Ecology and conservation of the endangered Clanwilliam sandfish (*Labeo seeberi*) in the Olifants-Doring River system, South Africa

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DECLARATION

I, Cecilia Cerrilla (CRRCEC001), hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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DECLARATION OF INCLUSION OF PUBLICATIONS

I, Cecilia Cerrilla (CRRCEC001), confirm that my supervisors and co-authors have given their permission to include aspects of the following publication in my PhD thesis:

> ***Cerrilla C****, Flemming L, Griffiths CL, Impson D, Jordaan MS, Kajee M, Paxton BR, van der Walt JA, Whitehead TO, Shelton JM. 2024. Using a head-start conservation intervention to boost spawning numbers of the endangered Clanwilliam sandfish. Conservation Science and Practice (6) e13065. DOI: 10.1111/csp2.13065.*

My supervisor has testified that I made substantial contributions to the conceptualisation and design of the published articles and that I independently executed the study and wrote the associated manuscript, with support in the form of comments and suggestions from listed coauthors. The published elements are part of a greater body of work presented in Chapter 4. See Appendix B 3 for co-author permission letter.

Signed by candidate

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Cecilia Cerrilla (CRRCEC001)

3-June-2025

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STATEMENT OF CONTRIBUTIONS BY OTHERS

Chapter 1 | This chapter was my own work. My supervisor, Emer. Prof. Charles Griffiths, and my co-supervisor Dr Jeremy Shelton provided feedback on earlier drafts.

Chapter 2 | This chapter was my own work. My supervisor, Emer. Prof. Charles Griffiths, and my co-supervisor Dr Jeremy Shelton provided feedback on earlier drafts.

Chapter 3 | This chapter was my own work. My supervisor, Emer. Prof. Charles Griffiths, and my co-supervisor Dr Jeremy Shelton provided feedback on earlier drafts.

Chapter 4 | This chapter was my own work. My supervisor, Emer. Prof. Charles Griffiths, and my co-supervisor Dr Jeremy Shelton provided feedback on earlier drafts. Elements of this chapter were published in *Conservation Science and Practice* in January 2024 (DOI: 10.1111/csp2.13065). I am the primary author, with L. Flemming, C.L. Griffiths, D. Impson, M.S. Jordaan, M. Kajee, B.R. Paxton, J.A. van der Walt, T.O. Whitehead, and J.M. Shelton as co-authors. I conceptualised and designed the research, independently conducted the study, and analysed the data. I wrote the manuscript and addressed reviewers' comments. Co-authors contributed to the publication by participating in fieldwork and providing specialised input and comments for the final published manuscript. Two anonymous reviewers provided feedback during the peer-review process, which was used to improve the final manuscript. The publication incorporates only one year's return data, whereas this chapter expands upon that by incorporating two years' return data. As such, the published elements form part of a greater body of work, which is presented in Chapter 4. Co-authors gave permission for the published content to be included in this chapter (Appendix B 3).

Chapter 5 | This chapter was my own work. My supervisor, Emer. Prof. Charles Griffiths, as well as my co-supervisor Dr Jeremy Shelton provided feedback on earlier drafts.

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"If you want to go fast, go alone. If you want to go far, go together." – Unknown origin.

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ABSTRACT

Freshwater ecosystems, though disproportionally rich in biodiversity, are the most endangered globally. Vertebrate population declines in fresh waters are outpacing those on land, with fishes facing particularly high extinction rates. In South Africa, freshwater fishes are the most threatened species group, and invasive alien fishes (IAF) pose the greatest threat, followed by ecosystem modifications, like dams and water over-exploitation. Within the Cape Fold Ecoregion (CFE), itself a hotspot of endemic range-restricted species, lies the Olifants-Doring River system (ODRS), which has the largest number of threatened fish species in southern Africa. The Clanwilliam sandfish (*Labeo seeberi;* hereafter 'sandfish'), a large-bodied cyprinid endemic to the ODRS, migrates annually from the mainstem Doring River into tributaries for spawning. Despite its migratory life history and potentially important role in nutrient cycling as the system's only large detritivore, its ecology has been understudied. Although a major population decline was observed in the latter half of the 20th century, no comprehensive study has been conducted to understand the full extent of the species' range contraction and fragmentation, or the corresponding implications for the species' long-term survival. This thesis evaluates the habitat preferences of critical sandfish life stages, tracks distribution shifts over time, and assesses the efficacy of an ongoing head-start conservation intervention in a key spawning tributary.

Chapter 1 provides a literature review that highlights the global value of freshwater fishes and discusses major threats to freshwater fishes globally and in South Africa. Background is provided on the Olifants-Doring River system, and the ecology, life history, distribution and current threats to sandfish are discussed, as is the Biedouw River as a key spawning tributary for sandfish. Finally, the Saving Sandfish Project is introduced, and rationale given for the research questions addressed in each chapter.

Chapter 2 assesses the habitat requirements (depth, water velocity, substrate size, and cover) of key developmental stages by weighting habitat use data by the relative abundance of available habitat – yielding a measure of true preference. Surveys were conducted at three 500 m reaches in the Biedouw River during the spawning, larval, and juvenile stages. Habitat measures were recorded where sandfish were observed and along evenly spaced transects. Habitat Utilisation Curves and Habitat Availability Curves were produced for each variable using Kernel Density Smoothing, and Habitat Preference Curves (HPCs) generated using an adaptation of the forage ratio. For each survey, a composite reach-averaged HPC per habitat variable was produced to represent preference over the greater sampling area.

Spawning adults exhibited a peak preference for moderate depths (around 18.2 cm), high velocities (around 0.61 m/s), and pebble substrate. Larvae preferred the shallowest depths (0 cm), lowest velocities (0 m/s), and sand and gravel substrates. Juveniles preferred deeper habitat (around 37.4 cm), very low velocities (0 m/s), and sand and gravel substrates. While spawning adults and juveniles apparently preferred open water, and larvae preferred vegetative or rocky shelter, the inherent bias against visually detecting fish using shelter requires that these results be interpreted with caution. The chapter concludes with a series of management recommendations to ensure appropriate habitat is available for each life stage, including the maintenance of the ecological reserve, implementation of strategic water releases into critical nursery pools during the summer months, and removal of non-native plants across the catchment. This chapter contributes to the broader understanding of how life-stage-specific habitat preferences can guide targeted habitat conservation and flow management strategies for migratory freshwater fishes.

In Chapter 3, historical and current sandfish occurrence and abundance records are used to evaluate shifts in distribution since 1936 and inform future management of the species. Historical records gathered from the Freshwater Biodiversity Information System (FBIS) and the grey literature were used to construct decadal distribution maps from the 1930s onwards. Data from previous fish surveys in the ODRS conducted in 2001, 2011, and 2013 were obtained and utilised to generate sandfish distribution and abundance maps over the last two decades. In October 2021, a large fyke net was used to resample 10 ODF survey sites along the Doring mainstem. In March-April 2022, five tributaries of the Doring River with historical sandfish records (Gif, Kransgat, Biedouw, Tra-Tra, and Matjies Rivers) were sampled with smaller fyke nets. Captured fish were identified and a subsample measured (total length). Data were also obtained from Oorlogskloof Nature Reserve (ONR) personnel following their semi-annual survey of the Oorlogskloof River gorge in March 2022. The 2021 and 2022 surveys were compared with historical data to determine differences in species composition and size classes present. Sandfish size distributions at mainstem sites were also compared with those from

past ODF mainstem catches. Finally, the species' current total area of occupancy (AOO) was calculated and mapped, and its distribution assessed for overlap with protected areas to evaluate the level of protection currently afforded to the species.

Sandfish were found across the Olifants River mainstem between the 1930s and 1970s, with records confined to the reach below Bulshoek Dam in the 1980s. Sandfish have not been recorded from the Olifants River system since. Since the onset of fish surveys in the Doring River system in the 1980s, sandfish have been recorded in the Doring mainstem and in the Gif, Brandewyn, Oorlogskloof-Koebee, Kransgat, Biedouw, Tra-Tra, and Matjies Rivers. While the upper reaches of the Doring appear to be a mainstem stronghold, the lack of subadults indicate that recruitment is likely functionally absent. Non-native fishes comprised 83% of mainstem catches by abundance, dominated by bluegill (*Lepomis macrochirus*) and black basses (*Micropterus* spp.) Two novel invaders were recorded for the first time in the Doring River system: common carp (*Cyprinus carpio*) and Mozambique tilapia (*Oreochromis mossambicus*), with banded tilapia (*Tilapia sparrmanii*) also present in low numbers. Sandfish were present in five of the 10 mainstem sites surveyed in 2021. Between 91.1 – 99.7% of catches in the Matjies, Biedouw, Kransgat and Oorlogskloof Rivers consisted of native fishes, as did 72.6% of the catch in the Gif River and 0.3% in the Tra-Tra. Non-native fishes present in the tributaries included *L. macrochirus*, *M. punctulatus*, *M. dolomieu*, and *T. sparrmanii*. The highest densities of sandfish were found in the Kransgat and Oorlogskloof Rivers. Sandfish sampled in tributaries were smaller in size, on average, than mainstem fish, although those sampled from the Koebee more closely approximated the mainstem average. The Gif, Matjies, Kransgat, and Oorlogskloof Rivers hosted moderate to high numbers of small and intermediate size classes. The conservative AOO estimate was 7.86 km^2, covering 36 sub-quaternary catchments in the Doring River system and spanning 430.2 river-kilometres. Catchment overlap with protected areas was generally low (12.8%) and mostly concentrated around the Matjies River. Management recommendations deriving from these results include the identification of tributaries where flow augmentation or IAF eradication efforts may be most effective for sandfish recovery, the establishment of stewardship agreements with private landowners where rivers fall outside of protected areas, and the expansion of native fish refugia in tributaries. The chapter concludes by identifying several research gaps that could positively inform future management of the species. These findings highlight the importance of long-term monitoring for mitigating threats and identifying opportunities for intervention.

Chapter 4 evaluates the efficacy of head-starting efforts in the Biedouw River aimed at increasing juvenile survival to reduce extinction risk. Juvenile sandfish were rescued from drying pools in the Biedouw River and relocated to six off-stream reservoirs to be reared to a larger size in 2020, 2021, 2022, and 2023. They were released back into the wild in 2021, 2022, and 2023. Most (90%) head-started fish were PIT-tagged prior to release. A passive PIT-tag detection antenna was deployed near the mouth of the Biedouw River in 2022 and 2023 to assess return rates during the annual migration. Mortality during rescues, growth and survival in the reservoir environment, and return rates relative to wild run size were evaluated as measures of the programme's success. A generalised linear mixed model (GLMM) was used to evaluate the influence of several conditions at release on the probability of fish returning in 2022 and 2023.

A total of 36,104 juvenile sandfish were rescued. The mortality rate of sandfish during the rescue process from 2021-2023 was 0.69%. Growth rate in the reservoir environment varied by reservoir and season, with most growth occurring between the months of November and March. Between 2021 and 2023, 2,829 sandfish were recaptured from reservoirs and released to the wild, ranging in size from 98 – 325 mm TL. After one year, the estimated survival rate at Enjo Reservoir was 67.9% (95% CI: 38.5 – 97.3%). Seventy-seven tagged fish were detected by the PIT tag antenna in 2021, as were 221 fish in 2022, corresponding to return rates of 7.7% and 16.4%, respectively. Seventy of the fish detected in 2022 were returning for the second consecutive year, indicating a minimum survival rate of 90.9% for these fish in the wild between their first and second year post-release. Ninety-eight (9.9%) of the 2021 release cohort deferred their first migration to 2023, indicating that annual return rate may be dependent on inter-annual environmental variation, and that subsequent-year return rates may be higher than first-year return rates. The return of 221 unique fish detected in 2023 represents a minimum 123% increase in run size compared to the 180 wild sandfish observed migrating in 2021 during a comprehensive visual survey. The GLMM predicted that for every centimetre increase in size at release, the odds of return increased by 11.0% ($p < 0.001$). Fish released after the migration were predicted to have a 90.4% lower chance of return than those released during the migration ($p < 0.001$).

The timing of PIT tag detections was also used to evaluate the environmental cues to migration onset, which were preceded by flood events. Additionally, observations of spawning events were temporally linked to water

temperature and flow data recorded by a submerged Levelogger to define the range of temperatures and discharges at which sandfish spawned in 2021 and 2022. Spawning appeared to take place as high flows subsided and water temperatures increased in spring. During spawning events, water temperature and discharge varied between 14.3 and 20.7 °C and 0.14 to 0.38 m^3/s, respectively. This research will positively inform future conservation efforts for sandfish and serves as a model for the conservation of freshwater fishes where there is an imminent risk of extinction by demonstrating the effectiveness of head-starting as a population augmentation tool.

Chapter 5 presents the overarching conclusions gleaned from this thesis, with a particular focus on management recommendations and future opportunities for research. This study demonstrated clear niche differentiation between three sandfish life stages, mapped temporal distribution shifts, and evaluated a pioneering head-start intervention programme as part of an evidence-based conservation approach for the species. These results highlight the importance of maintaining natural heterogeneity across the river networks sandfish rely on, identified locations to prioritise for different conservation actions, and provided insight into the factors that are most likely to maximise the impact of sandfish population augmentation efforts. Moreover, the insights gained into the species' ecology, including environmental cues to migration and spawning, will inform the ongoing recovery efforts for the species. The thesis concludes with promising avenues for future research. Overall, this thesis advances the field of conservation biology by integrating ecological research and applied interventions, providing a framework applicable to other imperilled freshwater species facing similar threats.

Keywords: freshwater fish; threatened species; habitat suitability; invasive species; distribution shifts; freshwater conservation; head-start; Cape Fold Ecoregion

Chapter 1

1 General Introduction

1.1 Global importance of freshwater fishes

Freshwater ecosystems are some of the most biodiverse on the planet. Although less than 0.03% of the total volume of all water on earth is freshwater, these ecosystems are home to more than 126,000, or 1 in 10, of known species of molluscs, fishes, insects, reptiles, plants, and mammals (Balian et al. 2008; Grooten & Almond 2018; Dudgeon 2019). Despite the comparatively miniscule volume of water they inhabit, one-third of known vertebrates (Balian et al. 2008), and over half of all known fish species (~18,000) occur in freshwater habitats (Hughes 2021; Fricke et al. 2023). The disproportionate share of biodiversity occurring within the confines of freshwater systems is due to the insular nature of these habitats, which has facilitated the evolution of an abundance of range-restricted species– many of which only exist in single catchments (Darwall et al. 2011). The inherent uniqueness of many fresh waterbodies renders them irreplaceable from a biodiversity standpoint.

Freshwater fishes span a dizzying range of sizes and morphologies, from a tiny minnow (*Paedocypris progenetica*) in the peat swamps of Indonesia weighing 0.004 g, to a dog-eating catfish (*Pangasius sanitwongsei*) on the opposite extreme weighing over 300 kg (Hughes 2021). They are found in all continents except Antarctica (Leroy et al. 2019) and exhibit a wide range of life histories and adaptations to myriad environmental conditions. Salmonids, one of the best-studied groups of fish on earth, illustrate this wide environmental tolerance through their migratory life histories: chum salmon (*Oncorhynchus keta*) can tolerate a remarkable range of salinities (McCormick 1994) and fry are capable of migrating hundreds of kilometres to the sea soon after hatching (Rounsefell 1958). Perhaps even more impressive are the migrations of young gilded catfish (*Brachyplatystoma rousseauxii*), which swim the entire width of South America (nearly 6,000 km) from the headwaters of the Amazon to its mouth, where they remain for several years before returning to the Andes to spawn (Barthem et al. 2017). Another notable example is the West African lungfish (*Protopterus annectens*) and their peculiar adaptation to arid conditions during the dry season. These large, eel-like fish burrow into mud and remain in a state of dormancy for up to eight months, without need of food or water, until rain inundates their habitats and they re-emerge (Sturla et al. 2002).

Due to the sheer number of species and their adaptations, fishes play important and diverse roles in freshwater ecosystems, influencing their functioning and structure through various mechanisms: fish can affect water clarity via intense phytophagous feeding, or by stirring up sediment; influence the distribution and abundance of primary producers; alter the size distribution of prey species via size-selective predation; and regulate nutrient release into both freshwater and terrestrial ecosystems via excretion and body decomposition (Northcote 1988). Their outsized role in freshwater systems is best illustrated by the concept of the trophic cascade, whereby notable shifts in communities result in impacts that reverberate across entire food webs. Perhaps the best-known example is the introduction of the piscivorous Nile perch (*Lates niloticus*) into Lake Victoria. Originally introduced in the 1950s and 1960s, it experienced a population explosion in the 1980s (Taabu-Munyaho et al. 2016), triggering the subsequent near-extirpation of endemic Cichlids comprising diverse feeding guilds (Paugy et al. 2017). This in turn led to the replacement of cichlids by detritivorous shrimp and zooplanktivorous cyprinids, an increase in aquatic insect larvae, and diet shifts in several bird species (Paugy et al. 2017). Fishes also play a critical role as prey: in southern Africa's Lake Kariba, the Nile crocodile *Crocodylus niloticus* consumes 225 tonnes of fish per year, 10% of what is removed annually by artisanal fisheries (Games & Moreau 1997). Additionally, fishes form critical links between the freshwater and terrestrial realms. In Alaska, salmon runs provide essential fat and protein for bears prior to hibernation, and their carcasses transport nutrients to riparian woodlands (Hughes 2021), while in the Pacaya-Samiria National Reserve in Peru, fruit-eating fishes disperse the seeds of 35% of the tropical floodplain trees that fruit during the flood season (Anderson et al. 2009). Finally, fishes serve as indicators of the health of freshwater ecosystems, acting as 'canaries in the coalmine' to signal when ecosystems have deteriorated to a point unfit for human use (Hughes 2021).

Despite the massive contribution of freshwater fishes to local and global economies, they are often overlooked. Twelve million tonnes of freshwater fish are harvested annually, making up 13% of the world's fish catch (FAO 2020). In the African Great Lakes alone, over one million tonnes of freshwater fish are landed each year (Hughes 2021). While the global monetary value of these fisheries is easy to appreciate (over US$38 billion per year; Hughes 2021), local benefits are less visible – though no less important. Over 200 million people around the world rely on freshwater fisheries as their primary source of animal protein, and freshwater fishes constitute a particularly valuable food source for vulnerable communities throughout Asia and Africa (Hughes 2021). In Bangladesh and Myanmar, 65% and 44% of fish production, respectively, derives from freshwater fisheries (FAO 2020). Moreover, this industry provides jobs to over 60 million people around the world – more than half of whom are women (Hughes 2021).

The value of freshwater fishes to the global economy is not restricted to commercial harvesting. The profits from recreational angling in freshwater can also be substantial and may even rival profits generated from commercial fishing. In 2011, freshwater angling in the United States generated over US$114 billion (more than four times the amount generated by commercial fishing) and accounted for more than 800,000 jobs (Tufts et al. 2015). Ninety million people in China partake in recreational angling every year, as do 39 million in the United States and nearly 26 million in Europe (Hughes 2021). The appeal of hooking iconic species plays a particularly important role in driving the growth of this industry. In riverside lodges in the Caprivi floodplains of Namibia, for example, 70% of revenue is attributed to angling tourism, largely driven by anglers hoping to catch one of the world's most sought-after freshwater angling species, the tigerfish *Hydrocynus vittatus* (Cooke et al. 2016). The benefits of angling also go beyond monetary value. The practice is increasingly being recognised and used as an effective rehabilitation therapy by the psychological and mental wellness sector (Hughes 2021). Moreover, anglers serve as sentinels on our world's rivers and lakes, as they are often the first to notice, and act on, problems arising in their fishing grounds (Hughes 2021).

Beyond commercial and recreational benefits, freshwater fishes also form part of the cultural identity of Indigenous peoples. In Australia, North America, and New Zealand, several freshwater eel, lamprey, and finfish species are valued not only as important food sources, but also for their roles in ceremonies, spiritual practices and tribal celebrations (Noble et al. 2016). These same species are also ecologically important; for example, long-finned eel (*Anguilla dieffenbachii*) and short-finned eel (*A. australis*) in New Zealand are apex predators and act as umbrella species due to their ability to occupy the full range of freshwater habitats. In North America, salmons (*Oncorhynchus* spp.) cycle nutrients across aquatic and terrestrial systems by virtue of their mass migrations. The frequent overlap between ecological and cultural significance of freshwater fishes means that Indigenous peoples have, over thousands of years, acted as stewards of biodiversity and sensitive ecosystems (Noble et al. 2016).

1.2 The freshwater biodiversity crisis

1.2.1 Global state of freshwater ecosystems

Despite the critical role that freshwater systems play in human well-being – as sources of life-giving water, food, transportation, and cultural services – they are the most imperilled ecosystems on earth. Since 1970, populations of freshwater vertebrates have declined by a staggering 83% (Almond et al. 2022). This compares to the overall decline among terrestrial, marine, and freshwater vertebrates of 69% over the same period (Almond et al. 2022). Freshwater species tend to be more highly threatened than their terrestrial counterparts, with recent figures estimating that 22.5% of 26,400 species assessed are threatened with extinction (Dudgeon 2019). Ironically, the same processes that drive high rates of speciation in these systems make freshwater biota particularly vulnerable to human impacts. The isolated nature of these systems, the unique adaptations of their species, and the limited mobility of most resident taxa mean that, when faced with novel human-linked pressures, those species are unable to cope or physically move to safety.

Although freshwater species face a higher risk of extinction than terrestrial species, they receive disproportionately fewer conservation resources. One contributing factor is that funding for threatened vertebrates is often linked to public interest, which tends to favour large, charismatic species – typically terrestrial animals like the giant panda or marine species like killer whales (Davies et al. 2018). Moreover, traditional conservation methods often used to protect terrestrial species are ineffective for riverine species. Protected areas, for example, have historically been designed for the protection of terrestrial biodiversity (Crivelli 2002) and the inclusion of freshwater systems within protected areas is rarely intentional (Abell et al. 2011). In South Africa's Western Cape, for example, most native freshwater fishes are not adequately protected by the current protected area network, as they remain exposed to the impacts of non-native fishes and upstream land-use practices (Jordaan et al. 2020b, Kajee et al. 2023b).

Freshwater ecosystems are also hotspots of endangerment due to the overlap between biological richness and the many forms of freshwater resource exploitation (Reid et al. 2019). More than half of the human population lives within three kilometres of a fresh waterbody (Almond et al. 2022), making these systems particularly vulnerable to overexploitation (Dudgeon 2019). About one-third of freshwater discharges around the world pass through urban, industrial, or agricultural infrastructure (Albert et al. 2021). Our global proximity to, and dependence on, fresh waters means that human needs most often take precedence and it is only after these needs have been met that water is allocated, or left over, to sustain aquatic biota (Dudgeon 2019). Since the middle of the 20th century, freshwater withdrawals have increased by more than threefold, impacting on the volume of discharge in half of the largest rivers on earth (Albert et al. 2021). Paradoxically, rampant water abstraction for human use has seriously compromised our own water security. Since 1960, there has been a 26.1% reduction in the availability of per capita freshwater resources (Vörösmarty et al. 2010), and nearly 50% of countries representing over 99% of the global population are projected to be water scarce by 2050 (Baggio et al. 2021). Human use of freshwater resources also has serious impacts on the integrity of freshwater ecosystems: water abstraction causes approximately a quarter of the planet's rivers to run dry before they reach the ocean (Albert et al. 2021), and 65% of global river discharge is considered to be under moderate to high threat (Vörösmarty et al. 2010). Similarly drastic impacts are also apparent in inland lakes. The most dramatic example is perhaps Lake Chad, which has lost more than 95% of its volume over the past six decades due to the combined effects of water withdrawals, drought, and desertification (Darwall et al. 2011).

One of the most visible and impactful ways in which we modify aquatic ecosystems is through the building of dams. Dams disrupt the movement of aquatic organisms, inundate spawning grounds, destroy nursery habitats, fragment previously connected rivers, and deprive downstream floodplains and estuaries of nourishing sediments (Day & Davies 2023, p. 332). Worldwide, only 37% of rivers longer than 1,000 km remain free-flowing (Grill et al. 2019), and nearly half of global river volume is considered to be moderately to severely impacted by flow fragmentation and regulation (Grill et al. 2015). The reservoirs created by the construction of dams globally retain more than 10,000 km^3 of water, equivalent to five times the standing volume of water in the planet's rivers (Nilsson & Berggren 2000). The conversion of flowing water systems to standing reservoirs can also act synergistically with other human impacts, such as when newly lentic conditions facilitate the spread of alien invasive species – in some cases increasing the likelihood of invasion by up to 300 times (Johnson et al. 2008). Several mitigation measures can be implemented to avoid or minimise the loss of river connectivity, and to restore connectivity where barriers exist. These include the addition of fish passage structures to constructed barriers, strategic dam operations to maintain environmental flows, barrier removal, and active groundwater management, among others (Thieme et al. 2024).

Invasive alien fishes (IAF) are considered one of the most serious threats to freshwater biodiversity. Predatory IAF can cause particularly devastating impacts in cases where indigenous prey have not evolved anti-predator adaptations ('prey naiveté'; Dudgeon 2019). Biota in freshwater systems are particularly vulnerable to this phenomenon due to higher biogeographic (and hence evolutionary) isolation between prey and predator than is experienced in terrestrial or marine systems (Anton et al. 2020). Exotic fishes have been recognised as particularly effective exploiters of naïve prey – a situation made even more damaging by the fact that indigenous fishes are particularly prone to being naïve towards exotic predators (Anton et al. 2020). In addition to elimination via predation, IAF may create or increase competition for scarce resources, or modify habitat such that it becomes less suitable for native fauna (Dudgeon 2019). Exotic species might also be vectors of novel diseases into biotic systems ill-adapted to their impacts (Dudgeon 2019). Finally, the possibility of hybridisation is a concern where close evolutionary histories exist between IAF and indigenous species (Dudgeon 2019).

While impacts arising from flow modification, water abstraction, pollution, and the introduction of non-native species are considered the most concerning immediate threats to freshwater biodiversity, climate change remains a major concern in the longer term. Projections in the latest IPCC report include the further intensifying of the global water cycle, with associated increases in the frequency and intensity of extreme events, such as droughts, floods, and severe storms (IPCC 2023). These have the potential to critically impact life cycles of aquatic species, due to their close links to hydrology (Dudgeon 2019). An increase in the frequency of droughts may cause shifts of perennial rivers to intermittent (Datry et al. 2016), and from intermittent to episodic. Moreover, if changing climates cause conditions to become unsuitable for locally adapted species (e.g., if waters become too warm for the thermal tolerances of certain fishes), movement to cooler habitats will be necessary. Species' ability to undertake these movements will be heavily constrained by the inherent isolation of rivers and lakes, the low mobility potential of many aquatic species, as well as by obstructions such as dams and natural topography (Dudgeon 2019).

A seminal paper by Dudgeon et al. (2006) identified the five most pressing threats to freshwater biodiversity as flow modification, overexploitation, destruction or degradation of habitat, water pollution, and invasion by exotic species. Since then, novel pressures have come to light that have the potential to severely impact freshwater biota. These emergent threats include infectious diseases, harmful algal blooms, contaminants, microplastic pollution, light and noise pollution, and freshwater salinisation, among others. While our understanding of the severity of each of their impacts is limited, it is expected that the combination of these stressors, together with those mentioned previously, will alter species' ranges, phenology, and survival; impact individual growth and reproduction; cause acute toxicity; and alter behaviour and physiology (Reid et al. 2019).

1.2.2 Global state of freshwater fishes

Freshwater fishes are in perilous decline worldwide, facing multiple and interacting stressors while equipped with limited adaptive capacity and mobility. Eighty freshwater fish species have already been declared extinct by the IUCN, with a further 10 categorised as extinct in the wild and 115 presumed extinct (Hughes 2021). Of the remaining species, nearly one-third are at risk of extinction (Hughes 2021). Freshwater fishes had the highest extinction rate of all vertebrates in the 20th century (Burkhead 2012), perhaps due to the inherent rarity of many species (Magurran 2009). A global assessment of the degree of freshwater fish biodiversity change in different river basins found that 170 of the 10,682 fish species considered have gone extinct within a river basin over the past two centuries (Su et al. 2021). Thirty-nine percent of freshwater fishes in North America are threatened with extinction, as are 38% of those in Europe (Dudgeon 2019). Across mainland Africa, 27% of species are threatened (classified as Critically Endangered, Endangered, or Vulnerable by the IUCN), and a further 18% are data deficient, highlighting the need for concerted biodiversity assessment efforts across the continent (Darwall et al. 2011).

Large river fishes have seen some of the most notable species declines (Dudgeon et al. 2006), as have migratory freshwater fishes. Since 1970, migratory fish populations have declined by 76% – equivalent to an average decrease of 3% per year – and over 21% of such species are classified as threatened (Deinet et al. 2020). This trend is particularly pronounced among sturgeon and anguillid species, whose populations have suffered losses of 91% and 92%, respectively, since 1970 (Deinet et al. 2020). Observed losses are spatially heterogenous, with the weakest declines observed in North America (-28%) and the strongest in Europe (-93%; Deinet et al. 2020). Potamodromous fishes (those that migrate within freshwater systems) have been more strongly affected than those that migrate between salt- and freshwater systems (-83% vs -73%, respectively; Deinet et al. 2020).

The European eel (*Anguilla anguilla*) provides one of the most dramatic examples of a precipitous migratory fish decline. In the 19th century, this species comprised one-third of the European freshwater catch by value, but by 1910, glass eels arriving in Europe had declined to just one percent of their historical numbers (Deinet et al. 2020). Anguillid distributions are declining even where they are not commercially exploited. In South Africa's KwaZulu-Natal province, the extent of occurrence of the four present eel species has declined between 35 and 82% since the 1950s (Hanzen et al. 2022). Fishes that undertake long migrations, such as *A. anguilla* (which swim up to 8,000 km to reach the Sargasso Sea; Deinet et al. 2020), are especially vulnerable to human impacts, as they rely on a vast range of high-quality, interconnected riverine (and marine) habitat to complete their life cycle. Threats arising at any point in their migrations have the potential to sever that fragile cycle, with catastrophic consequences. In the case of *A. anguilla*, overfishing, barriers to migration (such as dams), hydropower turbines, pollution, illegal poaching, disease, and climate change have collectively led to the species' near-disappearance over the last two centuries (Deinet et al. 2020).

