

Population crash of an endemic South African cyprinid: the role of non-native fish, drought and other environmental factors



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Plagiarism Declaration

1. I know that plagiarism is wrong. Plagiarism is using another's work and to pretend that it is one's own.
2. I have used the Conservation Biology Journal format as the convention for citation and referencing. Each significant contribution to, and quotation in, this project from the work, or works of other people has been attributed and has cited and referenced.
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Glossary

Acronyms

CFE: Cape Fold Ecoregion. A freshwater ecoregion, as described by Abell (2008) is “a large area encompassing one or more freshwater systems with a distinct assemblage of natural freshwater communities and species.” The CFE aligns roughly with the delineations of the Cape Floristic Region in South Africa.

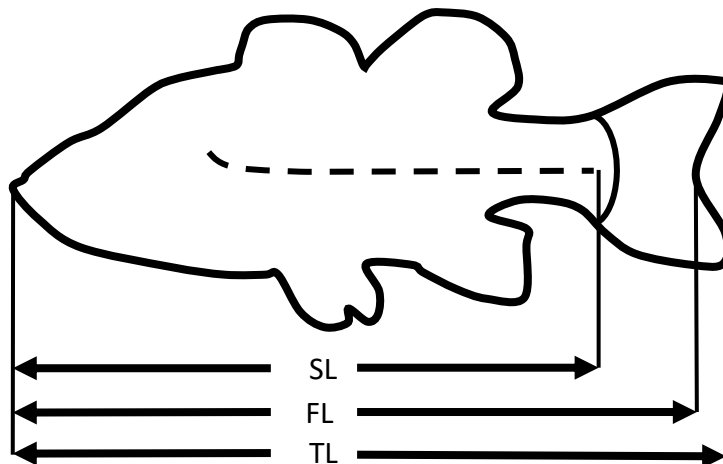
CPUE: Catch per unit effort. An indirect measure of the abundance of a species standardised by the effort (i.e. number of observation hours, number of samples taken, number of observers, and so on). In this case, CPUE uses the number of times a seine net was pulled through the water as the measure of effort, so CPUE values are standardised to reflect the number of fish per haul.

Fish length measurements:

FL: Fork length. The length of a fish measured from the snout to the end of the median caudal fin rays.

SL: Standard length. The length of a fish measured from the snout to the last vertebra. In other words, a measure of a fish that excludes the length of the caudal fin.

TL: Total length. The length of a fish measured from the snout to the end of the caudal fin.



msal: Metres above sea level.

ODRS: Olifants-Doring River system. Encompasses the Olifants and Doring Rivers and all associated tributaries in both catchments.

ONR: Oorlogskloof Nature Reserve.

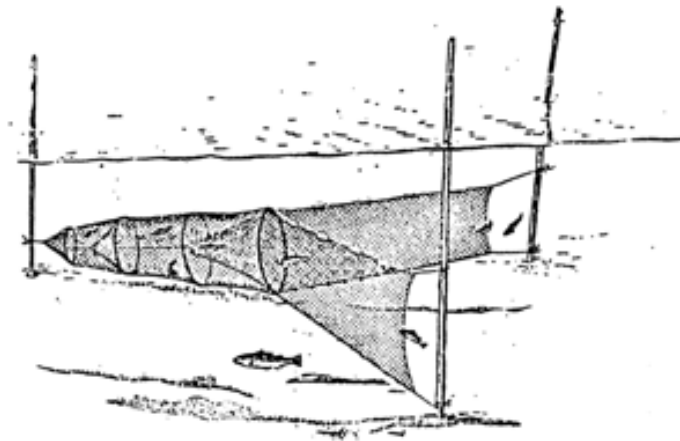
TAP: Total annual precipitation. Usually, the sum of all precipitation in a given year. In this case, the sum of all precipitation in the 12 months preceding each survey (beginning of March of one year to end of February of the next year).

YOY: Young-of-the-year. Otherwise referred to as age-0 fish. Refers to individuals born within the past year.

Definitions

Fingerling: Developmental stage of a young fish when, in addition to being able to feed themselves, they have developed working fins and scales. Typically the size of a finger.

Fyke net: A type of net used for passive sampling of fish consisting of a cylindrical or cone-shaped portion of netting mounted on rigid circular or square structures. Wings on either side help to guide fish toward the entrance of the net. Inside the cylindrical structure are a set of funnels/one-way valves that allow fish to swim deeper into the bag but make it difficult for them to swim out. Diagram courtesy of the Food and Agriculture Organization of the United Nations.



Invasive species: introduced biota that become widespread in their novel environments and harm economies, human health and ecosystems (Pfeiffer and Voeks 2008).

Non-native species: species occurring outside their natural range. “Non-native” may be used to describe species introduced outside their natural range within the borders of their native country. Non-native species need not necessarily harm ecosystems, human health or economies (National Park Service 2019).

Seine net: A type of net used for actively sampling fish. It is long and rectangular, often with a depression in the middle that acts as a pocket to more easily catch fish. Floats at the top and weights at the bottom help maintain its shape while operating. Two people operate the seine net, either walking or swimming while holding on to either end of the net (usually held open by rigid poles). To collect fish, the two samplers walk toward one another and once together, lift the seine net out of the water, trapping the fish inside the pocket. Photograph courtesy of Mandy Schumann.



Abstract

Native freshwater fish are in decline worldwide due to human impacts including impoundment, changes to water quality, over-abstraction, climate change and the introduction of non-native species. South Africa's Cape Fold Ecoregion (CFE) harbours exceptionally high freshwater fish endemism, yet the majority of these species are threatened with extinction. The Clanwilliam sandfish (*Labeo seeberi*), an endangered cyprinid, has declined across its range in the CFE and currently exists in only a handful of tributaries of the Doring River in severely fragmented populations. The last remaining recruiting sandfish population occurs in the Oorlogskloof River, making this tributary one of critical conservation value. In this study, I analysed a six-year dataset comprising fish survey data from 38 sites along 25 km of the Oorlogskloof River to characterise spatio-temporal variation in sandfish abundance and size structure and evaluate the relative influence of different environmental factors on sandfish population trends. The environmental factors considered included other fish in the system, especially three non-native fishes (banded tilapia *Tilapia sparrmanii*, smallmouth bass *Micropterus dolomieu* and bluegill sunfish *Lepomis macrochirus*), temperature, precipitation, habitat characteristics and a range of physico-chemical factors. The results show that sandfish have experienced a 93% decline in the sampled stretch of the Oorlogskloof River since 2013 and that the ongoing drought may be preventing recovery. They also suggest that banded tilapia do not adversely affect the Oorlogskloof River sandfish population, while predation of juveniles by smallmouth bass and/or bluegill sunfish apparently reduces the abundance of sandfish, especially of juveniles, where these non-native fish are present. Future studies should attempt to isolate the relative impacts of the different invasive fish species and examine habitat requirements of different sandfish life stages. Management of the river's sandfish population should focus on precautionary actions such as continued monitoring and landowner engagement, as well as clearing dams of non-native species high in the catchment, to prevent the further upstream invasion of smallmouth bass and bluegill sunfish. Proactive measures, such as eradicating smallmouth bass and bluegill sunfish from the Oorlogskloof River, are also necessary to restore connectivity of suitable sandfish habitat in the Doring River system.

1. Introduction

1.1 Threats to freshwater systems

Of all water found on earth, only 3% is freshwater, yet these systems contain up to 40% of all known fish species (WWF 2019). They are also some of the most threatened ecosystems on the planet: vertebrates have declined by at least 83% in freshwater ecosystems, compared to a combined decline of 60% among terrestrial, freshwater and marine ecosystems (Grooten & Almond 2018). Freshwater biodiversity is strongly affected by invasive species, human impacts on water quality and quantity and habitat modification (Grooten & Almond 2018). Examples of direct human impacts on freshwater systems include destructive activities such as excavation of riverbeds, impoundment, water over-abstraction for agriculture and the deliberate and accidental introduction of non-native species (Dudgeon et al. 2006). Indirectly, humans impact freshwater systems via the passive spread of non-native riparian plants that draw large amounts of water from rivers and through deforestation which can trigger increased sedimentation loads in waterways (Dudgeon et al. 2006). Moreover, changes in temperature, precipitation patterns and flow dynamics brought about by climate change exacerbate the more conspicuous threats already mentioned (Dudgeon et al. 2006).

Freshwater fish populations worldwide have experienced some of the largest observed declines compared to other vertebrates (Grooten & Almond 2018). In the late 20th century, at least 20% of freshwater fish species worldwide became threatened, endangered or extinct (Revenge et al. 2000). Moreover, faunal extinctions in freshwater systems occur at five times the rate of terrestrial extinctions, and this pattern is expected to continue in the coming decades, with up to 3,000 freshwater fish species predicted to become extinct between 2003 and 2033 (Ricciardi & Rasmussen 1999; Cambray 2003). Due to rivers and wetlands being patchy and naturally fragmented ecosystems, many freshwater fish species are rare or range-restricted, making them particularly vulnerable to human-induced perturbations (Magurran 2009). The major threats facing freshwater fish include those already mentioned for freshwater ecosystems as a whole, i.e. water extraction, overexploitation, pollution, the construction of dams, drought and the introduction of non-native invasive species (Darwall et al. 2009). In the Mediterranean, drought is considered a major threat to over 75% of threatened fish species (Smith & Darwall 2006), whereas in South Africa, invasive species

are considered the single greatest threat, impacting almost 85% of threatened species (Darwall et al. 2009).

Invasive fish are particularly problematic when introduced to communities poorly adapted to predation pressures, where they predate upon and competitively exclude native species naïve to these stresses (Paxton et al. 2002; Cox & Lima 2006). In these cases, non-native introductions might result in changes in the abundance and distribution of native fish, altered trophic relationships between species in the system or extinction of native fish altogether (Goldschmidt et al. 1993; Townsend 1996). One of the most dramatic and well-known examples of non-native fish impacts on native species is that of the decline and likely extirpation of hundreds of cichlid species after the introduction of Nile perch *Lates niloticus* (Linnaeus, 1758) into Lake Victoria in eastern Africa (Goldschmidt et al. 1993). Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), one of the most widespread invasive fishes, has been introduced to at least 99 countries (Gherardi 2010) and affects native fishes through hybridisation, competition for space and food and predation (Stanković et al. 2015). Its negative impacts are global, with examples ranging from North America, to Japan, Europe, Australasia, southern Africa, Chile and Sri Lanka, among others (Stanković et al. 2015). Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852), another global invader, has been successful in a wide diversity of environments due to its phenotypic plasticity in novel habitats. It has been implicated in the disappearance and decline of native fishes in Venezuela, Central America, Mexico, Australia, and various Pacific islands (Russel et al. 2012).

In South Africa, species restricted to rivers and wetlands, especially freshwater fishes, are declining at a faster rate than their terrestrial counterparts, due largely to pressures such as habitat loss, pollution, poor management of dams and invasive species (Skowno et al. 2019). Freshwater fish are the most threatened taxon in the country, with 36% of native species and 66% of endemic native species listed as threatened (Skowno et al. 2019). Of the various threats facing freshwater fish, the threat posed by invasive fish is considered by many to be the most serious (Cowx 2002). Since 1726, at least 55 freshwater fish species have been introduced into non-native areas in South Africa (Ellender & Weyl 2014), making the country one of six listed global hotspots for fish invasion (Leprieur et al. 2008). Non-native fish introduced to South Africa, or translocated within its borders, have been known to hybridise with native fish, introduce and spread disease and parasites, compete with native species and predate on native biota (Ellender & Weyl 2014).

1.2 Threats to freshwater fish in the Cape Fold Ecoregion

South Africa's Cape Fold Ecoregion (CFE; Abell et al. 2008) is a global biodiversity hotspot that harbours an exceptional concentration of endemic species under high threat (Myers 1990). The region is particularly rich in endemic freshwater fishes restricted to just one or two adjacent river systems (Tweddle et al. 2009). It also has the highest number of threatened fish species in southern Africa (Tweddle et al. 2009). Of the 23 fish species naturally occurring in the CFE, 20 of them are endemic to the region and of the endemic species, 14 are listed as Vulnerable, Endangered or Critically Endangered according to IUCN Red-List criteria (Ellender et al. 2017).

The Olifants-Doring River system (ODRS), located within the CFE (Figure 1), is arguably the most critical catchment in South Africa for freshwater fish conservation: eight of the 10 species native to the system are both endemic and threatened (Impson et al. 2002). The species in this system face various threats typical to freshwater systems worldwide including habitat loss and fragmentation by dams, loss of migratory corridors, over-abstraction and the presence of introduced species (Woodford et al. 2005; Tweddle et al. 2009). However, in a series of surveys carried out between 1972 and 1980, Cape Nature Conservation (now known as CapeNature, the provincial governmental conservation organisation for the Western Cape) found that the primary reason for the decline in the populations of native species in the Olifants River was the presence of non-native fish (Gaigher et al. 1980). This is unsurprising considering that CFE streams were largely without piscivorous fishes before the introduction of exotic fish species (Skelton 2001), and that up to 90% of the ODRS is currently invaded by non-native fish (Van der Walt 2014). Since the early 18th century, 20 non-native freshwater fish have become established in the CFE, 11 of which exist within the ODRS (Van der Walt 2014; Weyl et al. 2014). They now outnumber the ten species native to the ODRS (Van der Walt 2014).

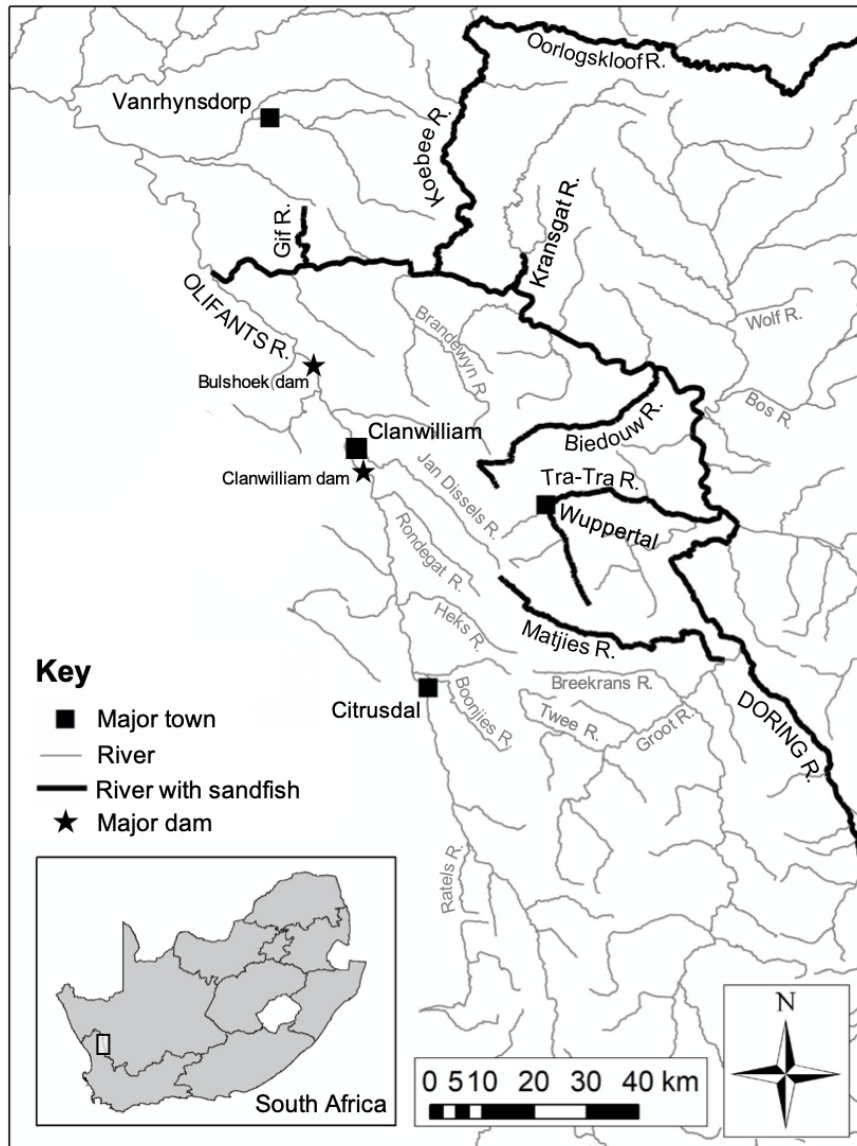


Figure 1. Map of the Olifants Doring River System with key rivers labelled. Rectangle in insert represents ODRS extent within South Africa. Rivers with capitalised labels are mainstems; other rivers are tributaries of those mainstems. Rivers in bold indicate rivers where sandfish are still found.

