. Lastly, Chapter 4 provides the general discussion by summarising the findings and addressing broader implications of the study. Subsequently, gives recommendations, detail management options and guide future research. This information is anticipated to contribute towards enhancing the long-term persistence of the remnant native freshwater fishes in the Krom River system.

# CHAPTER 2

## Historical and current distribution patterns of the freshwater fishes in the Krom River system in the Eastern Cape Province, South Africa

### Introduction

Despite comprising a relatively small proportion (0.8%) of the global aquatic habitats, freshwater ecosystems have a high diversity of species and provide several important ecosystem services (Abell et al., 2008; Balian et al., 2008; Collen et al., 2014; Harrison et al., 2016; Darwall et al., 2018; Reid et al., 2018; Nyathi et al., 2022). These fragile habitats are relatively small, and are more endangered environments than terrestrial and marine ecosystems. Freshwater ecosystems are among the most heavily disturbed globally, threatened by multiple stressors which include habitat destruction and modification, overexploitation, pollution and the introduction of non-native species (Dudgeon et al., 2006; Tweddle et al., 2009; Clavero et al., 2010; Geist, 2011; Bellard et al., 2016). Studies have been conducted to evaluate the effects of threats to native fishes globally, and it has been observed that native fishes face a high risk of extinction due to the interactions of these multiple impacts (Ogutu-Ohwayo, 1990; Skelton, 1990; Marchetti & Moyle, 2001; Cambray, 2003; Brook et al., 2008; Tweddle et al., 2009; Clavero et al., 2010; Reid et al., 2013; Ellender et al., 2017; Shelton et al., 2018). With the declines in population sizes and loss of biodiversity, it is important to understand the biotic and abiotic factors that influence the distribution of threatened fishes (Jackson et al., 2001; Sifundza et al., 2020). Some regions, particularly the Mediterranean-type ecosystems, are more vulnerable to these impacts (Abell et al., 2008; Hermoso & Clavero, 2011; Heilpern et al., 2018;). For example, the Iberian Peninsula in southern Europe and freshwater basins in California in the USA are characterised by a high number of endemic fish species (Marchetti & Moyle, 2001; Clavero & Garcı´a-Berthou, 2006; Clavero et al., 2010; Moyle et al., 2011; Maceda-Veiga, 2013). In these regions, the spread of invasive species has been associated with predation and competition (Clavero et al., 2004; Marchetti et al., 2004a), resulting in noticeable population declines and range reductions of the native aquatic biota (Clavero et al., 2010; Hermoso & Clavero, 2011; Moyle et al., 2011).

The Mediterranean ecoregion in southern Africa, known as the Cape Fold Ecoregion (CFE), is a biodiversity hotspot which harbours a unique assemblage of endemic freshwater fishes (Skelton, 2001; Weyl et al., 2014; Ellender et al., 2017;), and so this region has received much attention and a considerable research focus (Chakona & Swartz, 2013; Chakona et al., 2013; Weyl et al., 2014; Kadye et al., 2016; Chakona & Skelton, 2017; Ellender et al., 2017). The distribution patterns and population sizes of freshwater fish species are influenced by both abiotic and biotic factors. For example, biotic interactions such as predation and competition play a significant role in the fish community structure in river systems (Jackson et al., 2001; Kambikambi et al., 2019; Sifundza et al., 2020). Endemic fish in this region are threatened by habitat destruction such as the clearing of areas for agricultural purposes and by invasion from non-native species such as salmonids, *Salmo trutta* (brown trout) and *Oncorhynchus mykiss* (rainbow trout), the centrarchids *Micropterus* spp. (bass) and *Lepomis macrochirus* (bluegill sunfish), and the extralimital clariid, *Clarias gariepinus* (sharptooth catfish) (Ellender & Weyl, 2014). These non-native species have severe impacts on native biota through predation and competition for resources, and have been identified as the primary conservation concern for the unique fish fauna (Tweddle et al., 2009; Chakona et al., 2022). As a result of these multiple impacts, native freshwater fishes have experienced severe reduction in both population size and historical distribution ranges (Clark et al., 2009; Chakona et al., 2020a). For example, Cambray (2003) noticed severe depletion of the smallscale redfin (*Pseudobarbus asper*) in the Gamtoos River, and observed similar trends unfolding in adjacent river systems. Furthermore, some species, such as *Cheilobarbus capensis*, have been extirpated from the Berg River system (Clark et al., 2009), whereas remnant populations in the Breede River system are highly fragmented (Chakona et al., 2020). Many of the endemic species in the CFE are characterised by isolation and fragmented distributions, due to the effects of these multiple threats (Chakona et al., 2020).

The discovery of unique genetic lineages, and the description of new species within the highly threatened fish species in the CFE, highlights the need to re-evaluate the distribution, ecology and biology of the fishes of this region, to inform better conservation and management (Revenga et al., 2005; Kadye et al., 2016; Chakona & Skelton, 2017; Chakona et al. 2019). Studies of two redfin minnows that were described within the past eight years, the giant redfin, *Pseudobarbus skeltoni*, and the Verlorenvlei River redfin, *P. verloreni*, have helped to shed some light on their distribution patterns, habitat use and trophic ecology (Chakona & Swartz, 2013; Chakona et al., 2014; Kadye et al., 2016; Chakona et al., 2019).

The Krom River system contains three native freshwater fishes, *P. senticeps* (Krom River redfin), *Sandelia capensis* and a unique lineage of the Cape Galaxias, *Galaxias zebratus* *sp*. 'Joubertina' (Skelton, 2001; Chakona & Skelton, 2017; Chakona et al., 2018). The Krom River redfin is endemic to this system, whereas *Galaxias zebratus* sp. 'Joubertina' also occurs in the adjacent Gamtoos River system (Chakona et al., 2018). A recent study revealed that *Sandelia capensis* comprises three allopatric lineages, the west coast lineage, the Klein River system lineage and the southern lineage (Bronaugh et al., 2020). The southern lineage is distributed from the Swartkops River system to the Breede River system, and thus its range encompasses the Krom River system. The 2016 IUCN Red List assessment indicated that the native fishes of the Krom River system are highly threatened (Chakona et al., 2022). *Pseudobarbus senticeps* is listed as Critically Endangered and *Galaxias zebratus* 'Joubertina' is Endangered (Chakona et al., 2018). *Sandelia capensis* is currently listed as Data Deficient due to uncertainties on the species and geographic boundaries of the lineages identified in this species (Chakona et al., 2013; Bronaugh et al., 2020). Knowledge of the past and present distribution patterns of threatened species is crucial for identifying causes for range shift and for exploring effective management options to preserve remnant populations (Kadye et al., 2016; Chakona et al., 2019, 2020a; Sifundza et al., 2020). The aim of this study was to document past and present distribution of the fishes of the Krom River system, and to provide appropriate conservation measures to protect these narrow-range endemics.

## Materials and Methods

### *Fish sampling and distribution patterns*

To assess the distribution of the freshwater fishes in the Krom River system, non-destructive sampling was conducted using seine and fyke nets. All sampled fishes were identified on site and returned to their habitat alive. At each locality the sampled habitats were visually inspected and characterised, based on a modified Wentworth scale (Bovee, 1986), as silt (< 0.05 cm), sand (0.05–2 cm), gravel (2–6 cm), cobble (6–25 cm), boulder (25 cm–100 cm) or bedrock (> 1 m). The predominant substrate types were noted and recorded. Stream flow was also visually assessed, and characterised as fast, moderate or static flow. Riparian vegetation was also characterised as either woody or herbaceous.

Additional data on the distribution of the fishes of the Krom River system were obtained from various sources, including the National Research Foundation-South African Institute for Aquatic Biodiversity (NRF-SAIAB) database, studies published in peer reviewed literature and unpublished reports.

***NRF-SAIAB database***

The NRF-SAIAB in South Africa, houses the largest collection of fish and other aquatic taxa, collected largely in southern Africa as well as other regions on the continent and other countries. These records comprise mainly species distributional data with georeferenced location details and dates on which sampling was done or observations were made. Information on abundance is rarely recorded, and thus, for the purposes of this study, only presence-absence information was presented. At the time of this study, the NRF-SAIAB collection had over 62672 records of freshwater fishes, with the oldest collection record dating back to 1875. The database contained 150 records of indigenous freshwater fishes from the Krom River system. The locations for these historical records are shown in Figure 2.1.

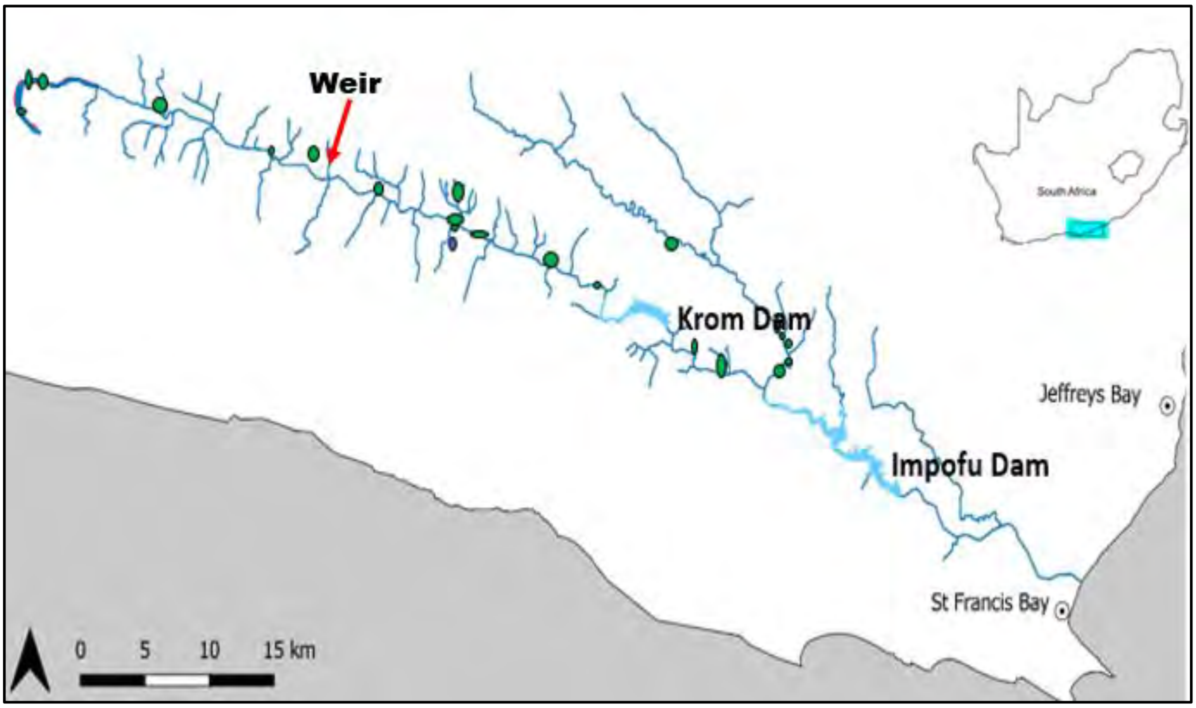


Figure 2.1: The sampled localities where freshwater fishes were historically distributed across the Krom River system in the eastern Cape Fold Ecoregion (CFE).

***Waters and Cambray 1997***

Waters and Cambray (1997) discovered a new population of the Cape galaxias, *G. zebratus*, in the upper sections of the Krom River, effectively extending the range of the species, whose eastern-most distribution had previously been thought to be the upper Gamtoos (Kouga) River system. Observations by Cambray et al. (1995), have updated the Krom and Gamtoos River systems records and extended the known range of *G. zebratus* at that time by approximately 600 km.

***Chakona et al., 2015, 2018***

Chakona et al. (2015, 2018) published distribution data for an extensive survey that they undertook in the Krom River system in 2011. They sampled 30 localities across an approximately 109 km stretch of the river, from the Upper Krom to the Krom Dam, which marked the lower limit of the sampled localities (Figures 2.1 & 2.2). Sampling was done using a 3 m seine net, and all species collected or encountered were recorded.