The construction of dams and other in-stream barriers negatively impacts indigenous freshwater fishes in several ways. Perhaps the most obvious is the direct blocking of migration routes, decreasing (or in some cases eliminating) species' ability to reach critical spawning grounds. In the Yangtze River, for example, dams have prevented Chinese sturgeon (*Acipenser sinensis*) from reaching their historical spawning grounds, leading to a 50% reduction in numbers of spawning fish (Zhuang et al. 2016). In addition to physically blocking migration routes, dams can also decrease survival and reproductive success of certain species by altering the flow regimes to which they are adapted. Examples include the alteration or elimination of hydrographic cues that several species use to spawn, the modification of stray rates of salmonids to non-natal rivers, and delays in egg hatching by coregonine fishes leading to starvation (Lytle & Poff 2004). Consider the Paraná River in Brazil, where the juveniles of the streaked prochilod (*Prochilodus lineatus*) rely on flooding to facilitate dispersal into lagoons. Damming has led to a decreased incidence of flooding downstream, preventing the species from reaching these critical nursery grounds and substantially increasing local extinction risk (Gubiani et al. 2007). In South Africa, despite progressive water resources legislation, river connectivity and fish migrations remain largely ignored in the construction of in-stream barriers (O'Brien et al. 2019). Very few dams and gauging weirs are equipped with fish passage facilities, and of these, only about 20% are known to be functional (Bok et al. 2007). These and other similar examples illustrate why dams are the primary reason that 20% of freshwater fishes are threatened with extinction (Day & Davies 2023, p. 332).

One of the more insidious threats indigenous freshwater fishes must contend with is the invasion of IAF into novel habitats. Many invasive alien fishes have been introduced intentionally to new environments to enhance the recreational angling potential of rivers (Cambray 2003b). Introductions were carried out to 'improve the biodiversity' of novel waterbodies for anglers (Hey 1926), with no regard to potential consequences for indigenous fauna. In fact, the goal was often explicitly to eliminate indigenous species, which were thought to interfere with the successful establishment of desirable angling species, such as trout or perch (Hey 1926). In a 1926 report detailing findings from a survey aiming to identify the most suitable river systems in South Africa for IAF introductions, Hey (1926) wrote that it was "possible that perch, in lower reaches [of the Berg River], might expedite the extermination of the Witte Vis and thus replace a very coarse and useless fish by a sporting and highly edible species."

Once IAF are introduced into a waterbody, they can spread naturally throughout river systems, may be actively distributed by farmers and anglers, or are transferred to new basins via inter-basin water transfer schemes (Cambray 2003; Shelton et al. 2017). The result is often the catastrophic loss of biodiversity in the recipient ecosystem. Impacts can manifest at the genetic level (hybridisation), the individual level (changes in behaviour and vital rates), the population level (disease transmission and changes in distributions), the community level (localised extinctions, food web restructuring), and the ecosystem level (alteration of biochemical cycles; Cucherousset & Olden 2011).

In New Zealand, the introduction of alien trout and their subsequent predation of benthic insects caused a trophic cascade, which resulted in the exclusion of indigenous galaxiids from prime foraging areas and their subsequent decline (Mcdowall 2003). In South Korea, the highly piscivorous North American largemouth bass (*Micropterus nigricans*[*]) reduced the abundance of five threatened *Barbus* species by 99% where bass were present (Gratwicke & Marshall 2001). In Lake Victoria, populations of the introduced Nile perch (*Lates niloticus*) ballooned from 1% of total annual harvest to 97% in just nine years, leading to the disappearance of over 60% of the lake's endemic fish species (Witte et al. 1992). Similarly in Mexico, IAF reduced the population of the Picote de Tequila (*Zoogoneticus tequila*) to such an extreme that the sole population was confined to a single pool measuring just 4 m across, only to be declared extinct in the wild by 2013, although a captive breeding and reintroduction program

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[*] Until recently, the scientific name *Micropterus salmoides* referred to the largemouth bass. However, a recent genomic study (Kim et al. 2022) reclassified the largemouth bass as *Micropterus nigricans*, assigning the name *Micropterus salmoides* to the Florida bass instead. All references to largemouth bass in this thesis have been updated to reflect the revised and currently accepted nomenclature.

is attempting to bring this species back from the brink (Koeck 2019). Indeed, the impacts of IAF are so damaging that eight freshwater fishes feature in the list 'One Hundred of the World's Worst Invasive Alien Species' (Lowe et al. 2000). Of these, seven were introduced primarily or partially for sport (Cucherousset & Olden 2011). The problem of IAF is compounded by the fact that many of the species were introduced as early as the 19th century, before any environmental impact studies, or baseline surveys, could be carried out against which to compare current trends (Cambray 2003b). Known impacts on indigenous fish species may therefore be underestimating the extent of true damages wrought.

While climate change can often feel abstract and temporally removed, it already potentially threatens half of all freshwater fish species (Darwall & Freyhof 2015). In lentic systems, the incidence and severity of eutrophication events may increase, reducing habitat suitability for resident fishes (Carosi et al. 2023). Drought frequency is also likely to increase under predicted climate change scenarios, especially so in Mediterranean climate regions. This is already taking place in Australia, where droughts severely threaten flow regimes in rivers, with consequent impacts on the migratory fishes that rely on them (Deinet et al. 2020). Increasing water temperatures decrease concentrations of dissolved oxygen, exacerbate the impacts of toxic contaminants (Ficke et al. 2007), facilitate the spread of disease (Johnson & Paull 2011), alter phenology (Krabbenhoft et al. 2014), and decrease survival (Bassar et al. 2016). Fish are ectotherms, relying on movement for thermoregulation, and as the waters they inhabit start to warm, they will increasingly be forced to move to higher altitudes and latitudes (Comte et al. 2013). Where this is possible, many species will find themselves isolated to headwater refugia in fragmented populations; where it is not, local species extinctions are likely. Where conservation resources are limited, the impacts of climate change on vulnerable fishes may be especially severe. In South Africa, a severe shortage of aquatic scientists at the provincial level (Impson 2016) hampers the monitoring of freshwater ecosystem responses to climate change and the implementation of timely conservation actions.

The interactions between climate change and invasive species have garnered considerable scientific attention, and it is becoming clear that, in many cases, the former will almost certainly exacerbate the impacts of the latter. One model posits that climate change will favour the dispersal of IAF through extreme events such as floods (as in the invasion of banded tilapia *Tilapia sparrmanii* into a South African tributary; Cerrilla et al. 2022), enhance their vital rates (thereby increasing colonisation success and aiding their establishment), and expand suitable habitat for invasives, ultimately intensifying invasion pressure in novel systems (Walther et al. 2009). Indeed, while climate change is predicted to result in distributional changes among freshwater fishes, not all species will stand to be 'losers' of this process. Many invasive warm-water species, such as centrarchids and cyprinids, actually stand to 'win' under future climate scenarios (Comte et al. 2013). The common carp (*Cyprinus carpio*), for example, is an excellent coloniser under highly modified ecosystems (such as those suffering increasing drought frequencies) due to its wide ecological tolerances (Koehn 2004). The highly piscivorous smallmouth bass (*Micropterus dolomieu*) is likewise predicted to benefit from a warming world, to the detriment of indigenous species. In northern Ontario, where the species' northern range is limited by temperature, modelling of future climate scenarios reveals that *M. dolomieu* will be able to survive in nearly every lake in the province by 2100, leading to the potential extirpation of more than 25,000 local populations of four indigenous cyprinids (Jackson & Mandrak 2002). It is quickly becoming clear that such trends may represent the norm, rather than the exception.

1.2.3 State of freshwater fishes in South Africa and the Cape Fold Ecoregion

1.2.3.1 Freshwater fish diversity in South Africa and the Cape Fold Ecoregion

Of the over 18,000 fish species found in freshwater ecosystems, nearly 3,000 are found in Africa (Lévêque et al. 2008). Approximately 1% of the world's freshwater fish species can be found within the borders of South Africa, which is classified as one of the world's 17 megadiverse nations (Skowno et al. 2019). There are currently 106 valid freshwater fish species and 18 unique genetic lineages or molecular operation taxonomic units (MOTUs) recognised in South Africa, with cyprinids accounting for just over half of all valid species (Chakona et al. 2022). The country is also known to host high levels of endemicity across taxa, with nearly half (49%) of all freshwater fishes classified as endemic to South Africa (Skowno et al. 2019).

The Cape Fold Ecoregion (CFE; Abell et al. 2008) is recognised as one of eight freshwater ecoregions in the country, which are defined on the basis of the distribution ranges of native fishes (Chakona et al. 2022). The CFE coincides broadly with the Cape Floristic Region, a widely recognised biodiversity hotspot known to host an exceptional concentration of endemic species (Myers et al. 2000). Forty-one (33%) of the country's freshwater fish taxa can be found within the CFE, with 92% of these endemic to the region (Chakona et al. 2022). Most species within the CFE are narrow-range endemics restricted to single river systems, or even to single tributaries, which accounts for the relatively low taxonomic richness of individual river systems (each containing from zero to 10 species; Ellender et al. 2017).

1.2.3.2 Trends and threat status of South African freshwater systems

Only 33% of river lengths in South Africa are considered to remain in a natural, or near-natural, condition, and 64% of all river ecosystem types are classified as threatened (Skowno et al. 2019). Rivers have the second highest proportion of ecosystem types that are classified as Critically Endangered (42%) in the country – second only to wetlands (61%; Skowno et al. 2019). Between 1999 and 2001, river condition in South Africa declined by 11%, and 67% of total river length is now considered to be in a degraded state (Skowno et al. 2019).

Following the global trend, species confined to freshwater ecosystems in South Africa are declining more rapidly than their terrestrial counterparts, with 17% of freshwater taxa classified as threatened, compared with 13% of terrestrial taxa (Skowno et al. 2019). Freshwater fishes exhibit one of the steepest declines in cumulative Red List Index status in the country, indicating that species in this group are becoming more threatened over time at a faster rate than other South African species groups (Skowno et al. 2019). Indeed, freshwater fishes are considered to be by far the most threatened species group within South Africa, with 36% of freshwater fish species (and 66% of endemics) threatened with extinction (Skowno et al. 2019; Chakona et al. 2022).

Of the eight aquatic ecoregions within South Africa, the CFE contains the largest proportion (70%) of threatened freshwater fishes – a consequence of the prevalence of endemic range-restricted species (Chakona et al. 2022). Of the 40 fish taxa native to the CFE, five are Critically Endangered, 14 Endangered, and nine Vulnerable (Chakona et al. 2022). Within the CFE, the Olifants River system is particularly valuable from a conservation perspective, as it hosts the largest number of threatened species in southern Africa, with 70-75% of all native fish species classified as threatened (Darwall et al. 2011).

1.2.3.3 Threats to freshwater fishes in South Africa and the CFE

According to the 2018 National Biodiversity Assessment, the most prominent threats to freshwater fishes in South Africa are invasive species (including diseases), natural-systems modifications (such as dams), and pollution (Skowno et al. 2019). Thirty-six percent of native freshwater fishes and MOTUs are impacted by invasive species (as are 81% of ToCC[*]), 26% are impacted by dams and other water management activities (as are 63% of ToCC), and 27% are impacted by agriculture and forestry effluents (as are 70% of ToCC; Skowno et al. 2019; Chakona et al. 2022). These three key threats have one feature in common: all of them cause fragmentation of fish populations by physically creating barriers, or by degrading rivers to such an extent that it effectively creates barriers to movement. The greatest threats to fishes in the CFE are invasive fish species and excessive water abstraction (Chakona et al. 2022).

Because South Africa suffers from water scarcity, water-supply reservoirs are a common feature across the country. Consequently, more than two-thirds of exploitable water is removed from rivers every year to sustain agricultural, industrial, and urban needs (Day & Davies 2023, p. 319). Nearly every river in the country has been impounded, converting many perennial rivers to seasonal torrents (Day & Davies 2023, p. 319). Instream dam walls represent impassable barriers to indigenous fish fauna – particularly species with migratory life stages. Populations of large migratory cyprinids, including yellowfishes (*Labeobarbus* spp.) and labeos (*Labeo* spp.), are especially vulnerable, given river connectivity and fish passage structures are rarely incorporated into constructed barriers (O'Brien et al. 2019). In the CFE's Olifants River, for example, the Bulshoek Dam (built in 1919) and Clanwilliam Dam (built in 1932; Figure 1.1) have fragmented populations of the Clanwilliam yellowfish (*Labeobarbus seeberi*) and altered flow-dependent spawning cues (Cambray et al. 1997).

Water management and over-abstraction is predicted to become more damaging to biodiversity in the future, as drier climates drive the over-exploitation of aquifers. Depletion of these underground water sources may well cause the extinction of many range-restricted species inhabiting rivers and wetlands that depend on aquifers to maintain base flows (Darwall et al. 2009). This is especially concerning in winter rainfall regions like the CFE, where excessive water abstraction leaves many rivers with little to no flow in the dry summer months (Darwall et al. 2009). The establishment and widespread distribution of thirsty alien plant species, such as *Eucalyptus* spp., *Pinus* spp., and *Acacia mearnsii*, in riparian areas further compounds this problem by substantially reducing flows (Le Maitre et al. 2020).

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[*] Taxa of Conservation Concern (ToCC) is the term used by Skowno et al. (2019) in the National Biodiversity Assessment to refer to taxa that are categorised according to the IUCN Red List criteria as Critically Endangered, Endangered, Vulnerable, Data Deficient, or Near Threatened.

Figure 1.1. Clanwilliam Dam in January 2024. Photograph by Jeremy Shelton.

In addition to modifying natural hydrological conditions, impoundments encourage the spread and establishment of IAF. The conversion of flowing waters into lentic habitats throughout South Africa results in the extirpation of fluvial species, such as cyprinids, while simultaneously providing ideal habitat for the proliferation of limnophilic species (such as non-native cichlids and centrarchids) and providing "stepping-stone" habitats for their spread throughout river systems (Johnson et al. 2008; Chakona et al. 2022). Moreover, inter-basin transfer schemes between reservoirs have allowed the direct transfer of alien fishes between the Berg, Breede, and Eerste River systems in the Western Cape (Darwall et al. 2009).

Invasive fishes are one of the greatest threats to indigenous freshwater fishes in South Africa, impacting 36% of indigenous species (Chakona et al. 2022). Indeed, South Africa is one of six global fish invasion hotspots, defined as areas where non-native fishes comprise more than a quarter of fish species (Leprieur et al. 2008). In South Africa, 21 non-native fish species are naturalised in the country (Weyl et al. 2020). Twenty-four species of non-native fishes have thus far been introduced into the waters of the CFE, 16 of which have become established across more than 90% of the region's river habitat (Marr et al. 2012). Many of these introductions were carried out in the 20th century by conservation departments, which established hatcheries to rear North American centrarchids and salmonids for the benefit of anglers (Cambray 2003b). Black basses (*Micropterus* spp.) have had a particularly destructive impact on indigenous CFE fishes (Table 1.1).

Table 1.1. A selection of studies providing evidence for negative impacts of non-native *Micropterus* spp. and *Oncorhynchus mykiss* on indigenous fishes in the Cape Fold Ecoregion.

Native species	Location	Non-native species	Impact	References
Cape kurper (*Sandelia capensis*) Eastern Cape redfin (*Pseudobarbus afer*) River goby (*Glossogobius calidus*)	Blindekloof stream, Swartkops River system, Eastern Cape	*M. nigricans* *M. dolomieu*	Where *Micropterus spp.* present, native species absent.	Ellender et al. 2011
Breede River redfin (*Pseudobarbus burchelli*)	Witte River, Breede River system, Western Cape	*M. dolomieu*	*P. burchelli* absent or in low abundances in reaches invaded by *M. dolomieu*.	Shelton et al. 2014
Breede River redfin (*P. burchelli*) Cape kurper (*S. capensis*) Cape galaxias (*G. zebratus*)	Upper Breede River system, Western Cape	*O. mykiss*	Mean densities of native species 89-97% lower in streams invaded by *O. mykiss.*	Shelton et al. 2015
Berg River redfin (*Pseudobarbus burgi*) Cape kurper (*S. capensis*) Cape galaxias (*G. zebratus*)	Berg River, Western Cape	*O. mykiss*	*G. zebratus* absent from pools invaded by *O. mykiss*. *P. burgi* segregated from *O. mykiss* by depth gradient. *S. capensis* potentially compete with *O. mykiss.*	Woodford & Impson 2004
Clanwilliam redfin (*Pseudobarbus calidus*) Fiery redfin (*P. phlegethon*) Clanwilliam rock catlet (*Austroglanis gilli*) Cape galaxias (*Galaxias zebratus*) Clanwilliam yellowfish (*Labeobarbus seeberi*)	Rondegat River, Olifants River system, Western Cape	*M. dolomieu*	*P. calidus*, *P. phlegethon*, *A. gilli* and possibly *G. zebratus* extirpated from lower reach by *M. dolomieu;* collapse in recruitment of *Labeobarbus seeberi.* After removal of *M. dolomieu*, large increase in indigenous fish density.	Woodford et al. 2005; Weyl et al. 2013; Castañeda et al. 2020
Cape galaxias (*G. zebratus*)	Driehoeks River, Olifants River system, Western Cape	*M. nigricans*	*G. zebratus* was less abundant (and selected deeper, faster-flowing microhabitats) where *M. nigricans* was present.	Shelton et al. 2008
Clanwilliam sandfish (*Labeo seeberi*)	Oorlogskloof River, Doring River system, Western Cape	*M. dolomieu*	Small *L. seeberi* mostly absent and only few large adults present in *M. dolomieu*-invaded reach.	Cerrilla et al. 2022

Largemouth bass (*M. nigricans*), smallmouth bass (*M. dolomieu*), and spotted bass (*M. punctulatus*) were imported from North America in 1928, 1937 and 1939, respectively, for angling purposes (Ellender et al. 2014). The introduction of each species was planned with its habitat requirements in mind: all *Micropterus* spp. were introduced into areas too warm for non-native salmonids; *M. nigricans* specifically was destined for lentic environments, whereas *M. dolomieu* was stocked into flowing waters and *M. punctulatus* into waters too turbid for *M. dolomieu* (Khosa et al. 2019). Government-supported stocking programmes continued until the early 1990s, supplemented by direct stocking by anglers (Khosa et al. 2019). The devastating impacts of *Micropterus* spp. and other IAF on indigenous CFE fishes have been extensively documented (Table 1.1). In most cases where *Micropterus* spp. are present, small indigenous species and juveniles of larger-bodied species are either absent, or extremely scarce. In the CFE's Olifants-Doring River system (ODRS), *Micropterus* spp. are established along 81% of stream habitat. Here, their distribution is limited by the presence of barriers such as waterfalls, cascades, and chutes (van der Walt et al. 2016). Small indigenous species are consequently mostly restricted to small reaches of headwater refugia, beyond the reach of bass (Day & Davies 2023, pp. 284).

While less intensively studied, the omnivorous bluegill (*Lepomis macrochirus*), also a North American centrarchid, is also widely established across the CFE and elsewhere in South Africa. Attaining up to 200 mm standard length (SL), it was introduced as a forage fish for *M. nigricans* (Skelton 2024). Although *L. macrochirus* is less predatory than *Micropterus* spp., it has had severe impacts on indigenous fish populations (Darwall et al. 2009). This prolific breeder tends to overcrowd waterbodies with dwarfed individuals (de Moor & Bruton 1988), often outcompeting indigenous fishes, and in some cases, even outcompeting bass (Darwall et al. 2009). They are believed to prey on the juveniles of indigenous species (Paxton et al. 2002). Despite their comparatively small body and gape size, individuals as small as 131 mm TL can consume a 50 mm TL fish (Leonard Flemming, unpublished data; Figure 1.2).

Figure 1.2. Dissected *L. macrochirus* (131 mm total length; TL) showing 50 mm TL juvenile sandfish (*Labeo seeberi*), removed from its gut. Photograph by Otto Whitehead.

Climate change predictions across the CFE indicate that water temperatures will increase substantially, while total runoff will decrease, leading to heightened extinction risk for the many fragmented and at-risk species in the region (Dallas & Rivers-Moore 2014). Given these forecasts, 14 of 20 indigenous fishes in the CFE have been classified as being Critically or Highly Vulnerable to the effects of climate change. The most vulnerable species are concentrated in the Olifants-Doring, Berg, and Breede River systems. While the cold-water *O. mykiss* is expected to experience range contractions as waters warm, the three warm-water *Micropterus* spp. are classified as Less Vulnerable to the effects of climate change (Shelton et al. 2018). In fact, a recent study found that there was a considerable invasion debt for *M. nigricans* and *M. dolomieu* in South Africa; in other words, given current climate change projections, the distribution of these IAF are likely to expand considerably across the country. The CFE was found to be the most highly suited for *M. dolomieu*, while most of South Africa will provide suitable habitat for *M. nigricans* under future predicted conditions (Khosa et al. 2019).

1.3 The Olifants-Doring River system

The Olifants-Doring River system (ODRS) straddles the Cape Fold and Karoo Freshwater Ecoregions and comprises two main branches: the Olifants River and its tributaries, and the Doring River and its tributaries (Figure 1.3). The Olifants River is about 250 km long and rises in the Agter Witzenberg Mountains, where it drains Table Mountain Group quartzitic sandstone (DWS 2006). It is located in a winter rainfall area, receiving 200 – 1,500 mm mean annual precipitation (MAP) and flows along the western flanks of the Cederberg Mountains. Most of its water comes from a series of perennial tributaries in the east, although minor flow is contributed by seasonal tributaries in the west (DWS 2006). The river eventually drains into the Atlantic Ocean. Two major instream dams are located along the lower Olifants River mainstem: Bulshoek Dam (storage capacity 7.5 million m^3) and Clanwilliam Dam (127 million m^3; DWS 2006). Major land-use in the catchment includes commercial agriculture, viticulture, rooibos cultivation, and livestock farming (DWS 2006). Large-scale water abstraction results in highly modified flows, causing the once-perennial river to dry up in some sections during the summer months. Summer flows below the Clanwilliam Dam are augmented by hypolimnetic releases (DWS 2006), which are typically characterised by poor water quality (Winton et al. 2019). No flow releases are made from Bulshoek Dam downstream (DWS 2006). Invasive alien fishes are a major problem in the Olifants River, especially in the mainstem, where they dominate the aquatic fauna (DWS 2006). Destructive farming activities have modified the natural riverscape, increased sedimentation, and reduced water quality (DWS 2006).

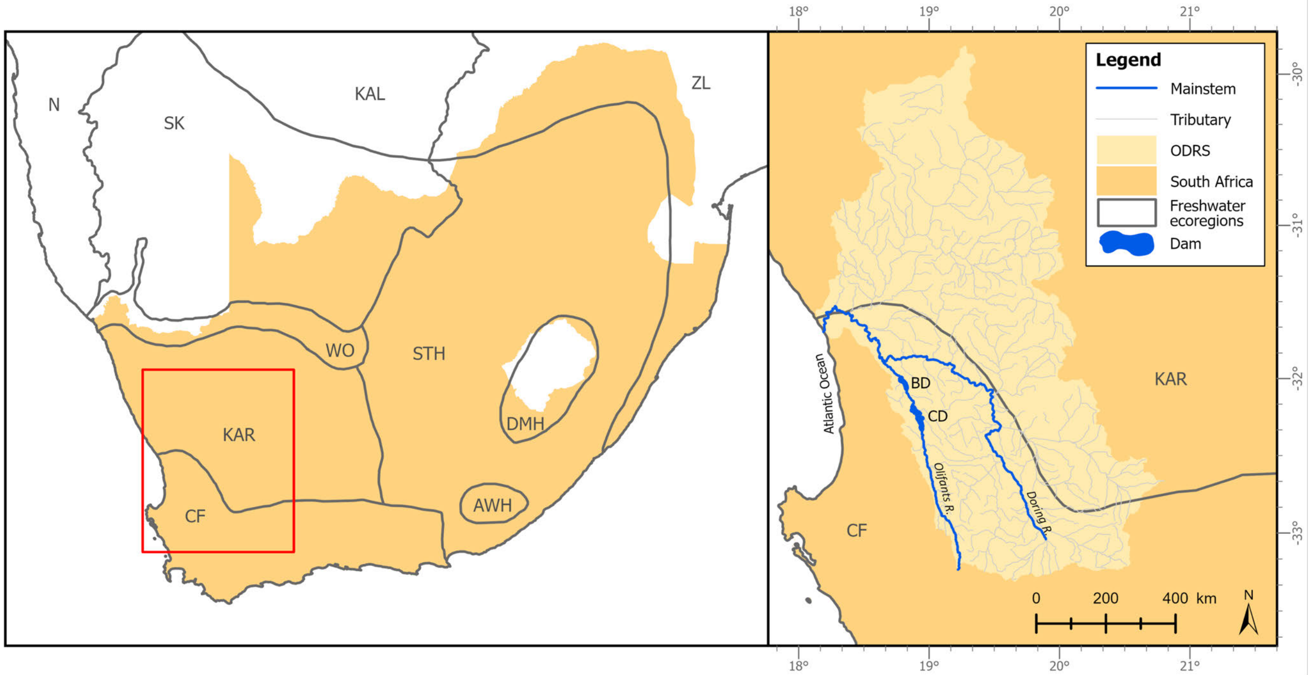

Figure 1.3. Olifants-Doring River system in the context of South Africa and the freshwater ecoregions of southern Africa (*sensu* Abell et al. 2008). Ecoregion abbreviations: AWH = Amatolo-Winterberg Highlands, CF = Cape Fold, DMH = Drakensberg-Maloti Highlands, KAL = Kalahari, KAR = Karoo, N = Namib, SK = Southern Kalahari, STH = Southern Temperate Highveld, WO = Western Orange, ZL = Zambezian Lowveld. Other abbreviations: BD = Bulshoek Dam, CD = Clanwilliam Dam, ODRS = Olifants-Doring River system.

The Doring River's headwaters lie in the Hex River Mountains, from where it flows along the eastern flanks of the Cederberg Mountains, before joining the Olifants River approximately 310 km from its source (and 100 km from the Olifant's outflow into the Atlantic Ocean; DWS 2006). Major tributaries include the Tankwa, Tra-Tra, Biedouw, Brandewyn, and Oorlogskloof-Koebee Rivers. The Doring River system flows seasonally: high winter baseflows drop off steeply in spring, with flow ceasing entirely for several months each summer (although flow might resume temporarily after summer thunderstorms; Paxton et al. 2002). Large pools persist throughout the summer.

The mainstem forms a distinct ecotone between the eastern and western drainages. The Western Folded Mountains ecoregion to the west drains the eastern slopes of the Cederberg Mountains and receives 200—1,500 mm MAP. The landscape is mountainous, and vegetation consists of Sandstone Fynbos (DWS 2006). The Greater Karoo ecoregion drains the arid region to the east, which receives 0—500 mm MAP, mostly in late summer to winter. The area is characterised by low relief and Central and Great Nama Karoo vegetation (DWS 2006). The western tributaries supply acidic, low-salinity water during winter, while the eastern tributaries supply saline water during summer. The fish fauna of the Doring River are uniquely adapted to these variable conditions (DWS 2006). Land-use in the catchment includes agriculture (mostly in the headwaters) and livestock farming in the lowlands (DWS 2006). Unlike the Olifants River, the Doring River is mostly free-flowing: the only in-stream obstruction is a large weir several meters tall at Brakfontein Farm in the upper reaches of the mainstem (Paxton et al. 2002). While water abstraction is limited compared with the productive Olifants River, it does have a considerable effect on summer flows due to the intermittent and arid nature of the system. Although the Doring River system is naturally seasonal, the lower Doring River and many of its tributaries now cease to flow for longer periods in summer than would occur naturally

(DWS 2006). During this time, aquatic fauna becomes restricted to large, isolated pools which retain water through the summer (DWAF 2005). Invasive alien fishes, which are considered a top threat to indigenous fishes in the Cape Floristic Region (Darwall et al. 2009), occur along the entire mainstem of the Doring River and in the lower reaches of its tributaries, causing the disappearance of small species and juveniles of larger species along large sections of the system (DWS 2006, van der Walt 2014). Alien plants, including black wattle *Acacia mearnsii*, oleander *Nerium oleander*, and mesquite *Prosopis* spp., occur throughout the catchment, especially where farming occurs, further impacting on flows (DWS 2006).

While recent data on water quality in the Doring River system is scarce, a 2005 report by the Department of Water Affairs and Forestry provides some insight. Water quality varies depending on location along the mainstem: in the upper Doring, water is considered suitable for agriculture and domestic use, while in the lower Doring, water quality is classified as "good at the end of winter and marginal at the end of summer." This is mostly attributed to elevated salinities during periods of high flow from Karoo rivers, which mostly occur after summer thunderstorms in the Karoo, and towards the end of summer, when water is concentrated in isolated pools. Data on water quality in the various tributaries is largely lacking (DWAF 20025).

1.4 The Clanwilliam sandfish

1.4.1 Ecology and life history

Species of the monophyletic *Labeo* genus (Cyprinidae) occur across Africa and southeast Asia and are adapted to a range of environments, including lacustrine and fluvial conditions (Paxton et al. 2012; Skelton 2024). There are around 60 *Labeo* species in Africa, many growing to large sizes (>150 mm SL; Skelton 2024). Most are strong swimmers that undertake mass upstream migrations to spawn (Skelton 2024). Labeos have inferior mouths with well-developed lips, which are used to feed on algae, periphyton and detritus from the substrate (Skelton 2024). There are four *Labeo* species in South Africa's Orange-Vaal, Tugela, and Olifants-Doring river systems: moggel (*Labeo umbratus*), Orange River mudfish (*Labeo capensis*), Tugela labeo (*Labeo rubromaculatus*) and Clanwilliam sandfish (*Labeo seeberi*). They tolerate low oxygen environments well and several are known to thrive in impoundments (Gaigher 1984; Potts et al. 2005; Skelton 2024).

Labeo seeberi (hereafter 'sandfish'[*]) feeds on algae, detritus, and small invertebrates by grubbing in soft sediments and grazing off rocks with their papillate lips (Skelton 2024), leaving distinctive scalloped trails where they have fed (Paxton et al. 2016; Figure 1.4). Mainstem adults have been known to reach sizes of up to 600 mm TL (Paxton et al. 2002). As the only large, detritivorous fish species in the ODRS (Skelton 2024), the sandfish likely plays a considerable role in riverine food webs and nutrient cycling. Its feeding strategy and size suggest it may substantially influence energy flow and the breakdown of organic matter in these ecosystems. Adults prefer large, deep pools with sandy substrate in the mainstems (Skelton 1987). While sexual maturity has not been specifically studied, evidence suggests that they reach this stage at around 250 mm (Jubb 1967). One spent female in the Oorlogskloof River measured 287 mm TL (Paxton et al. 2002). Size at sexual maturity of the closely related *L. umbratus* varies by location, ranging from 140 to 320 mm in males and from 200 to 370 mm in females (Gaigher

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[*] 'Sandfish' is used in lieu of *Labeo seeberi* to avoid confusion with the Clanwilliam yellowfish (*Labeobarbus seeberi*).