1.3 Threats to the Clanwilliam sandfish

The Clanwilliam sandfish *Labeo seeberi* Gilchrist & Thompson, 1911 (hereafter “sandfish”) is one of the most charismatic freshwater fish in the CFE. Its mouth is located on the underside of its head, consistent with its benthic feeding habit, and it has well-developed papillose lips, making the adults, and even small juveniles easily recognisable (Skelton 2001; Figure 2). It is one of the few large native cyprinids in the ODRS, reaching 550 mm SL (standard length; the length of a fish measured from the snout to the last vertebra; Impson

1997). It is also one of the most threatened fish in the ODRS. While it is currently listed as Endangered, it was, until 2015, considered Critically Endangered, and its population is decreasing (Jordaan et al. 2017). Once widespread throughout the mainstem and tributaries of the ODRS, it is now believed to be extinct from the Olifants River system entirely (Jordaan et al. 2017). The remaining subpopulations are severely fragmented and are now only found in low numbers within the Doring River system in the mainstem and in six tributaries: the Biedouw, Tra-Tra, Kransgat, Matjies, Gif and Oorlogskloof-Koebee Rivers (see Figure 1). Prior to non-native fish invasion, the Doring River constituted a critical refuge for remaining sandfish populations, and as the last large, undammed, free-flowing river in the CFE it represents indispensable habitat for this endangered species (Jeremy Shelton, pers. comm.) However, subadults and juveniles are largely absent from the majority of their natural range in the Doring River system, indicating widespread recruitment failure (Jordaan et al. 2017).



Figure 2. Adult (top) and juvenile (bottom) Clanwilliam sandfish. Photographs by Riaan Van der Walt (top) and Otto Whitehead (bottom).

Their decline from historical numbers is believed to have been precipitated by a combination of factors, including the predatory impacts of black bass species (a collective term for species belonging to the *Micropterus* genus from North America) introduced into the ODRS in the 1930s (De Moor & Bruton 1988), water over-abstraction and the construction of large dams that block their upstream breeding migrations (Jordaan et al. 2017). In 1938, thousands of sandfish were seen massed below the Bulshoek Dam (constructed 19 years earlier; Paxton et al. 2002) in their attempt to move up the rapids for spawning (Harrison 1976). More recently, the region has experienced a severe and prolonged drought believed to have been brought on by climate change and there is concern that this could be negatively impacting remaining populations (Jeremy Shelton, pers. comm.) The last known recruiting population of sandfish is found in the Oorlogskloof River where it is partially protected from non-native invasive fish species by a natural barrier. The tributary is a refuge of critical importance for the survival of this highly threatened species (Jordaan et al. 2017).

Non-native species occur along almost the entire extent of the Oorlogskloof River where the sandfish is present, leaving just under 3 km of sandfish habitat free of invasives. Smallmouth bass *Micropterus dolomieu* Lacépède, 1802 and bluegill sunfish *Lepomis macrochirus* Rafinesque, 1819 exist in the Koebee/Oorlogskloof River up to a natural barrier located 2.7 km south of the Oorlogskloof Nature Reserve (ONR) border, and banded tilapia *Tilapia sparrmanii* Smith, 1840 exist down the length of the Oorlogskloof River from its confluence with the Groen River at the upper border of the ONR.

1.4 Study objectives

In order to effectively manage and adequately concentrate conservation efforts, it is necessary to understand the population trajectory and current status of the sandfish, as well as the relative impacts of various threats on the last recruiting population of sandfish in the ODRS. This study examined the temporal and spatial patterns in sandfish abundance and size structure along a 25 km stretch of the Oorlogskloof River in relation to the presence of other fish species and environmental variables in order to assess the relative impact of these factors on the river's sandfish population.

In 2001, AAM Abrahams (Northern Cape Department of Tourism, Environment and Conservation, now known as the Department of Environment and Nature Conservation, the provincial governmental conservation organisation for the Northern Cape) and WAJ Pretorius (ONR manager at the time) called for continuous data collection to be undertaken in

the Oorlogskloof River in order to allow for monitoring of changes in fish populations and facilitation of management actions (Abrahams & Pretorius 2001). Since 2010, ONR staff have carried out systematic surveys to monitor the abundances and sizes of both native and non-native fish in a 25 km stretch of the Oorlogskloof River that centres around the ONR. However, until now, these data have not been comprehensively analysed, and native fish population status and trends have not been adequately assessed. Specifically, spatio-temporal patterns in sandfish distribution, abundance and population size structure have not yet been evaluated, nor has the influence of other environmental factors (including the presence of non-native fish). While the impacts of smallmouth bass on native fish in South Africa have been assessed extensively (Paxton et al. 2002; Woodford et al. 2005; Ellender et al. 2011; Shelton et al. 2014; Van der Walt 2014; Weyl et al. 2014; Van der Walt et al. 2016; see De Moor & Bruton 1988 for a comprehensive review of earlier studies), those of banded tilapia and bluegill sunfish have not yet been rigorously assessed. This constitutes the first study in South Africa that explicitly assesses the impacts of banded tilapia and bluegill sunfish on native fish, and the first study of all three species' impacts in the Oorlogskloof River.

The Oorlogskloof River is, by virtue of two barriers (one known and another as-yet unidentified), divided into three sections: the uppermost section contains only native fish, the middle section contains native fish and banded tilapia and the lowermost section contains native fish, banded tilapia, smallmouth bass and bluegill sunfish (Appendix 1). In this study I (1) evaluated changes in sandfish abundance and population structure between 2010 and 2018, and (2) compared the relative abundances and sizes of sandfish above and below the natural smallmouth bass and bluegill sunfish barrier (hereafter "barrier") and between pools where the non-native fish were either present or absent in order to assess their influence on the sandfish. I also considered the role of environmental variables, such as precipitation and temperature in influencing sandfish abundance and size. The former was particularly pertinent, as the area has been in the midst of a drought since around 2014, resulting in smaller, shallower, less connected pools throughout the length of the river during the summer months than in years with relatively high rainfall (in 2018 and 2019 nearly 50% of the pools were completely dry or too low to attempt to survey). My main questions were:

1. How has the relative abundance of sandfish changed over time?
2. What effect have other fish species (especially the non-natives banded tilapia, smallmouth bass and bluegill sunfish) had on sandfish abundance and size structure?

3. How do environmental factors (precipitation, temperature, physico-chemical variables) affect sandfish abundance and size structure?

My study is part of a larger sandfish conservation strategy involving the ONR, CapeNature, the Northern Cape Department of Environment and Nature Conservation, the Freshwater Research Centre, an independent filmmaker and other sandfish experts. The aims of the project are to better assess sandfish abundance and threats throughout the Olifants-Doring catchment, to increase sandfish recruitment in the Biedouw River through assisted translocation and to raise awareness of sandfish and the threats they face. In providing a rigorous assessment of sandfish responses to non-native fishes and environmental factors in the Oorlogskloof River, I am contributing much-needed context and information to aid in the management of the species in the ONR and elsewhere. This information will assist conservation practitioners in addressing the threats facing sandfish and help more effectively conserve this endangered species.

2. Methods

2.1 Study area

The Oorlogskloof River is a seasonal tributary of the Doring River located in the ODRS. It often ceases to flow in the summer months, forming a series of discrete pools. Its headwaters lie in the Roggeveld mountains near Calvinia at an altitude of 1,672 metres above sea level (masl; Figure 3; Abrahams & Pretorius 2000; Van der Walt 2014). From there, it runs west toward Nieuwoudtville and then turns sharply south, where it confluences with the Groen River at an altitude of 608 masl. It enters the Oorlogskloof Nature Reserve 1 km downstream of that confluence. The Oorlogskloof River confluences with the Koebee River at the border of the Northern Cape and Western Cape (south of the reserve border) at an altitude of 363 masl, and with the Doring River mainstem at an altitude of 116 masl. The 38 sampling sites (described under “Data collection”) span 24.5 km of river length.

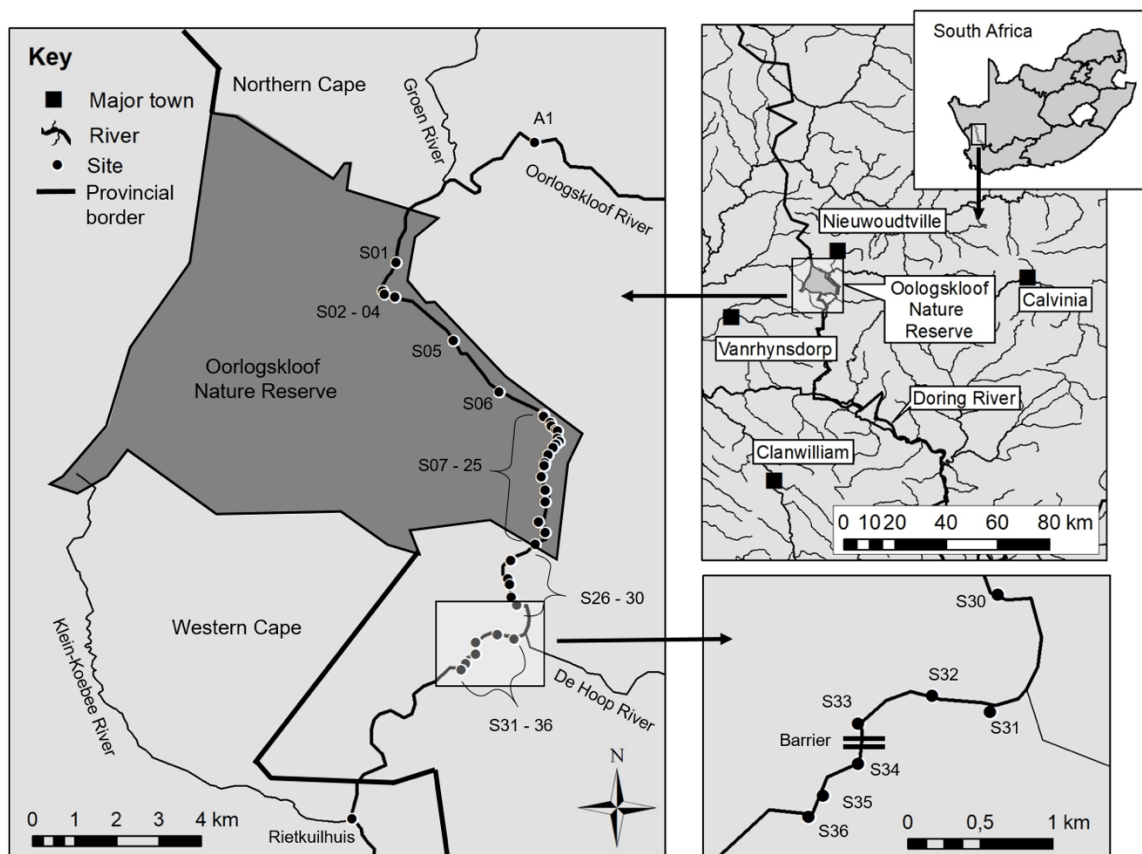


Figure 3. Location of sampling sites in the Oorlogskloof River in relation to the Doring River, nearby towns, and South Africa.

The landscape in the upper Oorlogskloof River catchment is comprised mostly of sheep farming, and the natural vegetation in this area is Vanrhynsdorp Shale Renosterveld and Bokkeveld Sandstone Fynbos (Rebelo et al. 2006). The river enters the ONR at the Oorlogskloof Gorge which runs along the eastern border of the reserve and is characterised by steep sandstone cliffs. Its relative inaccessibility has prevented the anthropogenic impacts typical of the upper catchment from impacting on the river in that area. In the upper reaches, the Oorlogskloof River consists of a series of falls and cascades, transitioning into a morphology of long shallow pools and riffles in the lower reaches (Van Der Walt 2014).

The ONR covers an area of roughly 5,755 hectares and is located in a botanical ecotone between the succulent karoo and fynbos biomes. Four vegetation types exist within its boundaries (Abrahams & Pretorius 2000). Karee (*Searsia lancea*) dominates the riparian vegetation (Abrahams & Pretorius 2000). Data from 1988 to 2019 indicate that total annual rainfall in the reserve ranges from 143 mm (2017) to 598 mm (1991), with an average of 395 mm per annum (ONR, unpublished data; refer to Appendix 2 for weather station location). Most rainfall occurs in May through August (ONR, unpublished data), although occasional summer and autumn thunderstorms in the Calvinia area augment flows during the drier months (Mandy Schumann, pers. comm.) Frost occurs sporadically, and snowfalls are infrequent. During the summer it is hot and dry, and strong winds are typical (Mandy Schumann, pers. comm.)

2.2 Study species

2.2.1 Native species

The Oorlogskloof River is home to four native fish species: Clanwilliam sandfish, Clanwilliam sawfin *Cheilobarbus serra* (Peters, 1864), Clanwilliam yellowfish *Labeobarbus seeberi* (Gilchrist & Thompson, 1913) and chubbyhead barb *Enteromius anoplus* (Weber, 1897; hereafter “sandfish”, “sawfin”, “yellowfish” and “chubbyhead barb”, respectively). The chubbyhead barb is listed as Least Concern in the IUCN Red List of Threatened Species, yellowfish and sawfin are both listed as Near Threatened and the sandfish is listed as Endangered. The populations of the latter three species are decreasing due to predation by invasive fishes, water over-abstraction, pollution from pesticides and fertilisers, localised livestock impacts, barriers to migration (i.e. dams) and invasion of the riparian zone by non-native tree species (Impson et al. 2017a, 2017b; Jordaan et al. 2017).

2.2.1.1 Ecology of the Clanwilliam sandfish

Arguably the most threatened of these, the sandfish, is endemic to the ODRS (Jubb 1967). While studies on growth, fecundity and sexual maturity requiring high mortality have not been carried out on sandfish due to their threatened status, males of 150 mm TL (total length; the length of a fish measured from the snout to the end of the caudal fin) and females of 250 mm TL have been found to be ripe and running (Bruce Paxton, pers. comm.). However, size at maturity likely varies with population density, habitat type and other factors (Dean Impson, pers. comm.) While not much is known about the biology of the sandfish in its natural habitat, captive juveniles can grow to 20 mm TL in three months and a 2 kg captive female can produce 80,000 eggs, indicative of an altricial life-history strategy (Impson 1997). They consume algae, detritus and small invertebrates by grubbing in sand and mud and grazing off rocks with specialised papillose lips (Skelton 1987, 2001). In 2019, they spawned in the Biedouw River in late August. The suspected breeding season is between August and September (Jeremy Shelton, pers. comm.)

Sandfish live in the Doring River mainstem and larger tributaries of the ODRS, favouring pools and deep runs (Skelton 2001). In the spring and summer, they migrate upstream into the tributaries in masses for breeding and spawn in spring at the end of the winter high-flow season when temperatures are increasing and flows decreasing (Skelton 2001; Paxton 2008). The tributary populations appear to be stunted in size compared to those in the mainstem; competition from overcrowding in small pools or low productivity in the headwaters may be causing this plastic phenotypic response (Roff 1992). The citrus industry around the Olifants River system abstracts large volumes of water in the summer for irrigation purposes, stressing a system that receives most of its rainfall during the winter (Impson 1997). Dams and weirs block the species' breeding migrations, alter the flow regime, and act as refuges for non-native fishes not adapted to strong flows (Skelton 2001; Paxton et al. 2002). The sandfish was once the most abundant of the three native cyprinids above the Olifant River's Clanwilliam dam, yet in 1967, surveys showed that the species had been entirely extirpated from this area (Impson 1997).

Compared to the Olifants River system, the Doring River and its tributaries are relatively undisturbed and undeveloped; here, non-native fish are considered to be the primary driver of sandfish population declines (Paxton et al. 2002). The last known recruiting population of sandfish is found in the Oorlogskloof River, a tributary of the Doring, which comprises less than ten percent of its native range (Impson 1997). It has been recommended

that the ecology of sandfish be better studied in order to more effectively manage the combined threats of habitat loss and non-native fish on remaining populations (Impson 1997).

2.2.2 Non-native species

Three non-native fish are also present in the Oorlogskloof River: smallmouth bass, bluegill sunfish and banded tilapia. Smallmouth bass (Figure 4) is a popular angling fish species native to eastern North America that can reach 550 mm SL and can live for more than fifteen years (Jubb 1967; Skelton 2001). Although adults may reach 2 kg, they typically weigh less than 500 g (De Moor & Bruton 1988). Juveniles prey on insects, crabs and small fish (Skelton 2001). Adults are primarily piscivorous but also consume crabs and frogs (Brown et al. 2009). As visual predators, they require clear water and cannot tolerate polluted or heavily silted rivers (De Moor & Bruton 1988; Brown et al. 2009). Smallmouth bass adapt easily to riverine conditions but also thrive in impounded waters (De Moor & Bruton 1988; Van der Walt 2014). This non-native invasive does well where bluegill sunfish and banded tilapia are also able to establish (Van der Walt 2014), since these smaller species provide an important food source.