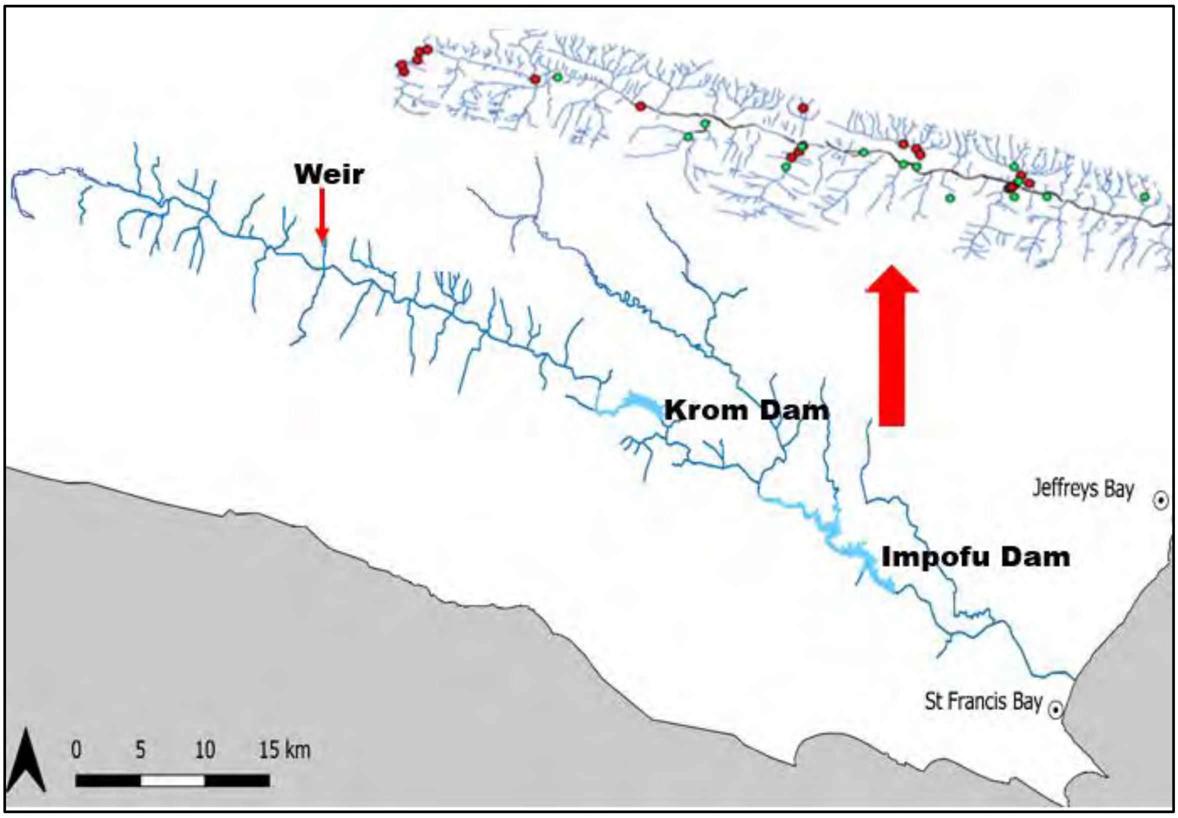


Figure 2.2: A map showing sampled sites for the historical records (1958–1983; green points) and current (2004–2019; red points) distribution records from recent surveys in the Krom River system.

### *Chakona and Skelton 2017*

*Pseudobarbus senticeps*, endemic to the Krom River system, was previously considered to be a synonym for the Eastern Cape redfin, *P. afer*, but recently Chakona & Skelton (2017) provided evidence for both the genetic and morphological distinctiveness of the Krom River redfin, resulting in its resurrection as a valid species and also provided evidence of a St Francis lineage (Gamtoos) described as a new species, *Pseudobarbus swartzi*, now re-described as *P. afer sensu stricto* (Peters, 1864) (Mandela lineage). The study provided more accurate distribution ranges for each of these species.

### *Chakona and coworkers (unpublished)*

Chakona and a team of researchers from the NRF-SAIAB recorded fish species that were sampled at 30 localities in March 2019. This is the most recent survey of the Krom River system, and it targeted several accessible localities on the mainstem section of the river, several tributaries, and some off-stream dams. The aim of their research was to map the distribution of freshwater fishes in the Krom River system upstream Krom Dam. Fish were sampled using seine and fyke nets. All sampled fishes were identified on site and returned to their habitat alive. At each locality, the sampled habitats were visually inspected and characterised based on a modified Wentworth scale (Bovee, 1986) as silt (< 0.05 cm), sand (0.05–2 cm), gravel (2–6 cm), cobble (6–25 cm), boulder (25 cm–100 cm) and bedrock (> 1 m). The predominant substrate types were noted and recorded. Stream flow was also visually assessed and characterised as fast, moderate or static flow. Riparian vegetation was characterised as woody or herbaceous.

## Results

### *NRF-SAIAB database*

Records in the NRF-SAIAB database indicate that historical sampling of the fishes of the Krom River system was done on an ad-hoc basis and was mainly confined to the mainstem section of the river (Table 2.1). The earliest record of a freshwater fish from this system is a collection of the holotype of *Pseudobarbus senticeps*, which was described by Smith in 1936. Surveys conducted between 1958 and 1983 only recorded two native freshwater fishes from this river system, *P. senticeps* (then *P. afer*) and *S. capensis* (Table 2.1). These surveys mainly focused on the section of the river above the Krom Dam and indicate that these two native species largely co-occurred in the mainstem sections of the river.

**Table 2.1:** Krom River system presence/absence data of fishes collected during the surveys conducted between 1958 and 1983. Three native fish species, namely the Krom River redfin *Pseudobarbus senticeps* (*Pse*), Cape kurper *Sandelia capensis* (*San*), Cape galaxias, *Galaxias zebratus* (*Gal*) and two non-native species, black bass *Micropterus salmoides* (*Mic*) and the bluegill sunfish *Lepomis macrochirus* (*Lep*).

| *Date* | *River* | *Latitude* | *Longitude* | *Pse* | *San* | *Gal* | **Mic* | **Lep* |
|---|---|---|---|---|---|---|---|---|
| 15/02/1958 | Krom | -33,9500 | 24,33333 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 14/06/1972 | Assegaaibosch | -33,9413 | 24,31888 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 14/06/1972 | Assegaaibosch | -33,9413 | 24,31888 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 15/06/1972 | Krom | -33,9413 | 24,31888 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 24/09/1972 | Assegaaibosch | -33,9413 | 24,31888 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 12/12/1973 | Assegaaibosch | -33,9333 | 24,31666 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 20/01/1975 | Assegaaibosch | -33,9419 | 24,31055 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 20/01/1975 | Assegaaibosch | -33,9413 | 24,31888 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 20/01/1975 | Assegaaibosch | -33,9413 | 24,31888 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 20/01/1975 | Assegaaibosch | -33,9333 | 24,31666 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 27/10/1976 | Krom | -33,9333 | 24,26666 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 27/10/1976 | Krom | -33,9500 | 24,31666 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 06/05/1982 | Krom | -33,9500 | 24,33333 | ✗ | ✓ | ✗ | ✗ | ✗ |
| 11/05/1982 | Krom | -34,0111 | 24,48333 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 12/05/1982 | Krom | -34,0111 | 24,48333 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 26/02/1983 | Krom | -33,9252 | 24,23972 | ✗ | ✓ | ✗ | ✗ | ✗ |
| 26/02/1983 | Wit Els | -33,9222 | 24,20805 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 26/02/1983 | Krom | -33,9091 | 24,15861 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 26/02/1983 | Krom | -33,9319 | 24,26000 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 26/02/1983 | Krom | -34,0391 | 24,57694 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 26/02/1983 | Krom | -33,9508 | 24,28388 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 26/02/1983 | Krom | -33,9252 | 24,23972 | ✓ | ✗ | ✗ | ✗ | ✗ |

** represents the non-natives* ✓ *represents presence* ✗ *represents absence*

***Published studies***

Following the discovery of the new population of *G. zebratus* by Waters & Cambray (1995), Chakona et al. (2015) undertook an extensive survey of the Krom and adjacent Gamtoos river systems to document a more accurate distribution range for this species and other freshwater fishes in these systems. This survey revealed that *G. zebratus* was confined to the upper sections of the Krom River system, and it did not co-occur with any of the native or non-native fish species (Table 2.2 & 2.3; Figure 2.3). *Pseudobarbus senticeps* (previously *P. afer*) was recorded from 20 localities, while *S. capensis* had a more restricted range, as this species was recorded at only nine localities. These two species co-occurred at five localities. These authors also recorded the presence of two non-native fish species, *Micropterus salmoides* and the bluegill sunfish *Lepomis macrochirus*, in mainstem localities.

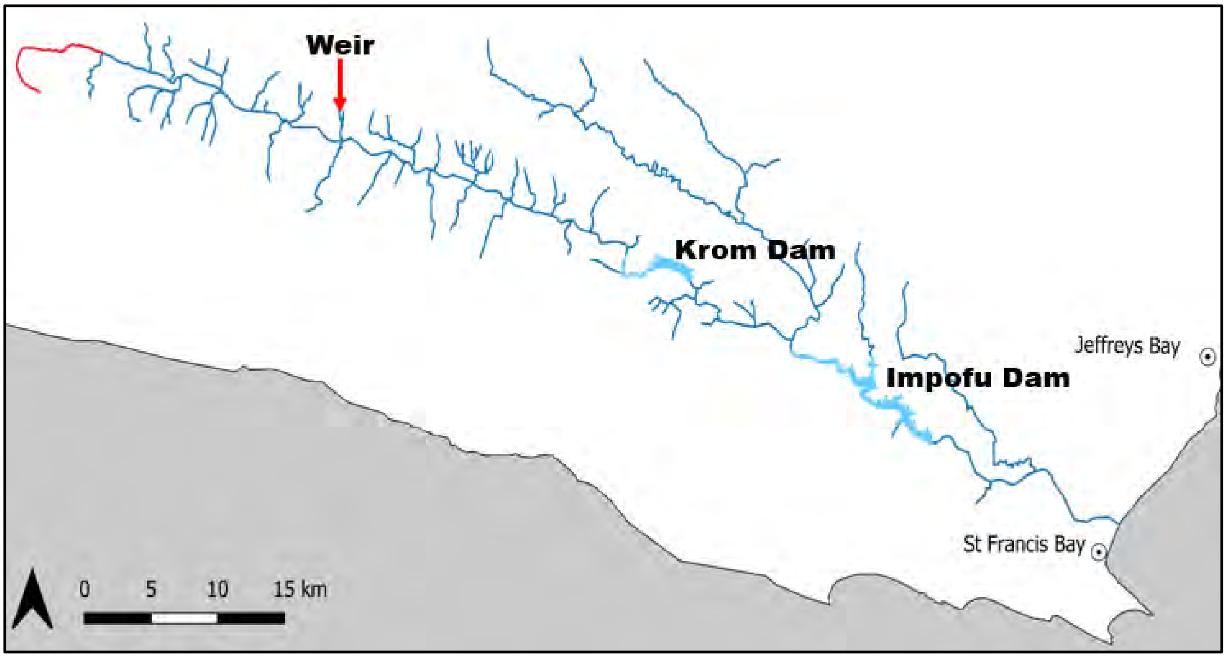


Figure 2.3: The localities where Cape galaxias, *Galaxias zebratus* 'Joubertina', was collected in the Krom River system by Chakona et al. in 2011.

**Table 2.2:** Freshwater fishes of the Krom River showing presence/absence data sampled during the February/March 2011 survey, showing the distribution patterns in the sampled localities of the system. Krom River redfin *Pseudobarbus senticeps* (*Pse*), the Cape kurper *Sandelia capensis* (*San*), Cape galaxias, *Galaxias* zebratus 'Joubertina' (*Gal*), black bass *Micropterus salmoides* (*Mic*) and the bluegill sunfish *Lepomis macrochirus* (*Lep*).

| *Date* | *River* | *Latitude* | *Longitude* | Pse | San | Gal | *Mic | *Lep |
|---|---|---|---|---|---|---|---|---|
| 02/02/2011 | Krom (upper pool) | -33,5227 | 23,56380 | ✗ | ✗ | ✓ | ✗ | ✗ |
| 02/02/2011 | Krom (farm open) | -33,5216 | 23,56420 | ✗ | ✗ | ✓ | ✗ | ✗ |
| 05/03/2011 | Krom | -33,5807 | 24,17597 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2011 | Krom (Dry river) | -33,5526 | 24,13219 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2011 | Krom (Dry river) | -33,5447 | 24,10287 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2011 | Krom | -33,5210 | 23,56444 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2011 | Krom | -33,5219 | 23,56403 | ✗ | ✗ | ✓ | ✗ | ✗ |

** represents the non-natives ✓ represents presence ✗ represents absence*

### *Chakona and co-workers (unpublished)*

Chakona and co-workers recorded the presence of instream physical barriers, visually assessed habitat and water quality, and undertook systematic sampling of the Krom River system and several of its tributaries to provide a snapshot of the current distribution of both native and non-native fishes in this system in 2019. Coordinates and main characteristics of the sampled sites are provided in Table 2.3. Only four species of freshwater fishes were recorded during the surveys. These were the two native species, the Krom River redfin *P. senticeps* and the Cape kurper *S. capensis*, and two introduced species, black bass *Micropterus* sp. and bluegill sunfish *L. macrochirus*. *Pseudobarbus senticeps* was the most common and widely distributed species in the system, and was recorded at 20 localities (four mainstem and 16 tributary localities) (Table 2.3; Figure 2.5). *Sandelia capensis* was less common and was recorded at only eight localities (four mainstem and four tributary localities) (Table 2.3; Figure 2.7). These two native species co-occurred at all the eight localities where *S. capensis* was recorded (Table 2.3), but *P. senticeps* was always more abundant than *Sandelia*. *Micropterus* sp. was recorded at six localities (four mainstem and 2 tributary localities) while *L. macrochirus* was recorded at five localities (four mainstem and one dam site) (Table 2.3; Figures 2.8, 2.9). Native fishes were not recorded at sites where non-native fishes were present, except at two localities in the Wit Els River, where juvenile *Micropterus* sp. were found amongst the native fish samples (Table

2.3). However, unlike the other localities where all size classes (i.e. young of the year, juveniles, subadults and adults) were present, only adult redfins were found in the Wit Els River where juveniles of largemouth bass occurred.