1984). While the lifespan of sandfish is currently unknown, *L. umbratus* and *L. capensis* can live to six and nine years, respectively (Skelton 2024). Large female sandfish are highly fecund, carrying up to 80,000 eggs (Impson 1997).

Figure 1.4. (a) Schools of adult Clanwilliam sandfish during a spawning migration in the Biedouw River and (b) characteristic feeding trails in the Gif River. Photographs by Jeremy Shelton.

Although the species' breeding biology has not been intensively studied, several sources report that adults residing in the mainstems migrate upstream into the tributaries to spawn in spring (as evidenced by a peak of ripe fishes around September; Gaigher 1973a). Reports from landowners in the Biedouw River valley confirm that mass movements of *L. seeberi* were observed in September and early October 2001 (Paxton et al. 2002), and more recent observations confirm that adults migrated into the Biedouw River to spawn between August and September of 2020–2023 (pers. obs.). It is thought that sandfish are synchronous rheophilic spawners, requiring specific flow and temperature conditions to successfully reproduce (Paxton et al. 2012). Poor rainfall years are therefore suspected to result in recruitment failure (Paxton et al. 2012). Studies of related Labeos suggest they are phyto-lithophils (depositing eggs in clearwater habitats on submerged plants, logs, gravel and rocks; Balon 1975; Potts et al. 2005), and that spawning is polyandrous, with several males pursuing a single female (Reid 1985). However, despite the sandfish's relatedness to the southern African group of Labeos, the unique winter rainfall regime of the Western Cape suggests that some biological traits may differ in meaningful ways, highlighting the need for further study (Paxton et al. 2012).

Although most information is known from the adult population of sandfish that reside in the mainstems, it should be noted that self-sustaining non-migratory populations appear to exist in several tributary headwaters – notably in the Oorlogskloof River (Cerrilla et al. 2022) and in the Biedouw River (pers. obs.). Adults residing in these populations may be growth-limited, seldom exceeding 250 mm in length (Paxton et al. 2002).

1.4.2 Distribution

The sandfish was historically widespread across the ODRS, which includes both the Olifants and Doring River mainstems and their tributaries. Although no confirmed distribution records exist for sandfish in the upper reaches of the Olifants River, a 1963 entry in the angling publication *Piscator* recounts a 1938 expedition during which a "great number" of juvenile sandfish could be seen at Keerom, a pool in the upper reaches of the Olifants River mainstem (Harrison 1963). This suggests that sandfish naturally occurred throughout the Olifants River in the first half of the 20th century. Recently hatched sandfish (1-2 cm TL) were also observed in the middle and upper reaches of the Olifants River in November and December 1963 (van Rensburg 1966). Adult sandfish were regularly caught in the Olifants mainstem during surveys in 1963 to 1964, although they were most commonly sampled downstream of Clanwilliam Dam (van Rensburg 1966). In surveys carried out in 1972- 1973 along the Olifants River mainstem, adult sandfish (and some immature sandfish measuring 12-20 cm FL) were recorded in the lower reaches of the Olifants River (below Clanwilliam Dam), but none were recorded in the Clanwilliam Dam itself, and few were recorded in the upper reaches at Citrusdal and Keerom (Gaigher 1973a). During the same surveys, "reasonable numbers" of small sandfish were recorded in the Doring River mainstem near its confluence with the Olifants River (Gaigher 1973a). In a series of surveys carried out between 1998 and 2001, sandfish were entirely absent from the Olifants River, although they continued to be recorded in the Doring River system (Paxton et al. 2002).

The pattern of historical records suggests that sandfish have suffered a considerable range contraction in the past century, disappearing entirely from the Olifants River and its tributaries over the course of mere decades. They presently occur in fragmented populations confined to the middle and upper reaches of the Doring River and some of its tributaries – namely the Gif, Oorlogskloof-Koebee, Kransgat, Biedouw, Tra-Tra, and Matjies Rivers (Jordaan et al. 2020a).

1.4.3 Threats

1.4.3.1 Historical threats

The disappearance of sandfish from the Olifants River and its tributaries is believed to be due to two major pressures: the construction of migration barriers and the introduction of IAF into the system (Darwall et al. 2009). The construction of the >7 m high Bulshoek Dam in 1919 was followed by the construction of the Clanwilliam Dam in 1932, which has since been raised to its current height of 43 m. These dam walls present unpassable barriers to migratory species like sandfish. Harrison (1963) recounts observations from his 1938 expedition:

> *"The irrigation barrages at Clanwilliam and Bulshoek have greatly increased the acreage of permanent water, whilst acting as complete barriers to upstream movement of fish, as is evidenced by the shoals of yellowfish, sawfin and sandfish massed below the barrages at the time of the spring spawning run."*

Moreover, the dams altered the flow regime of the downstream reaches, substantially impacting remaining sandfish habitat in the Olifants River (Paxton et al. 2002).

Several batches of *M. nigricans* were stocked into the Olifants River between 1933 and 1936, and they were well-established throughout the system by 1938 (Harrison 1963). Between 1943 and 1945, 1,050 young *M. dolomieu* were introduced into the Jan Dissels River, a tributary that joins the Olifants River directly below Clanwilliam Dam, and into the upper mainstem at Keerom (Harrison 1963). Four years later, *M. dolomieu* had become well established throughout the Olifants River system, and had largely displaced *M. nigricans* (Harrison 1963):

> *"This stream [Jan Dissels River] again seems to have resisted attempts to stock with trout, but it was kinder to the first introduction of smallmouth bass in August 1943… As in the cases of the Berg and Breede rivers, the smallmouth bass have nearly ousted the largemouth bass from their earlier dominance of the Olifants River."*

That sandfish were last spotted in the Olifants River in the 1970s (Gaigher 1973a; Paxton et al. 2002) is not surprising. After several decades of failed spawning runs, heavy predation of young fish by *M. nigricans* and *M. dolomieu* and altered conditions from water abstraction and inundation of fluvial habitat, sandfish appeared to have become extirpated from the Olifants branch of the ODRS.

1.4.3.2 Current threats

Although sandfish persist in the Doring branch of the ODRS, they do so in fragmented populations and declining numbers. Precipitation here is much lower than in the Olifants River catchment (DWS 2006), and large portions of the river flow through arid Karoo environments. Water abstraction therefore has a considerable impact on flows in the system: extensive abstraction for agriculture in the fertile Kouebokkeveld region in the headwaters is believed to have a large impact on the river's mean annual runoff (DWAF 2005), and smaller-scale abstraction throughout its length has a disproportionate impact due to the intermittent and arid nature of the system.

The top threats to remaining sandfish populations throughout the Doring River system are IAF and flow alterations associated with water over-abstraction. Three *Micropterus* spp. (*M. dolomieu, M. nigricans* and spotted bass *Micropterus punctulatus*) occur throughout the Doring mainstem, and they have invaded nearly every tributary in the system. Four tributaries have been entirely invaded, three have invasions along 96-97% of the length inhabited by fish, and a further two have invasions along 74-82% of fish-inhabited river length (van der Walt et al. 2016). A 2012 system-wide survey of the tributaries of the ODRS found that young sandfish were entirely absent where *Micropterus* spp. were present, with only individuals larger than 250 mm observed in bass-invaded reaches (van der Walt 2014). Surveys from 2001 confirmed that, although adults persisted in the mainstem and there was some evidence of reproduction, there were few to no surviving recruits to replace the ageing size classes (Paxton et al. 2012). This was attributed to predation by IAF (Paxton et al. 2012). Large densities of *L. macrochirus* throughout the Doring River system are also thought to play a substantial role in the displacement of indigenous fishes (Paxton et al. 2012).

These major pressures are compounded by local stressors. In the riparian zone of the Biedouw River confluence with the Doring River, for example, nutrient inputs from surrounding livestock degrade water quality, especially in the summer, when aquatic fauna are confined to isolated pools (Paxton et al. 2012). Closer towards the headwaters, invasion by alien trees, overgrazing, trampling, and agricultural activities such as clearing and ploughing destroy habitat, degrade water quality, and increase stream turbidity in critical downstream sandfish habitats (Paxton et al. 2012).

1.4.4 Biedouw River as key spawning tributary

The Biedouw River is a seasonal tributary of the Doring River, which rises in the Cederberg mountains in the western part of the Doring's drainage basin (Figure 1.5). It has a catchment of 329 km^2 and is approximately 50 km long, comprising roughly 33 km of fish habitat (van der Walt 2014). From the point where fish are first encountered (410 m above mean sea level; AMSL), the river runs through a steep and narrow gorge (which falls within Bushmans Kloof Private Nature Reserve) for approximately 4 km, after which the valley opens to a wide floodplain (Figure 1.6). For the remaining 29 km, the Biedouw River flows through the lowlands, before flowing into the Doring River at 202 m AMSL. The gorge section lies in the Fynbos biome (Cederberg Sandstone Fynbos), while the lower 29 km lies in the Succulent Karoo biome (Agter-Sederberg Shrubland and Tanqua Karoo; SANBI 2018b; Mucina & Rurtherford 2006). The area experiences winter rainfall, with most precipitation falling between May and August (Mucina et al. 2006; Rebelo et al. 2006). While the headwaters receive 393 mm MAP (Rebelo et al. 2006), the lower reach receives only 163 mm MAP and is characterised by a semidesert environment (Mucina et al. 2006). Mean annual temperature (MAT) in the Tanqua Karoo is 17°C, and the mean maximum and minimum monthly temperatures are 35.9°C (January) and 5.64°C (July), respectively.

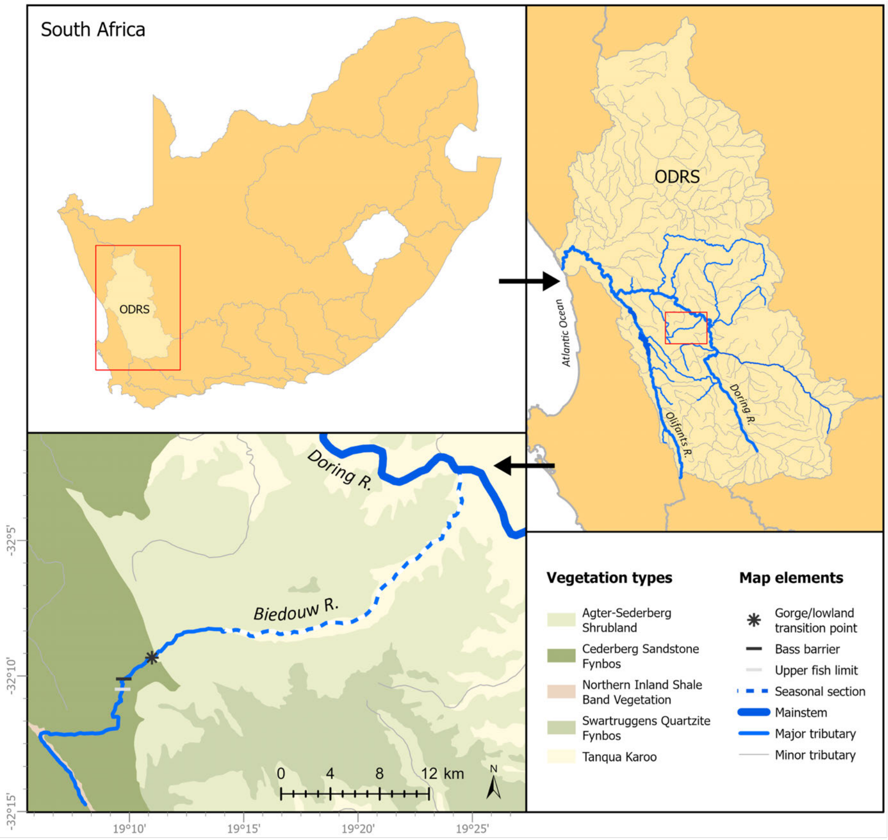

Figure 1.5. The Biedouw River, surrounding vegetation types (SANBI 2018a), and important features. Note that the upper limit of the seasonal section of the Biedouw River is an approximation, as this changes every year, depending on rainfall. No non-native fishes occur above the bass barrier, and no fishes at all occur above the fish barrier. The delineations in the map of South Africa (top left) correspond to primary catchments. ODRS = Olifants-Doring River system.

Figure 1.6. The Biedouw River valley facing upstream. The transition between gorge and lowland topography is clearly demarcated by the point at which the farmland begins. Photograph by Jeremy Shelton.

Land-use in the catchment is largely restricted to the lowland reaches, and includes agriculture, livestock farming, and eco-tourism (van der Walt 2014). Water is abstracted at various points throughout the river's length and stored in a series of small farm impoundments. While water flows in the gorge year-round, the downstream reaches are naturally seasonal. The lower reaches of the Biedouw River would have historically held water in isolated pools through the summer months (Lelie Hough*, pers. comm.). However, with the combined pressures of water abstraction and alien plants, including *Acacia mearnsii*, *Populus* spp., *Prosopis glandulosa* (van der Walt 2014) and *Eucalyptus globulus* (pers. obs.), the lower 25 km dries up completely each year by mid to late summer, leaving no refuge pools for fish to survive the summer.

When the river is flowing, 32.7 km of river length from the confluence to the gorge is invaded by *Micropterus* spp., comprising 97.4% of fish habitat (van der Walt 2014). Non-native bluegill and extralimital banded tilapia also periodically re-invade the lower reaches from established populations in the Doring River, and the former also potentially through downstream dispersal from an established population of *L. macrochirus* in the gorge. A small (<2 m high) waterfall in the gorge has prevented the expansion of IAF into the last remaining 900 m of fish habitat upstream (van der Walt 2014), where five indigenous species persist: Clanwilliam sandfish (Endangered), Clanwilliam yellowfish (*Labeobarbus seeberi*; Near Threatened), sawfin (*Cheilobarbus serra*; Near Threatened),

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* Lelie Hough was a long-time resident of the lower Biedouw River valley close to the Doring River confluence.

Clanwilliam redfin (*Pseudobarbus calidus*; Near Threatened), and Clanwilliam rock catfish (*Austroglanis gilli;* Near Threatened).

In addition to the resident sandfish population in the Biedouw gorge, migratory sandfish from the Doring mainstem utilise the lower 15 km of the Biedouw River as spawning grounds (Figure 1.7). Each year, around August and September, adult sandfish migrate *en masse* from the Doring River and into the Biedouw River. Individual sandfish remain in the Biedouw River for several days to several weeks, waiting for optimal spawning conditions. After spawning, the adults return to the mainstem, leaving behind their eggs. As the lower reaches ceased to flow, young-of-the-year sandfish would historically have over-summered in the remaining isolated pools, which once provided ideal nursery habitat during the early stages of development. In recent years, however, the species' life cycle has been severed at the juvenile stage. After hatching, young sandfish become isolated in shrinking pools with predatory *Micropterus* spp. and *L. macrochirus*. Those that survive predation eventually succumb to desiccation, as the lower 20—25 km of the Biedouw River dries up completely by mid-summer. If any young sandfish are flushed into the Doring River before flow ceases, they encounter the larger bass that reside there. Recruitment is therefore suspected to be functionally zero.

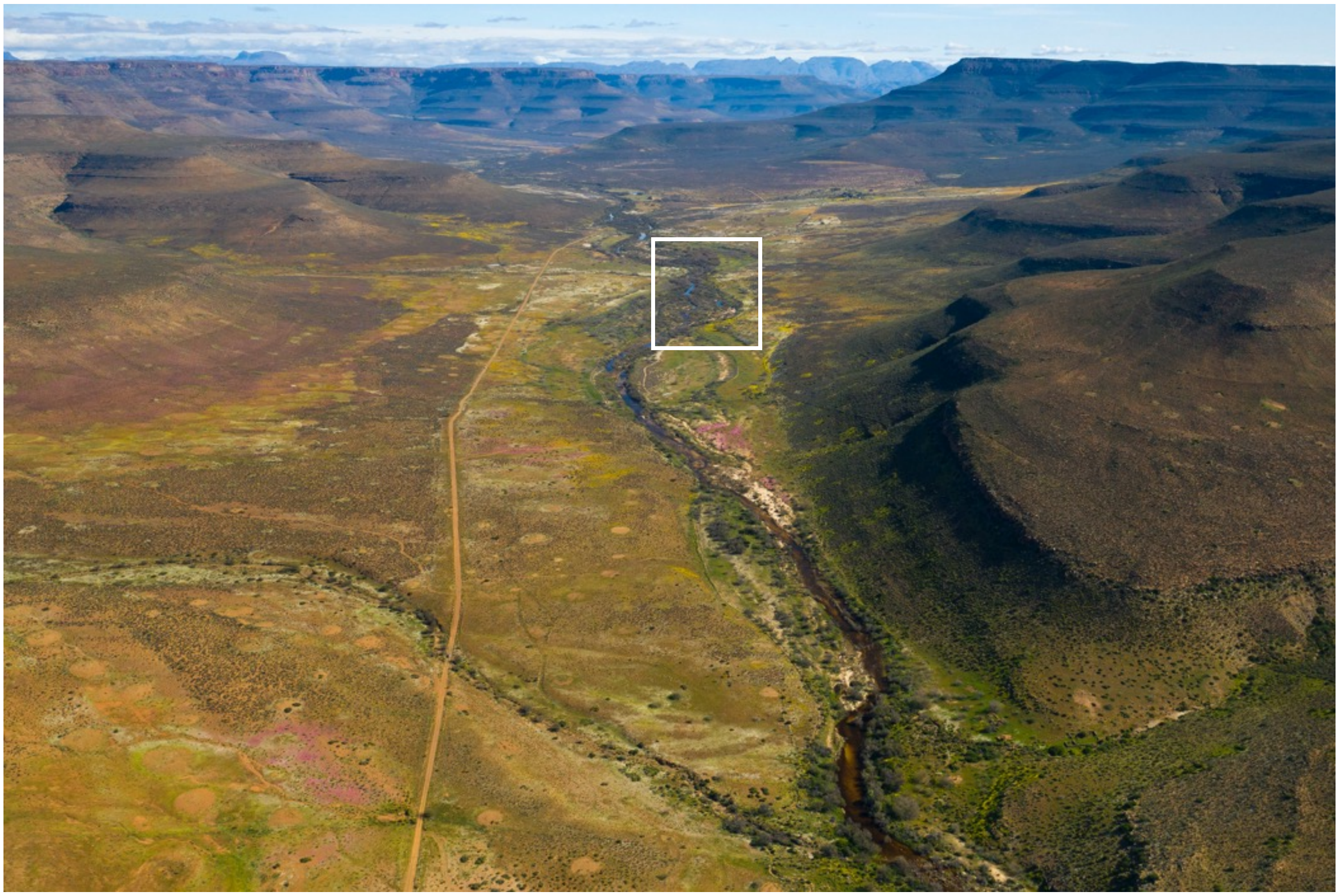

Figure 1.7. The Biedouw River valley facing upstream approximately 8 river-kilometres from its confluence with the Doring River. The section contained within the white rectangle includes the spawning riffles in what is known as "Jensen's Reach". Photograph by Jeremy Shelton.

While there are several confirmed resident populations of non-migratory sandfish in some tributaries (including the Oorlogskloof and Biedouw rivers), these are largely restricted to short headwater sections upstream of bass barrier waterfalls. Without successful recruitment into the core Doring mainstem population, the species is in imminent danger of extinction. The Biedouw River, as a critical spawning tributary, presents a unique opportunity to maximise juvenile survival and bridge the gap in the sandfish's broken life cycle.

1.4.5 The Saving Sandfish Project

The Saving Sandfish Project (SSP) was launched in 2019 with the aim of securing the short-term persistence of the Clanwilliam sandfish by maximizing survival of juveniles hatching in the Biedouw River via a 'head-start' programme. Head-starting involves the rescue and captive-rearing of young individuals to a less vulnerable life stage, before returning them to the wild (Alberts 2007). Every summer, members of the SSP team relocate juvenile sandfish from isolated pools in the Biedouw River to a series of off-stream farm reservoirs in the catchment. After growing to a size deemed less vulnerable to bass predation (at 1+ years), sandfish are re-captured from these reservoirs and released back into the Biedouw River, where they use the strong winter flows to re-join the Doring mainstem population. The smallest head-started fish were released at 98 mm TL, although efforts were made to prioritise the release of fish measuring at least 150 mm TL.

1.5 Rationale

Baseline biological and ecological information on freshwater fishes is essential for supporting conservation decision-making, yet such data are severely lacking across Africa (Darwall et al. 2011). A more thorough understanding is needed of shifting distribution patterns in response to major stressors, such as climate change, loss of habitat, and the spread of invasive species (Chakona et al. 2022). Long-term monitoring of migratory freshwater fishes is an especially important step in mitigating, or even preventing, further population declines moving forward (Deinet et al. 2020).

In the CFE, there is a conspicuous lack of research assessing fish movement, migrations, and eco-physiological requirements, despite the fact that these data would provide valuable insights into fish responses to environmental stressors (Ellender et al. 2017). Although the number of IAF impact studies in the region is growing, more nuanced investigations into their specific impacts on imperilled fauna are needed, especially given that IAF are considered the greatest threat to endemic fishes of the CFE (Ellender et al. 2017). In a report assessing the distribution of and threats to three endemic cyprinids in the ODRS (including sandfish), Paxton et al. (2002) recommended prioritising several research avenues: i) fish migration; ii) habitat utilisation by different life history stages; iii) identification of spawning requirements; and iv) diet composition and ecological requirements. They specifically recommended the implementation of tag-release monitoring surveys to address some of these knowledge gaps (Paxton et al. 2002). In the 1980s and 1990s, when the full extent of the decline of sandfish in the Olifants River was becoming apparent, Skelton (1987) and Impson (1997) recommended that the ecology and biology of sandfish be studied to better understand the combined impacts of habitat loss, fragmentation, and IAF predation on the species' distribution and survival.

Freshwater conservation capacity in South Africa is severely limited (Impson 2016), requiring managers to weigh trade-offs between competing objectives when making resource allocation decisions. In natural resource management, such decisions are frequently made under substantial uncertainty (Runge et al. 2011). Chapter 4, for example, describes a sandfish population augmentation programme implemented despite limited knowledge of individual homing behaviour and key demographic parameters (e.g., fecundity, survival, longevity). However, confronted with evidence of near-zero recruitment, managers chose to act despite the uncertainty, favouring timely intervention over the risk of inaction.

Effective management with limited resources requires identifying and separating "important" uncertainties – those where different actions are preferred under different assumptions and where choosing the wrong one could reduce performance – from those that do not influence the preferred action (Runge et al. 2011). In other words, some uncertainties are important for conservation decision-making, and others are not. For example, the habitat requirements of spawning, larval, and juvenile sandfish in the Biedouw River constitute an important uncertainty, since different management actions – such as habitat restoration or flow regulation – may be more or less effective depending on which life stage is most limited by habitat availability. Choosing the wrong focus could result in ineffective use of resources. Another important uncertainty is the current distribution of sandfish across the ODRS, which remains poorly understood due to limited and sporadic monitoring. Identifying source and sink populations would allow conservation actions to be tailored to local conditions by, for example, prioritising protective efforts like private stewardship agreements in areas where recruitment still occurs, while undertaking more active interventions such as population augmentation and habitat restoration in areas with low or failed recruitment.

To address these important uncertainties, this study aims to investigate three key aspects of the Clanwilliam sandfish's ecology and conservation: (1) habitat preferences and ecological requirements across life stages, (2) historical and contemporary shifts in distribution across the ODRS, and (3) the efficacy of a head-start conservation programme in a spawning tributary. This work directly responds to long-standing research priorities and conservation needs by improving our understanding of the species' behaviour and habitat use, contextualising current distributions against historical baselines, and generating evidence-based recommendations to support the adaptive conservation of the species.

It is hypothesised that different life stages will exhibit distinct habitat preferences, based both on their physiological differences and the varying ecological needs of larval, juvenile, and spawning sandfish. Additionally, the historical data are expected to show a substantial range contraction, with suspected recruitment failure reflected by the absence of subadult size classes in the Doring mainstem. Finally, it is anticipated that the head-start programme will moderately augment the population of spawning sandfish in the Biedouw River in the first year post-release, with return rates increasing in subsequent years. Together, these investigations aim to generate actionable knowledge to support the conservation of an endemic and endangered freshwater fish. By combining ecological research with applied conservation and programme evaluation, this study contributes a much-needed empirical foundation for decision-making around sandfish management in the Olifants-Doring River system.

1.6 Thesis overview

The overall objective of this dissertation is to provide an important resource that informs conservation practices not only for the Clanwilliam sandfish, but also for the plethora of imperilled freshwater fishes across southern Africa. The subsequent chapters present a comprehensive investigation into key aspects related to the ecology and conservation of the Clanwilliam sandfish in the Doring River system. Specifically, they evaluate the habitat preferences of critical life stages, historical and present distributions, and the efficacy of a conservation initiative.

Chapter 2 examines the habitat preferences of larval, juvenile, and spawning sandfish. In understanding the environmental conditions that influence habitat selection at different life stages, this chapter provides insight into the ecological requirements of sandfish during particularly vulnerable periods. It also defines the criteria of suitable nursery and spawning habitats. This information has the potential to inform water resource management and land-use practices in the Biedouw River catchment area to sustain sandfish during their annual occupancy in the lower Biedouw River. This will in turn contribute to the conservation of the river, benefitting other species of conservation concern.

Chapter 3 investigates the past and present distribution of sandfish. Through an analysis of historical records and contemporary surveys, this chapter aims to trace changes in sandfish distribution and abundance over time and shed light on key factors related to those trends. This historical perspective is crucial for understanding the impact of anthropogenic activities and environmental changes on sandfish populations over time. The updated surveys cover 10 historically surveyed sites along the length of the Doring River mainstem, as well as five key tributaries where sandfish still occur (the Gif, Kransgat, Biedouw, Tra-Tra, and Matjies Rivers). These surveys, which were carried out before the start of our head-starting efforts, should serve as a baseline against which to compare future trends in sandfish abundance within the Doring River mainstem. This work will therefore inform future efficacy assessments of the head-start intervention.

Chapter 4 critically evaluates the head-start conservation programme implemented for sandfish in the Biedouw River. Mortality rates of juvenile sandfish during the rescue process are assessed, as are growth and survival rates in the reservoir environment. Upon being returned to the Biedouw River, released sandfish were tagged with passive integrated transponder (PIT) tags. Return data collected from a passive PIT-tag monitoring system at the mouth of the Biedouw River are used to evaluate return rates of head-started sandfish, and generalised linear modelling is used to better understand the conditions at release that impact the likelihood of return in subsequent years. These data are essential input in a responsive management strategy, which seeks to employ the most effective methods for maximising juvenile survival via the head-start programme. In assessing the successes and challenges of this programme, the chapter provides insights into the effectiveness of head starting as a conservation tool for endangered freshwater fishes, offering recommendations for optimising future conservation efforts. PIT tag data are also used to investigate the abiotic cues that trigger the onset of upstream migrations and the timing of spawning events.

In the concluding Chapter 5, the key findings from the preceding chapters are synthesized and overarching conclusions are drawn, placing these important insights within a larger ecological and conservation context. This chapter aims to bridge the gap between specific research outcomes and their wider implications for environmental management. It also elevates the work's relevance beyond each chapter's immediate focus, demonstrating the potential impact of this research on both scientific understanding and practical conservation applications.

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Chapter 2

2 Ontogenetic variation in habitat preferences of Clanwilliam sandfish in the Biedouw River

2.1 Introduction

2.1.1 Rationale

Understanding habitat how aquatic biota interact with their environment is a critical first step in designing effective, targeted conservation strategies (Jithin et al. 2022)– an important consideration when conservation resources are often limited. This is especially relevant in South Africa, where there are dire capacity constraints in the freshwater conservation sector that severely limit the monitoring and conservation activities that can be undertaken at the provincial level (Impson 2016).

Like many of South Africa's freshwater fishes, the sandfish has historically been poorly studied, with most of the known ecological information confined to magazine entries, institutional reports, and field guides. The species entry for *Labeo seeberi* in *South African Red Data Book - Fishes* describes adult sandfish as favouring large, sandy pools, and juveniles as favouring the smaller pools and rocky runs of tributaries (Skelton 1987). Its listed diet includes microscopic crustaceans, algae, and diatoms. Its breeding biology has been poorly studied, but anecdotal observations suggest adults migrate upstream to breed in large aggregations, with a suspected breeding season of spring to early summer. Additional information published in the most recent edition of *A Complete Guide to the Freshwater Fishes of Southern Africa* includes a somewhat more detailed description of their feeding niche, stating it grazes algae from rocks and grub in soft sediments for detritus and small invertebrates (Skelton 2024). Although several reports and journal articles have since been published on the species, they deal mostly with distribution and relative abundance (Paxton et al. 2002; Ramollo et al. 2013; Jordaan et al. 2020b; Cerrilla et al. 2022), and in one case, population genetics (Rhode et al. 2024).

Although Skelton (1987) recommended that a study of the biology and ecology of sandfish be carried out, no study has yet investigated specific habitat requirements. Moreover, Cerrilla et al. (2022) recommended that detailed studies of the ecological requirements of different life stages should be prioritised in order to better understand the species' response to climate change. This chapter investigates the habitat preferences of sandfish at three critical life stages: during spawning, the larval stage, and the early juvenile stage. Anecdotal evidence from recent years suggests that sandfish spawn at very specific water velocities, although empirical data are lacking. The spawning success of a fish population is generally considered vital for its survival, but evaluations of the habitat requirements of young fish, including fry and juveniles, are often overlooked (Bovee & Cochnauer 1977). This is despite the fact that larval growth and survival rates, together with spawning success, are primary bottlenecks to recruitment in many non-salmonid fish populations (Mills & Mann 1985; Schiemer et al. 2003). Sandfish may be most vulnerable during the larval stage, as they have limited swimming power and co-exist with non-native predatory species upon hatching. Indeed, predation by adult bluegill (*Lepomis macrochirus*) has been implicated as an important source of mortality for freshwater larval fish in experimental treatments (Kim & Devries 2001). During the juvenile stage, sandfish continue to experience predation pressure and the additional stress of stranding as nursery pools in spawning tributaries shrink or dry out completely (pers. obs.). The spawning, larval, and juvenile phases are therefore considered to be the most critical in terms of ensuring the recovery and long-term persistence of the species.