Figure 4. Smallmouth bass in the Doring River. Photograph by Leonard Flemming.

In 1937 the Jonkershoek Hatchery in Stellenbosch, Western Cape, obtained smallmouth bass from the State Hatchery in Lewistown, Maryland (USA), where it was propagated for introduction into South African rivers as a sportfish (Jubb 1967). It was initially introduced in order to promote sportfishing below the trout zone and in order to control native species less favourable for angling (Skelton 2001). The first introduction of

smallmouth bass in the ODRS was into the Jan Dissels River in 1943. Reports from anglers indicated that by 1948 it had become well established, with observations of both adults and fingerlings in the system (Harrison 1963). It has since become extremely successful in the Western Cape, becoming established in the Olifants-Doring, Berg and Breede River systems (Jubb 1967; Skelton 2001). In most cases the presence of smallmouth bass has resulted in the decline or extirpation of native fish species (Paxton et al. 2002; Woodford et al. 2005; Ellender et al. 2011; Shelton et al. 2014; Van der Walt 2014; Weyl et al. 2014; Van der Walt et al. 2016; see De Moor & Bruton 1988 for a comprehensive review of earlier studies), leading to their listing as a category 1b invasive species under the National Environmental Management Biodiversity Act (NEMBA: Act 10 of 2004) Alien and Invasive Species Regulations (DEA 2014a, 2014b). A survey carried out before 1967 in the Jan Dissels River, where smallmouth bass were by then prevalent, revealed no other species of fish co-occurring with the bass (Jubb 1967). By some, it is considered the most destructive predator in the whole of the ODRS (Gaigher et al. 1980). Spotted bass *Micropterus punctulatus* (Rafinesque, 1819) and smallmouth bass together have invaded 52.4 km (73.8%) of the Koebee/Oorlogskloof River, leaving only 18.7 km (26.2%) bass-free, of which less than 3 km are entirely free of non-native species (Van der Walt 2014). Surveys carried out by Cape Nature Conservation showed that smallmouth bass was present at site S01 in the northern end of the ONR in 1983 and 1987 (Abrahams & Pretorius 2000). However, no subsequent surveys have recorded smallmouth bass above site S34, and they are believed to have been flushed from the system during high flows and high turbidity in 1988 (Abrahams & Pretorius 2000).

Bluegill sunfish (Figure 5) is an introduced predatory fish species from North America that can attain up to 200 mm SL and live for more than ten years (Skelton 2001). It can reach a weight of up to 1 kg, but rarely exceeds 200 g in most populations as it tends to crowd water bodies with dwarfed individuals (De Moor & Bruton 1988). Bluegill sunfish reach sexual maturity at two to four years and an 18 cm female can produce over 38,000 eggs (Scott & Crossman 1973). While they prefer shallow, warm, well-vegetated waters, they tolerate a wide range of water conditions and consume invertebrates and small fish (Jubb 1967; De Moor & Bruton 1988; Skelton 2001). An individual as small as 131 mm TL is capable of consuming 50 mm TL sandfish (Leonard Flemming, unpublished data; Appendix 3).



Figure 5. Bluegill sunfish. Photograph by Jeremy Shelton.

This North American native is a minor angling species and was originally introduced as a forage fish for largemouth bass *Micropterus salmoides* (Lacépède, 1802; Jubb 1967; Skelton 2001). Harrison (1963) notes that the species was not introduced deliberately into the Olifants River; rather, it likely became established after escaping from stocked farm dams in the area at some point before 1961. It is now widely distributed in rivers also stocked with black bass (Jubb 1967). In South Africa, it is found in the Northern Cape, Western Cape, Eastern Cape, KwaZulu-Natal, Mpumalanga and Free State (Skelton 2001). This prolific breeder is now considered a pest as it competes with native fish for food and preys on the young of native fish and more desirable angling fish (Jubb 1967; Skelton 2001; Weaver 2008). Moreover, the individuals that fill water bodies are undesirable as angling fish due to their dwarfed size (De Moor & Bruton 1988). Both bluegill sunfish and smallmouth bass invaded the Oorlogskloof River by moving upstream from the Doring mainstem up to the natural barrier. However, like smallmouth bass, bluegill sunfish were also recorded at site S01 in both 1983 and 1987, yet have been absent above the barrier in every subsequent survey (Abrahams & Pretorius 2000). They, too, are believed to have been flushed from the upper reach by high flows and high turbidity in 1988 (Abrahams & Pretorius 2000).

Banded tilapia (Figure 6) have a large native distribution ranging from the Orange River system in South Africa to the southern Congo tributaries (Skelton 2001). They attain a total length of nearly 230 mm SL and are omnivorous, consuming aquatic plants, zooplankton, small invertebrates and even small fish (Jubb 1967; Skelton 2001). They are aggressive when guarding their young; fish approaching a shoal of young banded tilapia are

attacked by the parents (Jubb 1967). Banded tilapia prefer standing waters, especially where there is plant cover along the edges, but are tolerant of a wide range of habitats (De Moor & Bruton 1988; Skelton 2001).



Figure 6. Banded tilapia. Photograph by Jeremy Shelton.

In 1941, banded tilapia specimens were sent to Jonkershoek Hatchery, where they were bred and subsequently distributed to farm dams throughout southern Africa (De Moor & Bruton 1988). Banded tilapia were stocked as an alternative to bluegill sunfish as forage fish for smallmouth and largemouth bass (Jubb 1967; De Moor & Bruton 1988; Skelton 2001). They likely became established in the Olifants River system after escaping from stocked farm dams before 1961, as they were not introduced deliberately into the river (Harrison 1963). With their wide environmental tolerances and broad dietary preferences, banded tilapia have proven to be a highly successful invader (De Moor & Bruton 1988). While it has been suggested that banded tilapia may compete with native species for resources and even prey on juveniles (De Moor & Bruton 1988), they have, unlike black bass, been found to co-occur with small native minnow species and juveniles of larger species (e.g. in the Swartkops River system, Ellender et al. 2011; and elsewhere in the ODRS, Van der Walt 2014). A 2001 survey of sites A1 through S36 yielded no banded tilapia specimens, suggesting that the invasion occurred sometime after 2001 (Abrahams & Pretorius 2001). Banded tilapia are believed to have invaded the Oorlogskloof River via the Groen River after a flood flushed individuals from the stocked Nieuwoudtville dam, through adjacent wetlands, and into the Groen River in 2007 (Mandy Schumann, pers. comm.).

2.3 Data collection

Oorlogskloof Nature Reserve staff and partners conducted surveys in 2010, 2013, 2014, 2015, 2016 and 2018 during the last week of February and first week of March. The sampling dates were chosen due to the difficulty of sampling during the winter months, when high flow makes it difficult and ineffective to use seine nets. The Oorlogskloof River typically ceases to flow during the summer months, separating into a series of discrete pools that facilitate the use of seine nets as an effective sampling method (Abrahams & Pretorius 2000).

A total of 38 pools were sampled in each survey (see Figure 3), with some exceptions mentioned below. Twenty-five pools were sampled within the borders of the ONR (pools S01 – S25), 12 pools were sampled directly downstream of the southern reserve border (pools S26 – S36 and Rietkuilhuis), and one pool (A1) was sampled 5.3 km upstream of the northernmost reserve pool (S01; Figure 7). The shortest distance between pools was 35 m and the longest distance was 5.4 km, with a median distance between pools of 263 m and a mean distance of 661 m. Site S01 was originally sampled in July 1982, and eight more times through 1999. Sites S01, S04, S05 and S21 were sampled in 1999 by AAM Abrahams and WAJ Pretorius. In February 2000 they sampled A1, S01 through S22 and four sites located 0.70 to 5.82 km downstream of Rietkuilhuis (Abrahams & Pretorius 2000). In March and September 2001, they sampled A1 through S36 (Abrahams & Pretorius 2001). The surveys were discontinued until 2010, when ONR staff carried out a survey of the 2001 sites. Rietkuilhuis was added to the surveys in 2013. Refer to Appendix 4 for site coordinates and elevations.

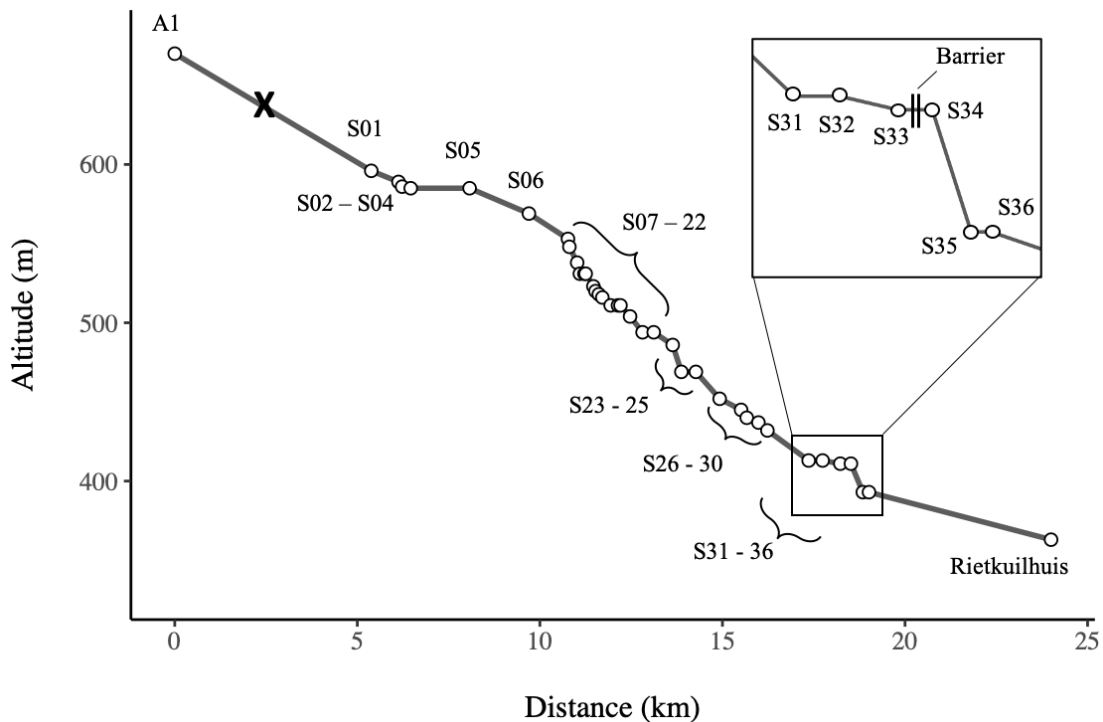


Figure 7. Distribution of sampling sites according to distance from the uppermost site (A1) and river gradient. Circles indicate sites, an “X” indicates where the Groen River conflues with the Oorlogskloof River (above which lies an unidentified barrier preventing upstream invasion by banded tilapia) and parallel vertical lines indicate the location of the natural smallmouth bass and bluegill sunfish barrier.

Seine sampling was carried out with a shade cloth seine net (length 3 m, depth 1.5 m), fitted with a weighted foot rope, a float rope across the top, and a 2 m deep pocket at the midpoint (80% mesh density). One to six seine hauls were conducted per site, depending on the amount of fish caught in the preceding hauls. The distance covered by the seine net before being pulled in depended on the size, depth and predominant substrate of the pool, as well as any obstructions such as edge vegetation and boulders. For example, in small shallow pools with sandy bottoms, the sampling team was able to walk the length of the pool before pulling the net up. In large, deep pools, one person would stand at the edge holding one end of the net while another swam out with the other end, moving in a wide arc until the ends of the net were brought together and pulled up by the person standing at the edge. If a pool was obstructed by significant amounts of reeds, branches or rocks, then a sandy patch was chosen as the sampling area. While the pattern of sampling varied between pools, the same pattern was carried out at each pool between years; this was possible because the sampling team consisting of rangers who are familiar with the area has not changed between surveys. Effort

(i.e. amount of seine hauls) was recorded from 2013 in order to standardise relative abundances between pools.

Fyke sampling was carried out with two-wing fyke nets (length 10 m, depth 1.5 m, mesh size 4 mm); a single net was set out in a pool overnight and fish were processed the following morning. Water did not flow strongly during the survey period; however, the fyke nets were generally set facing upstream so that any flow of water downstream would keep the pockets of the net open. As with the seine sampling, the position of the fyke nets depended largely on the shape and depth of the pools, as well as natural obstructions. Photographs of the fyke nets were taken every year in order to standardise sampling position per pool across years.

After each seine haul or fyke night, fish were placed into holding buckets and measured in trays with submerged rulers. Total length was recorded for all species. In 2010, only a subsample of captured fish was measured where the catch was exceptionally high. In all subsequent years, every individual caught was either measured or its length estimated from a representative individual: where the catch was high, individuals were grouped into size classes and a representative individual was measured per group. Fish were returned to the pools after being processed.

It is important to note that the two types of gear are selective for different types of fish. Seine nets tend to selectively sample smaller fish, as stronger, more agile swimmers may easily evade seines (Hayes 1989). Accordingly, fyke nets (a passive sampling strategy) tend to sample a higher proportion of large fish than do seines (Bruce Paxton, pers. comm.) Gear is also species-selective. In one study, fyke nets sampled a higher relative abundance of bluegill sunfish but a lower relative abundance of largemouth and spotted bass than did seines. Overall, fyke nets also caught a greater abundance of fish (Clark et al. 2007).

In every survey, sites S01 – S36 were seine sampled; A1 was only seine sampled in 2010 and 2013 (Table 1). Sites A1 and Rietkuilhuis were fyke sampled in every survey from 2013. Site S01 was also fyke sampled in 2013 and 2014 in addition to seine sampling. Due to logistical constraints, sites S07 and S35 were not sampled in 2010 and site S25 was not sampled in 2013. In 2018, following a prolonged drought, the following 16 pools were either too shallow to sample or entirely dry: S02 – S04, S08, S12, S14, S16, S17, S19, S22, S24, S27, S30 – S32 and S36. Due to the ongoing drought, no survey was carried out in 2019.

Table 1. Type of sampling carried out in each year’s survey by site. An “s” indicates a site was sampled by seine and effort was recorded; an “*” indicates a site was sampled by seine but effort was not recorded; an “f” indicates a site was sampled by fyke; an “sf” indicates a site was sampled by both seine and fyke; an “s (0)” indicates a site was sampled by seine but no fish were caught; and a “--” indicates the site was not sampled. Site “RKH” refers to Rietkuilhuis.

Site	Year						Site	Year					
	2010	2013	2014	2015	2016	2018		2010	2013	2014	2015	2016	2018
A1	s*	sf	f	f	f	f	S19	s*	s	s	s	s	--
S01	s*	sf	sf	s	s	s	S20	s*	s	s	s	s	s
S02	s*	s	s	s	s	--	S21	s*	s	s	s	s	s
S03	s*	s	s	s	s	--	S22	s*	s	s	s	s	--
S04	s*	s	s	s	s	--	S23	s*	s	s	s	s	s
S05	s*	s	s	s	s	s	S24	s*	s	s	s	s	--
S06	s*	s	s	s	s	s	S25	s*	--	s	s	s	s
S07	--	s	s	s	s	s	S26	s*	s	s	s	s	s
S08	s*	s	s	s	s	--	S27	s*	s	s	s	s	--
S09	s*	s	s	s	s	s	S28	s*	s	s	s	s	s
S10	s*	s	s	s	s	s	S29	s*	s	s	s	s	s
S11	s*	s	s	s	s	s	S30	s*	s	s	s	s	--
S12	s*	s	s	s	s	--	S31	s* (0)	s	s	s	s	--
S13	s*	s	s	s	s	s	S32	s*	s	s	s	s	--
S14	s*	s	s	s	s	--	S33	s*	s	s	s	s	s
S15	s*	s	s	s	s	s	S34	s*	s	s	s	s	s
S16	s*	s	s	s	s	--	S35	--	s	s	s	s (0)	s
S17	s*	s	s	s	s	--	S36	s* (0)	s	s (0)	s	s	--
S18	s*	s	s	s	s	s	RKH	--	f	f	f	f	f

In 2014, habitat and environmental variables were measured at all pools except A1, S28, S36 and Rietkuilhuis, which were omitted due to logistical constraints. Table 2 describes the habitat variables measured. A Multi-Parameter PCSTestr (35 Series; Eutech Instruments Oakton) was used to measure pH, conductivity, total dissolved solids and salinity.