**Table 2.3:** Presence and absence data of fishes in the Krom River system, surveyed in March 2019 showing the distribution in the system from the given locality names: the Krom River redfin *Pseudobarbus senticeps* (*Pse*), the Cape kurper *Sandelia capensis* (*San*), black bass *Micropterus* (*Mic*) sp. and bluegill sunfish *Lepomis macrochirus* (*Lep*).

| *Date* | *River* | *Latitude* | *Longitude* | Pse | San | Gal | *Mic | *Lep |
|---|---|---|---|---|---|---|---|---|
| 04/03/2019 | Krom (Du Toit) | -33,903 | 24,139 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 04/03/2019 | Jagerbos (Du Toit) | -33,9500 | 24,33333 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2019 | Krugerland | -33,8802 | 24,00475 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 05/03/2019 | Krugerland | -33,8765 | 24,00364 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2019 | Krugerland | -33,8737 | 24,01164 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2019 | Krugerland | -33,869 | 24,01272 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 05/03/2019 | Krom | -33,8679 | 24,01683 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 05/03/2019 | Dam | -33,8994 | 24,14597 | ✗ | ✗ | ✗ | ✗ | ✓ |
| 05/03/2019 | Krom | -33,8996 | 24,12575 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 05/03/2019 | Krom | -33,8996 | 24,12575 | ✗ | ✗ | ✗ | ✓ | ✓ |
| 05/03/2019 | Upper Krom 1 | -33,8651 | 23,96781 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2019 | Upper Krom 2 | -33,918 | 23,97903 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 05/03/2019 | Krom | -33,8846 | 24,07186 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 06/03/2019 | Wit Els | -33,9281 | 24,20283 | ✓ | ✓ | ✗ | ✓ | ✗ |
| 06/03/2019 | Wit Els | -33,9248 | 24,20658 | ✓ | ✗ | ✗ | ✓ | ✗ |
| 06/03/2019 | Wit Els | -33,9216 | 24,20869 | ✓ | ✓ | ✗ | ✗ | ✗ |
| 06/03/2019 | Krom | -33,9192 | 24,20806 | ✗ | ✗ | ✗ | ✗ | ✓ |
| 06/03/2019 | Weir | -33,9134 | 24,20819 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 06/03/2019 | Tributary | -33,9006 | 24,20875 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 06/03/2019 | Krom | -33,9147 | 24,19622 | ✗ | ✗ | ✗ | ✓ | ✗ |
| 06/03/2019 | Nguni Farm | -33,9207 | 24,25994 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 06/03/2019 | Nguni Farm | -33,9232 | 24,26664 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 06/03/2019 | Nguni Farm | -33,9265 | 24,26856 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 06/03/2019 | Nguni Farm | -33,9331 | 24,26756 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 06/03/2019 | Krom | -33,9373 | 24,27144 | ✗ | ✗ | ✗ | ✓ | ✓ |
| 06/03/2019 | Assegaaibosch | -33,9454 | 24,31378 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 06/03/2019 | Trib opp Assesgai | -33,9382 | 24,32008 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 06/03/2019 | Trib opp Assesgai | -33,9425 | 24,48425 | ✓ | ✗ | ✗ | ✗ | ✗ |
| 07/03/2019 | Krom | -33,9554 | 24,34833 | ✗ | ✗ | ✗ | ✓ | ✓ |
| 07/03/2019 | Dry tributary | -34,0021 | 24,50564 | ✗ | ✗ | ✗ | ✗ | ✗ |
| 12/03/2019 | Upper Krom | -34,8623 | 23,99142 | ✓ | ✗ | ✗ | ✗ | ✗ |

** represents the non-natives ✓ represents presence ✗ represents absence*

**Distribution maps**

Distribution maps were constructed from historical and current distribution data from various sources, including the NRF-SAIAB database and studies published in peer reviewed literature and unpublished reports, to compare the past and present distribution patterns of both native fishes and introduced fish species in the Krom River system. Continuous distributions (red solid lines) were prepared by interpolating between the discrete set of known data points from the distribution records.

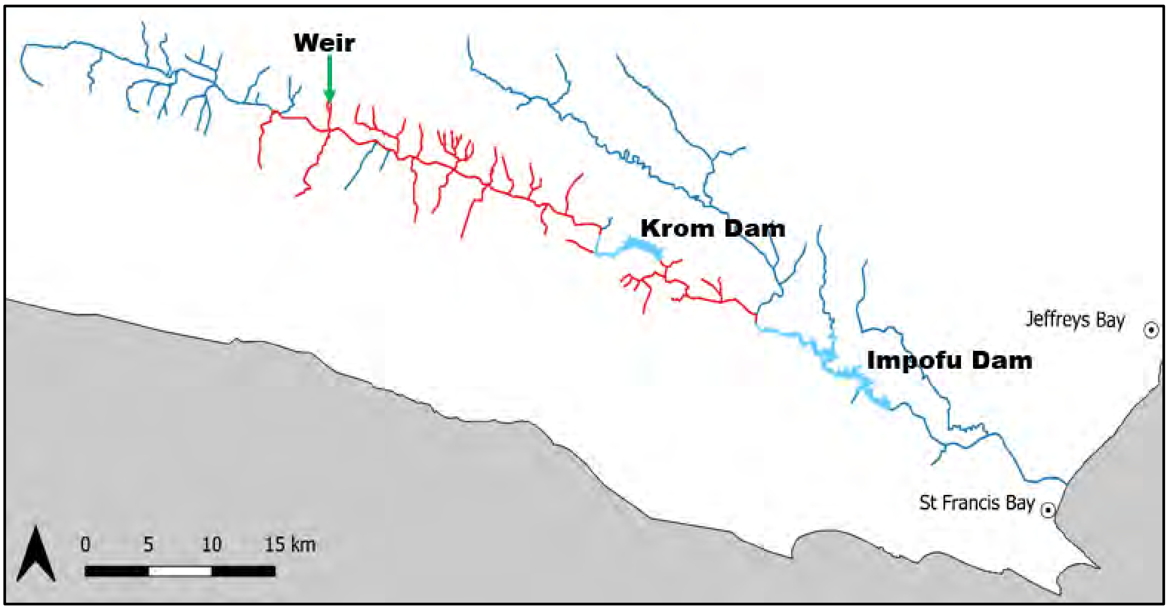


Figure 2.4: Map of the historical distribution pattern (shown in red) of *Pseudobarbus senticeps* in the Krom River system.

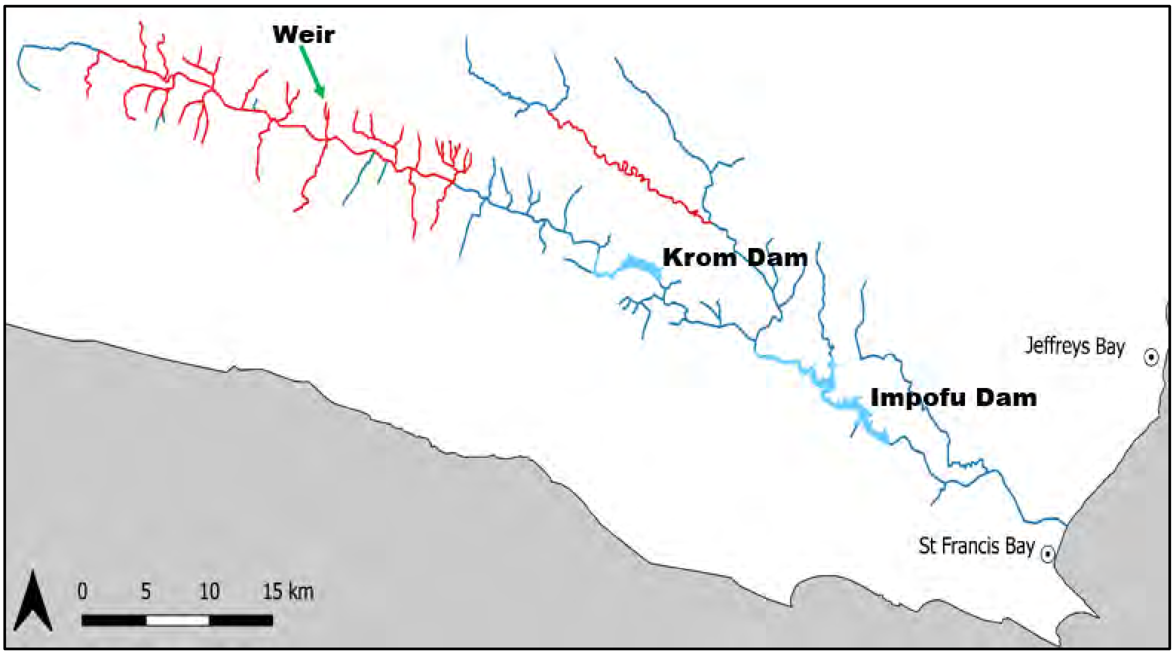


Figure 2.5: Map of *Pseudobarbus senticeps* distribution recorded in 2019, in uninvaded sections and all the sampled tributaries where the species was recorded in the Krom River system.

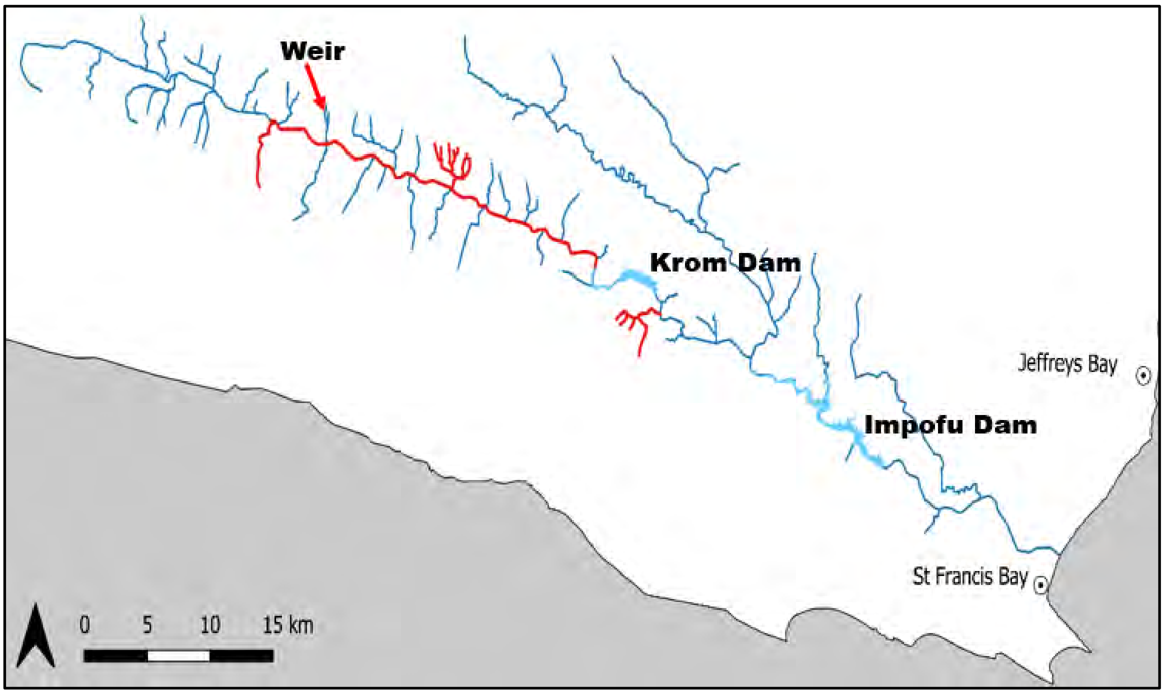


Figure 2.6: Historical distribution map showing sampled localities where *Sandelia capensis* was recorded in the Krom River system.