2.1.2 Habitat suitability criteria

One of the most widely used methods for assessing habitat requirements of aquatic organisms is the development of habitat suitability criteria (HSC). Habitat suitability refers to the physical conditions in which focal biota are likely to be found (Jowett et al. 2008), and habitat suitability criteria specifically define the range of suitable habitat parameters for a given organism (Bovee 1986). In aquatic environments, the instream habitat conditions of interest usually include depth, water velocity, and sometimes substrate and cover (Bovee & Cochnauer 1977; Jowett et al. 2008; Nestler et al. 2019). These habitat parameters are highly interrelated and can shift over time and space depending on discharge, as higher velocities associated with floods might redistribute substrate along a river, carry boulders and cobbles downstream, or deposit high volumes of sediment in novel locations. The availability of structural cover provided by different types of substrates may also be impacted by changes in flow, which can also change the distribution of plant cover. While these parameters do not encompass the totality of factors that determine an aquatic organism's behaviour or distribution, they are applicable to most stream conditions and known to strongly influence the microhabitat selection of stream fishes (Gorman & Karr 1978).

The development of HSC for a species is based on the assumption that individuals tend to select the conditions in a stream that they find most favourable, with relative abundance of individuals decreasing as conditions become less suitable (Bovee & Cochnauer 1977). The quantitative relationship between physical habitat conditions and species abundance can be used to build habitat suitability relationships represented as curves, which are often used as input into hydraulic models that predict how the abundance of suitable habitats will vary with changes in flow (Jowett et al. 2008). However, the curves themselves are highly informative and can be used to understand the specific habitat requirements of a species – or life stage – of interest by identifying the ranges of key habitat variables where it is most likely to occur.

Bovee (1986) defined three HSC categories. Category I criteria are generated without empirical data, relying instead on expert knowledge about the conditions a species is likely to favour. Category II criteria use microhabitat data collected at the precise locations where focal organisms are observed to create a utilisation function or curve. The curves generated with Category II data can reveal the types of conditions in which focal organisms are most often found, but are inherently biased by environmental conditions available at the time of observation. Category III criteria correct for this bias by weighting the utilisation function by the relative abundance of different types of available habitat, allowing for calculation of true habitat preferences. It is considered the most transferrable and scientifically defensible of the three approaches (Nestler et al. 2019).

The application of HSC for assessment of the habitat requirements of aquatic organisms is widespread (Nestler et al. 2019). In New Zealand, Category III criteria were developed for 27 species of common freshwater fishes (Jowett & Richardson 2008), while in Taiwan, they were used to develop a habitat preference index for an endemic river loach (Yu & Lee 2002). They have also been used in the western Himalaya to assess the impact of check dams on overwintering tadpoles (Jithin et al. 2022), to evaluate habitat preferences of juvenile salmon in Finland (Mäki-Petäys et al. 2002) and of macroinvertebrates in Poland (Szałkiewicz et al. 2022).

In 1991, Category III criteria were developed for the fish fauna of the Olifants River in South Africa's Western Cape to evaluate whether endemic fishes would have sufficient physical habitat to re-establish themselves, should non-native fishes be removed (Gore et al. 1991). In 2008, Category II criteria were used to assess instream habitat use of different size classes of sawfin (*Cheilobarbus serra*) and Clanwilliam yellowfish (*Labeobarbus seeberi*) in the Driehoeks River, a tributary of the Doring River (Paxton 2008).

The lexicon around the HSC methodology is extensive and can be misrepresented in the literature. The terms used in this study are defined as follows: habitat utilisation curves (HUCs) represent the habitats being used by focal organisms at the time of observation; habitat availability curves (HACs) represent the abundance of available habitats within the study area at the time measurements were taken; and habitat preference curves (HPCs; i.e., Category III curves) represent the habitat preferences of the focal biota, calculated as the ratio between habitat utilisation and habitat availability (Szałkiewicz et al. 2022). Habitat suitability refers to the subsequent subjective interpretation of habitat use and preference, which may incorporate measures such as optimum habitat ranges or composite suitability point indices (Jowett et al. 2008).

2.1.3 Objectives

In this chapter, Category III criteria were applied to generate habitat preference curves for spawning, larval, and juvenile sandfish. These curves were used to identify the habitat requirements of these critical life stages, with the goal of informing the management of the species and the habitats it depends on. The instream habitat parameters examined were depth, water velocity, substrate, and cover. Specifically, the objectives were to: i) evaluate how habitat use differed between life stages and ii) develop habitat preference curves for each life stage. To this end, habitat surveys were conducted across three reaches of the lower Biedouw River during periods when each life stage was present. The frequency of use of different depths, velocities, substrates and cover types by each life stage was compared with the availability of those habitat type to determine habitat preferences.

2.2 Methods

2.2.1 Study area

All surveys were carried out in the lower reach of the Biedouw River between September and November 2021. For a thorough site description of the Biedouw River, refer to Chapter 1 section 1.4.4.

2.2.2 Data collection

2.2.2.1 Microhabitat data

The habitat requirements of fish species can be defined at macro-, meso- and micro-habitat scales. The macrohabitat scale refers to river segments several kilometres in length (Wegscheider et al. 2020). Definitions of a mesohabitat can vary, with some defining it as a discrete morphological habitat unit (a pool, run, or riffle; Jowett et al. 2008), whereas others define it as a reach of up to 100 m in river-length, which encompasses several of these habitat units (Wegscheider et al. 2020). The microhabitat (or point) scale is more consistently defined as the immediate habitat surrounding a given point (Wegscheider et al. 2020), and measures no more than a few metres in extent (often a few centimetres). The assessment of habitat requirements in this chapter uses measurements taken at the micro-habitat scale to define reach-level habitat preferences at the mesoscale, that, when averaged together, can represent segment-level preferences at the macroscale. Here, a reach is defined as a continuous length of river 500 m in length made up of at least one riffle-run-pool sequence, and the segment encompasses the 10 km of river length bounded by the most upstream and downstream reaches.

Bias is minimised during site selection for habitat preference studies by choosing study sites that contain all conceivable combinations of available microhabitat conditions (Bovee 1986). In addition, a representative reach should include at least one pool/run/riffle morphological sequence (Jowett et al. 2008) based on the broad characteristics defined by Jowett (1993). To accomplish this, three 500 m reaches were selected in the lower 15 km of the Biedouw River, each of which contained a suspected spawning riffle (which constituted the upstream limit of each reach) and a variety of downstream pool, run, and riffle habitats (Figure 2.1). Although selection of the upper limit was non-random, the uniform extension of each reach for 500 m downstream was considered sufficient to minimise site selection bias in the rest of each reach. Each reach was surveyed three times: during spawning (Survey 1; 9-11 September 2021), during the larval phase (Survey 2; 4-6 October 2021), and during the juvenile phase (Survey 3; 20-22 November 2021). The spawning survey covered the end of the winter high-flow season, when flows are first starting to recede. Samplers monitored known spawning riffles daily and carried out the surveys when spawning took place. The larval survey took place at the start of spring, once flows had reduced drastically, 23-25 days after spawning. Larvae were suspected to be 13-15 days post-hatch at the time of Survey 2 based on field observations. The juvenile survey took place at the end of spring (72-74 days after spawning), when flows were low, but pools were not yet isolated. While the timing of the surveys was biologically informed and designed to coincide with the life stages of interest, they were somewhat dictated by personnel availability.

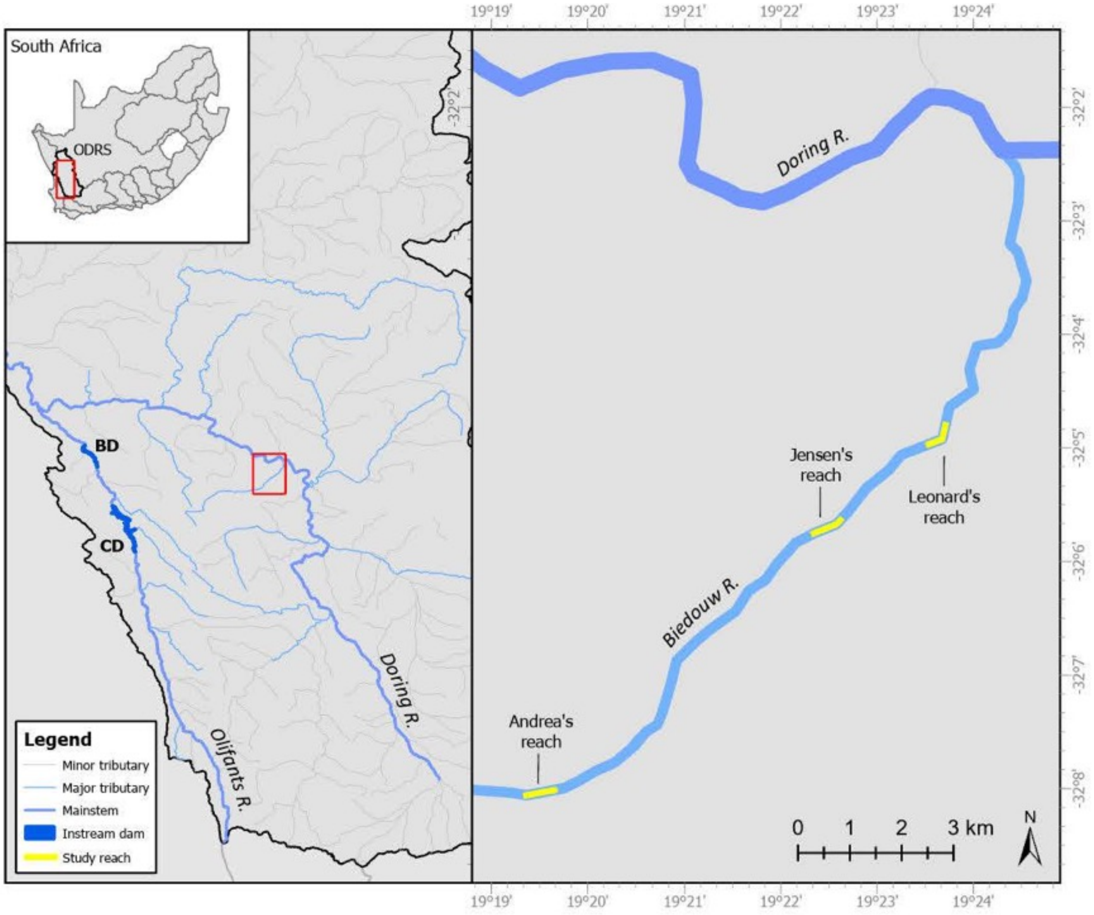

Figure 2.1. Site map of the three sampling reaches in the Biedouw River. BD = Bulshoek Dam; CD = Clanwilliam Dam; ODRS = Olifants Doring River System.

Two sets of data were collected over the course of these surveys: availability and sandfish utilisation data. Availability data represented the entire suite of habitats available to fish in any given reach at the time each survey was conducted, while utilisation data represented the habitat being utilised by fish during any given observation. Collection of both types of data allowed for the evaluation of sandfish habitat preferences during each life stage.

Direct surface observation of fish was used to collect the utilisation data, which causes minimum disturbance to fish, while allowing for the determination of the precise focal point of the observation (Bovee 1986). While this method is influenced by viewing conditions at the time of observation, it is very effective in small, clear streams (Bovee 1986). Direct surface observation was deemed appropriate in this case, as the channel was relatively shallow (0.5 – 99 cm) and narrow (1.69 – 23.78 m), the water was relatively clear (the stream bed could be seen in most cases), and there were very few in-stream visual obstructions. Moreover, spawning is best detected by direct observation due to the relatively short duration of this behaviour.

During surveys 2 and 3, two samplers positioned on opposite banks of the channel walked along the banks from the downstream limit of each reach towards the upstream limit, while scanning the water with glare-reducing polarized sunglasses. To avoid disturbing or altering the behaviour of sandfish during observation, the samplers remained on the banks and did not enter the water while scanning for fish. Wherever larval or juvenile sandfish were seen (during Surveys 2 and 3, respectively), a weighted marker tied to a numbered high visibility float was dropped at that location and the number of individuals, size class, and behaviour were recorded (Table 2.1; Figure 2.2). Only once these data had been recorded did one sampler enter the water briefly to place the weighted marker, while the second sampler monitored the fish from the bank to prevent duplicate observations.

Table 2.1. Size class and behaviour categories recorded during observations of sandfish.

Condition	Category	Definition
Size class	Larval	≤ 2 cm TL
	Juvenile	> 2 cm; ≤ 6 cm TL
	Subadult	> 6 cm; ≤ 15 cm TL
	Adult 1	> 15 cm; ≤ 30 cm TL
	Adult 2	> 30 cm; ≤ 45 cm TL
	Adult 3	> 45 cm TL
Behaviour	Schooling	Group swimming in the same direction in a coordinated manner.
	Swimming	Single individual moving without feeding, chasing, or spawning.
	Resting	Individual or group swimming passively (enough to stay in the same place or drift passively).
	Feeding	Individual or group actively feeding. Sandfish may feed in the sediment, on a rock, or around submerged vegetation.
	Chasing	Staging behaviour that precedes spawning in which one or more individuals chase another, without engaging in spawning itself.
	Spawning	Rapid and sudden burst of movement upstream by a group of adults, resulting in vigorous splashing. Rapid vibration of the dorsal fin of the leading sandfish (presumably a female) often observed.

Figure 2.2. Photograph of weighted floats being dropped at various points where sandfish were observed during sandfish microhabitat surveys in the Biedouw River in 2021. Photograph by Jeremy Shelton.

Observations of habitat use during Survey 1 differed slightly from other surveys due to the nature of sandfish spawning behaviour. In addition to two samplers walking the 500 m length of each reach during the daytime to record the habitat use of non-spawning adults (following the same protocols as in Surveys 2 and 3), additional observations were made in discrete sections of each reach that were known to the sampling team from prior observation to be spawning grounds, in order to record habitat use during spawning itself. During these spawning observation sessions, samplers positioned themselves along the length of the banks of the known spawning grounds (one per reach), which measured 100-200 m in river-length. Spawning activity at the spawning grounds at Leonard's reach was most challenging to observe, as it was deeper than the other two spawning grounds. The shallow conditions at Andrea's and Jensen's reaches facilitated observations there, as the dorsal and caudal fins of sandfish were largely visible in the shallow water. It is possible that some bouts of spawning were missed by observers during these periods, as several could take place simultaneously. However, the presence of several (two to five) observers ensured ample coverage of the spawning grounds.

Further adjustments were made to the sampling protocol during Survey 1 to avoid disturbing spawning sandfish. When a spawning observation was made, a photograph of the area was taken, and the location of the observation relative to reference points, such as distinctive river rocks, was marked and labelled on the photograph. The following morning, the samplers walked the length of the spawning grounds and dropped weighted markers at the locations indicated by the markers on the photograph. Although the delay in the collection of habitat information for spawning sandfish might impact the accuracy of these data, it was deemed a necessary concession to ensure sandfish spawned undisturbed. At no point did samplers enter the water while sandfish were spawning.

Behaviour categories were predetermined on the basis of prior observations by the project team. The threshold of each size class was based on the timing of known life history stage transitions of sandfish between the spawning season (early September) and the last habitat survey (late November). Based on prior observation, it was determined that sandfish larvae transitioned to actively feeding fry at around 2 cm total length (TL). The 6 cm threshold between juvenile and subadult was chosen to differentiate between different hatch-year cohorts; because no young-of-the-year sandfish would measure more than 6 cm by the end of the last survey (late November), any fish larger than 6 cm would thus belong to the previous year's hatch cohort. This cutoff was based on the team's monitoring of the young-of-the-year cohort during the sampling season and in previous years. The 15 cm threshold between subadult and adult 1 was chosen to help identify any reservoir-reared fish released between June and September 2021, as 15 cm was the lower threshold of releasable reservoir-reared fish in 2021 (few to no wild sandfish of this size class were expected due to sustained recruitment failure)[*]. The 30 cm threshold between adult 1 and adult 2 was used as an estimate of size at sexual maturity based on expert opinion (Dean Impson, pers. comm., 2021[†]), and on the size at sexually maturity of related *Labeo* species. Females of *L. umbratus* and *L. capensis* (both indigenous to the neighbouring Orange River system) reach sexual maturity at 200-370 mm standard length (SL) and 240 mm SL, respectively (Gaigher 1984; Skelton 2024). The final 45 cm threshold between the adult 2 and adult 3 categories was chosen to identify the oldest fish.

The identification of sandfish during the three surveys was facilitated by the absence of other cyprinid or other indigenous fishes in the study reach. The lower Biedouw River dries completely each summer, preventing the establishment of resident fish populations. Each year, this area is only repopulated starting in August, first by spawning sandfish, and later by non-native fishes. The three other cyprinid species indigenous to the Biedouw River – Clanwilliam redfin (*Sedercypris calidus*), Clanwilliam yellowfish and sawfin – are restricted to the IAF-free perennial gorge section more than 15 river-kilometres upstream (pers. obs.) The only other fishes sighted in the study reach during habitat surveys were adult bluegill, which were easily distinguished from sandfish by their size, body shape and lack of schooling behaviour. As sandfish were the only cyprinids present, and larval sandfish were consistently observed along shallow pool edges where they could be closely inspected, their identification was considered reliable.

Availability data were collected according to a uniform sampling design. Each 500 m reach comprised 50 transects 10 m apart. Transects comprised four sampling points distributed equidistantly across the channel, yielding a total of 200 availability sampling points per reach, and 600 per survey (Figure 2.3). The width of each transect was measured at the time of sampling.

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[*] Although external tags, such as Floy tags would have made it easier to distinguish reservoir-reared fish from wild fish through visual observationthey were not used to avoid causing unnecessary additional stress to the released fish.

[†] Dean Impson is a research associate at the Freshwater Research Centre in Cape Town, South Africa and was a fish scientist with the Scientific Services Unit of CapeNature from 1994 to 2019.

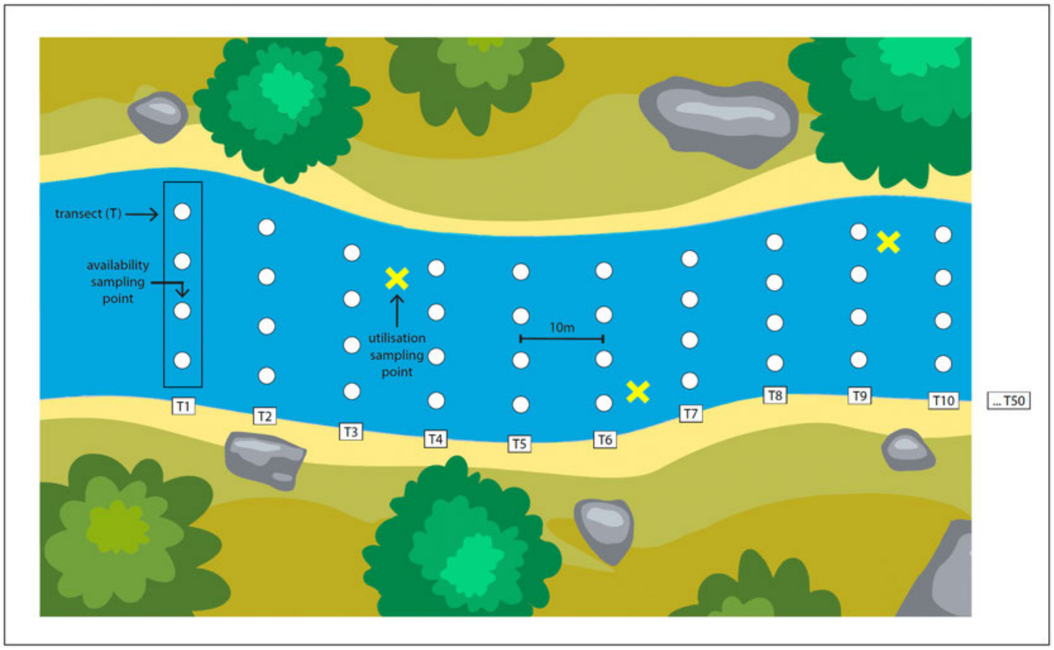


Figure 2.3. Study design of micro-habitat surveys. Each reach contained 50 transects, placed 10 m apart. Each transect comprised four equidistant availability sampling points (white circles). When an observation of fish was made, a weighted marker was dropped (yellow “x”). These comprised the utilisation sampling points. Environmental parameter measurements were taken at all sampling points.

Measurements at each availability sampling point were collected concurrent to measurements at each utilisation sampling point. After all fish markers had been dropped, the samplers moved in a grid pattern from the upstream-most transect in the downstream direction, measuring four environmental parameters at each sampling point: cover, depth, substratum particle size and water velocity (Table 2.2). The methods used to measure velocity and depth were selected due to their effectiveness in shallow rivers and their low cost. While alternative approaches – such as acoustic Doppler current profilers (Jowett et al. 2008) – are available, they were not necessary given the relatively shallow conditions of the Biedouw River.

Table 2.2. Environmental parameters measured at each sampling point and fish observation point.

Environmental parameter	Categories or units	Measurement tool	Definition
Cover	Categorical: woody matter, aquatic vegetation, rocky overhang, no cover	Visual determination	Type of structural cover in the water column if present at the sampling point.
Depth	Continuous: cm	Meter stick	Depth from substrate to surface.
Substratum particle size	Categorical if sand or bedrock. Numerical (to nearest cm) otherwise.	Meter stick	Size of substratum particle(s) at sampling point.
Water velocity	Continuous: m/s	Transparent velocity-head rod (TVHR)	Head depth (difference between superelevated depth on the upstream side of the TVHR and depressed depth on the downstream side) measured in the field to the closest centimetre. Head depth was then converted to water velocity using Equation 1.

Water velocity was measured with a transparent velocity-head rod (TVHR), a cost-effective method that allows for the efficient collection of a large number of data points (Fonstad et al. 2005). It consists of a transparent piece of Plexiglass, in this case measuring 105 (height) x 10 (width) x 1 cm (depth), with 1 cm tick marks (Figure 2.4). To take a measurement, the TVHR is placed into the water column with the bottom resting on the substrate and the broad face turned into the flow. The obstruction caused by the TVHR causes the water on the upstream side to become superelevated and the water on the downstream side to become depressed. The sampler stands downstream and records the upstream and downstream depths. As the TVHR is transparent, both measurements can be taken while standing downstream of the TVHR, precluding any impact of the sampler's position on the measurements. The difference between the upstream and downstream measurements is known as head depth, and can be converted to velocity (m/s) using a simple equation (Equation 1; Fonstad et al. 2005). This is discussed further under Section 2.2.3.1. Head depth was measured to the nearest 0.5 cm.

Figure 2.4. Photograph of the use of the transparent velocity-head rod (TVHR) used for the collection of velocity measurements during the 2021 sandfish microhabitat use surveys in the Biedouw River. It is placed into the water column with the broad face turned into the direction of the flow. Two measurements are then taken: the superelevated depth on the upstream face and the depressed depth on the downstream face. Photograph by Jeremy Shelton.

2.2.2.2 Spawning and egg observations

During the various spawning events that took place over the course of Survey 1 (September 8 – 10, 2022), opportunistic observations of sandfish spawning behaviour were made and recorded without a formal sampling protocol. Underwater footage was also taken during spawning and thereafter reviewed. In the days following spawning at the spawning grounds located within Andrea's Reach, the riffle was visited, and observations of eggs recorded. Eggs were approximately 2-3 mm in diameter and predominantly adhered to aquatic vegetation near the water's surface. Egg detection was carried out visually by carefully inspecting submerged vegetation. Four to seven days after spawning, a survey of 30 pools in the lower 15 km of the Biedouw River was carried out. During this survey, the substrate at the inflow of each pool was gently disturbed using a foot to kick up sand and fine particles into the water column. An aquarium net was then swept through the suspended material to collect any eggs. Any eggs captured within the net were counted and returned to the substrate. As sandfish were the only indigenous species present in the lower portion of the Biedouw River at the time, all eggs were assumed to belong to that species.

2.2.3 Data analysis

2.2.3.1 Data preparation

Each observation of sandfish was treated as a single data point, regardless of group size, as individuals in a school of fish are non-independent (Bovee 1986). While the group may show a preference for a specific habitat type, location of any individual fish within that school may be influenced more by schooling behaviour than by specific environmental conditions present at that location.

All adult size classes (A1, A2, A3) were combined into a single adult size class, and spawning adults were differentiated from non-spawning adults by the behaviour exhibited by the individual or group at the time of observation. If, for example, an A1 adult was observed feeding, it was classified as a "non-spawning adult" observation, while a group of six adults observed spawning was classified as a "spawning adult" observation.

Head depth was converted to velocity (m/s) using the velocity equation calibrated for measurements taken with a TVHR (Equation 1; Fonstad et al. 2005), where V is velocity, g is the acceleration due to gravity at 9.8 m/s^2, and h is the head depth in meters.

$$V = 0.728 \times ((2gh)^{0.5}) - 0.1126$$

Equation 1

Velocity measurements derived in this way from a TVHR are typically precise within 5% of measurements derived from a highly sensitive current meter (Fonstad et al. 2005). However, unlike more sophisticated current meters, TVHRs are unable to detect minimal velocities typical of slow-moving water at the edges of pools, as the head depth at such velocities is undetectable by eye (Fonstad et al. 2005). It was determined, however, that at such minimal flow the velocity experienced by fish is functionally zero, rendering the TVHR appropriate for the purpose of these analyses. In addition, at a head depth of zero, the velocity according to Equation 1 yields a negative velocity (-0.1126 m/s). In these cases, velocity was converted to a value of zero based on the reasoning that where there was no detectable difference in head, the velocity was functionally zero.

Although substrate size was measured to the nearest centimetre in all possible cases, sand and bedrock could not easily be measured, yielding two categorical substrate types among a continuous set of size data. To allow for standardised analyses, all substrate measurements were converted to substrate types according to a modified Wentworth scale (*sensu* Bain et al. 1985; Table 2.3). The only deviation from these conversions was the separation of the "sand" and "bedrock" categories. Bain et al. (1985) considered bedrock to occupy the same functional category as sand or silt. In the context of sandfish ecology, however, it is important that sand and bedrock be differentiated, as sandfish might utilise these substrates in different ways. For example, a young sandfish may be almost completely camouflaged against a background of sand, but not bedrock, and an adult may choose to consume algae by scraping it from bedrock, while choosing to consume detritus by sifting it from sand.

Table 2.3. The substrate type categories to which all substrate size data were converted, according to a modified Wentworth scale (Bain et al. 1985), with one deviation: sand and bedrock were retained as separate categories.

Substrate type	Size class (mm)
Sand	< 2
Gravel	2-16
Pebble	17-64
Cobble	65-256
Boulder	> 256
Bedrock	Fixed non-moveable rocky substrate

2.2.3.2 Habitat utilisation analyses

Simple non-parametric univariate tests were first carried out on the habitat utilisation dataset (the fish observations) to gauge differences in habitat utilisation between different life stages. As the data were non-normal, a Kruskal-Wallis Rank-Sum Test (R package 'stats' version 4.2.2; R Core Team 2022) was used to test the null hypothesis that depth utilised is equal across the four life stages of sandfish: spawning adult, non-spawning adult, larval, and juvenile. A Dunn's Multiple Comparison Test (package 'dunn.test'; Dinno 2017) was then carried out to test for differences between each two-group pairing. The same process was followed to test the null hypothesis that the velocity utilised by the four life stages of sandfish is equal.

A Fisher's Exact Test (R package 'stats' version 4.2.2; R Core Team 2022) with a simulated p-value was used to test the null hypothesis that there is no relationship between life stage and substrate type utilised. A Fisher's Exact Test is used in lieu of a Chi-Square Test when the sample size in one or more cross-categories is too small (e.g., if only two observations of larvae occurred over cobble). A core assumption of the Chi-Square Test is that all expected frequencies in the contingency table are greater than five. When the sample size of one or more cross-categories is too small, this assumption is violated, precluding the use of this test. When this is the case, a Fisher's Exact Test is used instead (McCrum-Gardner 2008). However, although the Fisher's Exact Test can handle small cross-category sample sizes, it is limited by the complexity of the contingency table. When the contingency table is too complex, it is recommended that some data either be grouped, or that one or more categories be excluded prior to running the test (McCrum-Gardner 2008). The grouping or exclusion of data must be biologically informed. In this case, the 4 (life stages) x 6 (substrate types) table created by the utilisation data was too complex for a Fisher's Exact Test. All data from non-spawning adults were therefore excluded, as these accounted for a small number (6.1%) of total observations and were less consequential than the three most critical life stages (spawning adult, larva, and juvenile). The resulting 3 x 6 contingency table was used as the input for a Fisher's Exact Test.

The test was carried out with a simulated p-value, which is useful when exact calculations are not feasible due to the size or complexity of the contingency table. The inclusion of this parameter causes the Fisher function to generate many random contingency tables containing the same row and column totals as the observed table, then calculates the proportion of simulated tables that are as, or more extreme, than the observed table's configuration. This proportion serves as the simulated p-value.

For similar reasons, a Fisher's Exact Test was again used to test the null hypothesis that there is no relationship between life stage and type of cover utilised. As for substrate, the "non-spawning adult" category was excluded prior to running the test to meet the complexity requirements of a Fisher's Exact Test. The resulting three (life stages) x four (cover types) contingency table was used as the input for the test. A simulated p-value was not necessary in this case.

2.2.3.3 Habitat preference analyses

While conventional habitat suitability criteria (HSC) are inherently univariate and do not account for imperfect detection, they remain widely used for identifying species' habitat requirements and as inputs for hydrological models (e.g., Dunn and Angermeier 2016; Szałkiewicz et al. 2022; Harris et al. 2024). Alternatives such as generalised additive models (GAMs) offer the advantage of incorporating interactions among habitat variables and can be more flexible in modelling complex ecological relationships (Jowett and Davey 2007). However, GAMs do not always outperform HSC in practice. One study compared the performance of HSC-based composite suitability indices (CSI) with GAMs and found that GAMs provided only marginal improvements in model fit. Given the transparency, ease of interpretation, and practical applicability of HSC for management, this approach was considered the most appropriate for this study.

2.2.3.3.1 Depth and velocity preference

To accurately assess habitat preference, availability data must represent the full range of conditions available to sandfish in proportion to their occurrence across the sampling area. Ideally, this is achieved by randomly distributing sampling points across the entire reach, ensuring that each habitat type is sampled in proportion to its actual spatial extent. However, in this study, availability data were collected systematically using 50 evenly spaced transects per 500 m reach, with four sampling points located equidistantly across each transect, regardless of channel width at that location, as in Shelton et al. (2008). As a result, wider parts of the channel were under-sampled relative to their area, and narrower parts over-sampled. To account for this, each availability sampling point was assigned a weight based on the surface area it represented. First, the surface area associated with each transect was estimated using the area of a trapezoid (Equation 2):

$$Surface\ area\ (SA) = h \times \frac{b_1 + b_2}{2}$$

Equation 2

Where h is the fixed distance between transects (10 m), and b_1 and b_2 are the estimated channel widths halfway between the transect of interest and the adjacent transects upstream and downstream, respectively (Figure 2.5). Because b_1 and b_2 were not measured directly, they were approximated as the average of the channel widths at the neighbouring transects. Each transect's surface area was then divided by four to assign an area to each of its four sampling points. Finally, in the habitat availability dataset, each sampling point was weighted by this estimated area (in square meters), so that the resulting frequency distribution of available habitat conditions was proportional to the spatial extent of those conditions in the reach.