Table 2. Habitat variables measured in 2014 at pools S1-S27 and S29- S35 (A1, S28, S36 and Rietkuilhuis excluded due to logistical constraints). These habitat characteristics were only used to analyse data from 2014, as they are liable to change inter-annually according to flow conditions.

Variable	Description	Measurement instrument
Pool length and width	Length and width of pool	Measuring tape or visual estimate depending on size and accessibility of pool
Depth class	Proportion of pool with certain categories of depth. The categories were ‘very shallow’ (< 0.1 m), ‘shallow’ (0.1 - 0.5 m) and ‘deep’ (> 0.5 m)	Visual assessment during lengthwise transects
Substratum size class	Proportion of pool bottom consisting of different types of substrates: silt, sand, gravel, small cobble, cobble, boulders and bedrock	Tactile assessment during lengthwise transects

2.4 Data analysis

Before analysing the data, I carried out a rigorous process of quality control. This included standardising entries, checking unusual entries (such as unexpected outliers) against original data sheets and liaising with ONR staff to address errors and inconsistencies.

2.4.1 Variation in sandfish abundance

2.4.1.1 Temporal patterns in sandfish abundance

Determining whether the abundance of sandfish fluctuated over time (Question 1) gives an indication of whether the populations are stable or if they suffered significant changes between years. For the following tests I used only seine data for which effort was recorded to standardise catch per unit effort (CPUE; all CPUE values reported hereafter refer to number of fish captured per pull of the seine net, i.e. fish/haul; see Table 1). This necessarily excluded data from 2010, leaving data from 2013, 2014, 2015, 2016 and 2018 as part of the analyses. Average CPUE values were calculated as the CPUE averaged by the number of pools sampled that year; in 2018, pools that were not sampled due to low water levels were included in the average as “0” values to account for the decrease in habitat available to sandfish and other species. I performed the Kruskal Wallis rank sum test

(confidence level = 0.05; R package ‘*stats*’ version 3.5.3; R core team 2019) to test for differences in sandfish CPUE among years, and Dunn’s (1964) test of multiple comparisons (R package ‘*dunn.test*’ version 1.3.5; Dinno 2017) to determine which years differed from one another. P-values reported for pairwise comparisons were adjusted with the Benjamini-Hochberg method in order to decrease the likelihood of a false positive (e.g. of concluding that there is a difference between years when there is none; Benjamini & Hochberg 1995). In addition to analysing all sandfish data together, I also ran separate tests for young-of-the-year (YOY) sandfish and for juvenile/adult sandfish to see if inter-annual variation in abundance varied with fish size. Young-of-the-year sandfish were classified as individuals measuring less than 80 mm TL and juvenile/adult sandfish were classified as individuals measuring 80 mm TL or greater (Bruce Paxton, pers. comm.)

2.4.1.1.1 Potential influencing factors

Annual precipitation influences flow, which in turn influences the size, depth and connectedness of pools within a river. Sandfish populations might be expected to respond to these factors (Question 3). I compared trends in sandfish abundance to trends in precipitation in the quaternary catchment (used as a proxy for flow within the Oorlogskloof River) in order to assess whether the former might be influenced by the latter. I calculated a single value for total annual precipitation (TAP) per year, averaging together the total accumulated precipitation recorded by ten different weather stations in the quaternary catchment over the twelve months preceding each year’s survey (hereafter ‘rainfall’). One weather station is located inside the ONR, two are in and around Nieuwoudtville and eight are located in the Calvinia area (Appendix 2). I also calculated a single temperature value per year, averaging together the maximum daily temperatures over the five months preceding each year’s survey from the single weather station in Calvinia for which temperature data were available (hereafter ‘temperature’; Appendix 2). These five months (October to February) represent the hottest months YOY would experience since hatching in August or September.

I also assessed whether the abundance of banded tilapia fluctuated over time in order to compare these patterns to those of sandfish and thereby gain insight into whether they might be influencing one another (Question 2). Since banded tilapia CPUE data violated the assumptions of the Kruskal Wallis rank sum test, I used the two-sample Wilcoxon rank sum test (R package ‘*stats*’ version 3.5.3; R core team 2019) to test the null hypothesis that

banded tilapia CPUE was the same between any two pairs of years. I used the same data as above: seine data from 2013, 2014, 2015, 2016 and 2018.

2.4.1.2 Spatial variation in sandfish abundance according to the presence of non-native species

I used a Pearson's Chi-Square test for independence (R package 'stats' version 3.5.3; R core team 2019) to assess whether there was a relationship between the presence of sandfish in a pool and the presence of any of the three non-native fish in that same pool (Question 2). Data from all years were included; a species was considered present if it was present in either a fyke or seine sample from a particular pool during that year's survey. I then used the two-sample Wilcoxon rank sum test (R package 'stats' version 3.5.3; R core team 2019) to assess whether median sandfish CPUE differed between pools where any of the three non-native fish were present. Only seine data from years where effort was recorded was included in the analysis (i.e. pools A1-S36 in 2013 and pools S01-S36 in 2014, 2015, 2016 and 2018).

2.4.1.3 Identifying top predictors of sandfish abundance

I assessed whether sandfish abundance varies randomly or might be attributable to factors such as the presence of non-native fish or abiotic factors in order to establish the primary threats to the species in the Oorlogskloof River (Questions 2 and 3). For the following analysis, I used seine data from surveys during which effort was recorded. This necessarily excluded pools where only fyke samples were taken (i.e. Rietkuilhuis in all years and A1 in 2014 – 2018) and data from 2010, where no effort was recorded (see Table 1).

I used a Generalised Linear Mixed Model (GLMM; R package 'glmmADMB' version 0.8.3.3; Fournier et al. 2012; Skaug et al. 2016) to determine the best predictors of sandfish abundance (the response variable). The initial set of predictors included presence of other fish in the pool (i.e. banded tilapia either present or absent, sawfin either present or absent, yellowfish either present or absent and chubbyhead barb either present or absent), rainfall and temperature, since these were considered factors most likely to influence sandfish abundance. Location relative to the barrier (i.e. above or below), presence of smallmouth bass and presence of bluegill sunfish could not be included as explanatory variables in the model due to a low sample size of sandfish present in one level of each of the variables, but the

influence of these parameters were assessed using univariate tests (see above). A species was classified as present if it was caught by either seine or fyke sampling in that pool. I controlled for effort (i.e. number of seine pulls per pool) by including the log of the effort as an offset (the response variable would therefore more accurately be called sandfish relative abundance). I logged the offset term because the model was fitted with a log-link function. The year in which a pool was sampled was included as a random term in the model to account for the lack of independence of sampling carried out at different pools within the same year. Likewise, 'site' (sample location) was included as a random term to account for the lack of independence of surveys carried out in the same pool across years. In order to address overdispersion of the count data under a Poisson distribution, I fitted the model with a Negative Binomial distribution (Lindén & Mäntyniemi 2011).

Model selection was carried out using classical backwards stepwise regression to a minimum adequate model, a method appropriate for disciplined hypothesis testing with small amounts of model reduction (Bolker et al. 2008). At each step, I generated an analysis of deviance table (R package '*car*'; Fox & Weisberg 2011) and removed the variable with the highest p-value greater than 0.1. All variables with a p-value lower than 0.1 were retained in the model, but only variables with a p-value lower than 0.05 were classified as significant predictors of sandfish size.

2.4.2 Variation in sandfish size

Seine and fyke data from all pools in all years were included in the following analyses in order to facilitate meaningful comparisons between pools. Rietkuilhuis, for example, which was only sampled by fyke, contained the vast majority of sandfish sampled below the barrier. Excluding fyke samples would therefore exclude most meaningful size data for sandfish below the barrier.

2.4.2.1 *Spatial variation in sandfish population size structure*

Predation by smallmouth bass elsewhere in the CFE has been shown to be size selective (Weyl et al. 2013). To examine size selective predation as a mechanism for smallmouth bass impact on sandfish, I split sandfish into two size classes based on the size at which they are likely to be vulnerable to predation by smallmouth bass (200 mm TL, based on Weyl et al. 2013; Question 2). Small sandfish were classified as those vulnerable to

smallmouth bass predation (i.e. individuals measuring up to 200 mm TL) and large sandfish were classified as individuals larger than 200 mm TL. I used Pearson's Chi-Square Test for Independence (R package 'stats' version 3.5.3; R core team 2019) in order to determine whether there was an association between river location (i.e. whether an individual was sampled above or below the natural barrier south of the ONR) and sandfish size category (i.e. whether sandfish were small or large).

2.4.2.2 Identifying top predictors of sandfish size

I used a Linear Mixed Model (LMM; R package 'lme4'; Bates et al. 2015) to determine the best predictors of sandfish size (Questions 2 and 3). Before refining the model, site location relative to the barrier, presence/absence of certain fish species at the site (banded tilapia, sawfin, yellowfish and chubbyhead barb), rainfall and temperature were included as explanatory variables. Barrier (i.e. if a sample was taken above or below the barrier) was used as a proxy for smallmouth bass and bluegill sunfish presence because these species have invaded the river from downstream up to the barrier and because there is significant spatial overlap in the presence of these two species in the below-barrier pools. A species was classified as present if it was caught by either seine or fyke sampling in that pool. I included site and year as random terms due to the non-independence of samples within these factors. The response variable (sandfish total length) was square root transformed to meet the assumptions of normality inherent to an LMM. Model selection was carried out using backwards stepwise regression to a minimum adequate model, retaining only variables with p-values lower than 0.1.

2.4.3 Influence of environmental variables on sandfish abundance and size

The 2014 dataset, which included site-specific information on habitat and environmental parameters, allowed for a more detailed assessment of environmental factors potentially influencing variation in sandfish abundance and size among sites (Question 3). At each site, pH, conductivity, total dissolved solids (TDS), salinity, pool length and width, depth class and substratum size class were measured or classified (see Table 2 in "Data collection" for details).

I used a GLMM (R package '*glmmADMB*' version 0.8.3.3; Fournier et al. 2012; Skaug et al. 2016) to determine the best environmental predictors of sandfish abundance since the residuals of the abundance data were not normally distributed (model 1). I fitted the GLMM with sandfish abundance as the response variable, controlling for varying effort per pool by including the log of effort as an offset term. Only seine data in years where effort was recorded were included in the model (i.e. seine data from 2013, 2014, 2015, 2016 and 2018). In order to address overdispersion in the data, I used a Negative Binomial distribution and included an observation-level random effect. As each observation consisted of a single abundance value per site, the random effect was essentially that of site.

As the residuals of the size data were approximately normally distributed, I used an LMM (R package '*lme4*'; Bates et al. 2015) to determine the best environmental predictors of sandfish size (model 2). The LMM was fitted with sandfish size as the response variable. Data from all years were included in the model, as size is not dependent on sampling effort. I included site as a random effect, as observations (i.e. individual sandfish) were clustered within discrete sites.

For both models (models 1 and 2), I grouped the seven substrate types into three groups: fine (silt and sand), medium (gravel, small cobble and cobble) and coarse (boulders and bedrock) in order to avoid overfitting the model. As TDS, conductivity and salinity were all highly correlated with one another ($r > 0.90$), I chose salinity to represent this group of variables in the final models. Pool area was estimated as the product of pool length and width; as pools are not rectangular, this measure was treated as relative. The explanatory variables included in the models before refinement thus included: proportion of pool in the 'deep' (>0.5 m) class (hereafter 'depth'), proportion of fine substrate, proportion of medium substrate, proportion of coarse substrate, pH, salinity and pool area. The following sites were excluded from the analyses as they were missing values for one or more of the explanatory variables or excluded from sampling due to logistical constraints: A1, S01, S02, S20, S21, S26, S27, S28 S34, S35 and S36. Model selection for both models was carried out using backwards stepwise regression to a minimum adequate model, retaining only variables with p-values lower than 0.1. All statistical analyses were performed in R version 3.5.3 (R Core Team 2019).

3. Results

3.1 Variation in sandfish abundance

3.1.1 Temporal patterns in sandfish abundance

Overall, every native species declined in relative abundance from 2013 to 2018 (Figure 8). Sawfin demonstrated the largest decline, from an average of 220.27 (\pm 39.23 SE) CPUE per pool in 2013 to a low of 25.35 (\pm 6.69 SE) CPUE in 2016. Yellowfish CPUE remained low throughout the surveys, averaging 0.14 individuals per pool per year. The remaining analyses focus on the patterns in sandfish abundance specifically, and on the factors that may be contributing to that decline.

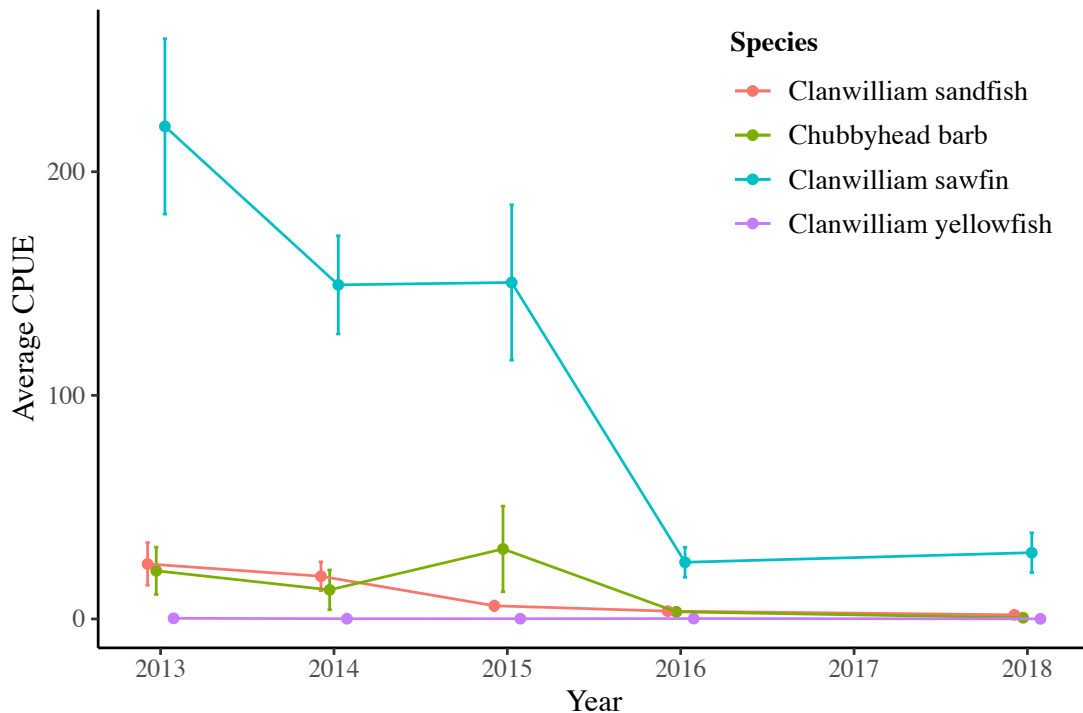


Figure 8. Average catch per unit effort (CPUE) \pm standard error (SE) of native fish between 2013 and 2018. Catch per unit effort was averaged among the pools sampled by seine that year ($n = 37$ pools in 2013, 36 pools in all other years) with one exception: in 2018, pools that were not sampled due to low water levels were included in the average as “0” values to account for the decrease in space available to fish.

Sandfish CPUE differed between years (Kruskal Wallis rank sum test, $X^2 = 27.14$, $p < 0.001$; Table 3). It was highest in 2013 and lowest in 2018, experiencing a nearly 93% decrease over five years (Table 3; Figure 9). Specifically, sandfish CPUE differed between

2018 and every other year (Dunn’s test of multiple comparisons; 2013 and 2018, $p < 0.001$; 2014 and 2018, $p < 0.001$; 2015 and 2018, $p < 0.05$; 2016 and 2018, $p < 0.05$), between 2013 and 2015 (Dunn’s test of multiple comparisons, $p < 0.05$) and between 2013 and 2016 (Dunn’s test of multiple comparisons, $p < 0.05$). In 2015, CPUE was 23.9% of what it was in 2013; in 2016, CPUE was 14.2% of the 2013 catch; in 2018, CPUE was 7.4% of the 2013 catch. Catch per unit effort of YOY sandfish also differed between years (Kruskal Wallis rank sum test, $X^2 = 50.40$, $p < 0.001$), experiencing a 99.7% decline between 2013 and 2018. Young-of-the-year CPUE in 2013 differed from every other year and CPUE in 2018 differed from every other year. In 2014, YOY sandfish CPUE was 10.7% of what it was in 2013 (Dunn’s test of multiple comparisons, $p < 0.01$); in 2018, YOY sandfish CPUE was 0.3% of the 2013 catch (Dunn’s test of multiple comparisons, $p < 0.001$). Catch per unit effort of juvenile/adult sandfish also differed significantly between years (Kruskal Wallis rank sum test, $X^2 = 10.61$, $p < 0.05$). The only pair of years that differed in terms of juvenile/adult sandfish CPUE were 2014 and 2018 (Dunn’s test of multiple comparisons, $p < 0.05$), with 10.5% of juvenile/adult sandfish sampled in 2018 compared to 2014.