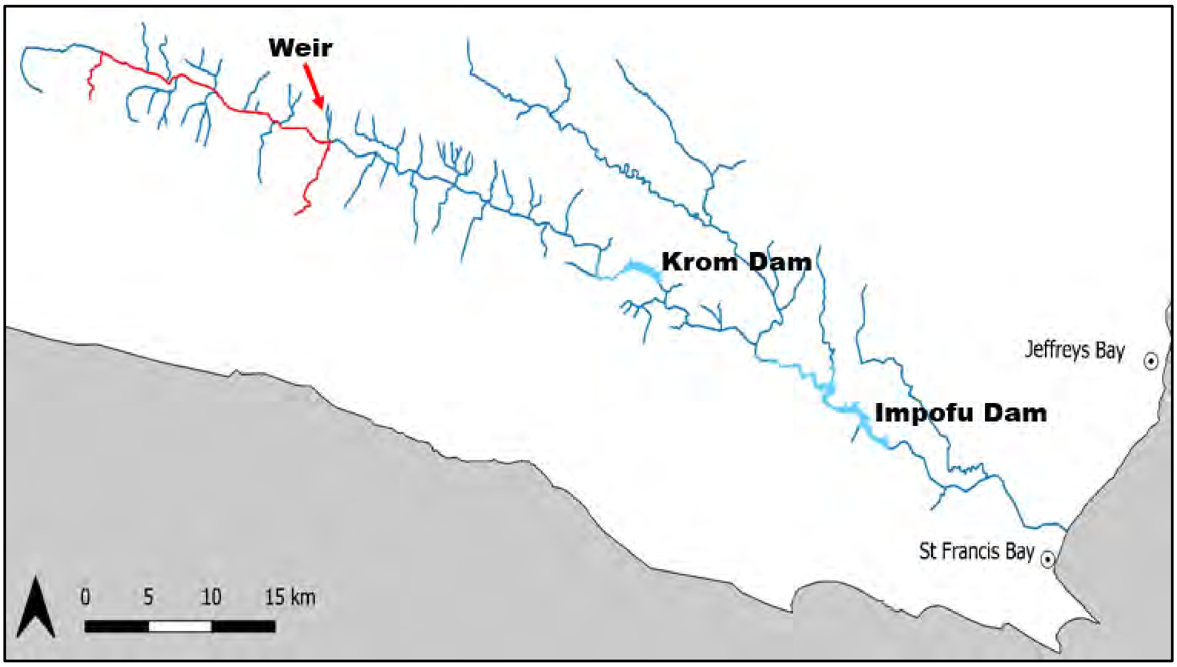


Figure 2.7: Map showing the distribution of the Cape kurper *Sandelia capensis* (South Coast lineage), represented by the Krom population, recorded in 2019 from sampled localities in the Krom River system.

**Non-native fish species in the Krom River**

Figures 2.8 to 2.10 show the distribution ranges of the two non-native fish species sampled during field surveys conducted in the Krom River system up to 2019. The invasion of rivers, particularly by non-native piscivore such as *Micropterus salmoides*, has been identified as the primary reason for the decline in distribution ranges of native fish species. The presence of native fish species in the Krom River system has been affected by intensification of human activities and they now inhabit only small proportions of the inferred historical range. All the remaining native populations are now extremely fragmented and isolated, due to the presence of dams, degradation of suitable habitats, and the presence of non-native predators (Figures 2.8 – 2.10) in the mainstem localities.

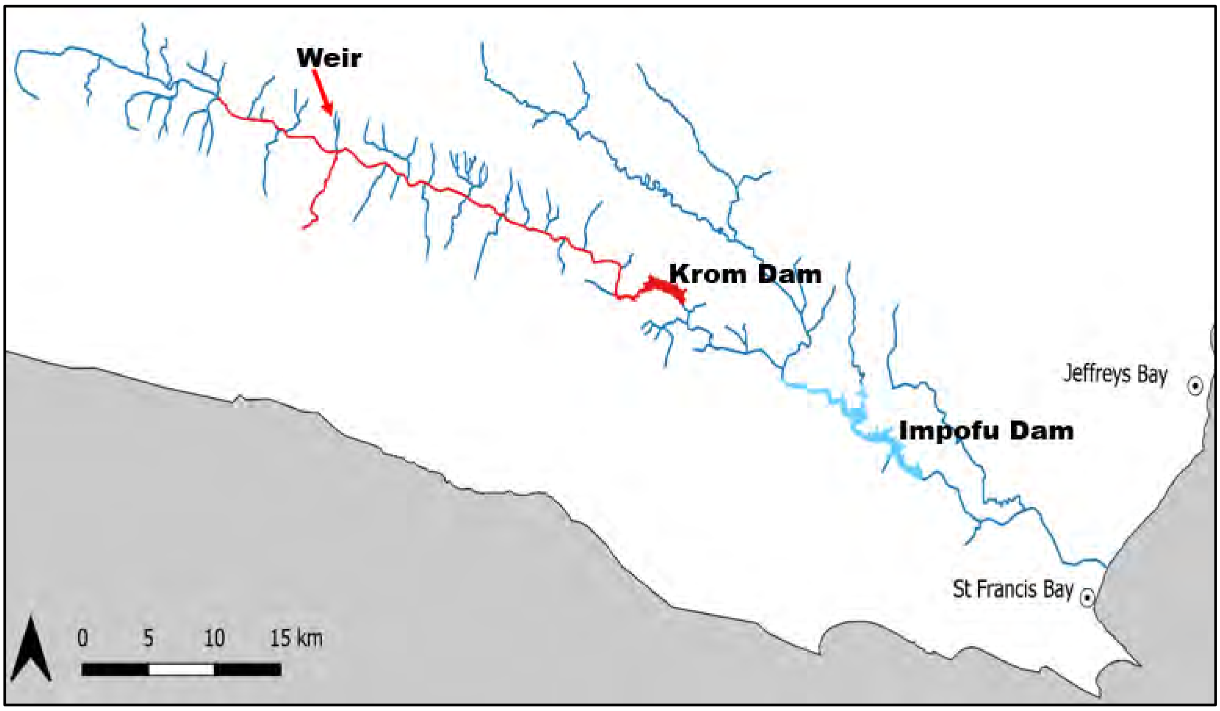


Figure 2.8: Distribution of the largemouth bass *Micropterus salmoides* recorded at mainstem localities and tributaries of the Krom River system in 2019.

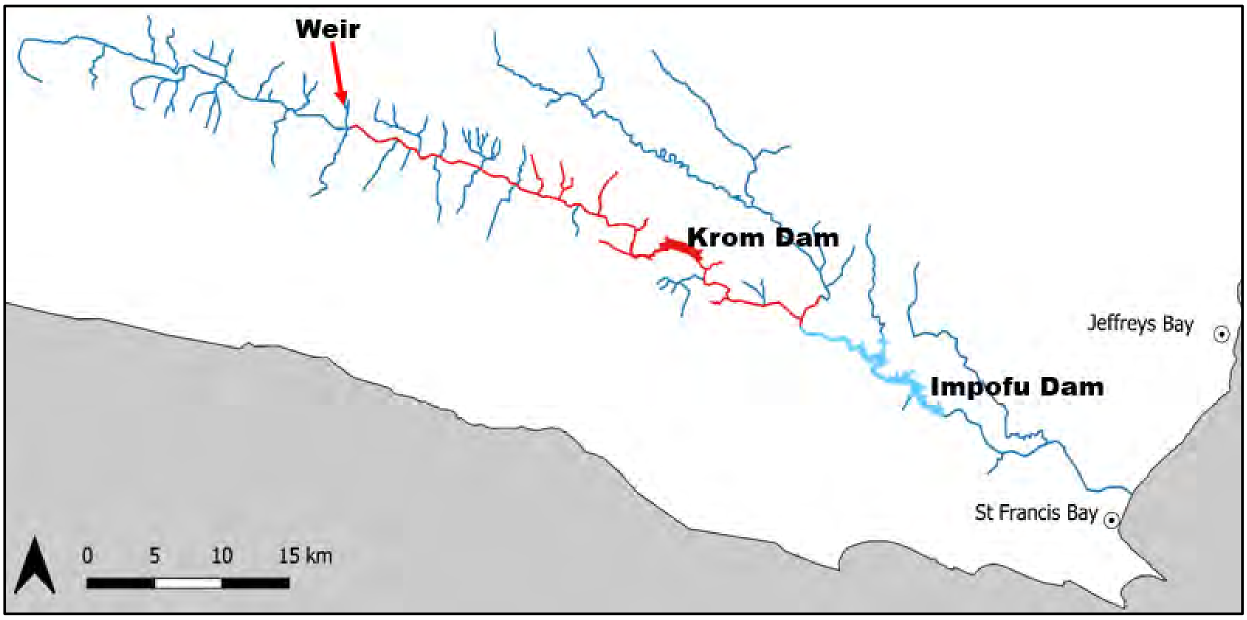


Figure 2.9: Map of the Krom River system showing localities in the mainstem and dam site, where the non-native bluegill sunfish *Lepomis macrochirus* was recorded in 2019.

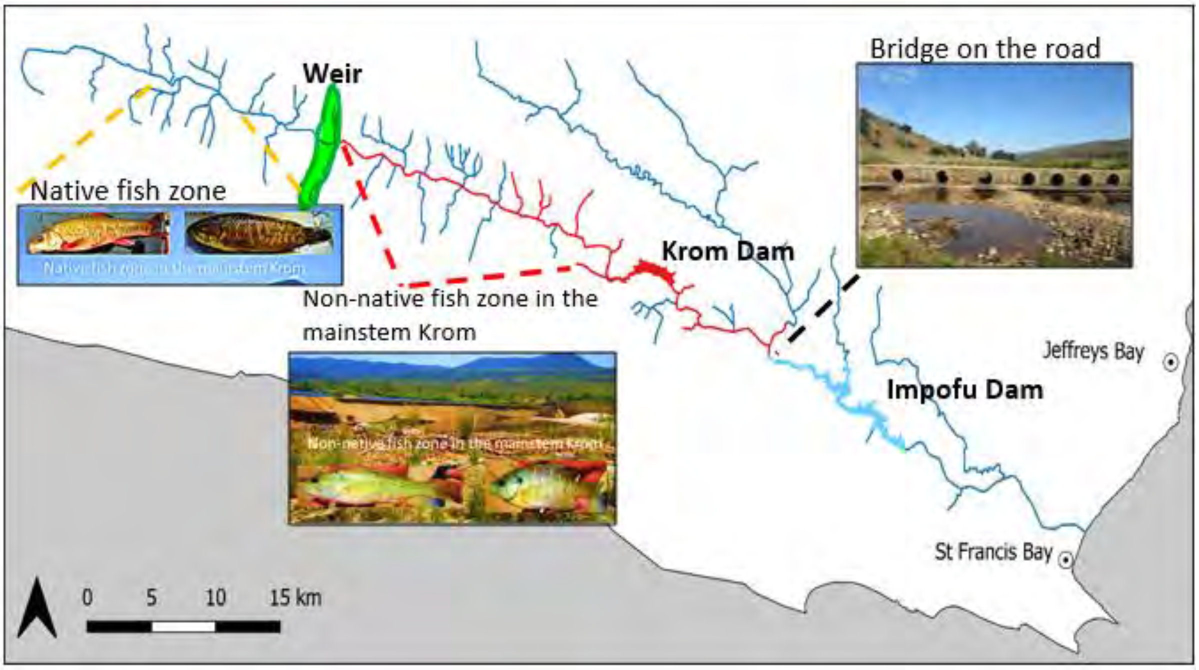


Figure 2.10: Distribution map of the fishes in the Krom River system showing the location of man-made instream physical barriers (weirs and dams), localities where the non-native fishes (*Lepomis macrochirus* and *Micropterus salmoides*) were recorded and their potential distribution range across the system.