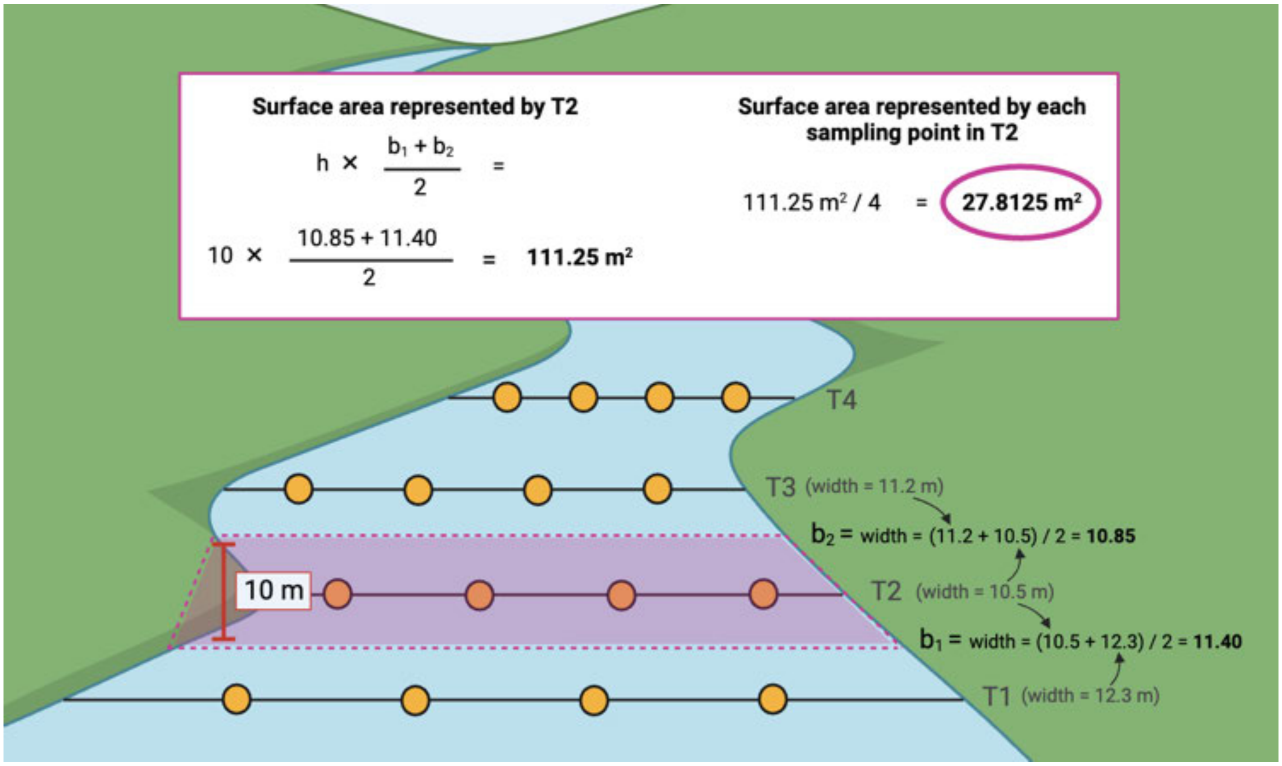


Figure 2.5. Schematic illustrating the process used to calculate the surface area represented by each systematic sampling point in the habitat availability dataset. Because each transect contained the same number of sampling points, regardless of channel width, individual sampling points represent different surface areas depending on the width of the transect. To correct for this, each transect's surface area was estimated using a trapezoidal approximation, where base$_1$ (b_1) and base$_2$ (b_2) represent the estimated channel widths at the midpoints between the transect of interest and the adjacent transects downstream and upstream, respectively. Since these midpoint widths were not directly measured, they were approximated as the average of the channel widths at the neighbouring transects. The height (*h*) of the trapezoid was fixed at 10 m, corresponding to the distance between transects. Each transect's surface area was then divided by four to assign a representative area to each of its four sampling points.

Utilisation data from non-spawning adults were excluded from the habitat preference analyses due to the small sample size (n = 13). Survey 1 therefore encompasses data for all spawning adult observations, survey 2 encompasses data for all larval observations, and survey 3 encompasses data for all juvenile observations.

Habitat Utilisation Curves (HUCs) and Habitat Availability Curves (HACs) were generated using the same method for depth and velocity, as both are continuous variables. All curves were first generated separately for each reach within a survey, resulting in three HACs and three HUCs per survey. Each HAC and HUC was produced via Kernel Density Smoothing (KDS), a method that replaces each sampling point with a Gaussian kernel and sums these kernels to estimate the overall density distribution. The resulting KDS curves closely resemble the pattern of a frequency histogram generated from the same data. This method is commonly used to produce continuous forage ratio functions (e.g., Hayes and Jowett 1994; Jowett 2002; Jowett et al. 2007; Paxton 2008).

Kernel Density Smoothing requires selecting a bandwidth, which controls the degree of smoothing: larger bandwidths produce smoother curves, while smaller ones yield more detailed, irregular curves (Appendix A 1). This is akin to choosing a bin width when creating histograms – narrow bins show finer patterns but may be too noisy for easy interpretation, while wider bins average over more data and may obscure finer patterns. Bandwidth selection is subjective and can influence both the appearance and interpretation of the resulting curves. Automatic bandwidth selection methods estimate an optimal bandwidth based on the data's spread and sample size. For example, the "nrd" method available in the geom_density function (package 'ggplot2'; Wickham 2016) calculates bandwidth using the standard deviation – however, it performs poorly on skewed data. For this reason, manual bandwidth selection is often preferred, aiming to produce biologically interpretable curves. To ensure comparability between life stages, the same bandwidth was applied across all analyses for a given variable (e.g., depth or velocity), though not between variables due to their differing units (cm and m/s, respectively). In this study, a bandwidth of 5 was used for depth (cm) and a bandwidth of 0.08 was used for velocity (m/s). While bandwidth affects curve dispersion, it has little effect on peak locations – i.e., where the preference index is highest – making it suitable for identifying general habitat preferences rather than precise optimal ranges. Appendix A 1 illustrates the above points in detail.

All HACs and HUCs were normalised to a maximum of 1 by dividing all y-ordinates by the maximum y-value, where 0 represents unsuitable habitat and 1 ideal habitat (Mäki-Petäys et al. 2002; Jowett and Davey 2007; Jowett et al. 2008; Szałkiewicz et al. 2022; Jithin et al. 2022). Normalising to 1 allows for comparisons between reaches and surveys and between HACs and HUCs, even when raw frequencies might differ.

Habitat preference is a function of utilisation and availability. To interpret preference meaningfully, utilisation data must be considered in the context of the habitat that is available to the fish. For example, if 54% of juvenile fish are observed at depths of 30-40 cm and only 15% at 50-60 cm, this might suggest a strong preference for shallower water. However, if 54% of available depths also fall within 30-40 cm and only 15% within 50-60 cm, then juveniles are simply using habitat in proportion to its availability – indicating no real preference.

The most commonly used preference index is the forage ratio (w; Equation 3). The numerator is the proportion of fish observations in habitat unit i and the denominator is the proportion of habitat unit i available in the reach. In other words, w is the relative use of a habitat divided by its relative availability (Jowett et al. 2008).

$$w = \frac{u_i/\sum u_i}{a/\sum a_i}$$

Equation 3

The forage ratio provides a simple way to quantify habitat preference but does not produce a continuous curve. To derive smooth Habitat Preference Curves (HPCs), KDS was used to generate continuous frequency distributions of utilisation (HUCs) and availability (HACs; Hayes & Jowett 1994; Jowett 2002; Jowett and Davey 2007). Preference was then calculated by dividing the smoothed utilisation density by the smoothed availability density at each point along the depth or velocity gradient. This ratio was plotted as a function of the shared x-axis (depth or velocity), resulting in a continuous HPC for each reach.

Kernel Density Smoothing can produce extremely small density values at the tails of distributions, particularly in areas where few or no data were collected. These small values may arise from limited sampling at marginal depths or velocities (Jowett and Davey 2007), or as an artefact of the smoothing process itself. Even when no data exist at the extremes, Gaussian kernels fitted to nearby values can extend into these regions, resulting in imperceptible but non-zero density estimates (e.g., on the order of $1e^{-18}$). When HPCs are derived by dividing smoothed HUCs by smoothed HACs, dividing two very small values can produce spuriously high preference ratios. This can lead to misleading results, where, for example, depths with no true preference appear to have extremely high preference values simply because both the utilisation and availability densities are near zero, and the ratio becomes exaggerated. In such cases, artificial peaks in the HPC can obscure genuine preference patterns, highlighting the importance of identifying and addressing tail artifacts when producing these curves.

To address the issue of spurious peaks in HPCs, a threshold was applied during the calculation of preference values: any points where the HAC density was less than 1×10^{-16} were excluded from the HPC. This ensured that preference values were not distorted by divisions involving extremely small denominators, which can artificially inflate preference estimates at the tails of the distribution. This adjustment was applied only to depth, as spurious peaks did not arise in the velocity HPCs. This threshold did not exclude meaningful data, as none of the 260 observations of spawning, larval, or juvenile sandfish occurred at depths greater than 60 cm. Thus, any apparent preference beyond this depth is clearly not representative of true habitat use but is rather an artefact of the smoothing process. This process is illustrated in detail in Appendix A 2.

Once individual HPCs were created for each reach within a survey, a composite reach-averaged HPC was generated to represent habitat preference over the greater sampling area (Jowett and Davey 2007; Jowett et al. 2008). Reach-specific HUCs, HACs and HPCs are valuable for illustrating differences in available and preferred habitat between individual reaches, while reach-averaged HPCs enable clear between-group comparisons by summarising overall preferences at a given life stage. To calculate reach-averaged HPCs, the y-values from each reach were averaged at each shared x-value. For example, at a depth of 15 cm, spawning adults had a normalised preference of 0.375 at Andrea's Reach, 0.998 at Jensen's Reach, and 0.674 at Leonard's Reach. The reach-averaged preference value at 15 cm would therefore be 0.682. Once reach-averaged preference values had been calculated for all x-values, they were normalised to 1 and plotted.

For both depth and velocity, the Kolmogorov-Smirnov (KS) two-sample test (R package 'stats' version 4.2.2; R Core Team 2022) was used to test whether habitat use differs from habitat availability for each life stage based on data pooled across all three reaches.

2.2.3.3.2 Substrate preference

As discussed in Section 2.2.3.1 and Table 2.3, all continuous substrate size values were converted according to a modified Wentworth scale. Although substrate types are categorical, they are often assigned numeric values in order to allow for habitat preference analysis (e.g., Mäki-Petäys et al. 2002; Heath et al. 2015). A numeric substrate class of 1-6 was assigned to each substrate type (Table 2.4).

Table 2.4. Substrate types and assigned numeric substrate class to allow habitat preference analysis.

Substrate type	Substrate class
Sand	1
Gravel	2
Pebble	3
Cobble	4
Boulder	5
Bedrock	6

It is important to note that although a linear scale was assigned to the substrate types, the increase in size from one substrate type to the next is non-linear (see Table 2.3). Interpretation of results should therefore be carried out with caution, especially when interpreting preference at points between substrate classes. Despite these considerations, it is still useful to conduct a habitat preference analysis, as it facilitates a visual comparison of differences in substrate preferences between life stages.

The bandwidth selected for the analysis of substrate utilisation and availability was 0.5, as it produced a smooth curve that neither under-smoothed nor over-smoothed the data. HUCs and HACs were generated via KDS as for depth and velocity, although no spurious peaks were present in this case.

A Fisher's Exact Test (R package 'stats' version 4.2.2; R Core Team 2022) with a simulated p-value was used to test the null hypothesis that there is no relationship between utilised and available substrate types for each life stage. Data across all three reaches were pooled.

2.2.3.3.3 Cover preference

Cover data comprised four discrete categories: no cover, vegetative cover, rocky overhang (rocky cover) and woody matter (woody cover). As there is no linear relationship between different types of cover, habitat preference analysis could not be conducted as for depth, velocity, or substrate. Instead, bar plots were generated for utilisation data and for availability data, and preference plots generated by dividing utilisation values by availability values. All plots were normalised to one, as before.

It is important to note that the collection of cover utilisation data is inherently biased, as fish that are actively seeking shelter under vegetation, rocky overhangs, or woody matter are less likely to be sampled than those in open water. This is especially true for larval fish, which would be especially difficult to observe while utilising cover. This can result in very small sample sizes of utilisation observations for certain categories of cover. If these small samples are coupled with small samples of available habitat in the same category, it may produce a spuriously high preference for that cover category, which is a result of small sample sizes more than being reflective of reality. This is analogous to the spurious peaks present in some depth and velocity HPCs prior to their removal. This consideration, together with the inherent bias of sampling fish under cover, means that interpretations of cover preference should be viewed with caution and treated as indications of preference rather than definitive results.

A Fisher's Exact Test (R package 'stats' version 4.2.2; R Core Team 2022) was used to test the null hypothesis that there is no relationship between utilised and available cover types for each life stage. Data across all three reaches were pooled. All work was carried out with appropriate permits and ethics approval (Appendix B 1; Appendix B 2).

2.2.3.4 Weighted usable area (WUA)

Weighted usable area (WUA) is an index commonly used in hydraulic modelling (i.e., PHABSIM) that quantifies the habitat available to an organism (Bovee 1986). It is calculated by weighting the wetted area of a river reach by the suitability of that area for the organism's use (Paxton 2008). The typical output is a figure plotting WUA across a range of measured or modelled river discharges, illustrating how available habitat changes with flow conditions. Although hydraulic modelling is beyond the scope of this survey, WUA was calculated for each sandfish life stage to quantify and compare suitable habitat availability during key periods of the species' life cycle (in m^2/m of channel length).

Velocity and depth values were categorised into bins based on observed distributions. For each bin, a suitability index (SI) score ranging from 0 (unsuitable) to 1 (optimal) was assigned, calculated from the relative frequency of sandfish observations within that bin. Substrates, classified into categorical types according to the Wentworth scale, did not require binning; SI scores were calculated directly for each substrate category based on the relative frequency of sandfish observations within each substrate type.

Independent suitability index scores for depth (SI_{depth}), velocity ($SI_{velocity}$), and substrate ($SI_{substrate}$) were then assigned to each point in the habitat survey and multiplied together to produce the composite habitat suitability index (HSI; Jowett and Davey 2007; Jowett et al. 2008):

$$HSI = SI_{depth} \times SI_{velocity} \times SI_{substrate}$$

Equation 4

Weighted usable area per point (WUA_i) was calculated by multiplying its composite HSI score by the surface area of its corresponding cell. Survey-level WUA was then obtained by summing the WUA values across all availability survey points across the three survey reaches (Bovee 1986):

$$WUA_{survey} = \sum WUA_i$$

Equation 5

Because hydraulic modelling falls beyond the scope of this study, survey-level WUA values are presented as a single value, in square metres, representing the total area of suitable habitat available within the sample reaches during a particular survey. To allow comparison of relative suitable habitat availability between surveys, weighted usable area is also expressed as square metres per metre of channel length. This approach offers a snapshot of habitat availability under the specific flow conditions present at the time.

2.3 Results

2.3.1 Habitat utilisation

A total of 273 sandfish observations comprising 1,234 individual fish were made over the course of the study, ranging in group size from 1-30 individuals (Table 2.5). While maximum group size varied between life stages, mean group size was fairly consistent.

Table 2.5. Total number of observations, individual fish, and group sizes according to life stage. Number of fish does not necessarily refer to number of unique individuals, as spawning adults, for example, could be counted more than once if they engaged in more than one spawning observation in a given evening. Observations of non-spawning adults, larvae, and juveniles would most likely correspond to unique individuals, since they exhibited limited movement during our observations, and therefore were mostly likely only counted once upon initial observation, before samplers moved on.

Survey	Life stage	Number of observations	Number of fish	Min-max number per group	Mean number per group ± SE
1	Non-spawning adult	13	49	1 – 11	3.77 ± 0.95
1	Spawning adult	68	300	2 – 12	4.41 ± 0.23
2	Larva	116	466	1 – 24	4.02 ± 0.43
3	Juvenile	76	419	1 – 30	5.51 ± 0.66

Non-spawning adults were most often observed resting, with three observations of schooling, and one each of chasing and swimming (Figure 2.6). Spawning adults were, by virtue of their categorisation, always observed spawning in groups of two or more. Larvae were most often observed swimming alone, followed by resting. Juveniles showed the most even distribution of behaviours and were most often observed resting, followed by feeding, schooling, and finally swimming.

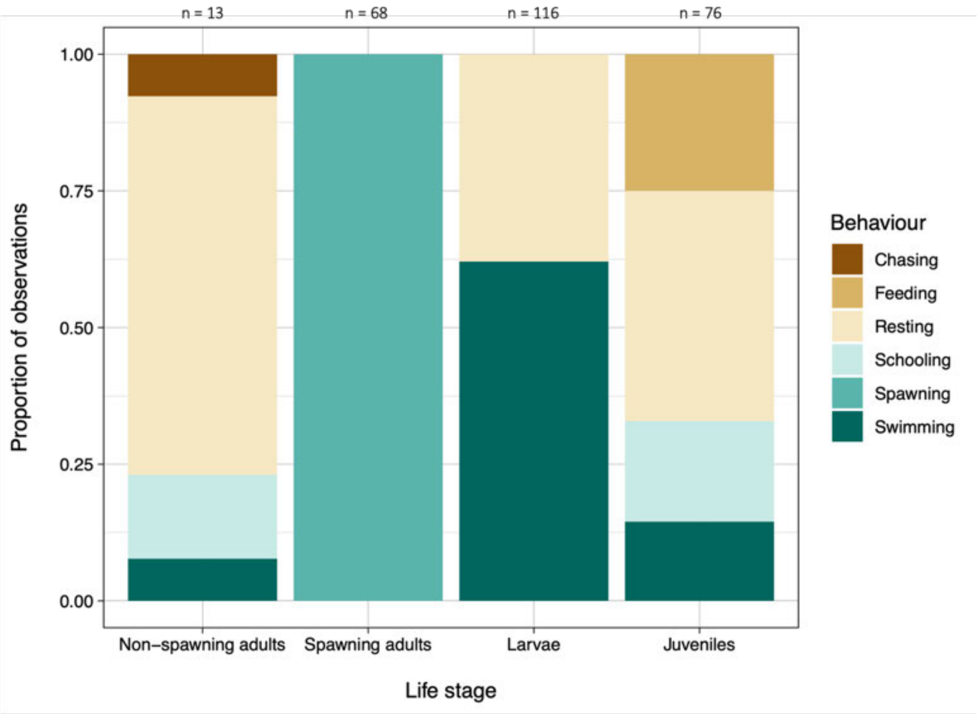


Figure 2.6. Behaviours displayed by groups of sandfish observed over the course of three surveys. Non-spawning and spawning adults were observed during the same survey (Survey 1).

The depth utilised by different life stages was not equal ($X^2 = 141.73$, df = 3, $p < 0.001$). All life stages utilised significantly different depths, except for non-spawning adults and spawning adults, and non-spawning adults and juveniles (Table 2.6). Non-spawning adults utilised the widest range of depths, with similar mean depths utilised as juveniles (Figure 2.7). Larvae utilised the shallowest depths.

Table 2.6. Results of Dunn's Multiple Comparison Test showing which pairwise groupings differ significantly from one another in depth utilised (* = p < 0.05, ** = p < 0.01, *** = p < 0.001). Mean depth utilised by each life stage is also reported.

Life stage	Mean depth utilised ± SE (cm)	Comparison	Z-value	p-value
Non-spawning adult	30.31 ± 5.99	Spawning adults	1.005	0.158
		Larvae	-4.520	< 0.001***
		Juveniles	1.240	0.107
Spawning adult	18.24 ± 1.57	Larvae	-6.664	< 0.001***
		Juveniles	4.052	< 0.001***
Larva	9.75 ± 0.53	Juveniles	-11.480	< 0.001***
Juvenile	27.74 ± 1.14	--		

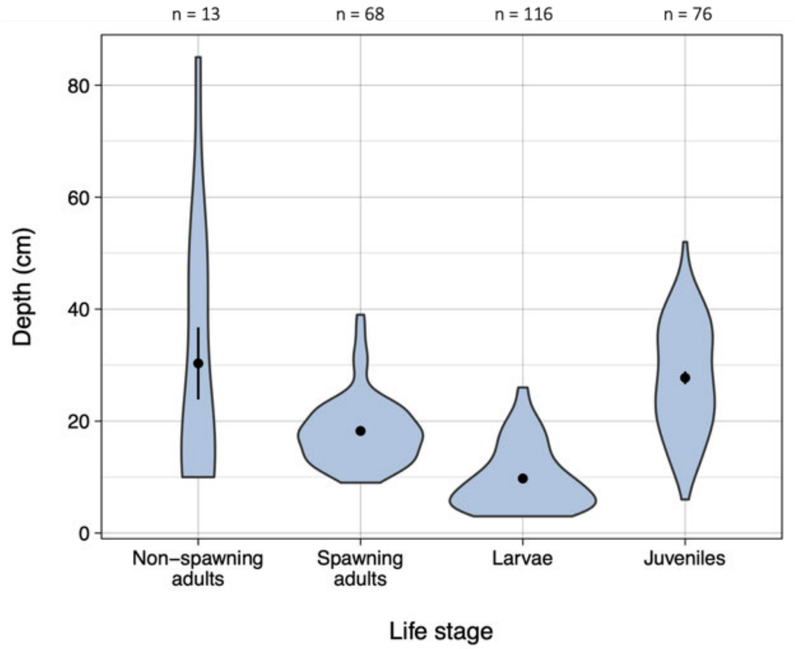


Figure 2.7. Violin plot depicting the distribution of depths utilized by each life stage. Dots and error bars represent mean depth occupied ± SE. The width of each violin plot at any given point along the y-axis is indicative of the density of data points at that specific value: wider sections of the plot indicate a higher density of data points at that given depth, whereas narrower sections indicate lower densities.

The velocity utilised by different life stage was not equal (X^2 = 211.27, df = 3, $p < 0.001$). Pairwise comparisons among all life stages differed significantly from one another except for larvae and juveniles (Table 2.7). Spawning adults utilised the widest range of velocities, as well as the fastest mean velocity (Figure 2.8). Larval and juvenile fish utilised the lowest mean velocities within a relatively narrow range.

Table 2.7. Results of Dunn's Multiple Comparison Test showing which pairwise groupings differ significantly from one another in velocity utilised (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Mean velocity utilised by each life stage is also reported.

Life stage	Mean velocity utilised ± SE (cm)	Comparison	Z-value	p-value
Non-spawning adult	0.182 ± 0.036	Spawning adult	-2.741	0.003**
		Larva	4.288	< 0.001***
		Juvenile	3.628	< 0.001***
Spawning adult	0.390 ± 0.017	Larva	13.645	< 0.001***
		Juvenile	11.494	< 0.001***
Larva	0.010 ± 0.003	Juvenile	-1.121	0.131
Juvenile	0.024 ± 0.006	--	--	--

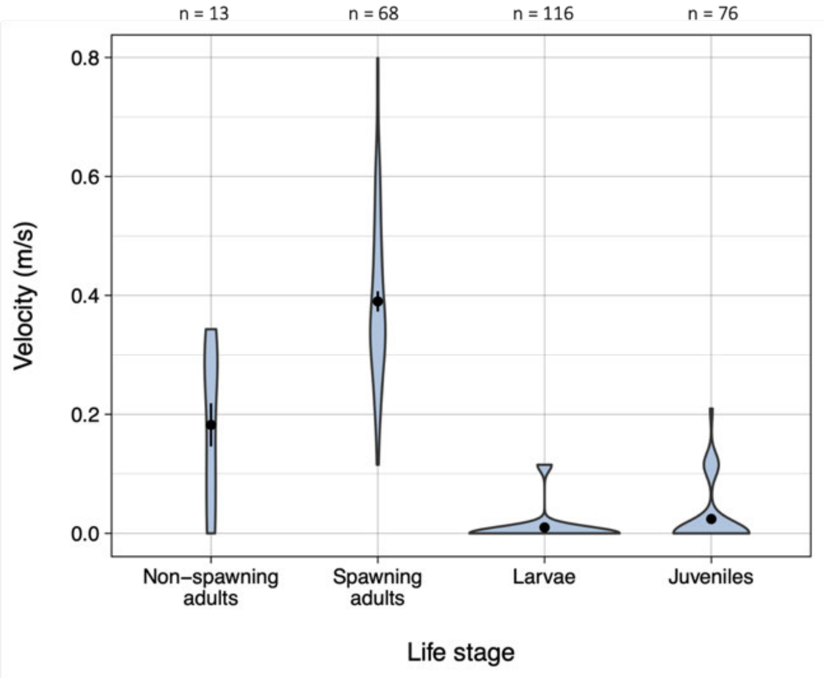


Figure 2.8. Violin plot depicting the distribution of velocities utilized by each life stage. Dots and error bars represent mean velocity occupied ± SE.

Forty-two percent of all observations occurred over sand, followed by cobble (23%), bedrock (21%), pebble and boulder (5% each), and gravel (3%; Table 2.8). Most juvenile and larval observations occurred over sand (53% and 57%, respectively), whereas cobble comprised the largest proportion of substrate utilised by both non-spawning and spawning adults (31% and 57%, respectively; Figure 2.9). The Fisher's Exact Test indicated that there was a statistically significant association between life stage (spawning adult, larva, and juvenile) and substrate type utilised ($p < 0.05$).

Table 2.8. Proportion of sandfish observations occurring at each substrate and cover type, grouped by life stage.

	Substrate						Cover			
Life stage	Sand	Gravel	Pebble	Cobble	Boulder	Bedrock	None	Vegetative	Rocky	Woody
Non-spawning adult	0.154	0.077	0.154	0.308	0.154	0.154	0.692	0.231	0.000	0.077
Spawning adult	0.103	0.000	0.132	0.574	0.088	0.103	0.794	0.191	0.000	0.015
Larva	0.569	0.052	0.017	0.112	0.043	0.207	0.526	0.336	0.138	0.000
Juvenile	0.526	0.026	0.013	0.105	0.013	0.316	0.803	0.066	0.105	0.026

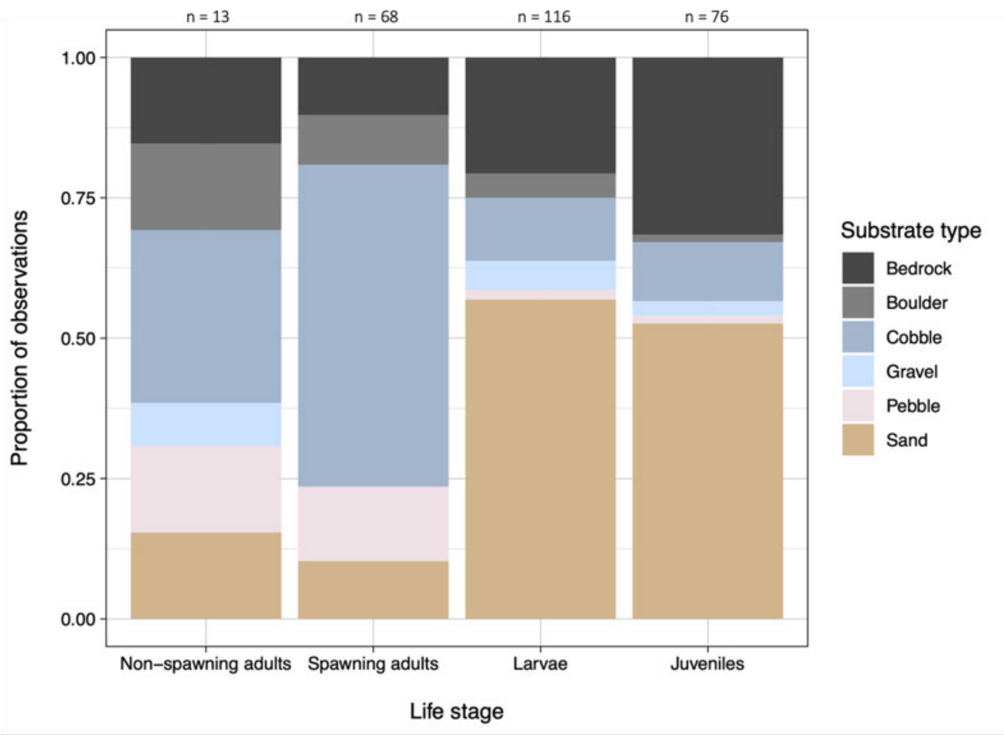


Figure 2.9. Proportion of observations occurring over each substrate type, grouped by life stage.

Sixty-eight percent of all observations occurred in open water (i.e., not associated with cover), followed by observations under vegetative cover (22%), rocky overhangs (8%), and woody matter (2%; Table 2.8). Larval fish were observed utilising the highest proportion of cover (47%) compared to other life stages (Figure 2.10). There was a relationship between life stage (spawning adult, larva, and juvenile) and cover type utilised (Fisher's Exact Test, $p < 0.05$). Sample sizes of observations made under each cover type are listed in Table 2.9.