Table 3. Compact letter display (CLD) of Dunn Kruskal Wallis multiple comparison displaying years that did and did not differ from one another in sandfish catch per unit effort (CPUE). Groups not sharing a letter differed significantly in their CPUE values ($p < 0.05$). Catch per unit effort was averaged among the pools sampled by seine that year ($n = 37$ pools in 2013, 36 pools in all other years) with one exception: in 2018, pools that were not sampled due to low water levels were included in the average as “0” values to account for the decrease in space available to sandfish. Young-of-the-year sandfish are those measuring less than 80 mm TL; juveniles/adults are those measuring 80 mm TL or more.

Year	Sandfish category								
	All			Young-of-the-year			Juvenile/Adult		
	Avg. CPUE	± SE	CLD	Avg. CPUE	± SE	CLD	Avg. CPUE	± SE	CLD
2013	24.60	9.53	a	19.90	8.94	a	4.67	2.15	ab
2014	19.10	6.44	ab	2.12	0.61	b	17.00	6.34	a
2015	5.89	2.08	b	3.49	1.81	b	2.40	0.71	ab
2016	3.49	1.14	b	2.03	1.00	b	1.46	1.00	ab
2018	1.83	0.71	c	0.05	0.04	c	1.78	0.68	b

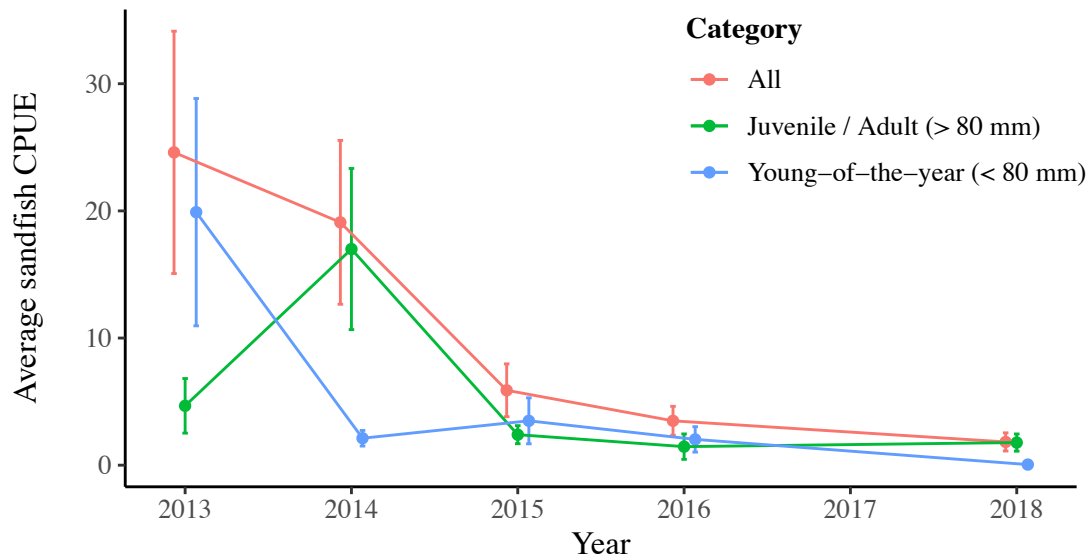


Figure 9. Average sandfish catch per unit effort (CPUE) \pm SE over time, split into three categories based on size (all, juvenile/adult, and young-of-the-year). Only data from seine samples was used in order to standardise catch per unit effort. Data from 2013, 2014, 2015, 2016 and 2018 was included. Catch per unit effort was averaged among the pools sampled by seine that year ($n = 37$ pools in 2013, 36 pools in all other years) with one exception: in 2018, pools that were not sampled due to low water levels were included in the average as “0” values to account for the decrease in space available to fish.

3.1.1.1 Potential influencing factors: temperature and precipitation

During the study period, average rainfall varied considerably (Figure 10a). Between 2010 and 2014, average rainfall remained above the nine-year average, only dropping below that average in 2015. Average rainfall continued to drop over time; in 2018, average rainfall was less than half of that recorded four years previously. During the same period, average maximum daily temperature varied minimally, with a difference of less than two degrees Celsius between the years with the highest and lowest average temperatures (Figure 10b).

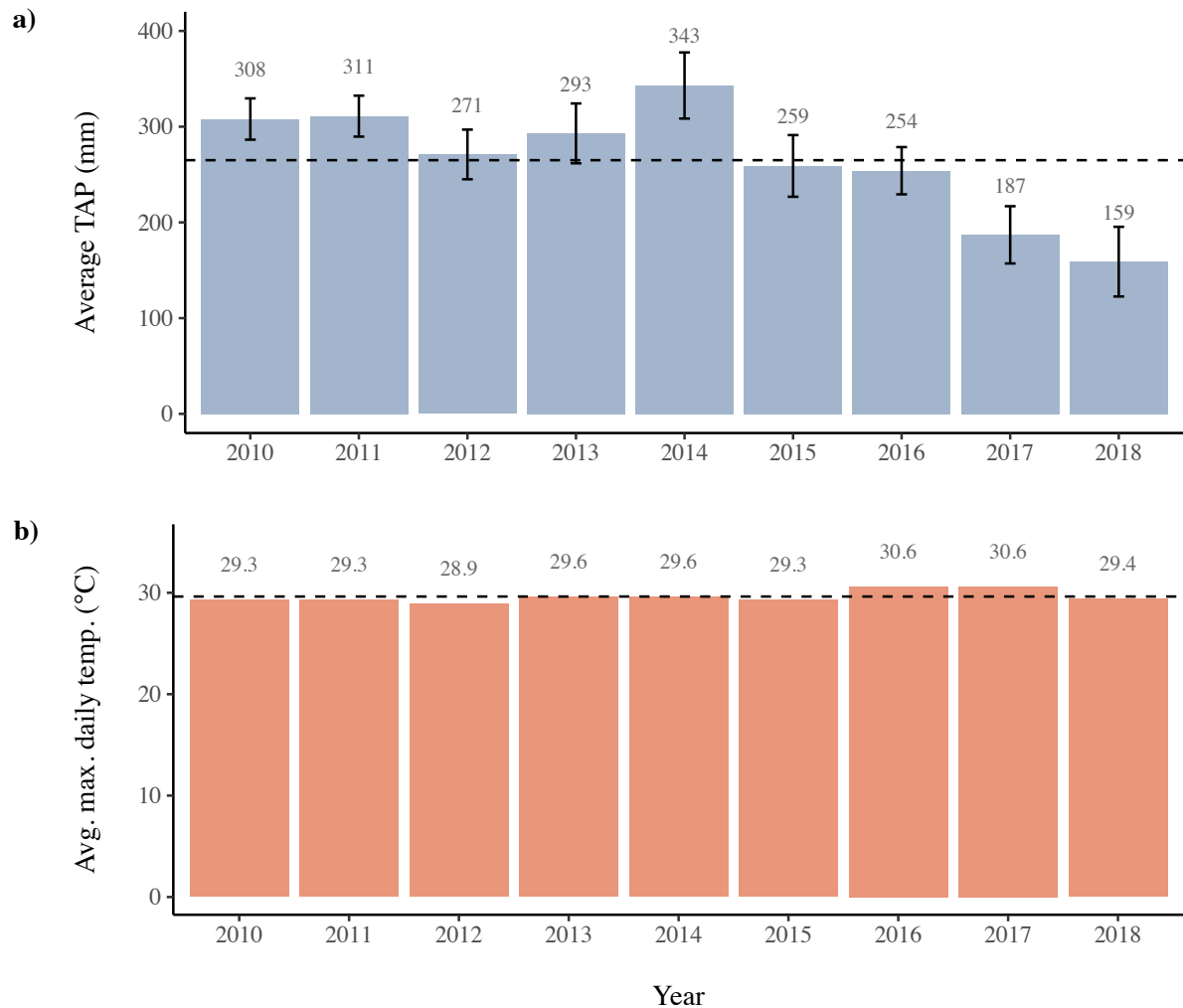


Figure 10. (a) Average total annual precipitation (TAP; mm; average of TAP recorded at ten weather stations in the Oorlogskloof catchment) \pm SE for the 12 months preceding each fish survey and (b) average maximum daily temperature ($^{\circ}$ C) over the five months preceding each survey from a single weather station in the catchment for which these data were available. The dotted lines represent average TAP between 2010 and 2018 (a) and the average October – February maximum daily temperature between 2010 and 2018 (b). No error bars reported for b) as the values come from a single weather station.

3.1.1.2 Potential influencing factors: non-native fish

Banded tilapia CPUE also differed significantly between certain years (Kruskal Wallis rank sum test, $X^2 = 11.24$, $p = 0.02$; Table 4; Figure 11a). It was higher in 2013 than in both 2016 (two-sample Wilcoxon rank sum test, $W = 867$, $p < 0.05$) and 2018 (two-sample Wilcoxon rank sum test, $W = 864$, $p < 0.05$). Catch per unit effort was also higher in 2015 than in both 2016 (two-sample Wilcoxon rank sum test, $W = 835$, $p < 0.05$) and 2018 (two-sample Wilcoxon rank sum test, $W = 831$, $p < 0.05$).

Table 4. Compact letter display (CLD) of Mann-Whitney U pairwise comparisons displaying years that did and did not differ from one another in banded tilapia catch per unit effort (CPUE). Groups not sharing a letter differed significantly in their CPUE values ($p < 0.05$). Catch per unit effort was averaged among the pools sampled by seine that year ($n = 37$ pools in 2013, 36 pools in all other years) with one exception: in 2018, pools that were not sampled due to low water levels were included in the average as “0” values to account for the decrease in space available to fish.

Year	Banded tilapia		
	Avg. CPUE	\pm SE	CLD
2013	39.20	10.54	a
2014	22.90	7.12	ac
2015	92.60	61.35	a
2016	10.60	5.47	bc
2018	11.98	3.63	bc

Bluegill sunfish CPUE from seine samples where they are known to occur (pools S34, S35 and S36; i.e. below the barrier) ranged between 0.67 and 9.33 individuals per pool per year (Figure 11a). Raw counts of bluegill sunfish from fyke samples at Rietkuilhuis, however, revealed higher abundances; from 2013 to 2016 there was a general decrease in catch from 314 to 56 individuals over a single fyke night per year, thereafter recovering to 593 in 2018 (Figure 11b). It is important to note that these samples are from a single site that is connected to the rest of the river during regular winter flows and therefore do not represent an isolated population of bluegill sunfish. Samples of smallmouth bass from both fyke and seine nets were extremely low throughout the surveys (only 15 individuals were sampled over five years; Figure 11); relative patterns in abundance are therefore difficult to describe.

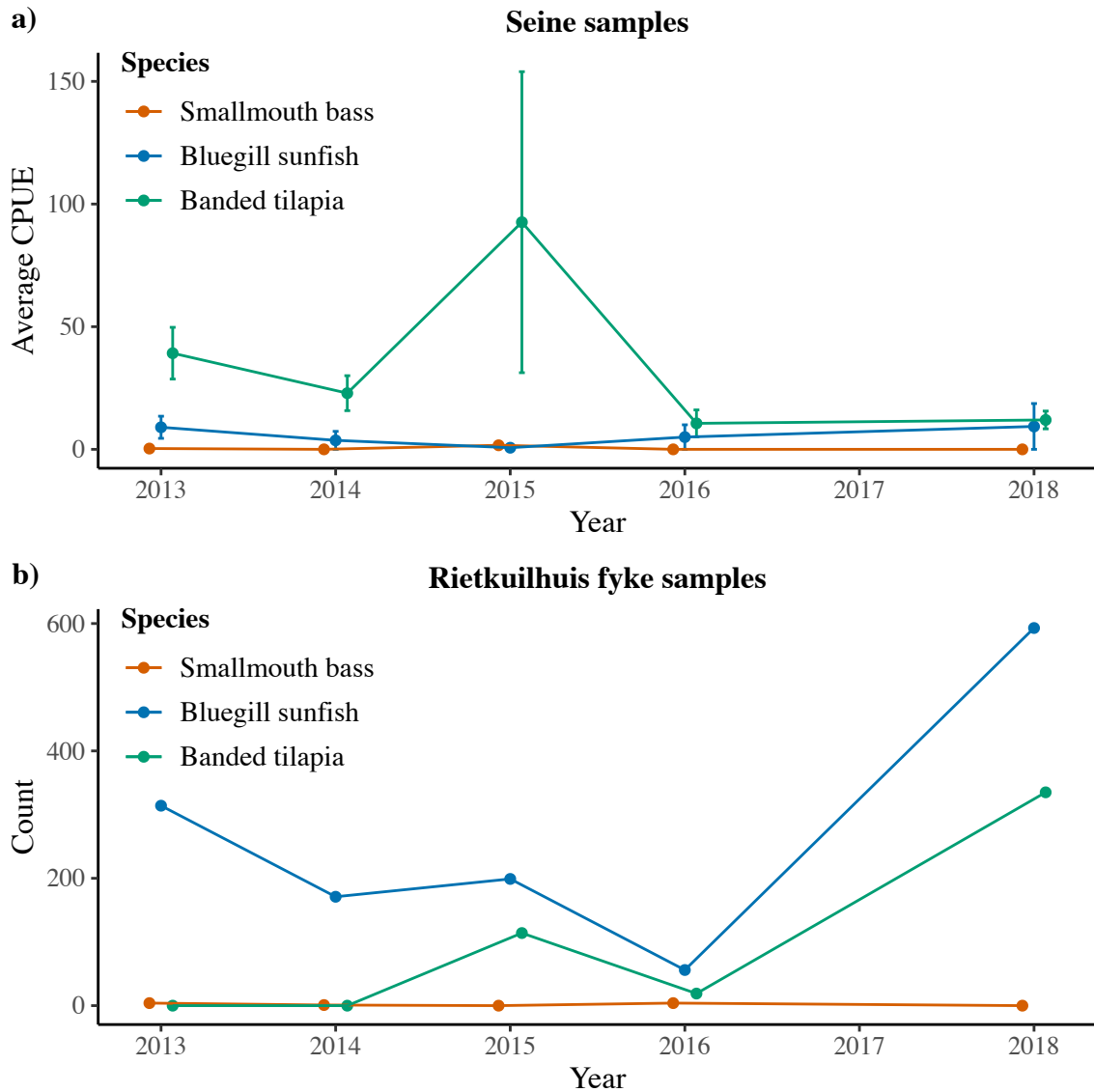


Figure 11. Average catch per unit effort (CPUE) \pm SE of smallmouth bass, bluegill sunfish, and banded tilapia from seine samples over time (a) and raw count of the same species from fyke samples at Rietkuilhuis over time. Note the difference in vertical axes. Sample size (i.e. number of pools) per year for the seine data (a) was three for smallmouth bass and bluegill sunfish and 36 for banded tilapia (except for 2013, when it was 35); sample size for the fyke data (b) was one for each species. No error bars were necessary for (b) as the values come from a single pool per year.

3.1.2 Spatial variation in sandfish abundance according to the presence of non-native species

The Chi-Square test for independence indicated that in pools where there were either smallmouth bass or bluegill sunfish present in either fyke or seine catches, sandfish were more likely to be absent ($X^2 = 3.96$, $p < 0.05$; $X^2 = 12.29$, $p < 0.001$, respectively). Sandfish were absent from 66% of the pools in which smallmouth bass were present ($n = 6$) and from 75% of the pools in which bluegill sunfish were present ($n = 12$). However, where there were

banded tilapia present, sandfish were more likely to also be present ($X^2 = 8.21$, $p < 0.001$). This pattern holds even when pools below the barrier are excluded to isolate the effect of banded tilapia from that of smallmouth bass and bluegill sunfish ($X^2 = 7.93$, $p < 0.01$). Of the 145 pools above the barrier in which banded tilapia were present over the seven survey years, sandfish were present in 77%.