## Discussion

Most river systems in the Cape Fold Ecoregion (CFE) are moderately to severely degraded due to impacts such as water extraction, sedimentation and pollution (Shelton et al. 2017), and due to their linear network, rivers are especially susceptible to fragmentation effects of barriers. Population fragmentation is considered a major threat to aquatic biodiversity (Nilsson et al., 2005; Liermann et al., 2012). This is due to the fact that, loss of population connectivity and disruption of gene flow are likely to increase the risk of extinction caused by inbreeding depression and loss of within-population genetic diversity. The loss of genetic diversity through fragmentation by weirs has been demonstrated by Meldgaard et al. (2002). The construction of physical barriers such as dams and weirs in the Krom River system has resulted in the fragmentation of native fish populations, particularly the Critically Endangered *P. senticeps*.

The findings of this study indicate that current distribution ranges of the three taxa have decreased, and the ranges are now fragmented compared to their historical distribution. This study found that the weir as shown on the map (Figures 2.10) formed the upper limit for non-native fishes. Largemouth bass and bluegill sunfish occurred immediately below the weir, and at almost all mainstem localities downstream of the weir. Most of the remnant populations of native species, redfins and Cape kurper occurred immediately above the weir. This suggests that the construction of instream physical barriers and complete water abstraction points have played a major role in the fragmentation of geographical ranges of native fishes in the Krom River.

Although, the weirs and their associated hydrological impacts may have contributed to significant decline in the historical distribution ranges of native fishes in the Krom River system, they have certainly prevented range expansion of non-native fish species into many of the tributaries and uninvaded sections of the river. In a similar case to the Gamtoos River system, *Galaxias zebratus* 'Joubertina' has not recently occupied the lower tributaries of the Krom River systems from upstream, and this suggests that lower altitude tributaries might not have suitable habitat.

Non-native fish species have been intentionally introduced into South African aquatic systems for aquaculture purposes, as angling species and as biological controls (Cambray & Jubb, 1977), where they have spread rapidly and successfully established in new regions (Ricciardi & Cohen, 2007). For example, the largemouth bass were first imported in 1928 (Ellender et al. 2014), and the bluegill sunfish was introduced as a source of food for the bass species (Harrison, 1952). Most of the introduction and spread of non-native fishes are mediated by human activities (Minchin et al., 2013; Ellender & Weyl, 2014; Kiruba-Sankar et al., 2018). Non-native species invasions have adverse ecological effects and threaten biodiversity (Strayer, 2010). The spread and establishment of non-native piscivores has been implicated in many studies as one of the major causes for large-scale decline in the distribution ranges of native fishes in many river systems in the CFE and neighbouring ecoregions (Didham et al., 2007; Kadye et al., 2013; Kadye & Booth, 2014; Chakona & Skelton, 2017; Ellender et al., 2017, 2018; Chakona et al., 2019a, 2020a). There are major conservation implications associated with non-native fish introductions (Dextrase & Mandrak, 2006), including extinctions of native biota related to direct predation and competition, habitat modification, alteration of food webs and hybridisation with native species (Cucherousset & Olden, 2011). Previous studies on predation impact have shown local extirpations of redfin minnows in habitats invaded by largemouth and smallmouth bass, and trout that are known to be visual predators, with preference for slower flowing, turbid waters (Skelton, 2001; Lowe et al., 2008; Russell, 2011; Shelton et al., 2018). Therefore, if these non-native fish species manage to invade sections that harbour the remnant populations of native fish species, they will likely have a negative influence on the species. Comparisons of historical and recent data indicate that non-native fishes were initially restricted to a localised area, and have gradually managed to establish and expand their ranges in the mainstem and lower sections of the Krom River system. The distribution of the native species has been severely fragmented by the recent introduction of non-native fish species. Based on the results, ongoing habitat destruction and decline in distribution ranges of native species in the Krom River is therefore likely due to these multiple impacts.

Although the constructions of physical barriers can have negative impacts on native fish populations, whereby habitat fragmentation and reduced hydrological connectivity can severely restrict distributions (Pess et al., 2008; Haddad et al., 2015; Cole et al., 2016). Instream barriers are used as a management strategy to control the spread of non-native fish species, to establish and maintain connectivity for native fish species. Converting the bridge (Figure 2.10) to a fish barrier will enable alien fish management projects to be implemented upstream of the bridge, thereby increasing available habitat for the threatened fish in the headwaters of the system.

The impacts of agricultural activities and non-native plants are well documented as biodiversity threats (Harding et al., 1999; Walser & Bart, 1999). Non-native plants have been associated largely with the alteration of river hydrology through increased water loss (Cullis et al., 2007) and reduced river flow (Chamier et al., 2012). Furthermore, landscape changes are hypothesised to have driven vicariant evolution by fragmenting species distributions that were formerly continuous (Barratt et al., 2018). The effects of agricultural land-use activities in the Krom River system have not been studied in detail, but previous studies have shown and argued that land use and land cover transformations through farming practices, road and bridge construction in this catchment may alter habitat integrity (Nsor, 2008; Rebelo, 2012). More recently, humans have converted a large proportions of forests into croplands (Ryan et al., 2014; Adole et al., 2018). The Krom River is impacted by agricultural activities, including crop and livestock farming, with large scale deciduous and citrus fruit farming in the Krom catchment. Previous studies have shown that agricultural runoff from such activities in agricultural lands may affect the structure, community composition and function in aquatic ecosystems. For example, agricultural activities have been found to alter water quality by increasing both organic and inorganic nutrient input (Haigh et al., 2008; Harding et al., 1999; Stauffer et al., 2000). Walser and Bart (1999) reported that agricultural runoff decreased fish biomass, specifically for species that use coarse substrate such as pools and riffles for feeding and spawning. In addition, excessive water abstraction for agricultural activities may alter river flow and result in sections below water-take off points becoming completely dry.

From the results of this study, eradication of non-native fishes from the mainstem section would facilitate recovery and establish connectivity of the fragmented populations of redfins and the Cape kurper. For instance, elsewhere in the CFE, eradication of non-native *Micropterus* spp. resulted in rapid recolonization and establishment of native fishes in the rehabilitated section of the Rondegat River (Weyl et al., 2014; van der Walt et al., 2019). There is also a need for increased awareness of the nature of this problem among the communities that live within the Krom River catchment. In particular, there should be engagement with land owners as part of effective and long-term measures to protect the remaining populations of threatened species. Close collaboration and exchange of information between various user groups, including landowners, policy makers, government officials and water specialists and their inclusion in decision making during planning, for example, for alien clearing and the rehabilitation, will help to ensure buy-in and setting up of long-term sustainable measures.

Information on past and present distributions of threatened species is crucial for identifying appropriate intervention measures to preserve remaining populations and possibly to initiate rehabilitation programs to allow recovery and re-establish connectivity among isolated populations (Fausch et al., 2006, 2009; Fullerton et al., 2010; Strecker et al., 2011; O'Hanley et al., 2013). The information is also important for demarcating Important Fish Areas or fish sanctuaries as part of the National Freshwater Ecosystem Priority Areas (NFEPA) initiative (Nel et al., 2011). Therefore, the results from this study can contribute to this body of knowledge that decision makers can draw on, as one of the primary objective of NFEPA is to provide strategies for prioritizing South Africa's freshwater ecosystems rehabilitation and conservation.

# CHAPTER 3

## Implications of incomplete taxonomy on conservation status assessment and delimitation of important fish areas

### Introduction

Growing evidence from molecular studies indicate that taxonomic diversity of freshwater fishes of southern Africa and elsewhere on the continent remains severely underestimated (Chakona et al., 2018; Arroyave et al., 2019; Jirsová et al., 2019; Braganca et al., 2020; Englmaier et al., 2020; Kambikambi et al., 2021; Mazungula & Chakona, 2021; Mutizwa et al., 2021). The incomplete documentation of taxonomic diversity presents challenges for biodiversity management, as it has implications for assessing species extinction risk and therefore the development of guidelines for prioritisation of important fish areas (Nel et al., 2011). As the IUCN Red List Assessment criteria exclusively focusses on formally described and valid species, cryptic species are likely to be inappropriately managed if they remain undescribed, because they will not receive conservation attention.

This is the case for several species of freshwater fish in southern Africa where molecular studies have uncovered genetically distinct lineages or candidate species, but where many of these remain undescribed (Swartz et al., 2007, 2009; Chakona et al., 2018, 2020b; Mutizwa et al., 2021). South African taxonomy on freshwater fishes has received considerable attention since the early 1900s (Skelton, 1996). Evidence from the 2016 national assessment of some of the unique genetic lineages of freshwater fishes in South Africa indicates that a number of them may be much more threatened than is documented, because they have narrow distribution ranges, are highly fragmented, and occur in landscapes with active or ongoing threats (Chakona et al., 2022).

Challenges related to poor documentation of fish taxonomic diversity have also been reported in many regions globally. For example, Shao et al. (2021) uncovered nine distinct genetic lineages clustering into eight geographic populations of bagrid catfish, *Tachysurus albomarginatus sensu lato* in East Asia, China. This discovery has been the catalyst for the delineation of the complex as eight putative species (Cheng et al., 2021). This species complex was previously assessed and listed as Least Concern (LC) in the Chinese freshwater fish Red List (Zhang & Cao, 2021). The discovery of unrecognized diversity within the different taxa has many implications for biodiversity conservation (Mace, 2004; Köhler et al., 2005; Bickford et al., 2007). The same pattern has been observed in the status of most threatened native fish species throughout the Iberian Peninsula, prompting a re-evaluation of current freshwater conservation measures for the area (Maceda-Veiga, 2013; Miranda & Pino-del-Carpio, 2016; Zhang et al., 2021), and the need to design and implement conservation plans for the poor conservation status of Iberian freshwater fish species. For example, analyses integrating morphological, molecular and geographic distribution data help in the identification of cryptic vertebrates such as fishes (Amaral et al., 2013; Steward et al., 2021). Similar trends of hidden diversity in freshwater fishes have been revealed using DNA-based studies in the Neotropical region (Junk, 2007; Reis et al., 2016; Ramirez et al., 2017).

The growing evidence from this type of study highlights the need to investigate the implications of incomplete taxonomy on conservation status assessments and prioritisation. This study focussed on case studies of species complexes of freshwater fishes whose taxonomy has been recently resolved, as well as two complexes with lineages that await formal recognition as distinct species. We focussed on well-studied species with up-to-date distribution information from recent surveys. The study taxa used were *Pseudobarbus afer sensu lato* (sl), *Enteromius anoplus* sl, *Amphilius natalensis* sl, *Sandelia capensis* and *S. bainsii*. Swartz et al. (2007) were the first authors to show that *P. afer*, as it was known at that time, was a species complex comprising four genetically distinct and allopatrically distributed lineages. Chakona & Skelton (2017) subsequently redescribed *P. afer*, revalidated *Pseudobarbus senticeps*, described a new species, *Pseudobarbus Swartzi*, and provided more accurate distribution ranges for each of these species. The taxonomic status of the fourth lineage, the Forest lineage, is presently being investigated (Chakona, 2022 pers. comm). Kambikambi et al. (2021) undertook a comprehensive genetic and morphological study of *E. anoplus* sl, and provided evidence that led to the redescription of *E. anoplus sensu stricto* (ss), the revalidation of *E. oraniensis* and *E. cernuus*, and the description of a new species, *E. mandelai*. Similarly, through use of an integrative taxonomic approach, Mazungula & Chakona (2021) showed that *A. natalensis*, as it was known at that time, was a complex of five genetically and morphologically divergent lineages with allopatric distribution ranges, namely *A. natalensis* ss and *A. laticaudatus*, which were redescribed, and *A. zuluorum*, *A. engelbrechti*, *A. marshalli* and *A. leopardus*, that were described as new species to science. The pattern of existence of undocumented diversity was also uncovered in the two anabantids that are endemic to South Africa, *S. capensis* and *S. bainsii* (Bronaugh et al., 2020; Chakona et al., 2020b).

The aim of this study was to therefore demonstrate how an inadequate knowledge of taxonomy can affect the assessment of extinction risk and potentially misdirect conservation prioritisation. Specifically, the study examined how change in taxonomy or recognition of undescribed genetic lineages in faunal listings affects range size and IUCN Red List risk category. Findings from this preliminary assessment emphasize the need for improving taxonomic knowledge through accurate delimitation of species boundaries, particularly within wide ranging taxa, in order to inform effective conservation practices and careful biodiversity management decisions.