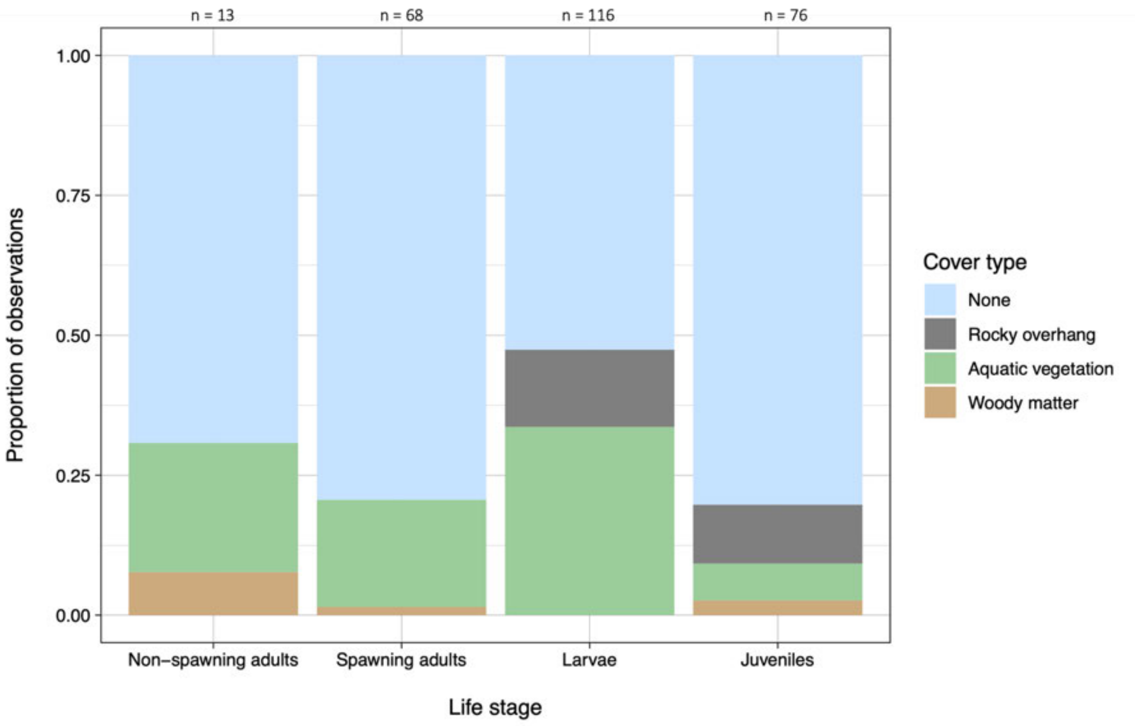


Figure 2.10. Proportion of observations corresponding to each cover type, grouped by life stage.

Table 2.9. Sample sizes of observations made under each cover type, grouped by survey and reach.

Survey	Reach	Total observations	None (n)	Vegetation (n)	Woody matter (n)	Rocky overhang (n)
1	Andrea's	24	17	6	1††	0†
	Jensen's	29	22	7	0†	0†
	Leonard's	15	15	0†	0†	0†
2	Andrea's	59	33	15	0†	11
	Jensen's	35	13	18	0†	8
	Leonard's	22	15	6	0†	1††
3	Andrea's	43	33	3††	0†	7
	Jensen's	15	13	0†	2††	0†
	Leonard's	18	15	2††	0†	1††

† sample sizes of 0
†† small sample sizes of 1-3

2.3.2 Habitat preference

2.3.2.1 Depth

Throughout the three surveys, Andrea's Reach covered the largest wetted area, with Jensen's and Leonard's reaches covering similar areas in any given survey (Table 2.10). Within each reach, wetted area changed slightly from survey 1 to survey 2, increasing by a small amount at Andrea's Reach and decreasing at Jensen's and Leonard's reaches. The largest changes occurred from survey 2 to survey 3, with the reaches shrinking by a cumulative 8,024 m^2 in wetted area.

Table 2.10. Total surface area covered by each reach over the course of the three surveys. The change in area from one survey to the next is given in the last column. All surveys were carried out in 2021.

Reach	Survey	Date	Total area (m^2)	Change (m^2)
Andrea's	1	9-10 Sept	13,394	--
	2	4-6 Oct	13,523	+ 129
	3	20-22 Nov	10,105	-3,418
Jensen's	1	9-10 Sept	10,395	--
	2	4-6 Oct	9,390	-1,005
	3	20-22 Nov	6,917	-2,473
Leonard's	1	9-10 Sept	10,030	--
	2	4-6 Oct	9,453	-577
	3	20-22 Nov	7,320	-2,133

Available depths ranged from 1- 99 cm (mean = 31.7 ± 0.8 SE) in survey 1, from 1- 94 cm (mean = 24.2 ± 0.8 SE) in survey 2, and from 0.5- 88 cm (mean = 18.9 ± 0.7 SE) in survey 3. Andrea's Reach consistently had the highest proportion of shallow (< 30 cm) habitat, as illustrated by the high degree of positive skew in the distribution of the reach's HACs (Figure 2.11 a, e, i). Leonard's Reach had the highest proportion of deep (> 60 cm) habitat, as illustrated by the lower degree of positive skew (Figure 2.11 c, g, k). Jensen's Reach fell somewhere in the middle, offering a higher proportion of deep habitat than Andrea's Reach but a lower proportion than Leonard's Reach (Figure 2.11 b, f, j). The deepest habitats (> 80 – 99 cm) were available only at Leonard's Reach across surveys.

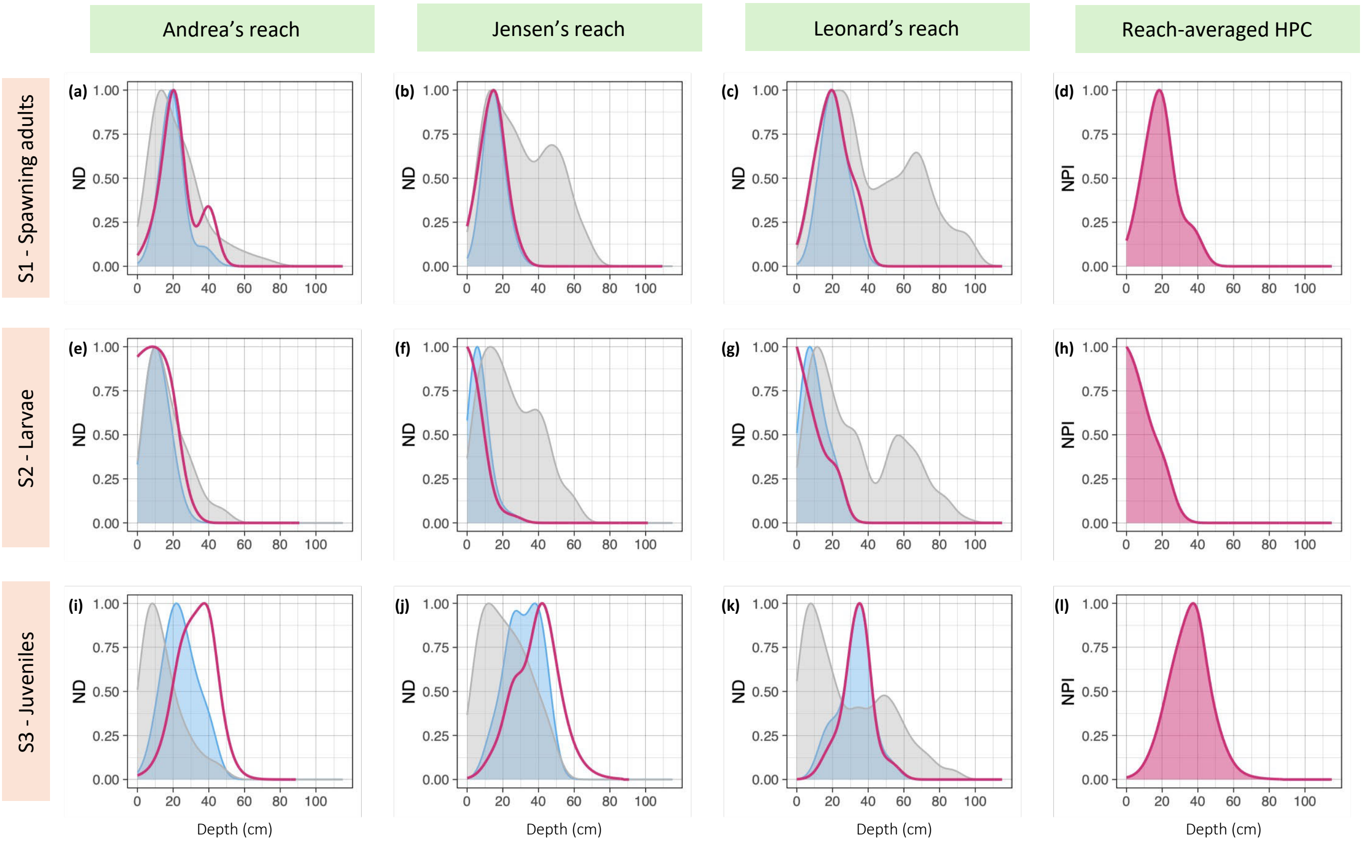


Figure 2.11. Depth habitat availability (HAC; grey), utilisation (HUC; blue), and preference (HPC; pink) curves for spawning adults (survey 1; panels a-c), larvae (survey 2; panels e-g), and juveniles (survey 3; panels i-k). Panels (d), (h), and (l) show the reach-averaged HPC for each life stage, calculated by averaging the HPCs across the three reaches sampled in each survey. These summarised curves illustrate the overall depth preferences for each life stage in the sampling area. Curves were generated using Kernel Density Smoothing (KDS; bandwidth = 5). Spurious peaks in HPCs were removed following the methods described in section 2.2.3.3.1 and Appendix A 2.

Although depths of up to 99 cm were available to spawning adults, average depth utilised was 18.20 cm (± 0.74 SE) and never exceeded 39 cm. The average depth utilised by larvae was 9.75 cm (± 0.53 SE) and never exceeded 26 cm, despite available depths of up to 94 cm. While there were available depths of up to 88 cm during survey 3, juveniles were only observed at a maximum depth of 52 cm, with an average utilised depth of 27.70 (± 1.14 SE).

Within each survey, there was some variation in the position of HPCs across reaches, but the general shape stayed largely consistent. Spawning adults showed a preference for slightly shallower habitat in Jensen's Reach than in Andrea's and Leonard's reaches (Figure 2.11 a-c). Larvae preferred the shallowest depths across reaches, although the range of preferred depths was narrowest in Jensen's Reach and widest in Andrea's Reach (Figure 2.11 e-g). Juveniles' depth preferences were largely consistent across reaches, with a somewhat narrower depth preference in Leonard's Reach (Figure 2.11 i-k).

Averaging depth preferences across reaches showed that peak depth preferences differed notably between life stages. Spawning adults' depth preference peaked at 18.2 cm, larvae's at 0.0 cm, and juveniles' at 37.4 cm (Figure 2.11 d, h, l). Depth utilisation differed significantly from available conditions for all life stages, indicating true preferences (Table 2.11). Spawning adults and larvae preferred proportionally shallower habitat than what was available, while juveniles preferred proportionally deeper habitat than what was available.

Table 2.11. Comparison between available habitat and habitat utilised by sandfish of different life stages. The Kolmogorov-Smirnov D-statistic is reported. Alpha = 0.05. Asterisks denote significant differences ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***). Sample size (N) represents m^2 for available habitat and observation count for utilised habitat.

	Depth (m) available		Depth (m) utilised			Velocity (m/s) available		Velocity (m/s) utilised		
Life stage	N†	Mean ± SE	N†	Mean ± SE	D-statistic	N†	Mean ± SE	N†	Mean ± SE	D-statistic
Spawning adult	16912	30.8 ± 0.16	68	18.20 ± 0.74	0.44***	16909	0.19 ± 0.00	68	0.39 ± 0.02	0.62***
Larva	16184	23.7 ± 0.14	116	9.75 ± 0.53	0.39***	16183	0.12 ± 0.00	116	0.01 ± 0.00	0.49***
Juvenile	12172	20.6 ± 0.15	76	27.7 ± 1.14	0.40***	12170	0.05 ± 0.00	76	0.02 ± 0.01	0.12

† The sample sizes for available depth and velocity differ slightly due to the process by which the availability datasets were mutated into a format appropriate for KDS curve generation and the KS test. These differences in sample size do not influence the analyses, as the KS test was applied to depth and velocity separately.

2.3.2.2 Velocity

Velocities ranging from 0- 1.35 m/s (mean = 0.21 ± 0.01 SE) were available in survey 1, from 0- 1.18 m/s (mean = 0.13 ± 0.01 SE) in survey 2, and from 0- 0.61 (mean = 0.07 ± 0.00 SE) in survey 3. Throughout the study period, the most abundant velocities were largely the slowest, narrowing notably from one survey to the next (Figure 2.12). Andrea's and Jensen's reaches had a lower proportion of the slowest velocities than Leonard's Reach in survey 1 (Figure 2.12 a-d). By survey 3, all available velocities were restricted to the lowest values (Figure 2.12 i-k).

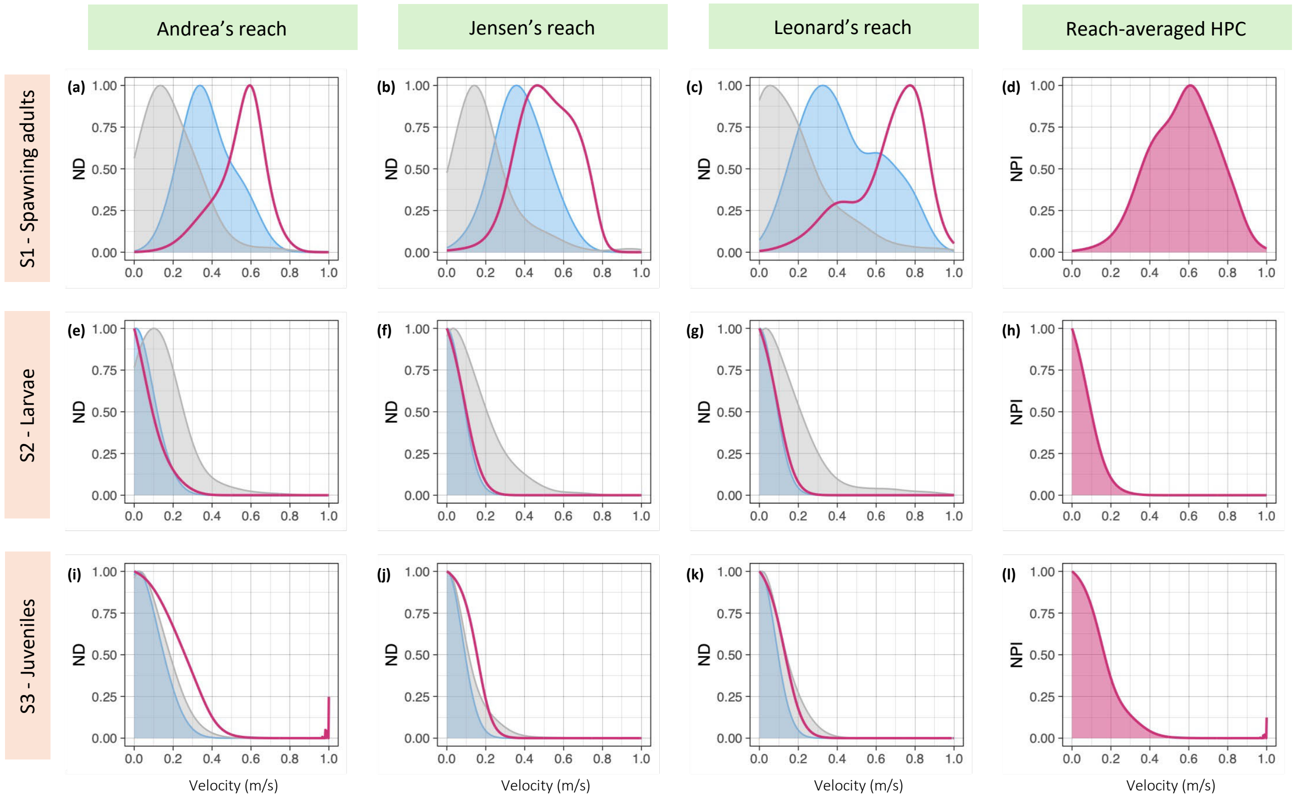


Figure 2.12. Velocity habitat availability (HAC; grey), utilisation (HUC; blue), and preference (HPC; pink) curves for spawning adults (survey 1; panels a-c), larvae (survey 2; panels e-g), and juveniles (survey 3; panels i-k). Panels (d), (h), and (l) show the reach-averaged HPC for each life stage, calculated by averaging the HPCs across the three reaches sampled in each survey. These summarised curves illustrate the overall velocity preferences for each life stage in the sampling area. Curves were generated using Kernel Density Smoothing (KDS; bandwidth = 0.08).

Spawning adults utilised, on average, faster velocities than were available: mean velocity utilised was 0.357 m/s (± 0.018 SE) compared with the mean of 0.209 m/s available. Velocity use by spawning adults never exceeded 0.800 m/s, despite the availability of up to 1.347 m/s. The average velocity utilised by larvae was 0.010 m/s (± 0.003 SE) and never exceeded 0.115, despite available velocities of up to 1.177 m/s. While available velocities reached 0.608 m/s in survey 3, juveniles only utilised a maximum velocity of up to 0.210 m/s (± 0.006 SE).

The highest between-reach variation in velocity preference occurred in survey 1, where spawning adults appeared to prefer a wider range of velocities in Jensen's and Leonard's reaches than in Andrea's Reach, with an apparent preference for faster velocities in Leonard's Reach than in either Andrea's or Leonard's reaches (Figure 2.12 a-c). Larvae showed a consistent preference for the slowest available velocities across reaches (Figure 2.12 e-g). While juveniles also appeared to prefer the slowest velocities, there was some among-reach variation, with juveniles in Andrea's Reach preferring a wider range of velocities than in either of the other two reaches (Figure 2.12 i-k).

Reach-averaged velocity preferences varied notably between spawning adults and the other two life stages (Figure 2.12 d, h, l). Velocity preferences peaked at 0.61 m/s for spawning adults, while both larvae and juveniles showed a strong preference for the slowest velocities, with peaks at 0.0 cm. Velocity utilisation differed significantly from available conditions for spawning adults and larvae, but not for juveniles (Table 2.11). Spawning adults preferred faster velocities than were generally available, whereas larvae preferred the opposite.

2.3.2.3 Substrate

Across surveys, the most abundant available substrates were, on average, cobble (28.4%), sand (27.3%) and bedrock (25.7%; Table 2.12). There was a moderate amount of boulder available (11.3%) and low amounts of pebble and gravel (5.87% and 1.43%, respectively). The distribution of available substrates varied between reaches, with Andrea's Reach comprising primarily cobble and bedrock; Jensen's Reach comprising primarily sand, cobble, and bedrock; and Leonard's Reach comprising primarily sand and cobble (Figure 2.13). While substrate composition per reach stayed relatively consistent from one survey to the next, there were some exceptions: the proportions of boulder and cobble decreased notably in Jensen's Reach between the start and end of the study period, while the proportion of boulder also appeared to decrease in Andrea's Reach from survey 1 to survey 3.

Table 2.12. Proportions of substrate and cover types available during each survey. Each value indicates its proportion in terms of area (m^2) relative to the total area for that survey.

	Substrate						Cover			
Survey	Sand	Gravel	Pebble	Cobble	Boulder	Bedrock	None	Vegetative	Rocky	Woody
Survey 1	0.284	0.005	0.031	0.284	0.155	0.242	0.758	0.221	0.000	0.022
Survey 2	0.251	0.016	0.072	0.304	0.102	0.254	0.655	0.281	0.052	0.012
Survey 3	0.285	0.022	0.073	0.264	0.081	0.276	0.665	0.227	0.081	0.026

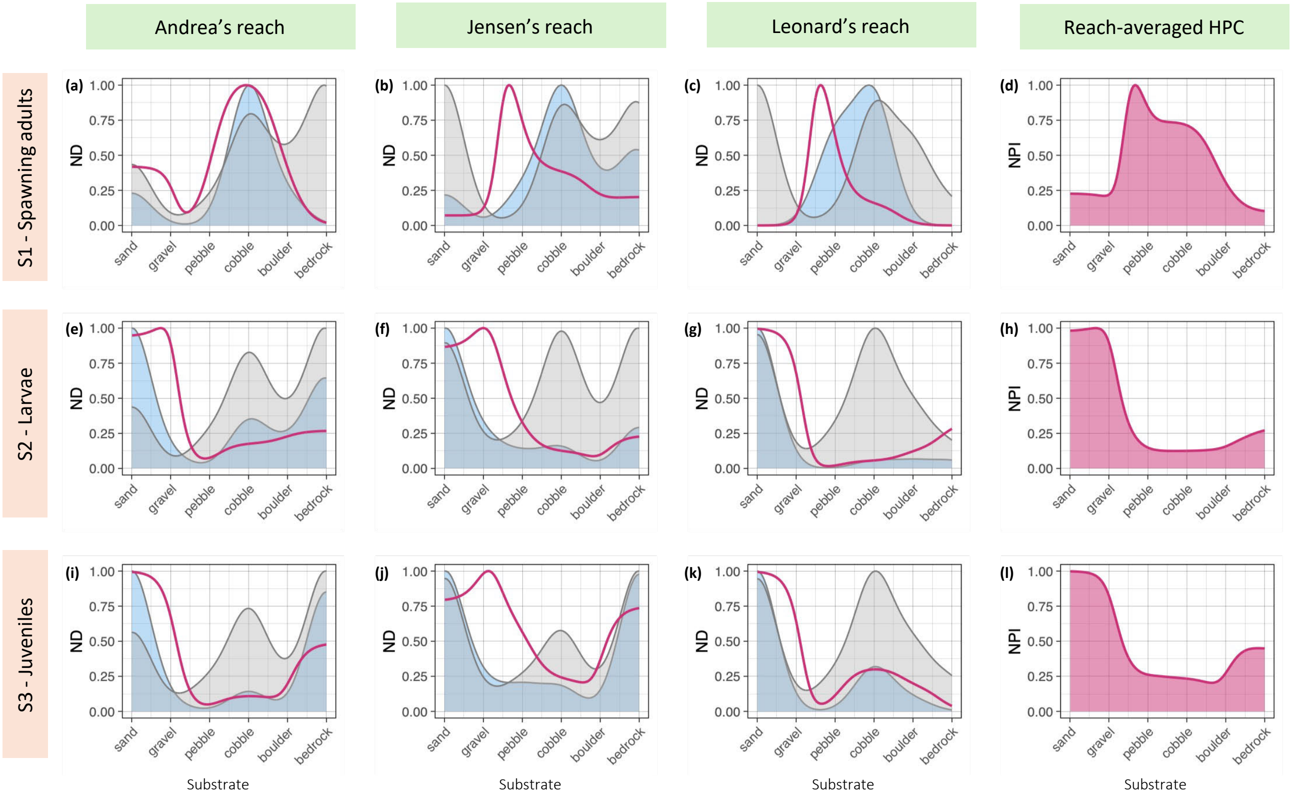


Figure 2.13. Substrate habitat availability (HAC; grey), utilisation (HUC; blue), and preference (HPC; pink) curves for spawning adults (survey 1; panels a-c), larvae (survey 2; panels e-g), and juveniles (survey 3; panels i-k). Panels (d), (h), and (l) show the reach-averaged HPC for each life stage, calculated by averaging the HPCs across the three reaches sampled in each survey. These summarised curves illustrate the overall substrate preferences for each life stage in the sampling area. Curves were generated using Kernel Density Smoothing (KDS; bandwidth = 0.5).

Spawning sandfish utilised cobble in the highest proportions, while larvae utilised mostly sand (Figure 2.13). Juveniles in Leonard's Reach also utilised sand in the highest proportions, but utilised sand and bedrock in roughly equal proportions in Andrea's and Jensen's reaches (Figure 2.13 i, j).

There was high variation in between-reach substrate preference in survey 1. While spawning adults showed the highest preference for cobbles in Andrea's Reach, they demonstrated the highest preference for pebble in Jensen's and Leonard's reaches (Figure 2.13 a-c). Larval preference was consistent across reaches, concentrated around sand and gravel (Figure 2.13 e-g). Juveniles showed a high preference for sand and gravel in Andrea's and Jensen's reaches, and moderate preference for bedrock (Figure 2.13 i, j). While they showed a similarly high preference for sand and gravel in Leonard's Reach, they did not show a similar preference for bedrock (Figure 2.13 k).

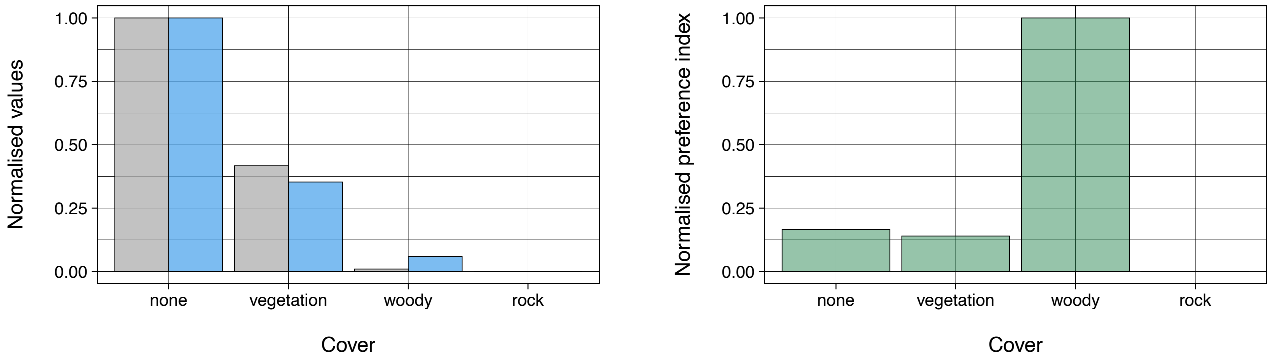
The calculation of reach-averaged substrate preference revealed similar preferences among larvae and juveniles, with peak preferences around sand and gravel (Figure 2.13 h, l). Spawning adults showed a peak preference for pebble, with a moderate preference for cobble. Sandfish of all life stages were distributed non-randomly across the different substrate types ($p < 0.001$ in all cases), indicating true preferences (Table 2.13).

Table 2.13. Comparison between available and utilised substrate and cover, grouped by life stage. Fisher's Test was used to test for differences between available and utilised habitat. Alpha = 0.05. Asterisks denote significant differences ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***).

Life stage	Substrate	Cover
Spawning adult	$p < 0.001$***	$p > 0.05$
Larva	$p < 0.001$***	$p < 0.001$***
Juvenile	$p < 0.001$***	$p < 0.01$**

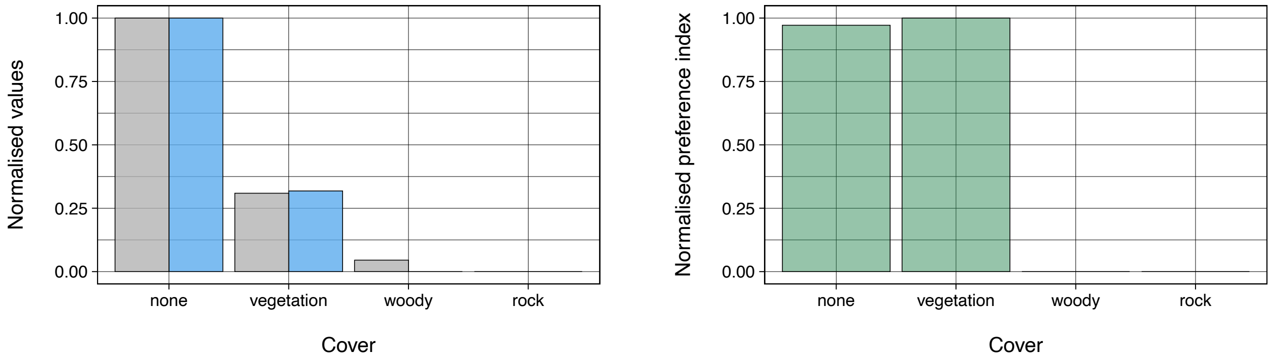
2.3.2.4 Cover

An average of 69.3% of available area across the three surveys had no cover present (Table 2.12). The most abundant cover type was aquatic vegetation, occurring over an average of 24.3% of available area. Rocky cover comprised an average of 4.4% of available habitat, followed by woody matter at 2.0%. The proportional distribution of available cover types was consistent across surveys, with no cover as the most abundant cover type, followed by vegetation, and then by woody and rocky cover, which occurred infrequently across the study area (Figure 2.14, Figure 2.15, and Figure 2.16).



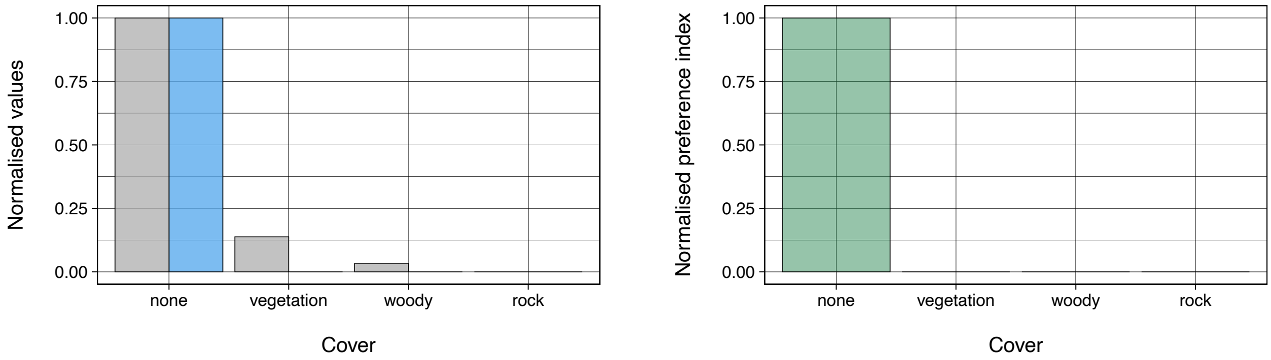
(a) Andrea's Reach

(b) Andrea's Reach



(c) Jensen's Reach

(d) Jensen's Reach

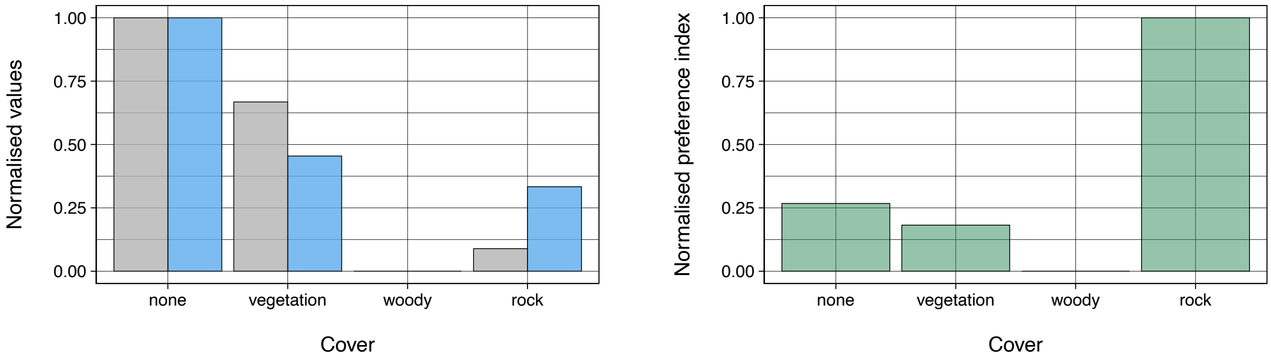


(e) Leonard's Reach

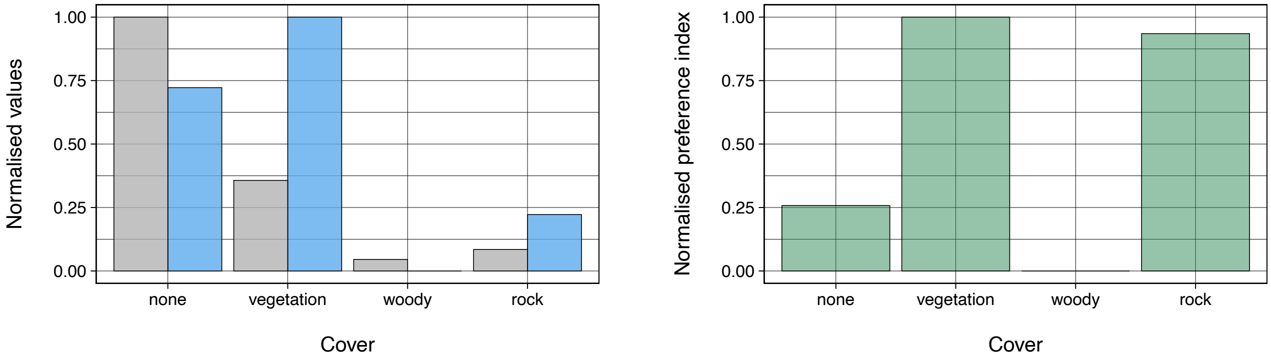
(f) Leonard's Reach

Figure 2.14. Cover Habitat Availability Plots (grey), Habitat Utilisation Plots (blue) and Habitat Preference Plots (green) of spawning adults in each reach during survey 1.