When I compared sandfish CPUE from seine catches in pools where smallmouth bass and bluegill sunfish were either present or absent, I found that sandfish were entirely absent if either smallmouth bass or bluegill sunfish were present (two-sample Wilcoxon rank sum test, $W = 414$, $p < 0.05$; $W = 819$, $p < 0.01$, respectively; Table 5). When considering the entire sampling range (i.e. A1 – S36), fewer sandfish were present where banded tilapia were present than where they were absent (two-sample Wilcoxon rank sum test, $W = 1616.5$, $p < 0.001$). However, when pools below the barrier are excluded in order to isolate the impact of banded tilapia, the relationship breaks down. There is no significant difference in sandfish abundance between pools where banded tilapia were present and where they were absent above the barrier (two-sample Wilcoxon rank sum test, $W = 1402$, $p = 0.06$).

Table 5. Results of Wilcoxon rank sum test assessing whether median sandfish abundance differs between pools where smallmouth bass, bluegill sunfish and banded tilapia are present and where they are absent. The section labelled “Banded tilapia * ” includes only pools A1 through S33 (i.e. only pools above the barrier); this was done in order to avoid the possible confounding effect of smallmouth bass and bluegill sunfish presence on sandfish abundance. Data were pooled across years (2013, 2014, 2015, 2016 and 2018).

Non-native species	Condition of non-native species	Mean sandfish CPUE	Median sandfish CPUE	n	p-value
Smallmouth bass	Present	0	0	3	0.032*
	Absent	12.3	2	161	
Bluegill sunfish	Present	0	0	6	0.002**
	Absent	12.5	2.3	158	
Banded tilapia	Present	11.3	3.5	121	< 0.001***
	Absent	20.5	0	43	
Banded tilapia *	Present	11.2	3.8	120	0.060
	Absent	14.3	1	30	

This apparently binary relationship between sandfish and smallmouth bass/bluegill sunfish is illustrated when sandfish count is plotted against the count of each of the three non-native species (Figure 12). Where there are smallmouth bass and/or bluegill sunfish in either

seine or fyke samples, there are almost always no sandfish present at the site. However, sandfish co-occurred with banded tilapia even at sites supporting relatively high banded tilapia abundances.

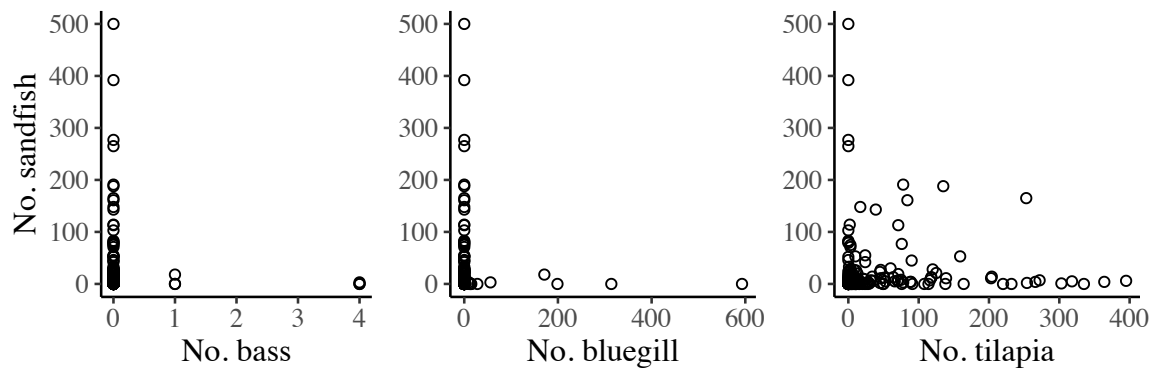


Figure 12. Relationship between the abundance of sandfish and the abundance of non-native fish. Data include both seine and fyke samples from all years (2010, 2013, 2014, 2015, 2016 and 2018) except pool S03 in 2015; this outlying data point (banded tilapia count = 2199, sandfish count = 21) obscured the overall pattern between banded tilapia and sandfish at lower abundances of the former; it was excluded to emphasise the overarching patterns.

3.1.3 Identifying top predictors of sandfish abundance

The initial GLMM fitted with sandfish relative abundance as the response variable included the presence of the three native fish, the presence of banded tilapia, rainfall and temperature as explanatory variables. The best model ultimately retained rainfall, sawfin presence and banded tilapia presence, although the latter was not a significant predictor of sandfish abundance ($p = 0.087$; Table 6). Temperature and presence of yellowfish and chubbyhead barb were ultimately excluded from the model, indicating that they are not strong predictors of sandfish relative abundance. The model predicted a greater abundance of sandfish in years with higher rainfall (Figure 13). It also predicted sandfish abundance to be nine times higher in pools where sawfin are present than in pools where sawfin are absent (Figure 13).

Table 6. Results of generalised linear mixed model (GLMM) selection examining predictors of sandfish relative abundance (catch per unit effort; CPUE). All explanatory variables with $p < 0.10$ were retained in the model (left-justified) but only those with $p < 0.05$ were considered significant predictors of sandfish size ($* = p < 0.05$, $** = p < 0.01$, $*** = p < 0.001$). Explanatory variables with $p > 0.10$ were removed from the model in a stepwise fashion, with the least significant variable being removed at each step (statistics given at the point of removal). Variables that were removed from the model are indented. The response variable is stated as “sandfish relative abundance” to indicate that it does not represent a raw abundance estimate, but rather abundance offset by varying effort at each pool.

Response variable	Fixed effects	Estimate	\pm SE	X^2	p-value
Sandfish relative abundance (CPUE)	Rainfall	0.005	0.002	9.309	0.002**
	Sawfin presence	2.193	0.728	9.072	0.003**
	Banded tilapia presence	0.698	0.408	2.930	0.087
	Chubbyhead barb presence	--	--	1.901	0.168
	Yellowfish presence	--	--	0.680	0.409
	Temperature	--	--	0.568	0.451
Random effects	Variance	SD			
Site	1.658	1.287			
Year	0.055	0.234			

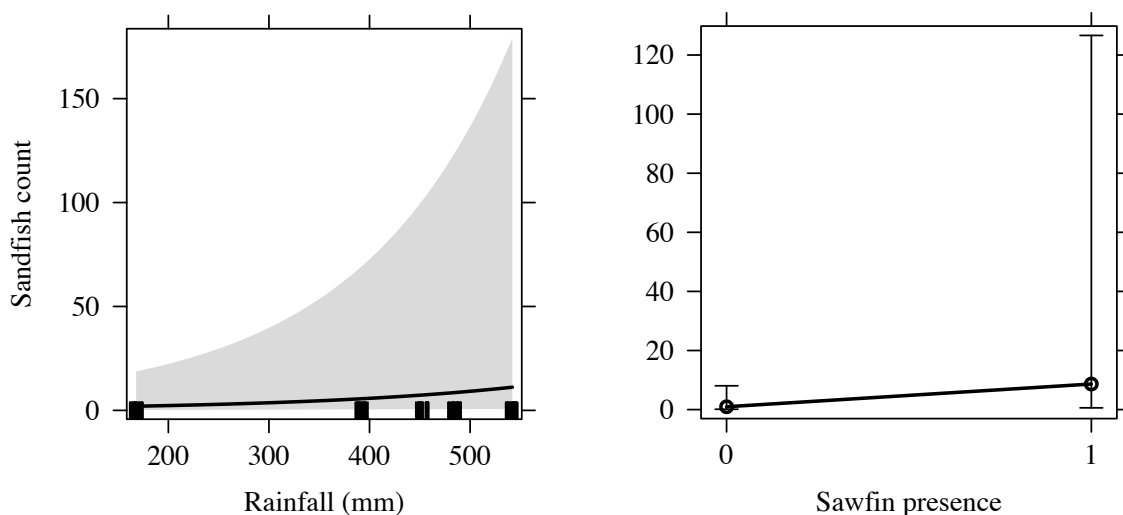


Figure 13. Effect plots of the two significant predictors retained in generalised linear mixed model (GLMM) examining predictors of sandfish relative abundance. For the latter plot, “0” on the horizontal axis indicates that sawfin were absent; “1” indicates that they were present. Shaded area and error bars represent 95% confidence intervals.

3.2 Variation in sandfish size

3.2.1 Spatial variation in sandfish population size structure

The sandfish sampled above the barrier exhibited a wide range of size classes from 10 mm to 360 mm TL, with most fish measuring between 40 mm and 120 mm TL and few fish measuring above 260 mm TL (Figure 14a). The vast majority of fish sampled above the barrier fell below the presumed size at reproductive maturity, with 77.8% of fish measuring less than 150 mm TL (the presumed size of males at reproductive maturity) and 99.3% of fish measuring less than 250 mm TL (the presumed size of females at reproductive maturity). Sandfish sampled below the barrier ranged between 38 mm and 471 mm TL, with all but four individuals measuring between 370 mm and 471 mm TL (Figure 14b). The smallest individuals (10 mm – 30 mm TL) were entirely absent below the barrier.

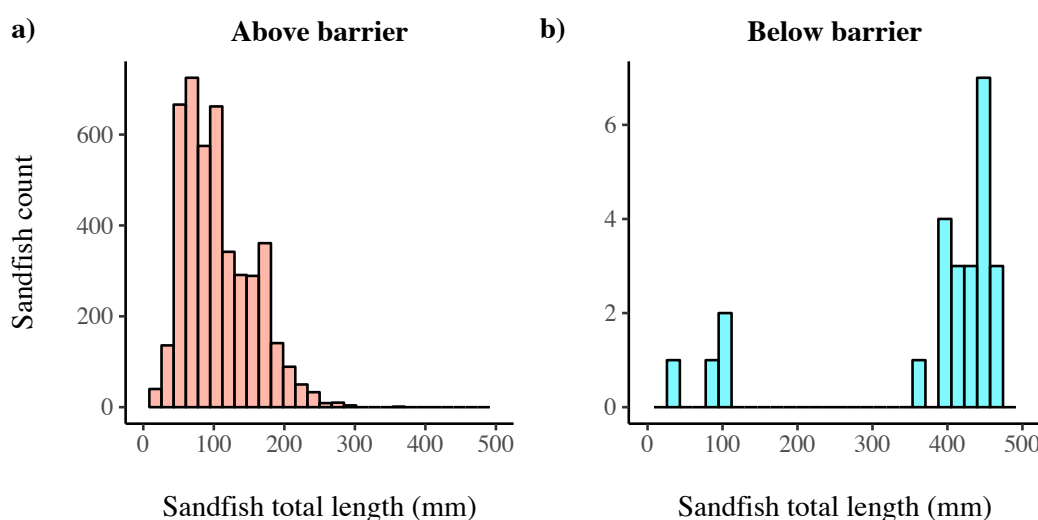


Figure 14. Sandfish length (TL) distribution (a) above (sites A1 – S33; $n = 4,626$) and (b) below (sites S34 – Rietkuilhuis; $n = 25$) the barrier. Data from all years (2010, 2013, 2014, 2015, 2016 and 2018) have been combined. Note difference in sample sizes and vertical axis scales.

River location had a highly significant association with sandfish size category (Pearson's Chi-Square test for independence, $X^2 = 194.36$, $p < 0.001$). Above the barrier, 92% of sandfish measured 200 mm TL or less, whereas below the barrier, only 16% of sandfish belonged to the smaller size class (Figure 15).

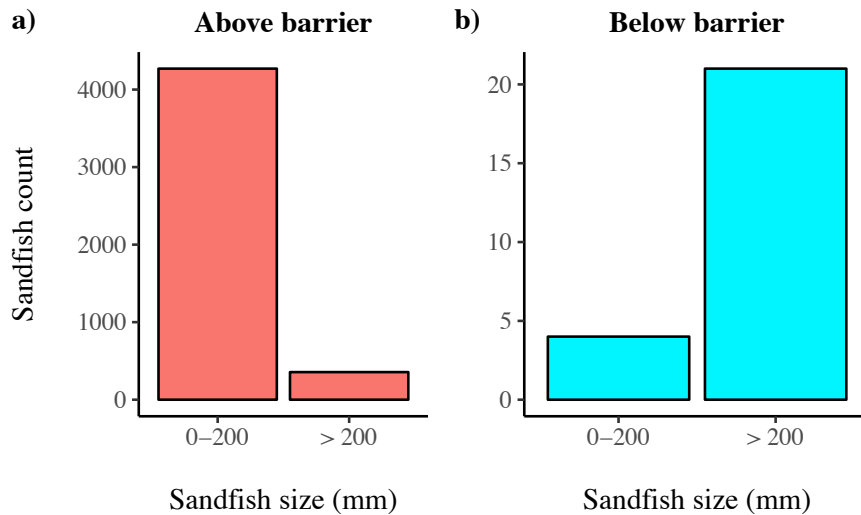


Figure 15. Counts of sandfish in two different size classes sampled (a) above (sites A1 – S33; n = 4,626) and (b) below (sites S34 – Rietkuilhuis; n = 25) the barrier. Data from all years (2010, 2013, 2014, 2015, 2016 and 2018) were combined. Note difference in sample sizes and vertical axis scales.

3.2.2 Identifying top predictors of sandfish size

The initial LMM fitted with sandfish size as the response variable included barrier, presence of each of the three native fish, presence of banded tilapia, temperature, and rainfall. The best model for sandfish size ultimately retained barrier, presence of chubbyhead barb, presence of sawfin and rainfall as explanatory variables. However, rainfall was not a significant predictor of sandfish size ($p = 0.055$; Table 7). Barrier had the largest effect size, with sandfish sampled below the barrier predicted to be 2.6 times larger than the sandfish sampled above the barrier (holding other fixed effects constant and within the same year; Figure 16). Mean sandfish size above the barrier was 106 mm TL while below the barrier it was 373 mm TL. Presence of chubbyhead barb and sawfin each had a very small effect on sandfish size relative to barrier.

Table 7. Results of linear mixed model (LMM) selection examining predictors of sandfish size (TL). All explanatory variables with $p < 0.10$ were retained in the model (left-justified) but only those with $p < 0.05$ were considered significant predictors of sandfish size (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Explanatory variables with $p > 0.10$ were removed from the model in a stepwise fashion, with the least significant variable being removed at each step (statistics given at the point of removal). Variables that were removed from the model are indented.

Response variable	Explanatory variable	Estimate	\pm SE	X ²	p-value
Sandfish size (TL)	Barrier	-5.833	1.379	17.902	< 0.001***
	Chubbyhead presence	-0.438	0.111	15.602	< 0.001***
	Sawfin presence	1.292	0.535	5.834	0.016*
	Rainfall	-0.005	0.002	3.682	0.055
	Yellowfish presence	--	--	2.442	0.118
	Temperature	--	--	1.091	0.296
	Banded tilapia presence	--	--	1.080	0.299
Random effects	Variance	SD			
Site	2.886	1.699			
Year	0.616	0.785			

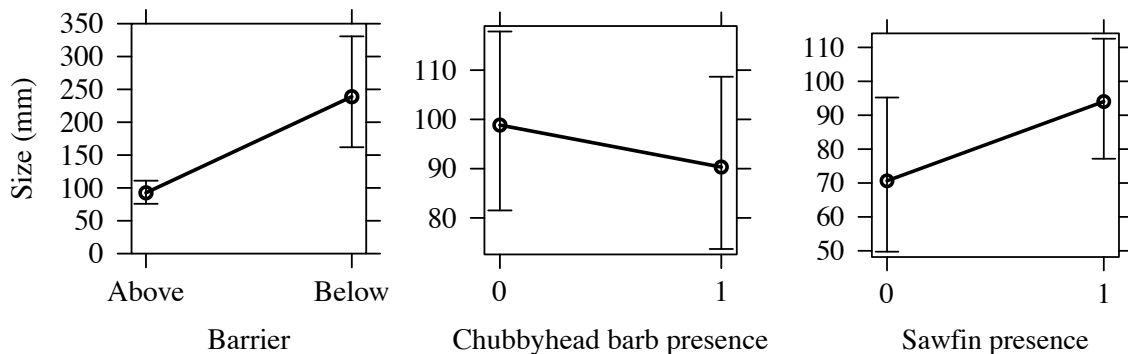


Figure 16. Effect plots of three significant predictors retained in linear mixed model (LMM) examining predictors of sandfish size (TL). For the latter two plots, “0” on the horizontal axis indicates that the particular species was absent; “1” indicates that it was present. Error bars represent 95% confidence intervals.

3.3 Influence of environmental variables on sandfish abundance and size

The initial GLMM fitted with sandfish relative abundance in 2014 as the response variable included pool area; depth; proportions of coarse, medium, and fine substrate; salinity and pH as explanatory variables. Pool area and depth were ultimately retained as significant predictors in the best model for sandfish abundance (Table 8). In both cases, the relationship

was negative: larger and deeper pools both predicted lower counts of sandfish (Figure 17). The standard errors of the estimates of both area and depth were large compared to the estimates.