## Methods

The data used for mapping the distribution ranges for each of the complexes and their lineages or species considered in this study was obtained from the NRF-SAIAB database and supplemented with information and data from the recently published literature for each of the case studies (as described in the introduction to this chapter). The risk of extinction was assessed following the IUCN Red List categories and criteria (IUCN, 2001, 2003). These assessments were done at two scales. Firstly, they included distribution data for the species complex, and secondly, they focused on each of the lineages or delimited species within each complex separately. For this study, Geospatial Conservation Assessment Tool (GeoCAT) based on the GPS points of species occurrence was used to calculate two range extent parameters. Distribution records were used to calculate the area of occupancy (AOO) in GeoCAT (Bachman et al., 2011). The extent of occurrence (EOO) was calculated based on the area of the maximum convex hull around all distribution points (Lehner & Grill, 2013). For each assessment, a csv file of the points was uploaded to this online program (see http://geocat.kew.org/) in order to calculate the Red List parameters on the geographic distribution of each species. The range extent calculations were used as expert knowledge of each species and their response to threats in accordance with the IUCN Red List Guidelines (IUCN, 2022). GeoCAT output for EOO and AOO are in $km^2$, within an IUCN default cell width of 2 km (Hernandez & Navarro, 2007). The key threats impacting each species or taxon were classified based on the IUCN Threat Classification Scheme Version 3.2 (www.iucnredlist.org).

## Results

For each of the 16 species and six known lineages (Table 3.1), the likelihood of extinction was assessed according to IUCN Red List Criteria: Version 3.2. Geographic range (Criterion B) was used in determining threat status for each species according to IUCN conservation status categories and threats (Appendix B).

**Table 3.1**: A list of the assessed native freshwater fish species and known lineages of South Africa, their calculated AOO & EOO and conservation status (Appendices A & C).

| **Species** | **Area of occupancy** | **Extent of occurrence** | **Threat category** |
|---|---|---|---|
| *Pseudobarbus afer complex* | 56 $km^2$ | 2,183 $km^2$ | Endangered (EN) |
| *P. senticeps* | 68 $km^2$ | 101.492 $km^2$ | Endangered (EN) |
| *P. swartzi* | 160 $km^2$ | 3,360 $km^2$ | Endangered (EN) |
| *Sandelia capensis* | - | - | Data Deficient (DD) |
| *S. 'capensis* South Coast' | 28 $km^2$ | 5,735 $km^2$ | Critically Endangered (CR) |
| *S. 'capensis* West Coast' | - | - | Not Evaluated (NE) |
| *S. 'capensis* Klein' | - | - | Not Evaluated (NE) |
| *Enteromius anoplus sl. (complex)* | 124 $km^2$ | 174,286.268 $km^2$ | Endangered (EN) |
| *E. anoplus ss* | 12 $km^2$ | 299.697 $km^2$ | Endangered (EN) |
| *E. cernuus* | 20 $km^2$ | 1,578 $km^2$ | Endangered (EN) |
| *E. mandelai sp* | 80 $km^2$ | 4,350 $km^2$ | Endangered (EN) |
| *E. oraniensis* | 12 $km^2$ | 286.380 $km^2$ | Endangered (EN) |
| *Sandelia bansii* | 92 $km^2$ | 8,812 $km^2$ | Endangered (EN) |
| *S. 'bansii* Kowie' | 64 $km^2$ | 3,302 $km^2$ | Endangered (EN) |
| *S. 'bansii* Keiskamma' | 16 $km^2$ | 151.514 $km^2$ | Endangered (EN) |
| *S. 'bansii* Buffalo' | 12 $km^2$ | 293.690 $km^2$ | Endangered (EN) |
| *Amphilius natalensis* | 712 $km^2$ | 414,579 $km^2$ | Endangered (EN) |
| *A. laticaudatus* | 16 $km^2$ | 3, 568 $km^2$ | Endangered (EN) |
| *A. leopardus* | 8 $km^2$ | 0.223 $km^2$ | Critically Endangered (CR) |
| *A. marshalli* | 28 $km^2$ | 1,741 $km^2$ | Endangered (EN) |
| *A. engelbrechti* | 8 $km^2$ | 0.433 $km^2$ | Critically Endangered (CR) |
| *A. zuluorum* | 24 $km^2$ | 542.099 $km^2$ | Endangered (EN) |

### *IUCN conservation status assessment*

Geospatial conservation assessment tool (GeoCAT) was used to determine the conservation status of each described species, and to assess their risk of extinction using the IUCN Red List Criteria. The tool has two estimators, the extent of occurrence (EOO) and the area of occupation (AOO). The EOO which is a measure of the geographic range size of a species, contained all sites of occurrence, shown with a polygon in the map (Appendix C), to give an indication of density of collections, whereas the measure of the area in which the species occurred was given by the AOO (Bachman et al., 2011).

## Discussion

Geographic range estimates AOO and EOO for the currently assessed taxa calculations of show ranges from 8 $km^2$ to 712 $km^2$. The taxa were classified as Critically Endangered and Endangered under threat categories for the newly described and revalidated species. This study determined that the majority of the assessed species (this study) ranked by IUCN are classified as highly threatened under the IUCN risk categories, which means that the species face a high risk of extinction. From this study it is clear that by considering species as widespread, local extinctions are overlooked, because of their relative size and range. For example, the genus *Amphilius* contains two species assessed as Critically Endangered and four classified as Endangered. However, due to the lack of taxonomic and distribution data for many species of different taxonomic groups, precise assessments on their extinction risks can only be approximated (Mimouni & Beisner, 2016). This is the case with the Data Deficient, *Sandelia 'capensis* West Coast' and *S. 'capensis* Klein' lineages, due to the paucity of information on the distribution of these species and lineages under study, a proper assessment of their conservation status was not made.

Conservation status of these widely distributed species complexes were determined to be of Least Concern or Near Threatened. For example, the recent conservation status for *E. anoplus* s.l. complex, assessed as Least Concern by Woodford in 2017, was rendered obsolete, because new information shows that the species is actually a complex with four genetic and morphologically distinct species. This new information shows that the species have narrow distribution ranges, restricted to a single river system or few localities. Their geographic range sizes are in continuous decline due to the multiple impacts of identified threats. The last IUCN assessment for *Pseudobarbus swartzi* was in 2017, when it was listed as Vulnerable. This was with an extent of occurrence of 2,254 $km^2$ and an area of occupancy of 36 $km^2$. The revision of the conservation status based on this study, has resulted in an elevation of the species to Endangered status. The results of this study show that this species has an AOO of 160 $km^2$ and EOO of 3.360 $km^2$. This might be due to its more restricted distribution range and the presence of alien predators, primarily *Micropterus* spp. and *Clarias gariepinus*, that have managed to invade the mainstem sections of the river (Chakona, Weyl & Ellender, 2017). Although the native fish species has dramatically declined and their distribution ranges have been reduced due to invasion by alien fish species and absence of suitable habitats. There may, however, be a possible increase in the remnant native fish populations, in the uninvaded natural habitat. For example, *Pseudobarbus senticeps'* last conservation status was assessed as Critically Endangered by Chakona & Skelton (2017). The results of the assessment from this study (Table 3.1), show that the species' area of occupancy has increased from 20 $km^2$ to 68 $km^2$. Based on the results, the species demonstrate increase in range, however is still being marked as highly threatened in the IUCN Red List. Conservation status of the Krom River redfin population might be upgraded to Endangered, due to the improved quantity and quality of spatial data.

Limited data pose a challenge to both the species conservation and taxonomy, since lack of distribution data may lead to under- or over-estimations of their geographic distributions. Accurate range, AOO and EOO estimates are of great importance because they are used in assessing the conservation status of these highly threatened fish fauna. The quality of taxonomic delimitation of these taxa should be guaranteed to prevent taxonomic confusions and implications of misidentifications in the IUCN Red Lists.

Freshwater fish biodiversity hotspots need to be prioritized for conservation, in order to minimize the impact of human activities on these animals (Cuttelod et al., 2009; Bellard et al., 2014; Mendoza-Ponce et al., 2020). Unfortunately, a lack of knowledge about many species' distribution and abundance has hindered the implementation of effective conservation strategies. Some of the species have a naturally disjunct distribution and there is limited data, therefore, the distribution ranges of these taxa and taxonomic knowledge is needed to ensure success in preserving target species. Recent advances in the field of freshwater fish biodiversity have allowed researchers to gain a better understanding of species' distribution and conservation status. Revalidation and description of new species and known lineages has resulted in the corresponding re-evaluation of their distribution ranges and conservation status for these taxa. This is because, the apparent expansion of the species known distribution range is countered by potential local extirpations.

This study supports the findings of the study by Chakona et al. (2022) who found that South African freshwater fish species are highly threatened. It is evident that these species have narrower distribution ranges with corresponding higher extinction risks than previously thought. The distribution patterns and survival of these species and identified lineages have been influenced by anthropogenic impacts, including invasions by piscivores (Kadye et al., 2020), and land use changes in different catchments (Bomhard et al., 2005). The IUCN Red List criteria has been implemented as the global standard for the evaluation of the conservation status of freshwater fishes, to assess a species' extinction risk (Mace et al., 2008), and these assessments have become integral to identify conservation targets to prioritize (IUCN, 2016).

There is growing need to broaden the definition of cryptic diversity and incorporate environmental data when assessing variation. The presence of cryptic species within the complex species in these taxa will affect the future species' conservation status on the IUCN Red List. The revision of their conservation status would most likely result in a possible elevation to Endangered or Critically Endangered due to their more restricted ranges, when considered with an independent assessment of newly emerging taxa. These species are in the highly threatened category, and all locations they occur in are under continuous degradation and threat from invasion by non-native species in the mainstem sections, and there is lack of management in their suitable habitats. These new species and lineages are currently known to occur only in single river system, urgent conservation priority is needed. These results demonstrate that the projected range reductions of threatened species and lineages would translate into loss of populations with unique genetic and ecological characteristics. It is therefore, important to improve on the species data and their distribution ranges, for better knowledge on the true extent of the extinct and extant biodiversity (Tapley et al., 2018).

The use of integrative taxonomic methods, and the discovery and description of new species as presented in this study, has laid the groundwork for future studies. There is a need to update scientific databases at museums which house natural history collections, as they play a valuable role in preserving biodiversity. The findings of this study demonstrate the value of combining data from different collections, as this study would not have been possible without the extensive museum collections from the NRF-SAIAB database. There is a need to gather more information on the distribution and abundance of these newly described and recognized undescribed genetic lineages. Even if the species is assessed as Data Deficient, the information is important and more useful to conservation assessments, for the species to get prioritisation rather than leaving the species as Not Evaluated. The assessments will help gauge trends as taxa are prioritized, and sufficiently update conservation status. The results of this study further emphasize the need to incorporate genetic and environmental data in IUCN assessments for securing the protection of highly threatened lineages and species and keep pace with assessing the newly described and revalidated species. The results will contribute to ongoing studies to resolve the genetic diversity, ecology and distribution patterns of freshwater fish species and lineages. However, there is still much taxonomic work to be done and a lot more to be discovered on the distribution of these taxa, evidenced by the fact that among recent studies discovering new fish species and lineages at the time of this study, some still await formal description.

# CHAPTER 4

# GENERAL DISCUSSION

Despite freshwater fish species being considered among the most highly threatened taxa globally, there is still insufficient information to support their conservation efforts of their biota (Carrizo et al., 2013; Jordaan, 2020; Hughes, 2021; Chakona et al., 2022). Therefore, a general understanding on the distribution, ecology and taxonomic status of the newly discovered or described fish species and lineages is imperative to provide expert guidance to policy makers and conservation planners on the conservation and sustainable use of freshwater fishes and their habitats for effective management strategies (Pressey, 2004; Forest et al., 2007; Chakona et al., 2017; Moore et al., 2017; Rudra, 2018; Nkosi et al., 2021). In line with these imperatives, this study assessed the distribution and conservation status of the freshwater fishes in the Krom River system, highlighting the importance of presence-absence data and habitat characteristics of the fish species in this system to guide their conservation.

To provide insight to these aspects, Chapter 2 mapped the changes in the current distribution patterns of native and non-native fish species in the Krom River compared to their historical ranges, and identified the principal drivers of changes affecting their distribution within the system. Based on the results, it was found that in general, the current distribution ranges of the native fish species have been reduced compared to their historical ranges. The recent surveys revealed the presence of three native fish species in the system the Krom River redfin, namely *Pseudobarbus senticeps*, the Cape kurper, *Sandelia capensis* and the Cape galaxias, *Galaxias zebratus* 'Joubertina'. Despite being historically abundant and widely distributed in the system (Chakona & Skelton, 2017; Chakona et al., 2018), these species are now confined to the upper reaches of the mainstem and in the upper tributaries where non-native species have not invaded and where water quality remains near-pristine. The records show that *P. senticeps* and *S. capensis* co-occurred, the former being more widespread than the latter. In comparison, although *Galaxias zebratus* 'Joubertina' is known to occur in the Krom River, this taxon was not caught from the survey conducted in 2019. Overall, the absence of native fishes within invaded sections of the study system suggest that that due to their small adult sizes (*P. senticeps* 120 mm SL, *S. capensis* 200 mm SL, and *G. zebratus* 75mm SL) (Skelton, 1993), these species were likely to be vulnerable to predation by piscivorous non-native fish species, and thus unlikely to coexist with invasive species for long.