(a) Andrea's Reach (b) Andrea's Reach



(c) Jensen's Reach (d) Jensen's Reach



(e) Leonard's Reach (f) Leonard's Reach

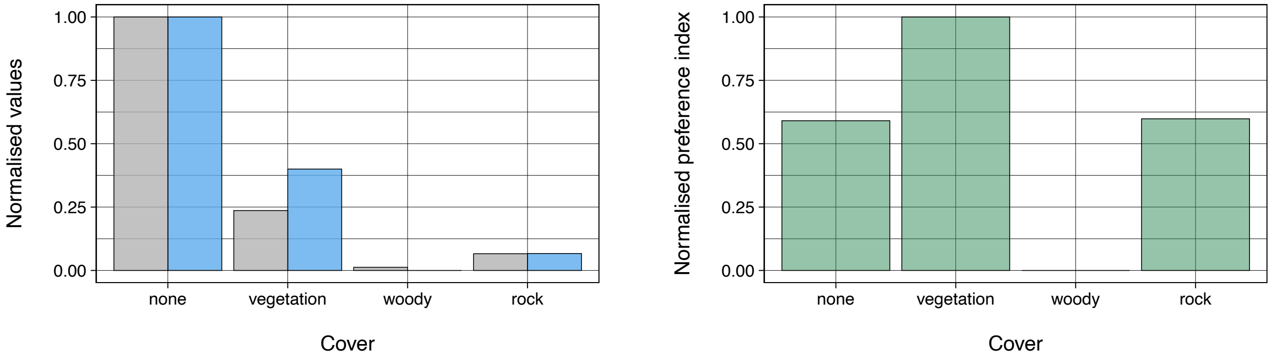


Figure 2.15. Cover Habitat Availability Plots (grey), Habitat Utilisation Plots (blue) and Habitat Preference Plots (green) of larvae in each reach during survey 2.

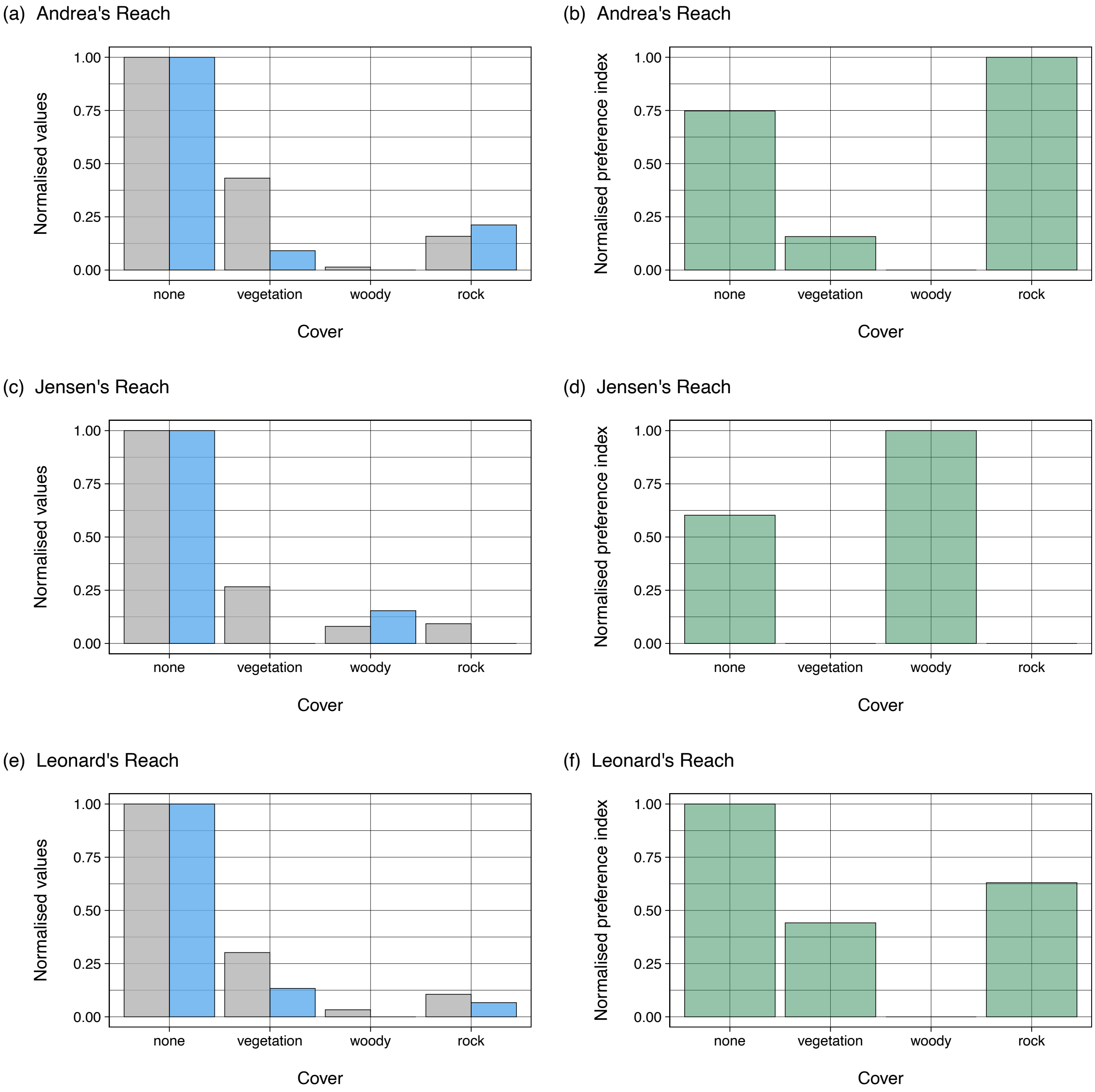


Figure 2.16. Cover Habitat Availability Plots (grey), Habitat Utilisation Plots (blue) and Habitat Preference Plots (green) of juveniles in each reach during survey 3.

Sandfish were observed most frequently in open water across all reaches and life stages (Figure 2.14, Figure 2.15, and Figure 2.16), except for larvae in Jensen's Reach, which were most often observed using vegetative cover (Figure 2.15). Utilisation of the woody and rocky cover types remained low throughout the study period.

There appeared to be a high degree of variation in between-reach and between-survey cover preferences (Figure 2.14 b, d, and f; Figure 2.15 b, d, and f; Figure 2.16 b, d, and f). Reach-averaged preference calculations revealed a high preference for no cover among spawning and juvenile sandfish, and high preferences for vegetative and rocky cover among larvae (Figure 2.17). Peak habitat preferences per life stage are summarised in Table 2.14.

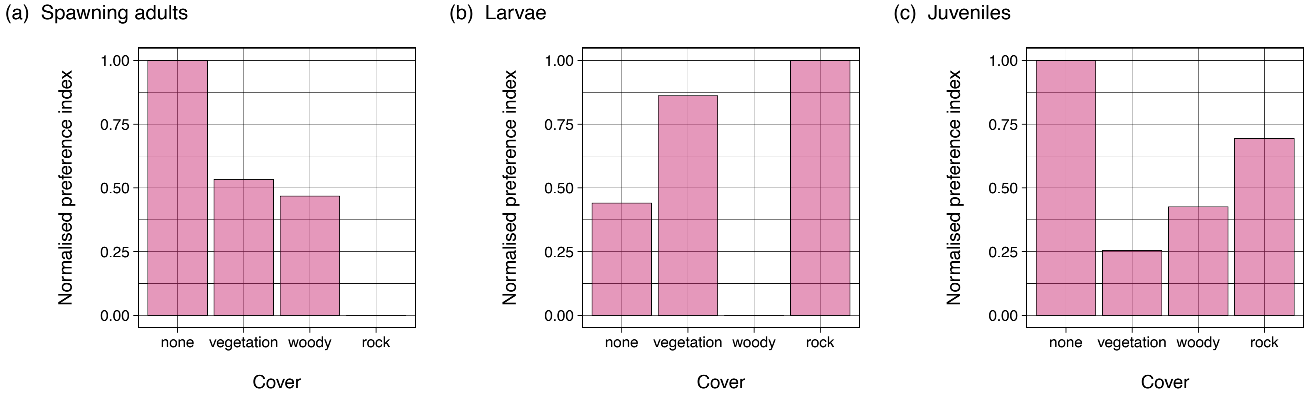


Figure 2.17. Reach-averaged cover preference per life stage. For each life stage, the HPC values from the three reaches were averaged together to produce a reach-averaged HPC, shown here.

Table 2.14. Reach-averaged peak habitat preferences per life stage (where normalised preference index = 1).

Life stage	Habitat parameter			
	Depth (cm)	Velocity (m/s)	Substrate	Cover
Spawning adult	18.2	0.61	Pebble	No cover
Larva	0.0	0.00	Sand and gravel	Vegetative and rocky
Juvenile	37.4	0.00	Sand and gravel	No cover

2.3.3 Weighted usable area (WUA)

Weighted usable area within the 1,500 m of river length covered by the three sampling reaches was 2,000 m^2 for spawning sandfish during survey 1, 1,526 m^2 for larvae during survey 2, and 4,765 m^2 for juvenile sandfish in survey 3. In terms of meters squared of WUA per meter of channel length, this translates to 1.3, 1.0, and 3,2 m^2/m in surveys 1, 2, and 3, respectively.

2.3.4 Spawning and egg observations

Over the spawning period (September 8 – 11, 2022), adult sandfish remained in slow-flowing pools during the day. Over the spawning period, schools of sandfish would begin moving towards shallow riffles in the hours preceding sunset. In Andrea's and Jensen's riffles they travelled upstream from the daytime pools until they reached the respective spawning riffles, while in Leonard's Riffle they were observed drifting downstream into the riffle immediately adjacent to P13. Once at the riffles, sandfish were relatively easy to observe, as in many cases the water was so shallow that their dorsal or caudal fins would protrude above the water's surface. Spawning began in the early evening and continued well into the night, becoming more vigorous after dark. At this time, sandfish lost all fear response, and one could approach to within a meter without eliciting a startle response.

Between spawning bouts, sandfish would rest in the lee of boulders and cobbles (alone or in small groups), presumably conserving their energy prior to spawning. During a spawning bout, one fish would make a sudden movement upstream, followed quickly by one or more other fish. Considerable splashing was observed as fish expressed eggs and milt in sometimes dense aggregations. Spawning bouts lasted from a few seconds to a minute. Sometimes it was difficult to see the configuration of the spawning aggregation, and all one could observe was the splashing movements or rapid vibration of one or more dorsal fins. Follow this link to view footage of spawning behaviour.

Approximately three days after a spawning event, dozens of eggs were observed attached to submerged vegetation at the spawning riffles or nestled into crevices among pebbles and between cobbles. While some eggs were entirely submerged, some protruded above the water surface, perhaps following a drop in water levels. Four to seven days after spawning, non-adhesive, non-buoyant eggs were collected from the sandy substrate located at the in-flows of 14 of the 30 pools surveyed. Egg counts per pool where eggs were found ranged between 1-48.

2.4 Discussion

2.4.1 Study context

Understanding a species' habitat requirements is critical to effectively manage habitat for the benefit of individual species, or species assemblages. It is also an essential input for species distribution models, which require knowledge of the specific associations between species and a suite of environmental variables to predict where those species might occur both now, and under future climate scenarios (Veech 2021). Habitat analysis can also yield insights into the carrying capacity of specific river systems, which in turn informs conservation targets and even fisheries allocations (Veech 2021). Many of the world's arid river systems now constitute complex land-use landscapes with competing water demands for both people and the environment. In light of this, an understanding of a species' physical habitat needs (especially flow-related parameters such as depth and water velocity) allows water resource managers to balance ecological health with societal needs when allocating water resources (Petts 1996).

Habitat preferences of larval fish vary between species, some inhabiting flowing mainstem habitat and others inhabiting more lentic backwater habitats (Nannini et al. 2012). Compared with larvae in the backwaters, mainstem-inhabiting larvae are vulnerable to displacement due to their inability to maintain position in high currents (Nannini et al. 2012). Such life-stage-specific vulnerabilities are particularly relevant when designing management actions for threatened species, which might suffer population bottlenecks at very specific points in their life cycle (Rosenfeld & Hatfield 2006). Understanding these nuances allows managers to target interventions at the appropriate temporal and spatial scale.

The evaluation of patterns of habitat utilisation (without reference to the proportionality of available habitat) enables the characterisation of habitat that is typically frequented by each life stage in the Biedouw River. Although it does not necessarily speak to true habitat preferences, it is nevertheless useful in a complex riverscape offering a heterogenous distribution of habitats. In other words, it enables the assessment of the habitat types that sandfish tend to use, given what the Biedouw River has to offer. An analysis of actual preferences – habitat use weighted by available habitat – adds an additional layer of insight. By accounting for the distribution of habitat types, it clarifies whether observed use reflects selection or simply availability. This distinction is particularly important in stochastic systems like the ODRS, where the composition of available habitats may shift dramatically with changing discharge. It also improves transferability of the habitat suitability criteria to other systems or flow regimes (Bovee 1986). However, preference functions developed under one set of conditions are not universal, and applicability beyond the sampling system must be explicitly validated.

This study evaluated ontogenetic patterns of sandfish habitat use and preference by comparing available microhabitat with that utilised by sandfish across three 500 m reaches in the Biedouw River during the spawning, larval and juvenile stages. The three life stages differed substantially in their habitat use and preferences, consistent with literature showing ontogeny-linked differences in habitat selection among stream fishes (Sempeski & Gaudin 1995; Mann 1996; Hedger et al. 2005; Ayllón et al. 2010). This has implications for the future management of sandfish, as any habitat restoration efforts must specifically consider the target life stage and its associated habitat requirements.

2.4.2 Microhabitat use and preference by life stage

2.4.2.1 Larvae

Larvae were observed in loose groups of, on average, four individuals, although their group size ranged widely between 1 – 24. They were never observed schooling, but rather moved independently, similar to larval Clanwilliam yellowfish (*Labeobarbus seeberi*) in the Olifants River (Cambray et al. 1997). Most of their time was spent swimming and resting, typical of yolk-sac larvae that lack fully-formed mouths and digestive structures prior to the transition to exogenous feeding (the point at which the yolk sac is almost or completely absorbed and when fish first ingest food orally and are capable of digestion; Balon 1986; Yúfera & Darias 2007).

Typical habitat utilised by larval sandfish in the Biedouw River can be broadly characterised as extremely shallow (<30 cm), sandy edges of pools and backwaters where there is no current, sometimes utilising the cover provided by aquatic macrophytes and rocky overhangs. Larval sandfish occupied the shallowest habitat compared with all other size classes (mean 9.75 cm), and they were never observed in water deeper than 26 cm. Although larvae were most frequently observed in open water, they utilised the highest proportion of cover compared with other life stages, perhaps owing to the function of submerged aquatic vegetation as a refuge from predators (Rozas & Odum 1988).

Larval habitat preference largely mirrored their recorded habitat use, with a high preference for extremely shallow areas (< 10 cm), no easily detectable flow, and a sandy substrate. They generally selected shallower depths, lower velocities, and smaller substrate particle sizes than were available in the sampling reaches. Larvae of *Labeobarbus seeberi* in the Olifants River have similarly been known to select placid, shallow edge areas over sandy substrates and, like larval sandfish, are not generally found in water deeper than 28 cm (Cambray et al. 1997). Larvae of several European cyprinid species in the Upper Rhône River in France (Copp 1992), the Danube River in Austria (Schiemer & Spindler 1989), and the River Frome in England (Mills & Mann 1985) exhibit similar preferences for shallow, low-flow environments. Indeed, while the habitat occupied by cyprinid larvae does vary by species, they are very frequently found in areas with virtually no flow (velocity < 0.02 m/s; e.g., Lightfoot & Jones 1979; Mann & Mills 1986). One contributing factor may be larvae's vulnerability to displacement by flow (Scott 1985) due to their poor swimming abilities in the first few weeks post-hatch. Despite the availability of similarly shallow riffle habitat, for example, larval sandfish were never found in these high-flow environments. The swimming capabilities of larvae develop along with changes in body shape (notochord flexion) and fin ray development, which provide support for developing muscle (Kopf et al. 2014). Until then, swimming abilities of larval fish remain extremely limited. Larval sandfish early in their ontogenies would therefore be expected to select shallow, low-flow habitats until the onset of morphological changes and resultant improvements in swimming capabilities. Together with other life-history characteristics, this tendency for larvae to select for low-flow environments helps place sandfish within life-history strategy 2 as defined by Kopf et al. (2014): flood recruitment specialists that spawn in response to high flows and exhibit altricial ontogenies, with underdeveloped larvae at hatching that have limited swimming capabilities and no parental care. Importantly, larvae's absence from higher-flow habitats may be more reflective of their physiological vulnerability to displacement (limiting their ability to remain and be observed in these environments) than of active habitat selection. Laboratory flume experiments (e.g., Kopf et al. 2014) would provide a controlled means to distinguish behavioural habitat selection from passive distribution driven by flow-induced displacement.

Ontogenetic variation in depth preference is also frequently observed among cyprinids. For example, young European cyprinids (including Ebro barbel *Luciobarbus graellsii*, South-west European nase *Parachondrostoma toxostoma*, European chub *Squalius cephalus,* Evrotas chub *Squalius keadicus*, and Iberian chub *Squalius aradensis*), tend to occupy shallower habitats than adults (Grossman & De Sostoa 1994; Santos & Ferreira 2008; Vardakas et al. 2017). In South Africa's Thukela River system, young Thukela labeo (*Labeo rubromaculatus*) tend to occupy shallow backwaters, while adults prefer deep pools (Skelton 2024). Size-specific selective pressures may help explain this trend. Shallow water offers a size refuge from predators, such as bass, leading smaller individuals to select for shallower habitat as an anti-predator response (Schlosser 1987). At the same time, larger fish are generally more exposed to predation by birds in shallower habitats, leading them to seek out deeper waters (Santos & Ferreira 2008). The relatively clear waters of the Biedouw, together with observations of talon marks on several adult fish, would support the conclusion that adult sandfish are vulnerable to bird predation.

The present study provides a snapshot of larval habitat preferences during a single point in time. It does not capture potential variation in habitat use driven by fluctuations in environmental conditions, such as in food availability or predation pressure. Larval fish, which typically experience very high mortality rates, must constantly balance the need to forage with the risk of being preyed upon – a trade-off that becomes especially critical when food is scarce (Jørgensen et al. 2014). When food availability is low, larvae are more likely to engage in riskier foraging behaviours to maintain sufficient ingestion rates. These compensatory behaviours increase their exposure to predators (Jørgensen et al. 2014). For example, larvae may venture into better-lit areas in search of food, increasing vulnerability to visual predators (Iwasa 1982), or swim more actively, making them easier targets for ambush predators (Visser and Kiørboe 2006). Larval sandfish were mostly encountered in shallow, sandy, low-flow habitats – areas that may reduce predator presence or enhance feeding efficiency under certain conditions. However, it is not known whether these preferences reflect context-dependent trade-offs. Further research should investigate how larval habitat use shifts in response to food availability and predation risk, especially given the relatively novel presence of predatory centrarchids in the study system.

2.4.2.2 Juveniles

Juvenile sandfish were observed in similar size groups as larvae, with a mean of 5.5 individuals and a range of 1 – 30. Schooling is known to reduce probability of capture of a given individual (Neill & Cullen 1974), perhaps explaining the higher mean and maximum group size of juveniles, when compared with adults. While larvae only exhibited two behaviours, juveniles exhibited four: swimming, schooling, resting, and feeding. This may be indicative of the development of more exploratory tendencies compared with larvae, likely due to improved swimming ability, which allow them to escape predatory fish more easily, and the onset of exogenous feeding, as evidenced by a high proportion of juvenile feeding observations. Compared with larvae, which preferentially occupied depths of 0 – 8.4 cm, juveniles utilised relatively deeper waters that covered a wider range of depths, further supporting this conclusion. Like larvae, the majority (80.3%) of juvenile observations occurred under no-flow conditions, which may indicate true preference, because a comparatively lower proportion (59.7%) of available habitat comprised zero flow during Survey 3. While substrate utilisation by juveniles largely mirrored that of larvae, a much higher proportion of juvenile observations occurred in open water, further lending support to the onset of exploratory behaviour. This is consistent with work showing that young cyprinids occupy increasingly larger areas of stream as development progresses and swimming abilities improve (Rincón et al. 1992). Overall, the data indicate that juvenile sandfish in the Biedouw River predominantly occur in areas of no flow over sandy or bedrock substrate, tending to occupy a relatively wide range of depths and are most frequently observed in open water.

Juveniles are frequently observed in open water in a relatively clear-water system that likely makes them vulnerable to predation by bass, a highly piscivorous pursuit predator that relies almost exclusively on visual information when hunting (Nyberg 1971; Sullivan & Atchison 1978). Studies elsewhere show that largemouth bass hunt more efficiently in simplified habitats with limited structure and complexity and have an attack success rate of 70-90% (Nyberg 1971; Savino & Stein 1982). Furthermore, work throughout the ODRS has shown that fish measuring less than 20 cm are generally not able to coexist with bass (Weyl et al. 2013; van der Walt et al. 2016; Cerrilla et al. 2022). Juvenile (< 6 cm) sandfish in open water with few to no visual obstructions would therefore be especially susceptible to bass predation. Vulnerability to bass predation may be further compounded by the lack of a shared evolutionary history between predator and prey. Prior to the introduction of bass, no analogous predators occurred naturally in the ODRS (Chakona et al. 2022; Skelton 2024). Consequently, indigenous fishes exhibit no appropriate defence response against such predation – a classic case of prey naiveté (Cox & Lima 2006). It is likely that the anti-predator behaviour of fishes indigenous to the ODRS evolved in response to predators such as sharptooth catfish (*Clarias gariepinus*) of the neighbouring Orange River system (only severed from the ODRS during the Tertiary; Dingle & Hendry 1984), which are nocturnal hunters (Bruton 1979). There is evidence that native fishes are able to co-exist with extralimital *C. gariepinus* elsewhere in South Africa (Woodford et al. 2024). In contrast, without an evolutionary drive to seek shelter in the daytime, juvenile sandfish would remain highly exposed to the relatively novel threat of bass predation.

Like larvae, juveniles preferred areas of no flow with sand and gravel substrates. However, the peak depth for juveniles occurred at 37.4 cm, despite the higher relative availability of shallower (< 25 cm) habitat, again demonstrating the value of considering available habitat when determining true habitat preferences.

2.4.2.3 Non-spawning adults

Maximum group size of non-spawning adults was smaller than that of either larvae or juveniles, a likely consequence of their lower relative vulnerability to fish predation, and thus a decreased need to aggregate in large schools. Alternatively, it could be a symptom of the decades of apparent recruitment failure in the Doring River system, which have resulted in a massive decline in numbers of sandfish migrating up the Biedouw River over time (Sarah Fransman[*], pers. comm.). Non-spawning adults spent most of their time resting, although a single instance of chasing, a pre-spawning behaviour, was observed.

Non-spawning adults occupied the widest range of depths compared with all other life stages, were observed at a higher average water velocity than the early life stages and showed the most even distribution of substrate use. Like other life stages, most observations occurred in open water. Habitat use by non-spawning adults in the Biedouw River can thus be categorised as relatively varied compared with the other size classes, comprising a moderate range of depths and velocities and a wide range of different substrates. This is likely because adults are more vagile and better able to withstand higher velocities compared with larvae and juveniles, allowing them to take advantage of the range of available habitat types in the Biedouw River. It is important to note that only 11

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[*] Sara Fransman is a long-term resident of the lower Biedouw River valley whose lived experience offers an important perspective of historical changes in the landscape. She has inhabited one of the farms close to the Doring River confluence for decades.

observations of non-spawning adults were made over the course of Survey 1. Results must therefore be interpreted with caution.

2.4.2.4 Spawning adults

The observed average spawning group size of 4.41 individuals may reflect current low population densities. When fish populations fall below a certain abundance threshold, they may experience slowed or even negative population growth due to disrupted reproductive behaviours and reduced opportunities for successful breeding. Contributing factors include lower fertilisation success, reduced mate availability, altered sex ratios, and fewer social interactions during spawning (Rowe & Hutchings 2003). Given the lack of baseline data, it is unknown whether past conditions would have supported larger spawning aggregations of sandfish or different reproductive strategies. This highlights the need for further research into sandfish reproductive dynamics, especially if reduced group sizes may be limiting recovery in the current population.

The observation of adhesive eggs among submerged vegetation and in the crevices among stones a few days after spawning lends support to the placement of sandfish within the phyto-lithophil reproductive guild (Balon 1975) as first suggested by Paxton et al. (2012). The eggs of *L. umbratus* are also adhesive and attach to grass on rocks (Skelton 2024). The subsequent sampling of eggs from many of the sandy in-flows of pools suggests that at least some eggs become dislodged and drift in the current until they settle in lower-flow environments, where they hatch approximately 10 days after spawning (pers. obs.)

Spawning adults utilised by far the widest range of water velocities and the highest mean velocity (0.390 m/s) compared with other life stages. They selected faster-flowing water than was generally available in each reach, substantiating previous observations of spawning taking place in riffle habitat. Studies targeting the spawning preferences of riverine cyprinids show that even among cyprinids with similar life histories, velocity requirements can differ (Melcher & Schmutz 2010). For example, although both barbel (*Barbus barbus*) and nase (*Chondrostoma nasus*) are large, migratory, rheophilic cyprinids found in the Austrian Danube River system, the former spawns in velocities of 0.6-1.0 m/s, while the latter spawns in higher flows of 1.0 – 1.1 m/s (Melcher & Schmutz 2010). In a review of the habitat requirements of European riverine non-salmonids, Mann (1996) found that phyto-lithophil cyprinids spawned in water velocities ranging from less than 0.2 m/s to 0.5 m/s. Habitat preference analysis revealed a maximum preference at 0.61 m/s for spawning sandfish. It is worth noting that velocities in the range of that preferred by *C. nasus* and *B. barbus* were not generally available to adult sandfish in any of the reaches at the time of the study. In all three cases (including sandfish), however, the spawning cyprinids preferred higher velocities than were generally available.

It is worth noting that velocity preference appeared to differ between reaches, with sandfish spawning in Leonard's Reach exhibiting a preference for higher velocities than in Andrea's and Jensen's reaches. This suggests that there may be interactions between key habitat parameters (including those not considered here, such as pH and quality of downstream habitat) that influence the choice of spawning location. Importantly, the observed preference for higher velocities in one reach raises questions about the availability of optimal spawning habitat elsewhere in the system. If sandfish historically had access to higher flows in the ODRS, which may have provided more suitable spawning conditions, then their current use of the Biedouw River could reflect habitat limitation rather than preference. Future studies should investigate velocity preferences of spawning sandfish in other tributaries and mainstem habitats (if these exist), and any future assessments should take additional habitat parameters into consideration.

Both *C. nasus* and *B. barbus* prefer water depths of 16-30 cm while spawning (Melcher & Schmutz 2010), comparable to the depth range preferred by spawning sandfish (approximately 15-25 cm). Pebble and cobble substrates frequently characterise the spawning habitats of phyto-lithophil cyprinids (Mann 1996). Most sandfish were observed spawning over cobbles, although preference analysis revealed a higher relative preference for pebble habitat. The substrate preference of spawning sandfish mirrored that of *C. nasus,* comprising a high preference for pebbles and a moderate preference for cobble, but differs somewhat from that of *B. barbus*, which prefer gravel and pebble substrate (Melcher & Schmutz 2010).

The closely related *L. umbratus* which occurs in the country's interior and northern river systems (Skelton 2024), has also been observed spawning over gravel and cobble substrates (Gaigher et al. 1975; Mitchell 1984). Like sandfish, adult *L. umbratus* require a major tributary in flood to spawn, undertaking major longitudinal migrations upstream to breed (Tómasson et al. 1984). This is typical of many labeines, which tend to breed at times of flood and spawn on floodplains over inundated vegetation (Daget & Durand 1981; Tómasson et al. 1984; Weyl & Booth 1999).

Like the other life stages, most spawning observations occurred in open water offering no cover. Spawning events began in the late afternoon, when the sun was low, and continued for hours after dark, which would have provided some protection from birds while spawning, perhaps obviating the need to seek cover. Furthermore, many of the locations where active spawning bouts were observed were so shallow that dorsal fins, caudal fins and even the dorsal surface of the body was visible above the water's surface, making shelter entirely inaccessible across much of the spawning grounds.