Table 8. Results of generalised linear mixed model (GLMM) selection examining habitat predictors of sandfish relative abundance in 2014. All explanatory variables with $p < 0.10$ were retained in the model (left-justified) but only those with $p < 0.05$ were considered significant predictors of sandfish size (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Explanatory variables with $p > 0.10$ were removed from the model in a stepwise fashion, with the least significant variable being removed at each step (statistics given at the point of removal). Variables that were removed from the model are indented.

Response variable	Explanatory variable	Estimate	\pm SE	X ²	p-value
Sandfish relative abundance	Pool area	-0.001	0.001	5.402	0.020*
	Depth	-2.853	1.346	4.495	0.034*
	Coarse substrate	--	--	1.850	0.174
	Fine substrate	--	--	0.780	0.377
	Medium substrate	--	--	0.677	0.411
	Salinity	--	--	0.130	0.718
	pH	--	--	0.188	0.665
Random effects	Variance	SD			
Site	3.643	1.909			

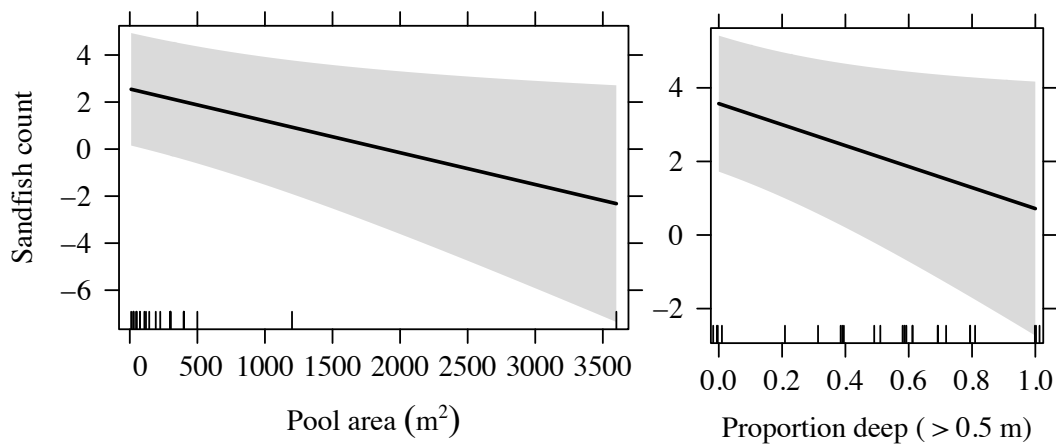


Figure 17. Effect plots of the two significant predictors of sandfish relative abundance in 2014 retained in generalised linear mixed model (GLMM). Shaded areas represent 95% confidence intervals.

The initial LMM fitted with sandfish size in 2014 as the response variable included pool area; depth; proportions of coarse, medium, and fine substrate; salinity and pH as

explanatory variables. The only significant predictor of sandfish size retained within the LMM was pool area (Table 9). Proportion of coarse substrate was also retained in the model but was not significant ($p = 0.097$). The effect plot for area indicates a negative relationship with sandfish size; sandfish sampled from larger pools are predicted to be smaller (Figure 18). However, the estimated among-site standard deviation is very large relative to the magnitude of the effect of area, suggesting that differences between sites account for more variation in sandfish size than do differences in pool area.

Table 9. Results of linear mixed model (LMM) selection examining habitat predictors of sandfish size in 2014. All explanatory variables with $p < 0.10$ were retained in the model (left-justified) but only those with $p < 0.05$ were considered significant predictors of sandfish size (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Explanatory variables with $p > 0.10$ were removed from the model in a stepwise fashion, with the least significant variable being removed at each step (statistics given at the point of removal). Variables that were removed from the model are indented.

Response variable	Explanatory variable	Estimate	\pm SE	X^2	p-value
Sandfish size	Pool area	-1.592	0.595	7.154	0.007**
	Coarse substrate	-29.712	17.913	2.751	0.097
	Medium substrate	--	--	0.977	0.323
	pH	--	--	0.679	0.410
	Salinity	--	--	0.700	0.403
	Depth	--	--	0.234	0.629
	Fine substrate	--	--	0.004	0.951
Random effects	Variance	SD			
Site	208.700	14.450			

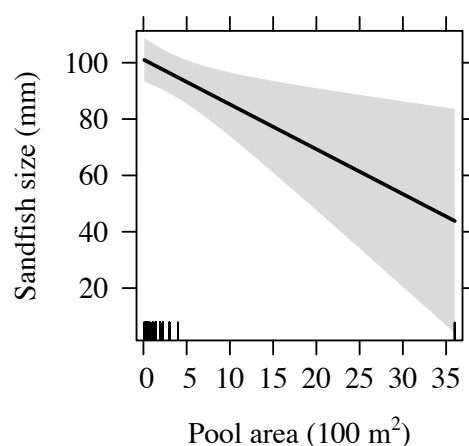


Figure 18. Effect plot of single significant predictor of sandfish size in 2014 retained in generalised linear mixed model (GLMM). Shaded area represents 95% confidence intervals. Note horizontal axis: values were scaled by a factor of 1/100 in order to address a scaling mismatch between the different explanatory variables in the model. A value of 15 on the horizontal axis therefore represents 1500 m².

4. Discussion

In this study I analysed data from six surveys carried out at 38 sites in the Oorlogskloof River over nine years in order to determine how the relative abundance of the endangered Clanwilliam sandfish has changed over space and time and how sandfish size differs according to several spatial factors. The potential influence of other fish species and environmental and habitat variables on variation in sandfish numbers and size was evaluated. Over the study period, the data suggests that the Oorlogskloof River population of sandfish declined by 93%. While both size classes experienced significant declines, most notable and concerning is the 99.7% decline of YOY sandfish over the study period. At sites where non-native smallmouth bass or bluegill sunfish were present downstream of a waterfall barrier, sandfish relative abundance was low relative to smallmouth bass-free and bluegill sunfish-free sites upstream, and small individuals (< 200 mm TL) were almost entirely absent. On the other hand, sandfish co-occurred with non-native banded tilapia, even where there were moderate to high banded tilapia abundances. Above the smallmouth bass and bluegill sunfish barrier, the sandfish population exhibited a relatively healthy size structure, with a wide range of sizes from 10 mm to 360 mm TL, although spawning-size individuals are notably scarce. Below the barrier, most sandfish sampled were large adults (> 370 mm TL). Larger, deeper pools generally had lower abundances of sandfish sampled, and larger pools were also associated with a smaller mean sandfish size. Other environmental variables were not significantly correlated with sandfish size or abundance.

4.1 Variation in sandfish abundance

4.1.1 Cautious interpretation of sandfish population decline

It is important that short term population fluctuations be compared against the context of long-term population trends and significant stochastic events. Monitoring native fish populations over several cycles of floods and droughts, for example, would help elucidate the role of these stochastic events in regulating sandfish population fluctuations. While the data collected here span nine years and constitute the most complete and continuous record of relative fish abundances in the Oorlogskloof River, it is critical that the variable nature of the environment where this study takes place and the relatively small timeframe under consideration be borne in mind. The apparent decline in sandfish numbers during the study

period must therefore be interpreted cautiously and with the understanding that an evaluation of current population trends will be better contextualised and more accurately understood when more data are added to the data set over a longer time frame in the years to come.

4.1.2 The role of other fish species

Bearing this in mind, that all three of the most abundant native fishes (sawfin, chubbyhead barb and sandfish) experienced declines during the study period is nevertheless cause for concern. While the focus of this present study is on sandfish, the similarity of these patterns should be borne in mind. Both size cohorts experienced substantial declines, yet the near-eradication of YOY sandfish from an average of 19.90 CPUE in 2013 to 0.05 CPUE in 2018 is especially alarming since it signals widespread recruitment failure. Furthermore, the juvenile/adult size cohort includes small fish vulnerable to bass predation (those measuring 80 mm to 200 mm TL), which could be driving the pattern of 89.3% decline from 2013 to 2018. Future studies should further separate sandfish into YOY (0-79 mm TL), bass-vulnerable juveniles (80-199 mm TL) and adults (200 mm TL +) in order to better understand the apparent decline in sandfish during the study period.

The drastic decline in YOY sandfish is unlikely to have occurred as a direct result of increased predation or competition by banded tilapia, since the relative abundance of this non-native species did not differ between 2013 and 2014, when YOY sandfish experienced the steepest drop in numbers. In addition, sandfish relative abundance did not differ between pools where banded tilapia were present and where they were absent and presence of banded tilapia was not a significant predictor of sandfish abundance in the GLMM. This suggests that banded tilapia are not impacting strongly on the sandfish population in this system. This result is consistent with studies that have found banded tilapia co-occurring with native minnow species in several streams elsewhere in the ODRS (Van der Walt 2014) and in the Swartkops River system, Eastern Cape (Ellender et al. 2011).

This is in stark contrast to the apparent impacts of smallmouth bass and bluegill sunfish. That sandfish were entirely absent from seine catches where either smallmouth bass or bluegill sunfish were present and that only very few sandfish were recorded from fyke samples containing smallmouth bass or bluegill sunfish (a total of 21 sandfish sampled from only two pools) are strong indications that these non-native species are almost entirely excluding the endangered sandfish from the invaded reach. This is consistent with several studies in South Africa that have found native fish to be abundant above a black bass-

excluding barrier but absent or scarce in the black bass-invaded reaches downstream. During a low-flow period in South Africa's Blindekloof stream in the Eastern Cape, for example, no other fish species were observed where largemouth and smallmouth bass were sampled (Ellender et al. 2011). In the Rondegat River, part of the ODRS, four of the five fish species native to the system were found to be entirely absent from the smallmouth bass-invaded reach (Woodford et al. 2005). Furthermore, the small native Clanwilliam redfin *Sedercypris calidus* (Barnard, 1938) and fiery redfin *Pseudobarbus phlegethon* (Barnard, 1938) occurred in a significantly higher proportion of tributaries located above physical barriers than below barriers where black bass were present (Van der Walt et al. 2016).

Analogous field studies of bluegill sunfish effects on South African freshwater fish are conspicuously lacking, although some studies suggest that while black bass may often co-occur with other invasives, including bluegill sunfish and banded tilapia, the consistency of observed impacts where black bass are present in the ODRS indicate that bass species are having the largest impact on native cyprinid populations (Van der Walt et al. 2016). On the other hand, it is evident from this study and others (Paxton et al. 2002) that the concentrations of bluegill sunfish in the Doring River and its tributaries can be considerably higher than those of black bass. Over the course of these surveys, 1,426 bluegill sunfish were sampled, compared to only 15 smallmouth bass. Bluegill sunfish might therefore be having disproportionate effects on sandfish abundance due to their comparatively high densities. It is also evident from dissections of bluegill sunfish in the Biedouw River that these predators, although smaller than black bass, are easily capable of consuming sandfish more than a third of their size in length (Leonard Flemming, unpublished data). Unfortunately, the strong spatial overlap between smallmouth bass and bluegill sunfish in the four below-barrier pools sampled in this study makes it difficult to isolate the effects of bluegill sunfish relative to those of smallmouth bass in the Oorlogskloof; sampling of more pools in the invaded reach is therefore recommended to more decisively separate the impacts of these non-native fish. If, however, the strong spatial overlap between the two species occurs throughout the invaded reach of the Oorlogskloof, it may be necessary to compare sandfish responses to these predators in rivers where smallmouth bass occur but bluegill sunfish are absent, and vice versa, if indeed such rivers exist.

It is worth noting that the presence of sawfin was correlated with relative abundance of sandfish, with nearly nine times more sandfish predicted to exist in pools where sawfin were present. While a detailed analysis of this relationship was beyond the scope of the present study, this large effect might indicate that both cyprinids thrive in similar

environments or may respond to threats in a similar fashion. Continuing to monitor temporal patterns in the sawfin population of the Oorlogskloof River is therefore important as they might serve as a warning to signal similar shifts in the sandfish population, and vice versa.

4.1.3 The role of precipitation and temperature

The recent drought experienced by the region is unlikely to have precipitated the 2013 - 2014 YOY sandfish decline, as rainfall – and therefore river flow – only started to decline between 2014 and 2015. However, it is possible that high flows brought about by above-average rainfall in 2014 may have suppressed spawning or flushed away eggs, larvae or even juveniles in the Oorlogskloof Gorge downstream into the smallmouth bass and bluegill sunfish zone, where they would have been vulnerable to predation. In the Driehoeks River, higher winter flows lasting later into the spring were hypothesised as a reason for the reduced recruitment (via suppression of spawning and/or reduced larval survival) of Clanwilliam sawfin in the 2005/6 spawning season (Paxton 2008). Proposed mechanisms included larval displacement into unfavourable habitats and the reduction in availability of resources due to lowered productivity of shallow environments (Paxton 2008). The drought conditions beginning in 2015 and continuing through the study period may have then played a role in preventing sandfish populations from recovering, either through reproductive effects (environmental interference with spawning migrations or spawning itself) or recruitment effects (environmental interference with egg and larvae survival; Humphries & Lake 2000). For example, drought conditions may have failed to trigger sandfish spawning, reduced the quality of spawning habitat, or the availability of habitat for young fish to grow and feed, thereby decreasing their survival.

Indeed, rainfall was correlated with sandfish relative abundance, indicating that fewer sandfish are present during years with low rainfall. Continuous flow monitoring (as per Paxton 2008) would greatly improve our understanding of how river conditions influence population fluxes from year to year. Furthermore, sampling during the spring and summer leading to an estimate of larval and juvenile abundances each year would give an indication of whether sandfish spawned in that particular year, either helping to confirm spawning failure as a cause of a lack of juveniles or signal post-spawning recruitment failure as a likelier cause.

Temperature was not a significant predictor of sandfish relative abundance in the GLMM. However, the measure of temperature used in this analysis has its limitations: it is an

average of daily maximum temperature taken over a period of five months preceding the surveys, and as such does not reflect the most extreme conditions experienced by YOY individuals during which they would presumably be most vulnerable. A count of the days during the same five-month period during which temperatures reached values above a long-term average would more accurately reflect degree of exposure to extreme conditions and may be a better measure of sandfish vulnerability to changes in temperature – an avenue to explore in future analyses.

It is important to emphasise here that there is significant uncertainty involved in interpreting sandfish population fluctuations against the relatively limited environmental data available, due primarily to our poor understanding of sandfish reproductive biology, spawning behaviour and sandfish population responses to high and low flow conditions. A more comprehensive collection of environmental data moving forward, especially as it relates to sandfish spawning ecology, is critical to our improved understanding of sandfish population responses to changing environmental conditions.

4.2 Variation in sandfish size

The wider size range represented in the population structure of sandfish sampled above the barrier, with many small juveniles (< 120 mm TL) and few large adults (> 250 mm TL), exists in stark contrast to the adult-dominated sandfish population structure sampled below the barrier, where all but four of the individuals measured more than 370 mm TL. Notably, very large individuals (larger than 350 mm TL) were also entirely absent from the above-barrier sampling sites, and fish larger than the presumed size of females at reproductive maturity (250 mm TL) were extremely scarce above the barrier. It is possible that the above-barrier sampling sites are unsuitable for large sandfish, as they tend to be smaller than the pools found downstream of the Oorlogskloof Gorge (i.e. the below-barrier pools). The increased inter- and intra-species competition in the smaller pools might be driving adult sandfish to favour the larger pools below the barrier. This interesting pattern warrants further study.

The meaningful difference arguably remains that of the conspicuous lack of small juveniles below the barrier. Coupled with the sudden drop in abundance of sandfish falling within the smallmouth bass-vulnerable size class below the barrier, and the fact that barrier was the strongest predictor of sandfish size in the LMM, my results suggest that this reach may be unsuitable for small sandfish due to the presence of non-native invasive predators,

and functions as a population sink within the system. Sites S33 and S34, the two pools immediately on either side of the barrier, are only 285 metres apart, yet these differences are apparent as soon as that barrier is crossed. An abundance of studies illustrates the destructive impact that non-native fish species, especially black bass, have had on small native species and juveniles of larger fish species in the region. In the Rondegat River in the ODRS, for example, only sawfin larger than 100 mm TL are able to co-exist with smallmouth bass (Woodford et al. 2005), and throughout the ODRS, only large-bodied native fishes over 100 mm FL (fork length; the length of a fish measured from the snout to the end of the median caudal fin rays) coexist with black bass (Van der Walt et al. 2016). A survey carried out in 2001 at 16 sites in the ODRS showed that in reaches free of smallmouth bass and bluegill sunfish invasion, smaller size classes of sandfish and sawfin dominated, whereas in invaded reaches, larger individuals (> 400 mm TL) were dominant (Paxton et al. 2002). A similar survey carried out between 2012 and 2014 found that individuals belonging to the three large cyprinid species in the ODRS smaller than 200 mm TL were entirely absent below black bass barriers (Van der Walt 2014).