Similar patterns have been observed in river systems in this ecoregion, such as the Berg and Witte rivers, which were historically populated with native species. However, populations of these native fishes have been outcompeted and extirpated due to invasion by non-native fishes (Clark et al., 2009; Shelton et al., 2014). A study by Shelton et al. (2015) shed some light on the predation impacts of non-natives in the Cape Floristic Region, with the findings of their study providing empirical evidence that the presence of introduced non-native fish species have deleterious impacts on the native fish populations. Furthermore, Van der Walt et al. (2016) showed that the top predators such as the largemouth bass reduced the abundance of native fish species such as the small-bodied cyprinid minnows, *Pseudobarbus phlegethon* and *Sedercypris calidus* (Skelton et al., 2018) in the Olifants-Doring River system, through predation and competition. Information on these invasion impacts has been instrumental in implementing conservations and management efforts to mitigate further impacts. For example, conservation intervention projects such as eradication of non-native species has been attempted. Specifically, rotenone, a piscicide, has been used to eradicate invasive piscivore population of smallmouth bass, *Micropterus dolomieu*, 'from the Rondegat River and has resulted in effective removal of this non-native fish species (Weyl et al., 2014, 2016a; Donaldson & Cooke, 2016; Sandodden et al., 2018). Therefore, in addition to limiting further introductions of non-native fishes into the Krom River, interventions such as eradiation could be attempted where possible. However, caution should be taken when using piscicides as they can have negative impacts, such as killing native fish populations and other aquatic organisms such as invertebrates (Woodford et al., 2013).

In addition to the presence of non-native fishes, there is concern on the potential impact of anthropogenic disturbances such as agricultural activities in the Krom River catchment. While there were no empirical findings related to this aspect during this study, evidence from the adjacent ecoregions and elsewhere have documented agricultural impacts on rivers and streams. For example, some studies have shown that agricultural runoff not only reduced fish richness and diversity in impacted sites (Walser & Bart, 1999; Staufer et al., 2000), but had potential implications on the structure and function of aquatic food web dynamics by altering the taxonomic composition of lower trophic groups, such as primary producers and aquatic invertebrates (Matomela et al., 2021). Therefore, high levels of land degradation that have been reported from the Krom River system as a result of unsustainable farming, land conversions and the presence of non-native plant species is concerning. Specifically, the over-abstraction of water for agriculture and construction of instream-barriers, which are also common impacts affecting river systems in this ecoregion (e.g. Skelton et al., 1995; Rouget et al., 2003; Nel et al., 2007), have potential impacts, such as altering the hydrology of this system and habitat loss. This has potential negative implications on the native fishes of this system considering that many of these now only occur in low-order tributaries that are more likely to be susceptible to the above-mentioned impacts.

Recent taxonomic revisions of freshwater fishes in the CFE and other freshwater ecoregions in southern Africa have assisted with resolving taxonomic conflicts within several currently recognised species (Chakona & Skelton, 2017; Chakona et al., 2018; Chakona et al., 2020b; Bronaugh et al., 2020; Kambikambi et al., 2021; Mazungula & Chakona, 2021; Mutizwa et al., 2021), and has facilitated the determination of the re-evaluation of the conservation status of previously synonymised species (e.g. Kadye et al., 2016; Chakona & Skelton, 2017; Woodford, 2017; Chakona et al., 2018; Chakona et al., 2019b; Chakona et al., 2022). Despite increasing attention in addressing the taxonomic barriers in freshwater biodiversity, the slow rate at which biodiversity is described hinders conservation status assessments (Lundberg et al., 2000; Godfray, 2002; Lipscomb et al., 2003; Wheeler, 2004; de Carvalho et al., 2007; Desforges et al., 2022). Consistent with this observation, the second data chapter (see Chapter 3) of this thesis assessed the implications of incomplete fish taxonomy and their impact on conservation status assessments. The conservation status of the taxa in this study were reassessed under the IUCN Red List categories using GeoCAT to determine the extent of occurrence, EOO and area of occupancy, AOO. Currently, *Sandelia capensis*, which is broadly described as occurring in several coastal rivers within the CFE (Skelton, 2001), is categorized as Data Deficient. Recent molecular taxonomic research has unravelled that this taxon comprises three allopatric lineages, *S. 'capensis* South Coast' which include the Krom River population, *S. 'capensis* Klein' confined to the Klein River and *S. 'capensis* West Coast' which occurs in the Langvlei, Verlorenvlei, Berg and Diep rivers (Bronaugh et al., 2020). This study's reassessment of *S. 'capensis* South Coast' categorised it as Critically Endangered. A similar pattern for other *S. capensis* lineages, species from other genera, such as two recently described *Amphilius* species, *A. leopardus* and *A. engelbrechti* (Mazungula & Chakona 2021), which were categorized as Critically Endangered. Three *Sandelia bansii* lineages, *S. 'bansii Kowie'*, *S. 'bansii Keiskamma'* and *S. 'bansii Buffalo'*, and 13 species (*Pseudobarbus afer sensu stricto* (ss), *P. senticeps, P. swartzi, E. anoplus* (ss), *E. oraniensis, E. cernuus, E. mandelai, Amphilius natalensis* sl, *A. laticaudatus*, *A. zuluorum*, *A. marshalli* and *A. leopardus*), which were previously thought to belong to genera that were considered widespread and thus categorised as Least Concern, were reassessed as Endangered in this study. The results of this study, thus, provided evidence that incomplete taxonomy negatively affects the conservation status decisions. Specifically, lack of information appears to have negative implications on the Red List assessments of species that are data deficient. Research has shown that data deficient species should be given more attention and afforded more research as they may often be threatened. The improved knowledge may help to make progress in predicting the likely status of these species (Pimm et al, 2014; Bland et al., 2015; Miqueleiz et al., 2022).

To conclude, informed scientific knowledge is essential to promote education and public awareness regarding the biological diversity and conservation crisis (Albert et al., 2020; Chakona et al., 2022). Because the land in the CFE is largely privately owned, successful implementation of conservation strategies requires the involvement of local communities in surveys for environmental education (Cambray & Pister, 2002). Appropriate measures need to be put in place, to ensure persistence of native fish populations and protect their habitats. The presence of instream barriers (weirs) needs monitoring as they are likely to contribute to further fragmentation of the highly endangered native fish species in the Krom River system. General fish monitoring and where possible, eradication of non-native fishes in invaded sections is one strategy that would facilitate recovery and establish connectivity of the fragmented remnant native fish populations in the Krom River. By documenting the potential impacts and conservation status of these threatened taxa, this study provided information to help bridge the information gaps and provide recommendations for future studies. This can be achieved through further studies on aquatic ecosystems to understand the ecological attributes and general life history strategies, behavioural and functional traits of these taxa following the invasions and consequences of these impacts on biodiversity (Diaz et al., 2001; Eros et al., 2009; Kadye et al., 2016; Catford et al., 2018). Moreover, detailed research is needed to evaluate ecological traits such as habitat associations, food web patterns and trophic ecology (Boecklen et al., 2011).

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## APPENDICES

Appendix A: Scientific and common names of fish species mentioned in the thesis.

| **Scientific Name** | **Common name** |
|---|---|
| *Amphilius natalensis* | Natal mountain catfish |
| *Amphilius engelbrechti* | Inkomati mountain catfish |
| *Amphilius laticaudatus* | Broadtail mountain catfish |
| *Amphilius leopardus* | Mottled mountain catfish |
| *Amphilius marshalli* | Slender mountain catfish |
| *Amphilius zuluorum* | uMkhomazi mountain catfish |
| *Cheilobarbus capensis* | Cape whitefish |
| *Clarias gariepinus* | Sharptooth catfish |
| *Enteromius anoplus* | Chubbyhead barb |
| *Enteromius cernuus* | Chubbyhead barb |
| *Enteromius oraniensis* | Chubbyhead barb |
| *Enteromius mandelai* | Chubbyhead barb |
| *Galaxias zebratus* | Cape galaxias |
| *Lepomis macrochirus* | Bluegill sunfish |
| *Micropterus salmoides* | Largemouth bass |
| *Oncorhynchus mykiss* | Rainbow trout |
| *Pseudobarbus afer* | Eastern Cape redfin |
| *Pseudobarbus asper* | Smallscale redfin, Kleinskub-rooiverkie |
| *Pseudobarbus senticeps* | Krom River redfin |
| *Pseudobarbus swartzi* | Gamtoos River redfin |
| *Salmo trutta* | Brown trout |
| *Sandelia bansii* | Eastern Cape rocky |
| *Sandelia capensis* | Cape kurper |

Appendix B: Summary of the five criteria (A–E) used to evaluate whether or not a taxon belongs in a threatened category (Critically Endangered, Endangered or Vulnerable) (adapted from Red List Guidelines 2022).

**A. Population size reduction.** Population reduction (measured over the longer of 10 years or 3 generations) based on any of A1 to A4

| | Critically Endangered | Endangered | Vulnerable |
|---|---|---|---|
| **A1** | ≥ 90% | ≥ 70% | ≥ 50% |
| **A2, A3 & A4** | ≥ 80% | ≥ 50% | ≥ 30% |

**A1** Population reduction observed, estimated, inferred, or suspected in the past where the causes of the reduction are clearly reversible AND understood AND have ceased.

**A2** Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

**A3** Population reduction projected, inferred or suspected to be met in the future (up to a maximum of 100 years) *[(a) cannot be used for A3].*

**A4** An observed, estimated, inferred, projected or suspected population reduction where the time period must include both the past and the future (up to a max. of 100 years in future), and where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

*based on any of the following:*

- **(a)** direct observation *[except A3]*
- **(b)** an index of abundance appropriate to the taxon
- **(c)** a decline in area of occupancy (AOO), extent of occurrence (EOO) and/or habitat quality
- **(d)** actual or potential levels of exploitation
- **(e)** effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

**B. Geographic range in the form of either B1 (extent of occurrence) AND/OR B2 (area of occupancy)**

| | Critically Endangered | Endangered | Vulnerable |
|---|---|---|---|
| **B1.** Extent of occurrence (EOO) | < 100 km² | < 5,000 km² | < 20,000 km² |
| **B2.** Area of occupancy (AOO) | < 10 km² | < 500 km² | < 2,000 km² |
| **AND at least 2 of the following 3 conditions:** | | | |
| **(a)** Severely fragmented **OR** Number of locations | = 1 | ≤ 5 | ≤ 10 |

**(b)** Continuing decline observed, estimated, inferred or projected in any of: **(i)** extent of occurrence; **(ii)** area of occupancy; **(iii)** area, extent and/or quality of habitat; **(iv)** number of locations or subpopulations; **(v)** number of mature individuals

**(c)** Extreme fluctuations in any of: **(i)** extent of occurrence; **(ii)** area of occupancy; **(iii)** number of locations or subpopulations; **(iv)** number of mature individuals

**C. Small population size and decline**

| | Critically Endangered | Endangered | Vulnerable |
|---|---|---|---|
| **Number of mature individuals** | < 250 | < 2,500 | < 10,000 |
| **AND at least one of C1 or C2** | | | |
| **C1.** An observed, estimated or projected continuing decline of at least (up to a max. of 100 years in future): | 25% in 3 years or 1 generation (whichever is longer) | 20% in 5 years or 2 generations (whichever is longer) | 10% in 10 years or 3 generations (whichever is longer) |
| **C2.** An observed, estimated, projected or inferred continuing decline AND at least 1 of the following 3 conditions: | | | |
| **(a)** **(i)** Number of mature individuals in each subpopulation | ≤ 50 | ≤ 250 | ≤ 1,000 |
| **(ii)** % of mature individuals in one subpopulation = | 90–100% | 95–100% | 100% |
| **(b)** Extreme fluctuations in the number of mature individuals | | | |

**D. Very small or restricted population**

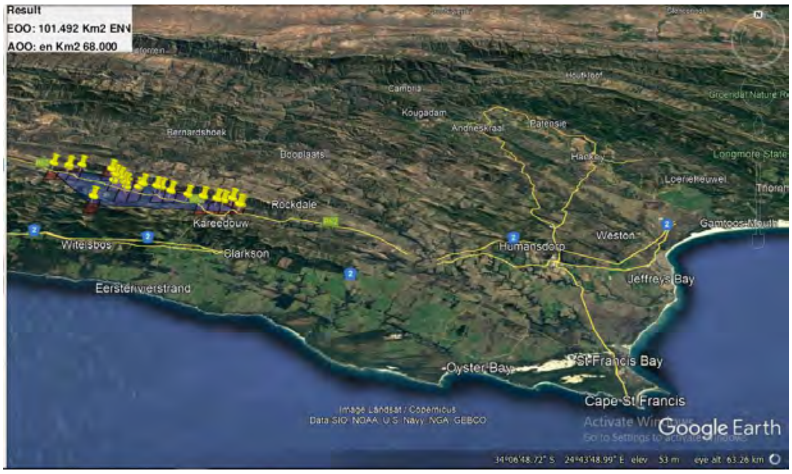
| | Critically Endangered | Endangered | Vulnerable |
|---|---|---|---|
| **D.** Number of mature individuals | < 50 | < 250 | **D1.** < 1,000 |
| **D2.** *Only applies to the VU category* Restricted area of occupancy or number of locations with a plausible future threat that could drive the taxon to CR or EX in a very short time. | - | - | **D2.** typically: AOO < 20 km² or number of locations ≤ 5 |

**E. Quantitative Analysis**

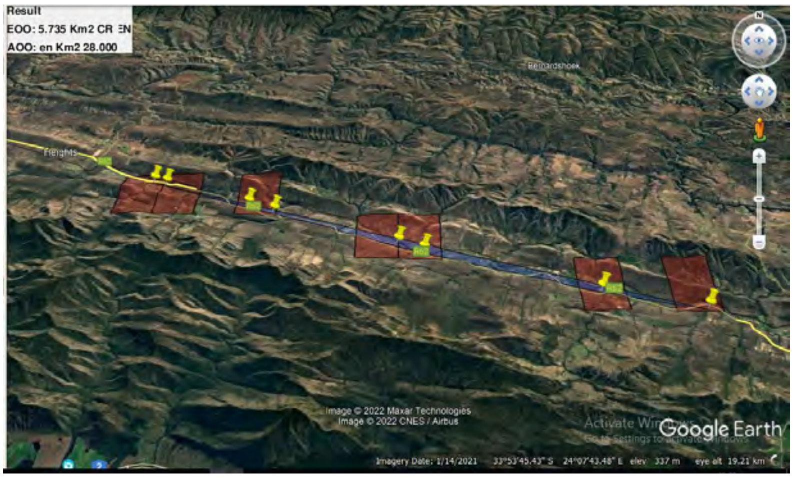
| | Critically Endangered | Endangered | Vulnerable |
|---|---|---|---|
| **Indicating the probability of extinction in the wild to be:** | ≥ 50% in 10 years or 3 generations, whichever is longer (100 years max.) | ≥ 20% in 20 years or 5 generations, whichever is longer (100 years max.) | ≥ 10% in 100 years |

1 Use of this summary sheet requires full understanding of the *IUCN Red List Categories and Criteria* and *Guidelines for Using the IUCN Red List Categories and Criteria*. Please refer to both documents for explanations of terms and concepts used here.

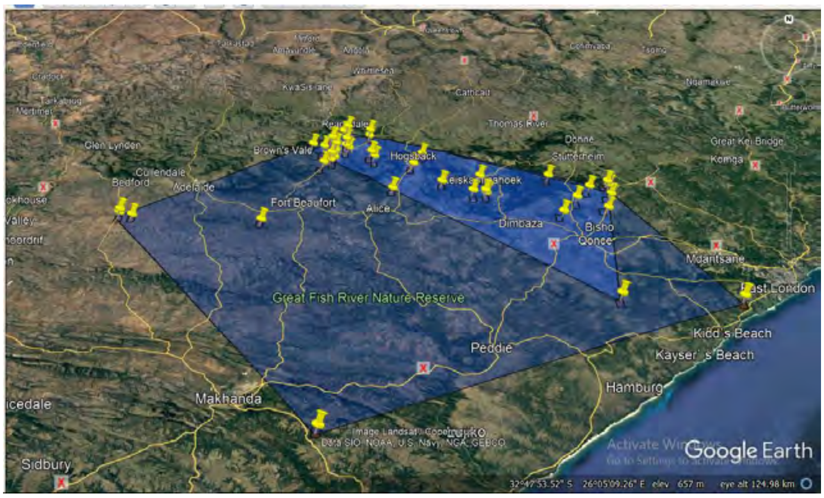
Appendix C: Geographic maps of the assessed taxa using GeoCAT with the results of geographic range estimates (EOO and AOO).




*Pseudobarbus senticeps* (Krom River redfin)



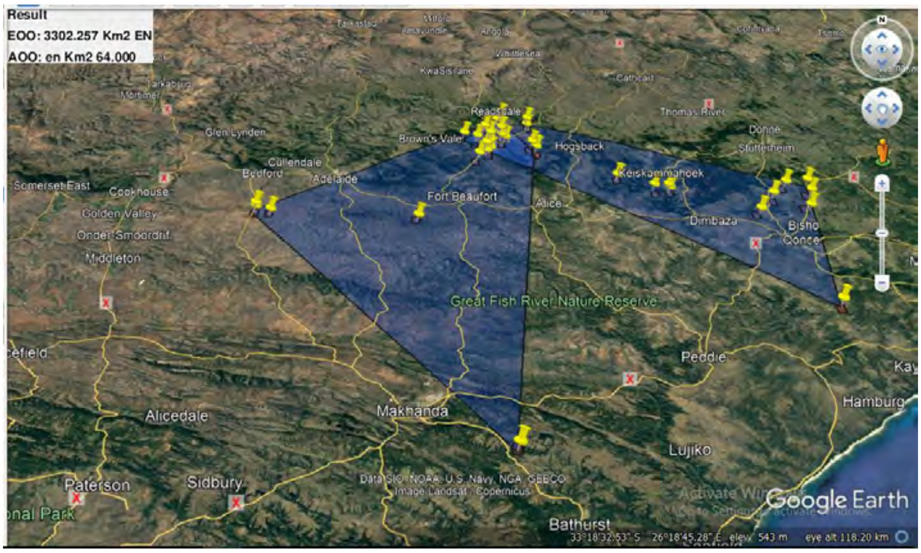

*Sandelia 'capensis* South Coast' (Krom population)




*Sandelia bansii* s.l. (Eastern Cape rocky)


*Sandelia "bansii* Buffalo"


*Sandelia "bansii* Keiskamma"



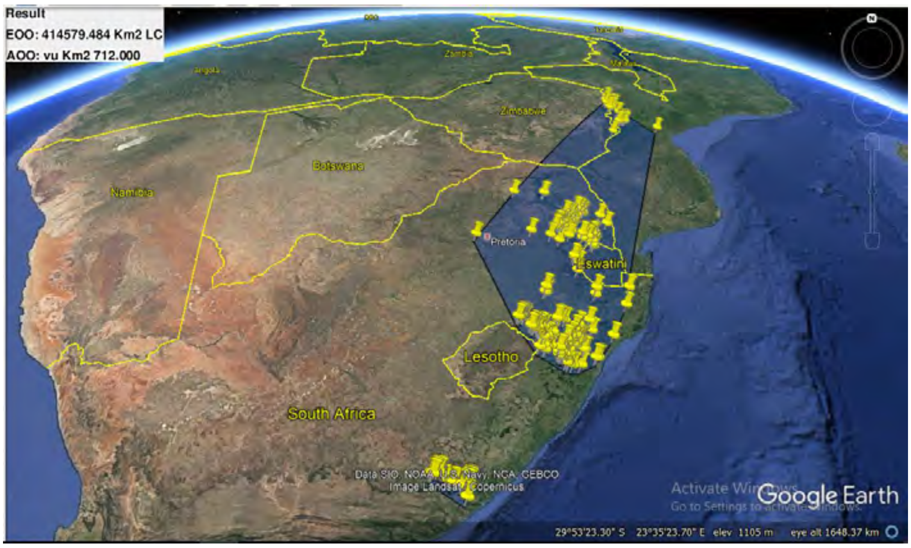

*Sandelia "bansii* Kowie"


*Amphilius engelbrechti*

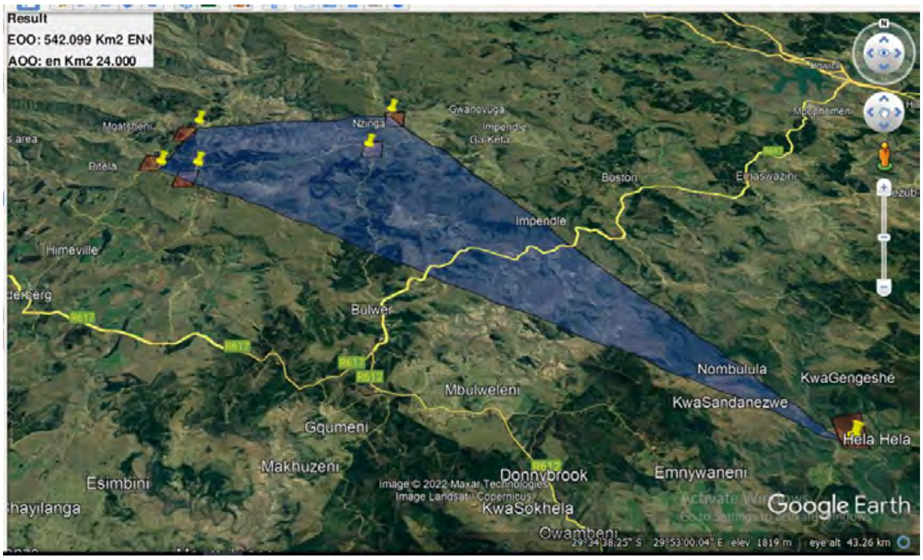

*Amphilius laticaudatus*


*Amphilius leopardus*

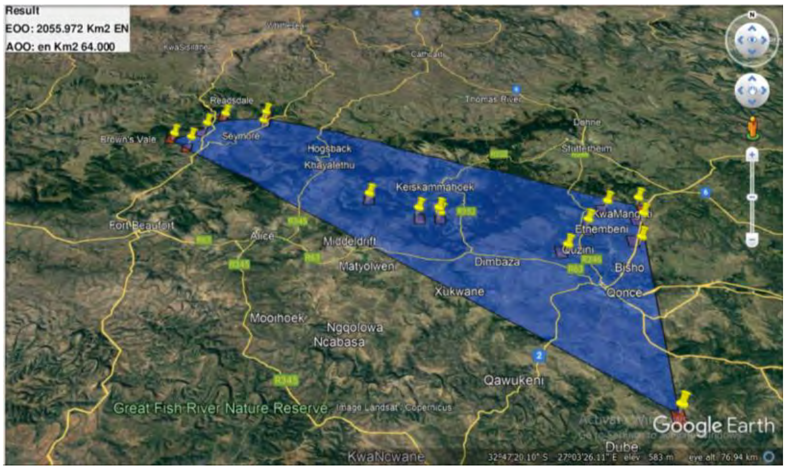

*Amphilius marshalli*



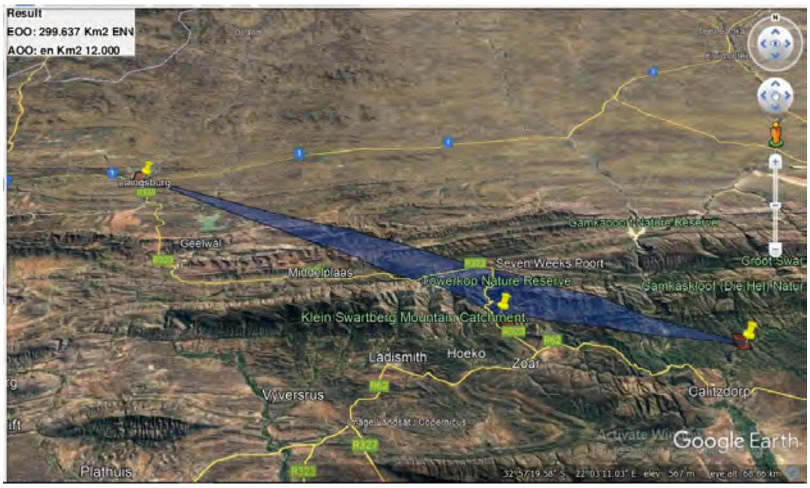

*Amphilius natalensis*




*Amphilius zuluorum*




*Enteromius anoplus*




*Enteromius anoplus* ss


*Enteromius cernuus*


*Enteromius mandelai* sp. Nov


*Enteromius oraniensis*