As mentioned previously, out of the non-native fishes present in the ODRS, black bass have been recognised as the primary threat to native fishes (Van der Walt et al. 2016). In this case however, due to the significantly higher concentrations of bluegill sunfish, coupled with the high spatial overlap between the smallmouth bass and bluegill sunfish in the sampled pools, it is difficult to conclude with any certainty which of the two non-native species plays the larger role in excluding juvenile sandfish in the invaded reach. Nevertheless, if, in addition to predation by smallmouth bass and/or bluegill sunfish, environmental conditions become unfavourable to spawning or larval development, a population crash such as has been observed in the Oorlogskloof River might seem inevitable.

4.3 Influence of environmental variables on sandfish abundance and size

The GLMM fitted with sandfish abundance in 2014 as the response variable showed that lower abundances of sandfish were associated with larger, deeper pools. Sampling becomes more difficult in larger and deeper pools as fish can more easily evade the net where there is more space to hide (Mandy Schumann, pers. comm.) The seine net, only 1.5 m deep, can easily sweep the entire vertical column of water in a shallow pool, but will fail to touch the bottom in a deeper pool, leaving plenty of space for escape or concealment. The lower

relative abundance of sandfish associated with larger and deeper pools may therefore be a better reflection of sampling bias than of patterns in abundance.

Similarly, the LMM showed that sandfish of a smaller mean size were associated with larger pools. Large fish in general evade seine nets more readily than smaller fish (Hayes 1989). In large pools where it is already difficult to operate the seine net, it might be easier for large fish to evade the net than it is for small fish, leading to an apparent lower relative abundance of large fish sampled in larger pools. Thus, given this potential sampling bias, the data from pools deeper than 1.5 metres, as well as the apparent negative association between sandfish and deep pools, should be treated with some caution. Fyke sampling in a subset of pools could help assess the consistency of seine sampling in pools of different sizes and depths.

4.4 Sampling recommendations and future directions

Fish sampling. Relationships between pool volume and sandfish abundance may be best elucidated using fyke nets (which are less susceptible to sampling bias in deeper pools) rather than seine nets and will allow for a more rigorous assessment of the relationship between these site characteristics and the abundance and size of sandfish. Other sampling alternatives, such as underwater and video surveys are not practical for the Oorlogskloof River; visibility differs between pools and between years depending on river location and recent flooding events (Mandy Schumann, pers. comm.) If a different type of sampling, such as additional fyke netting, is employed in the future, it is important that this be done in addition to seine sampling if possible, so as to preserve the continuity of the data set and maximise comparability to past conditions – continuing with the current seine sampling regime should be a top priority.

Flow and temperature monitoring. Furthermore, it would be useful and informative to measure several other environmental variables in a consistent manner moving forward. Continuous (i.e. daily or hourly) flow and water temperature data collected from at least August (i.e. the suspected beginning of the sandfish spawning season) through March when the surveys are carried out would allow for a rigorous temporal assessment of the role of these environmental conditions in regulating sandfish population patterns. Flow data have been collected at two locations along the Oorlogskloof River in the past but could not be included in the present study due to data gaps (both flow loggers failed due to suspected high concentrations of corrosive compounds in the river). Continuing to collect these data would

allow for a more accurate assessment of the effect of flow conditions on sandfish spawning and larval development compared to using rainfall as a proxy. These data will be especially useful in helping to interpret population responses during periods of drought and flooding. A temperature logger set to record hourly thermal data should be installed at the flow site.

Water quality and habitat. Additionally, pH and salinity should be sampled at each site during the annual survey. While these factors did not emerge as significant predictors of sandfish abundance or size, the sampling was limited to a single year and more nuanced analyses might be possible if these data were available over an extended period of time. The predominant substrate at each site should also be recorded, as it is relevant to the feeding ecology of the sandfish and might prove to influence the abundance and size structure of sandfish given adequate sampling size. Recording the approximate length, width and depth of sampling pools may also shed some light on the relationship between these characteristics and those of the sandfish population. For all future sampling, it should also be noted if a particular sampling site is an isolated pool, or if it is part of a larger network of interconnected pools at the time of sampling, as these characteristics might bias sampling and are liable to change year to year depending on precipitation and flow.

Sampling locations. Sampling more pools below the smallmouth bass and bluegill sunfish barrier would allow for a more meaningful and nuanced analysis of the relative importance of smallmouth bass compared to bluegill sunfish in regulating the sandfish population. However, there is the possibility that smallmouth bass and bluegill sunfish overlap too significantly in their habitat use to allow for such an analysis. In order to avoid unnecessary and inefficient effort allocation and expense, it might be useful to designate this additional downstream sampling to a trial period of two or three years, after which an analysis should be carried out to determine if there is enough spatial separation between the two non-native species to warrant continued sampling. If, however, smallmouth bass and bluegill sunfish co-occur in most below-barrier pools in the Oorlogskloof River, it may be necessary to sample sandfish response in rivers where they co-occur with only one of these non-native species.

Furthermore, sampling additional pools upstream of S01 where only native fish exist would help distinguish more thoroughly the role that banded tilapia plays, if any, in regulating sandfish abundance and size. Increasing sampling effort on both extremes of the sampling range will necessarily make the sampling process more difficult; it might therefore be necessary to decrease sampling effort within the ONR itself. If this is necessary, it is recommended that the discarded pools be chosen from the stretches where more concentrated

sampling takes place, such as between S07 and S20. However, the elimination of sampling sites should be avoided if budget and manpower allow for the additional proposed sampling. Changing the sampling regime may threaten the continuity of the dataset and jeopardise comparability to past conditions. However, if this is unavoidable, a detailed power analysis must take place in order to ensure that any remaining sampling allows for a large enough sample size to statistically detect a difference in sandfish abundance and size if one does indeed exist.

Stable isotopes. Furthermore, a rigorous stable isotope analysis of all the fishes in the Oorlogskloof River should be undertaken in order to trace the flow of nutrients within the system and thereby clarify the trophic relationships between the non-native piscivores and the native species. Dissections of stomach contents would also further this aim.

Sandfish biology. Finally, the biology of the sandfish remains understudied – primarily because these studies traditionally require sacrificing individuals which was considered inappropriate given their threatened status. One option for estimating sandfish age at reproductive maturity would be to carry out length-cohort analyses using otoliths to age closely related species, such as sawfin and yellowfish. These surrogates could provide approximations for sandfish reproductive traits until more precise, non-invasive ageing methods can be developed for sandfish themselves.

Furthermore, there are currently no rigorous assessments of environmental conditions favourable to spawning and larval development existing at this time. It is recommended that a concerted effort be made to better understand the spawning and development requirements of the sandfish by collecting continuous environmental data (as described above) and fine-scale habitat use data over the spawning window of the sandfish and observing the conditions under which they spawn. Such information, as well as the confirmation of whether or not spawning occurred in a particular year, would be invaluable in helping to elucidate the relative influence of abiotic factors compared to the threats posed by non-native fish in regulating the sandfish population.

5. Conservation recommendations and conclusions

The Oorlogskloof River sandfish population suffered a considerable population decline during the study period, led primarily by a decline in YOY numbers. Banded tilapia had no measurable effect on sandfish abundance or size and appeared not to be the main

factor responsible for the dramatic fall in sandfish numbers. The striking differences in sandfish abundance and size structure above versus below the smallmouth bass/bluegill sunfish barrier combined with an abundance of evidence from past studies suggest that size-selective predation by smallmouth bass – and likely bluegill sunfish – are the primary drivers of the decline in juvenile sandfish. Unfortunately, this study could not definitively separate the temporal or spatial effects of smallmouth bass and bluegill sunfish due to low sample size and spatial overlap between the two species. The ongoing drought may be preventing the recovery of the sandfish population above the barrier, either by preventing spawning or reducing the habitat quality and quantity for sandfish adults and juveniles. However, more precise data from flow loggers and other environmental considerations, as well as a more detailed understanding of sandfish spawning biology and fine-scale habitat requirements, are necessary to determine the extent to which these factors play a role in regulating the Oorlogskloof River sandfish population.

As the last known tributary with a recruiting population of sandfish, the Oorlogskloof River is of unparalleled conservation importance. The conservation of the sandfish within this tributary is therefore critical and must be prioritised. In South Africa, as is the case elsewhere, conservation is constrained by limited manpower and funding (Impson 2016), and as such, it is necessary to allocate effort and finances efficiently. Based on the findings from this study, it is recommended that no action apart from the current monitoring be currently taken against the banded tilapia in the Oorlogskloof River. Conservation efforts should rather be targeted at preventing the further invasion of smallmouth bass and bluegill sunfish past the current barrier. Preventative actions include the continuation of the current monitoring surveys, educational outreach with local landowners to prevent the deliberate stocking of the upper reach with these non-native species and consistent monitoring of municipal and farm dams that may overflow into the Oorlogskloof River during floods. Proactive measures are also necessary to preserve the smallmouth bass- and bluegill sunfish-free reach of the Oorlogskloof River as a sanctuary for juvenile sandfish. In 2017, CapeNature and the Northern Cape Department of Environment and Nature Conservation successfully cleared two farm dams in the ODRS of non-native bluegill sunfish and common carp (*Cyprinus carpio* Linnaeus 1758) using the piscicide Rotenone (Marr et al. 2019). A continuation of these efforts in other farm dams high in the Oorlogskloof catchment would safeguard the river from accidental introductions of non-native fish into the non-invaded reach. The eradication of smallmouth bass and bluegill sunfish from the Oorlogskloof River itself must also be taken into consideration; while the upper reaches of the river constitute a critical

refuge for young sandfish, the lower reaches act as a significant population sink, effectively isolating the upper Oorlogskloof population from the remaining populations in the Doring River mainstem and other tributaries. Restoring habitat connectivity between these systems is essential for the long-term survival and recovery of this endangered species. Furthermore, working with local landowners to prevent large-scale water abstraction, especially during times of water stress such as the summer months, is necessary to ensure that enough habitat remains available for sandfish to recover.

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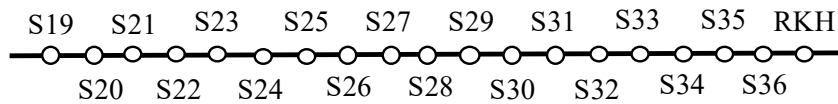
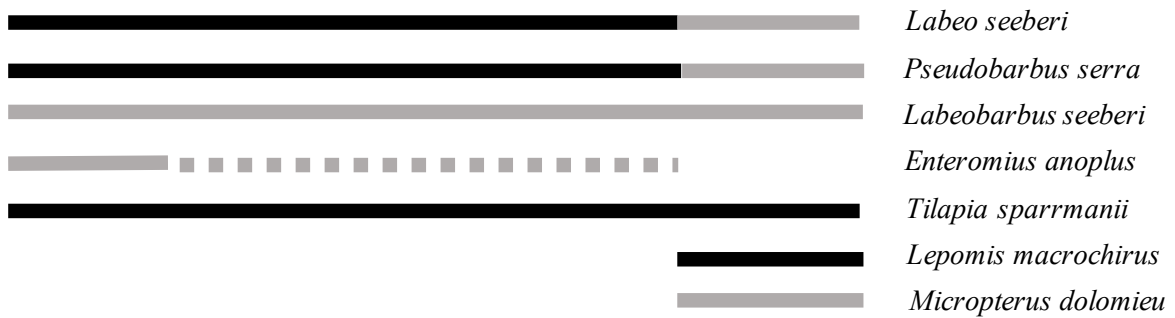
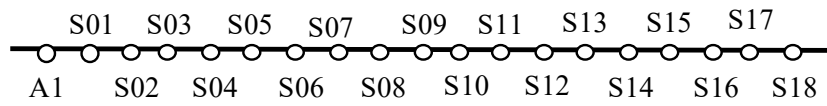
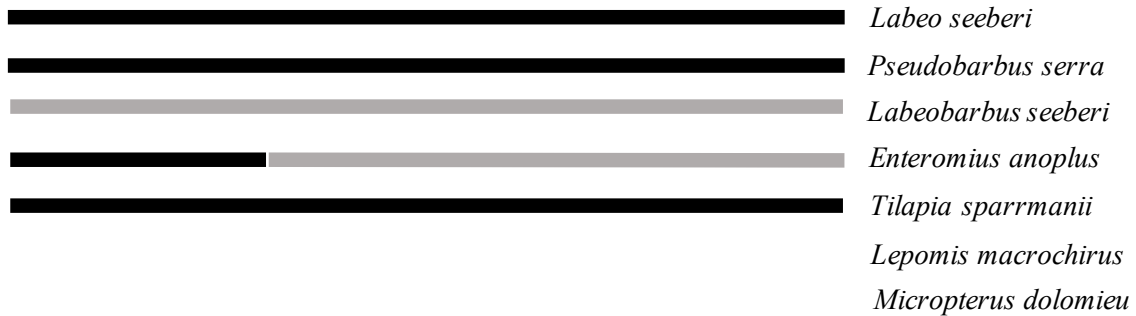
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Appendix 1. The distribution of native and non-native fishes in the Oorlogskloof River across sampling sites. Solid black lines indicate where a species is present, solid grey lines indicate where a species is present in lower relative numbers and dashed lines indicate that a species may exist (but has not yet been observed) at that location. Distance between sites is not to scale.



Appendix 2. Locations and sources of precipitation and temperature data used in analyses. SAWS refers to the South African Weather Service. SANBI refers to the South African National Biodiversity Institute. DENC refers to the Northern Cape Department of Environment and Nature Conservation.

Station name	Coordinates	Data	Source
Calvinia Bo Downes	-31.5820, 19.9560	Precipitation	SAWS
Calvinia Doega	-31.5240, 19.5350	Precipitation	SAWS
Calvinia Kalkgat	-31.5440, 19.8050	Precipitation	SAWS
Calvinia Wo	-31.4810, 19.7610	Precipitation, temperature	SAWS
Kreitzberg	-31.6250, 19.7990	Precipitation	SAWS
Nieuwoudtville SAPD	-31.3740, 19.1170	Precipitation	SAWS
Platberg	-31.5960, 19.6350	Precipitation	SAWS
Rooiwal	-31.7300, 19.9540	Precipitation	SAWS
Uitsig	-31.4340, 19.7970	Precipitation	SAWS
Oorlogskloof Nature Reserve	-31.4687, 19.0749	Precipitation	DENC
Hantam National Botanical Gardens	-31.3975, 19.1406	Precipitation	SANBI

Appendix 3. Dissected bluegill sunfish and stomach contents. Caught in the Biedouw River. Centre left is a juvenile sandfish. Dissection carried out by Leonard Flemming.



Appendix 4. Locations and altitudes of survey sites. RKH refers to Rietkuilhuis.

Site	Coordinates	Altitude (m)	Site	Coordinates	Altitude (m)
A1	-31.4390, 19.1105	670	S19	-31.5077, 19.1125	511
S01	-31.4645, 19.0810	596	S20	-31.5100, 19.1120	504
S02	-31.4705, 19.0781	589	S21	-31.5129, 19.1128	494
S03	-31.4713, 19.0785	586	S22	-31.5154, 19.1128	494
S04	-31.4718, 19.0807	585	S23	-31.5197, 19.1114	486
S05	-31.4811, 19.0932	585	S24	-31.5219, 19.1128	469
S06	-31.4920, 19.1029	569	S25	-31.5244, 19.1107	469
S07	-31.4969, 19.1124	553	S26	-31.5279, 19.1054	452
S08	-31.4972, 19.1124	548	S27	-31.5318, 19.1048	445
S09	-31.4986, 19.1139	538	S28	-31.5330, 19.1052	440
S10	-31.4993, 19.1141	531	S29	-31.5357, 19.1056	437
S11	-31.5001, 19.1151	531	S30	-31.5373, 19.1067	432
S12	-31.5003, 19.1154	531	S31	-31.5446, 19.1062	413
S13	-31.5019, 19.1154	523	S32	-31.5436, 19.1026	413
S14	-31.5026, 19.1158	520	S33	-31.5453, 19.0980	411
S15	-31.5032, 19.1151	518	S34	-31.5478, 19.0980	411
S16	-31.5039, 19.1145	516	S35	-31.5498, 19.0958	393
S17	-31.5054, 19.1134	511	S36	-31.5511, 19.0949	393