2.4.3 Weighted usable area (WUA)

Juveniles had the highest absolute (m^2) and relative (m^2/m) WUA compared to larvae and spawning adults, suggesting that suitable juvenile rearing habitat was more abundant than spawning and larval habitat. This is notable given that the wetted area across the three study reaches during the juvenile survey (survey 3) was 28% and 25% lower than in surveys 1 and 2, respectively. In other words, despite less total habitat being available, juveniles had 2.5 and 3 times more suitable habitat than spawning adults or larvae, respectively. This indicates that greater total wetted area does not necessarily translate to greater availability of suitable habitat, highlighting the importance of identifying the specific habitat requirements of each life stage and assessing how these align with the conditions present when that life stage is in the river. It may also reflect the broader environmental tolerances of juveniles compared to the more specific requirements of spawning adults and larvae.

However, it is important to recognise that juvenile habitat availability declines sharply as summer progresses and flows drop. By late summer, when the lower Biedouw River dries up entirely, WUA for juveniles would fall to zero. On their own, the WUA values presented here offer only a static snapshot. However, they could become a valuable comparative tool if additional spawning tributaries are identified. Calculating WUA across life stages in multiple systems would help determine which stage faces the most limiting habitat conditions. If, as these results suggest, suitable habitat is the most constrained at the time of spawning, then conservation interventions should focus on restoring or establishing additional suitable spawning areas (e.g., by adding appropriate spawning substrate to suitable areas; Barlaup et al. 2008). That said, while spawning WUA may be most limited at a specific point in time, juvenile habitat is ultimately the most constrained, as it disappears entirely by the end of summer without sufficient flow.

2.4.4 Study limitations

Several limitations in study design and methodology should be considered when interpreting the findings of this analysis. Two main limitations were linked to the use of visual observation as the sampling method: 1) the difficulty of detecting fish using structural cover, and 2) the potential for reduced visibility during evening spawning observations. The first concern introduces a key limitation in the cover analysis, as the method is inherently biased against visually observing fish utilising overhead cover. Confidence in the resulting analysis is therefore limited. The issue was further compounded by the small number of availability sampling points that included cover, along with the few observations of fish actively using cover – conditions that may have created spurious preferences. These results should thus be treated with caution. To better assess cover preference in future studies, a sampling method less sensitive to this bias should be employed. Electrofishing, for instance, has been shown to produce higher density and biomass estimates for cryptic species that tend to shelter under structural cover in streams, when compared with a visual observation method (Macnaughton et al. 2015). The second concern relates to the timing of spawning observations. Adult sandfish spawned from late afternoon into the evening. Observations of microhabitat use took place from 16:00 to 18:30, meaning that reduced visibility toward the end of this period may have limited the accuracy and completeness of behavioural observations. Some spawning activity and associated habitat use may therefore not have been fully captured due to the onset of darkness.

While it is likely that some sandfish were missed during the utilisation surveys, there is relatively high confidence that all three life stages were adequately represented—despite certain limitations. Adult sandfish congregated in discrete riffle sections during spawning (n^* = 68), which facilitated their observation. However, observers were limited to a single spawning riffle per evening and may have missed spawning activity occurring at other riffles on the same night. Larval sandfish (*n* = 116) were confined to pool edges and backwaters, making them relatively easy to detect. Nonetheless, due to their small size (<2 cm), some individuals were likely overlooked. The greatest confidence in survey coverage likely applies to juveniles (*n* = 76). By the time of their observation, the wetted area of the Biedouw River had contracted by approximately 30% since Survey 1, resulting in a narrow and shallow channel with an average width of 8 m and an average depth of 19 cm. Water clarity was excellent, and juveniles tended to form schools, which made them particularly easy to detect.

Another key limitation of this study is that sampling of available habitat was conducted uniformly – or systematically – across the study reaches. The primary drawback of this method is that it creates a "data pooling bias", where each sample enters the preference calculations as a frequency, but the frequencies do not represent the same area (Bovee 1986). If these samples are assumed to represent the true frequency of habitat types available in the study reach, this may lead to misleading preference calculations. To correct for this, an area-based weighting factor was applied to each availability sample to account for the different areas represented by each sample. This method is similar to the way that hydraulic models assign cell areas around sampling points when computing weighted usable area (WUA; see Figure 5.1 in Jowett et al. 2008). While this correction allows for meaningful interpretation of habitat preferences, these findings should be treated with some caution. Indeed, it is recommended that the study be repeated following accepted protocols. A consideration of different sampling approaches and their benefits and drawbacks is thoroughly discussed in Bovee (1986). The recommended approach for collecting availability data in the future follows that used by Jowett and Davey (2007): a random selection of

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[*] Sample size *n* represents number of observations, not individual fish; for example, a school of juveniles was recorded as a single observation.

cross-sectional transects with fixed intervals between points along each cross section. This ensures that availability data are collected in a manner that reflects the heterogeneity of the stream environment. Practically speaking, if the study were to be repeated in the lower Biedouw River, the following protocol should be followed:

1. Identify three 500 m reaches based on known spawning grounds that contain at least one pool/run/riffle morphological sequence (Jowett et al. 2008).
2. Using mapping software, project transects at intervals of 1 m down the length of each reach. Number these from 1-500.
3. Randomly select 50 transects to sample using a random number generator.
4. At each transect, measure habitat variables at fixed 1 m intervals across the channel. Additionally, take one measurement 10 cm from each bank to ensure adequate representation of shallow, near-shore habitats.

Small sample sizes, particularly for habitat use observations, represent another potential limitation. Small samples increase the likelihood of irregularities in habitat use curves (HUCs) and, by extension, habitat preference curves (HPCs). A result of this can be the emergence of multimodal distributions that may not reflect true biological preferences. Outliers in small data sets are particularly problematic, as they may produce spurious preferences (Jowett and Davey 2007). Bovee (1986) recommends a minimum of 150 observations in order to construct a reasonably smooth histogram to which an observed frequency distribution can be fitted. Unfortunately, this limitation is a consequence of the currently small and fragmented population of sandfish and thus could not be resolved within the scope of this study.

A further constraint of the wider application of this study is the univariate nature of the analysis, where each habitat variable (depth, velocity, substrate, and cover) was evaluated in isolation. Derivation of independent suitability criteria ignores potentially significant correlations and interactions among variables (Jowett and Davey 2007). For example, fish may use shallow water when overhead cover is available and deep water when it is not, but will not use shallow water without cover (Bovee 1986). Generalised additive models (GAMs) are a common multivariate alterative that resolve some of the key criticisms of conventional habitat suitability criteria (HSC). They allow for correlation between variables, can incorporate variable interactions, and can make quantitative predictions of absolute abundance or probability of occurrence (Jowett and Davey 2007). However, while GAMs offer a theoretical improvement over conventional HSC, in practice the benefits may be marginal and – like HSC – their performance depends heavily on the quality of the calibration data (Jowett and Davey 2007). To directly assess the utility of each method in the study system, both conventional HSC and GAMs could be applied to the same high-quality dataset, as in Jowett and Davey (2007).

Additionally, this study was limited to a single catchment – the Biedouw River – which is subject to a highly modified flow regime. Although habitat preference functions enhance the transferability of results beyond the original study conditions compared to simple habitat use functions (Bovee 1986), this transferability remains limited. In fact, neither HSC or GAMs transfer easily to other rivers (Jowett and Davey 2007). Additionally, because the preference distributions were created under non-natural conditions, the observed "preferred" habitats may simply represent the best options available within an already compromised system, rather than truly optimal conditions. This raises an important caveat: habitat suitability criteria developed under degraded conditions risk misrepresenting the true requirements of the species. Therefore, future investigations of sandfish habitat preferences should, where possible, incorporate additional rivers – especially those in more natural conditions.

However, this is currently constrained by the fact that the Biedouw River is the only known spawning tributary for migratory sandfish residing in the Doring mainstem.

Lastly, this study was conducted under a single set of flow conditions per life stage. Future studies should aim to repeat the habitat assessment under a range of flow regimes and incorporate hydraulic modelling approaches (e.g., PHABSIM). This would allow researchers and managers to predict how suitable habitat availability shifts with discharge and to better manage environmental flows for this species.

2.4.5 Implications and conservation opportunities

The life stage-specific habitat preferences identified in this study demonstrate the wide breadth of different habitats required for sandfish to complete their life cycle. These habitat requirements vary in time and space: spawning fish are dependent on a narrow range of high velocities and shallow water at reaches with appropriate pebble and cobble substrates as winter flows are subsiding, while larval and juvenile fish rely on low-flow environments with sandy substrates as nursery grounds through spring and summer. Maintaining natural spatial and temporal variability in key habitat parameters – especially discharge – is therefore crucial for supporting the diverse habitat requirements of sandfish at different life stages.

Flows in the Biedouw River are extensively modified by upstream abstraction. There are at least four perennial off-stream reservoirs in the Biedouw River catchment, which are either supplied by surface water (via piping or water diversion using earthen canals) or groundwater (via borehole groundwater abstraction). Several farms also use centre pivot irrigation supplied by water pumped directly from the river. As a result, discharge moving through the Biedouw River is highly dependent on the amount of water that is abstracted upstream, and this varies throughout the year, based on seasonal abstraction requirements, as well as natural variation in base flows. That spawning adults appear to have a range of velocity preferences across the river segment suggests they may be relatively robust to small modifications in flow. However, spawning adults' overall preference for shallow riffle habitat is a potential vulnerability, as substantial abstraction may render large swathes of optimal spawning habitat inaccessible during the narrow spawning period. It is possible that current abstraction practices may already be limiting spawning to fewer suitable spawning grounds than would naturally exist in the Biedouw River, although this is difficult to verify without a baseline. Given the extensive flow modifications, understanding how existing and future flow regimes affect the availability and quality of preferred spawning habitats is critical. To this end, these data should be incorporated into hydrological models that predict the distribution of different velocities and depths under varying discharges. Future water management strategies should incorporate these findings with species-specific flow requirements to support reproductive success and population resilience.

Perhaps the most observable impact of over-abstraction is the transition of the lower reach of the Biedouw River from a seasonal system that held water in permanent pools through the summer (Lelie Hough[*], pers. comm., 2021), to one in which at least the first 15 river-kilometres dry up completely every year. Sandfish appear to tolerate the low dissolved oxygen content and high salinity typical of isolated pools[†], they are entirely reliant on the availability of water in those pools through the summer months; without it, they become stranded and die.

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[*] Lelie Hough was a long-time resident of the lower Biedouw River valley close to the Doring River confluence.

[†] During the course of the 2022 Doring tributaries survey described in Chapter 3, hundreds of subadult sandfish were observed in an isolated pool with a dissolved oxygen reading of 0% and a conductivity reading of 1,172 µS/cm.

Sandfish population recovery and long-term sustainability depends on the availability of appropriate flows and water volume at key points during their life cycle. As one of the most important known spawning tributaries, the Biedouw River offers an important opportunity to intervene. There is a clear need to manage environmental flows[*] in the Biedouw River so that the requirements of water users in the valley are balanced with the need to maintain the ecological reserve[†]. In the context of the present study, enough water must remain in the system to support sandfish spawning and recruitment, while providing enough water for the various water users in the valley. Abstraction must be carefully managed and limited in the months preceding and including the spawning migration (July – September) to ensure appropriate discharge is available for large-scale spawning. In addition, strategic releases from storage waterbodies could be facilitated throughout the summer months to ensure that sufficient refuge pools are available to juvenile sandfish until winter flows resume. A relevant example comes from Australia, where reactive environmental flow releases helped sustain populations of the Murray hardyhead (*Craterocephalus fluviatilis*) following a bush fire, likely playing a role in preventing its extinction(Morrongiello et al. 2011).

In addition to water abstraction, the proliferation of non-native plants throughout the Biedouw Valley is suspected to substantially reduce baseflows year-round. Low densities of black wattle (*Acacia mearnsii*) are present in the gorge, dense stands of white poplar (*Populus alba*) and blue gum (*Eucalyptus globulus*) occur in the middle reaches, oleander (*Nerium oleander*) are present in large numbers in the lower reaches, and mesquite (*Prosopis* spp.) occur throughout the floodplain. Eucalypts in particular are excessive consumers of water, with a single *E. globulus* capable of transpiring up to 90 litres of water in one day in the species' native Australian range (Taylor et al. 2001). Mesquite can also have drastic impacts, with one hectare of land invaded with the species being associated with groundwater uptake of over 2,000 litres per day in South Africa's Northern Cape (Dzikiti et al. 2013). Given these severe impacts, concerted clearing efforts focused on the thirstiest non-native plants must be undertaken throughout the Biedouw Valley to increase year-round baseflows, especially given their likely impacts on summer flows, when juvenile sandfish are dependent on permanent pools. Together with the implementation of the ecological reserve, this should facilitate maintenance of the variable habitats that sandfish depend on during their life cycle.

Climate change constitutes a third important stressor affecting water volume in the sandfish's remaining range. Compared with other river systems in the Western Cape, the ODRS is predicted to experience more intense drying and more severe drought frequency in the future (Naik & Abiodun 2020). Like most cyprinids (Hontela & Stacey 1990), sandfish appear to rely heavily on flow and temperature cues to trigger spawning (see Chapter 4 section 4.3.4.3). A disruption to these cues might very well interfere with successful reproduction, causing further population declines and hindering population recovery. Evidence from the Oorlogskloof River, a tributary of the Doring River, suggests that prolonged droughts have the potential to inhibit sandfish population recovery through various mechanisms, including the suppression of spawning activity (Cerrilla et al. 2022).

A general increase in extreme rainfall events is predicted over large parts of southern Africa (Engelbrecht et al. 2013), with a consequent rise in catastrophic floods. Labeos are especially vulnerable to flash floods, which can smother eggs in silt, or leave larvae stranded by receding floodwaters (Skelton et al. 1991). In the Oorlogskloof River,

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[*] Environmental flows are defined as "the quantity, timing, and quality of water flows required to sustain freshwater and estuarine ecosystems and the human livelihoods and well-being that depend on these ecosystems" (Brisbane Declaration 2007).

[†] The "ecological reserve" refers to the water required to sustain the aquatic ecosystems associated with a given water resource. It is defined in conjunction with the "basic human needs reserve", which provides for the "essential needs of individuals served by the water resource in question and includes water for drinking, for food preparation and for personal hygiene"(NWA 1998).

a 99.6% decline in relative abundance of young-of-the-year sandfish between 2013 and 2018 was attributed to several high rainfall events. Unusually severe rainfall corresponding with the 2014 spawning season was implicated in the initial decline, potentially by suppressing spawning, covering eggs and larvae with silt, or flushing eggs and larvae into the bass and bluegill zone (Cerrilla et al. 2022). Soon thereafter, in January 2016, an extreme summer flood caused heavy sedimentation throughout the Oorlogskloof River, further hindering population recovery (Cerrilla et al. 2022).

The acute and severe impacts of extreme flooding also became apparent in the Biedouw River during the present study. In 2020, prior to the implementation of the head-start intervention, the estimated wild run size of sandfish in the Biedouw River was 180 (see Chapter 4 section 4.3.4.1). Nearly 8,000 young sandfish were rescued and relocated in the subsequent months. Similar numbers were rescued in 2021. In 2022, with the addition of 77 head-started sandfish to the spawning run, over 18,000 juveniles were rescued from the Biedouw River. In 2023, despite the addition of 221 head-started sandfish to the spawning run (compared with 2020), only 2,713 juveniles were rescued. The apparent decrease in juvenile survival between spawning and rescue can likely be attributed to the unprecedented flooding that swept through the Biedouw River starting on September 25, 2023, just two weeks after spawning occurred (and less than a week post-hatch). The high flows likely caused mass mortality of larval fish through heavy siltation, or by flushing larval fish from the Biedouw River system and into the Doring River, where they would have been vulnerable to predation and dangerously high flows. Given larval and juvenile preferences for low-flow environments, this record flood would have had a considerable impact on survival, reflecting in poor catches during the 2023 rescue efforts.

Although sandfish are naturally adapted to high levels of environmental stochasticity, they remain vulnerable to flow-related impacts wrought by water abstraction, decreased baseflows, and climate change. The last of these is perhaps the most difficult stressor to address. However, ameliorating the impacts associated with water abstraction and non-native plants will help buffer sandfish against the effects of climate change by increasing water availability in the catchment. Establishing population redundancy throughout the Doring River system is another form of “biological insurance” that will enhance the species’ resilience against the stochastic effects of climate change. For example, if sandfish continue to spawn successfully only in the Biedouw River, catastrophic flooding here during the spawning or nursery season might result in overall recruitment failure for that particular year. However, if other tributaries can be rehabilitated so that sandfish spawn in several other catchments beyond the Biedouw River, localised extreme events will have milder consequences.

2.4.6 Conclusions

This study constitutes the first assessment of Clanwilliam sandfish habitat use and preference. It demonstrates clear differences in the habitat preferences of spawning, larval and juvenile sandfish, substantiating the pattern of ontogeny-linked habitat selection among stream fishes in general (Sempeski & Gaudin 1995; Hedger et al. 2005; Ayllón et al. 2010), and cyprinids in particular (Mills & Mann 1985; Grossman & De Sostoa 1994; Mann 1996; Santos & Ferreira 2008; Vardakas et al. 2017). Specific preferences for depth, velocity, and substrate were identified; these tended to differ substantially between life stages, except for substrate and velocity preferences between larvae and juveniles. Preferences for spawning and nursery habitat in the Biedouw River were defined, providing a foundation for identifying, protecting, and restoring critical habitat for sandfish.

The clear differences in habitat requirements between life stages support the need to promote natural habitat heterogeneity to facilitate sandfish population recovery. While the maintenance of shallow, fast-flowing riffle habitat should be prioritised to maximise spawning output, appropriate low-flow, shallow, sandy habitat must also be available to larvae and juveniles to enable recruitment into the adult population.

The findings reveal several conservation opportunities. Environmental flows must be managed among all water users so that adequate discharge and water volume is available to sandfish during the spawning and nursery periods, and concerted efforts must be made to remove non-native plants to augment baseflows year-round. Building population redundancy across the Doring River system will be necessary to buffer sandfish against the effects of climate change. In addition, safeguarding and restoring longitudinal, lateral, vertical, and temporal river connectivity – as highlighted by Thieme et al. (2024) – will be critical in improving sandfish dispersal, spawning and recruitment. Given that natural and artificial barriers help limit the spread of IAF in the ODRS, enhancing longitudinal connectivity will require creative solutions that enable indigenous fish to migrate while preventing further IAF expansion ('selective fish passage', e.g., Kerr et al. 2021).

To this end, future research should build on this study by repeating the survey using the updated sampling protocol described in section 2.4.4. Incorporating multiple years with varying hydrological conditions will also help account for natural variability in habitat use. To improve understanding of evening spawning behaviour, future surveys should consider the use of night-vision equipment or infrared cameras to extend observations into low-light periods. Replicating this study in additional spawning tributaries is critical to refining sandfish habitat preferences and testing the transferability of results beyond the Biedouw River. This will first require identifying other tributaries that still support spawning migrations—ideally including systems in a more natural hydrological state. Comparative studies across rivers will also enable more robust evaluation of habitat modelling approaches. In particular, future work should explicitly test the performance of traditional habitat suitability criteria versus GAMs by applying both analytical methods to a shared, high-quality dataset. Finally, the habitat preference outputs developed here should be incorporated into hydraulic models to predict how the distribution of suitable habitats may shift under different flow scenarios, including those expected with climate change.

Chapter 3

3 Spatio-temporal shifts in sandfish distribution and population status: implications for future monitoring and management

3.1 Introduction

Effective conservation of threatened species requires an understanding of how their populations have changed over time and how particular stressors may be driving these changes. However, conservation action is often undertaken urgently and with limited data, particularly in the case of highly threatened and lesser-known species. To guide more impactful management of these species, we need to know where they occurred historically, how their distributions have shifted, and how these changes have impacted specific measures of population health. These data are critical for contextualising current population trends and shaping management plans moving forward, forming the backbone for threat status assessments that in turn determine the allocation of scarce conservation resources. For example, three of the five criteria used to evaluate a species' IUCN Red List threat category pertain to changes in population size or geographic range (IUCN Standards and Petitions Committee 2024). Establishing a baseline prior to specific impacts, such as European settlement, or the introduction of non-native species, is ideal, as it enables the comparison of current trends against a known starting point. Although these baselines are seldom available, it is possible to synthesise multiple lines of evidence – such as historical records and early fisheries reports – to approximate past distributions. Such reconstructions are not only valuable for assessing historical losses but also provide critical guidance for restoration and reintroduction efforts.

In addition to the historical context, current data are vital for assessing the status of remaining populations. Understanding population structure – such as the size distribution of fragmented fish populations – can provide insights into population dynamics and help identify source and sink populations. For example, populations lacking smaller size classes may indicate poor recruitment, while an absence of larger classes may indicate high mortality of adults or slow growth (Miranda et al. 2024). Repeated size structure assessments over time or space can also reveal whether a given management intervention has resulted in observable size changes in a population of interest (Miranda 2024). This is particularly useful as size is often used as a proxy for ecological attributes such as life stage and fecundity (Miranda et al. 2024). Importantly, the time of year in which a sample is taken must be carefully considered, as observed sizes may vary due to factors such as spawning timing and seasonal variability in growth rates.

Consistent monitoring of fish populations serves several other important purposes, including the detection of concerning trends in native fish populations, fluctuations in non-native species abundances, or the arrival of novel invaders. Early detection of invasive species is particularly valuable in facilitating rapid response programmes to mitigate potentially deleterious impacts. In addition to supporting management action, monitoring data inform broader conservation planning efforts, including evaluating the efficacy of protected areas and developing alternative methods where these are inadequate. Protected areas have traditionally been designed with a focus on terrestrial ecosystems (Crivelli 2002), often overlooking the unique dynamics of river networks that leave them vulnerable to upstream influences. As a result, freshwater systems may continue to experience negative impacts despite their inclusion within protected areas. Understanding where the distributions of threatened fish species overlap with protected areas is important for evaluating their effectiveness in mitigating specific threats, identifying population refuges, and revealing opportunities for conservation beyond formal protections.

3.1.1 Historical records

In the Olifants-Doring River system (ODRS), no comprehensive surveys of the system's fish fauna were carried out prior to large-scale human impacts. The first attempt to characterise the systems' fish fauna took place in 1937 (Harrison 1963), four years after the introduction of largemouth bass (*Micropterus nigricans*) into the Olifants River (Gaigher 1973b), five years after the construction of the Clanwilliam Dam, and 18 years after the construction of the Bulshoek Dam (both located on the Olifants River; Cambray et al. 1997). At this time, a collection of the fishes of the Olifants River[*] was carried out by the South African Museum "with a feeling of urgency, in case the spread of predatory bass might upset the natural balance of the indigenous fauna" (Harrison 1963).

Between this first collection in 1937 and the turn of the century, much of the information about the state of indigenous fishes in the Olifants River was found in anecdotal accounts and recollections in the angling magazine *Piscator* (e.g., Wells 1949; Brooks 1950). Surveys were also undertaken by conservation scientists from the Cape Department of Nature Conservation (CDNR), the conservation authority at the time, and the results recorded in internal reports (van Rensburg 1966; Gaigher 1973a), summarised in *Piscator* articles (Jubb 1961; Gaigher 1973b), or in one case published in the peer-reviewed literature (Gaigher et al. 1980). Despite the scattered and unstandardised nature of these surveys, observations, and collections, these records constitute valuable data sources about the historical distributions (if not abundance) of indigenous fishes, including sandfish, in the Olifants River.

Compared with the Olifants River, the Doring River and its tributaries are more remote and less accessible. As a result, fewer forays were made into that system by either anglers or scientists prior to 1980. Some sporadic records of sandfish in the Doring River system start to emerge in the literature from around 1980 (FBIS 2024), but it was only in 2001 that the first comprehensive survey of the Olifants-Doring River system (mostly focused on the Doring arm) was undertaken, commissioned by the Department of Agriculture and the Department of Water Affairs and Forestry (Paxton et al. 2002). These surveys came to be known as the Olifants-Doring Fish (ODF) surveys. In addition to reporting on sandfish occurrence, the ODF surveys also provided a measure of population status by collecting data on size distribution, presence of parasites, and signs of disease. Around the same time, surveys of the Oorlogskloof River, a tributary of the Doring River, were initiated by staff at the Oorlogskloof Nature Reserve (Abrahams & Pretorius 2000), although it was only in 2010 that these became a more regular occurrence (surveys were carried out in 2010, then annually from 2013 to 2018). Further ODF surveys took place in 2011 (February/March and November), and in 2013 (October). The information yielded from these surveys has made it possible to develop a conservation strategy for the species, as detailed in the Biodiversity Management Plan for the Clanwilliam sandfish (Paxton et al. 2012).

Taken together, these records, made up of scientific reports, anecdotal observations, museum collections, and dedicated fish surveys, offer insights into how sandfish distribution has changed over time, particularly following the introduction of invasive alien fishes (IAF) into the ODRS. Many of these data have been uploaded onto the Freshwater Biodiversity Information System (FBIS) and are available online for download (FBIS 2024).

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[*] Collections were limited to the Olifants arm of the ODRS, and no collections were made from the Doring arm at this time.

3.1.2 Current records

The pre-2000 records provide valuable historical context for how sandfish distribution has changed over time. However, it was only in 2001 when the first ODF survey took place that survey efforts became somewhat standardised, with representative sites along the mainstem established for repeat sampling into the future. In October 2021, eight years after the last of these surveys, those sites were revisited and another comprehensive survey of the Doring River mainstem undertaken to determine the sandfish's current distribution and population status. In February and March 2022, five of the six tributaries where sandfish were suspected to persist at the time, according to Jordaan et al. (2020; the Matjies, Tra-Tra, Biedouw, Kransgat, and Gif rivers) were also surveyed. A survey of the sixth tributary (the Oorlogskloof River) was carried out in March 2022 by staff of the Oorlogskloof Nature Reserve and the Northern Cape Department of Agriculture, Environmental Affairs, Rural Development and Land Reform (DAERL). The goal of these surveys was to gather updated distribution data of the indigenous fish species present in the Doring River, with a specific focus on sandfish.

3.1.3 Objectives

The aims of this chapter were to: i) collate all available sandfish occurrence records from the FBIS, the grey literature, and the various ODF surveys to produce decadal ODRS sandfish distribution maps to evaluate changes in distribution over time; ii) evaluate changes in sandfish distribution and population status over the past 20 years using presence/absence and occurrence data gathered during the various ODFS surveys; iii) compare fish species (native and non-native) composition between surveys and sites over the course of the ODF surveys; iv) assess differences in size distributions between sampling locations across the species' current range; v) consider the spatial overlap of the current range with protected areas; and vi) make recommendations for future surveys and research avenues.

3.2 Methods

3.2.1 Historical sandfish occurrence records: data collation

3.2.1.1 The Freshwater Biodiversity Information System (FBIS) and grey literature search

To evaluate changes in sandfish occurrence over time, all records from 1936 (when the first record appears) onward were downloaded from the Freshwater Biodiversity Information System (FBIS) on 27 March 2024. Only records verified by taxon specialists as part of the development of the Department of Forestry, Fisheries and the Environment (DFFE) Screening Tool for Fish were included, as this process excluded erroneous records (Kajee et al. 2023a). To do so, the "DFFE Screening Tool for Fish 2022" filter was selected before downloading sandfish records. The acquisition of these data necessarily includes only "presence" records and excludes any "absence" records that may be associated with the data sources in the FBIS.

Two records from iNaturalist (https://www.inaturalist.org) were excluded, as the geographic location setting was set to "obscured" within the application, resulting in the projection of the observation locations to a random location within a quarter-degree squared radius of where the observation actually occurred. After cleaning, a total of 262 sandfish records downloaded from the FBIS were retained, the bulk occurring after 2000 (Figure 3.1).

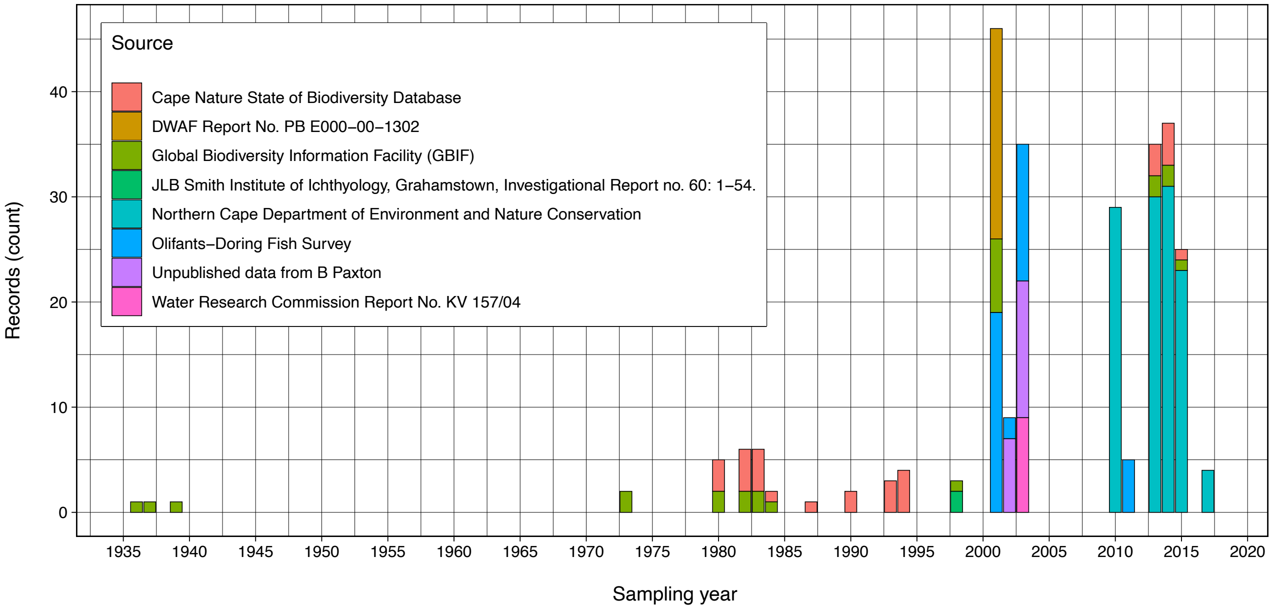

Figure 3.1. Dates and sources of sandfish records stored within the Freshwater Biodiversity Information System (FBIS) between 1936 and 2017 (FBIS 2024).

In addition to the FBIS records, a grey literature search was carried out with the help of taxon experts, who identified likely internal reports and other grey literature which were likely to contain additional occurrence records. Only records and observations which included at least the collection or observation year, as well as geographic coordinates, or the name of a well-known geographic reference site, were included. For example, an entry in

Piscator No. 57 references a March 1938 collection in the "Keerom pool", which "… was remarkable for the great number of small indigenous fish (*Barbus* and *Labeo*) which it contained and large collections were made with a minnow seine net" (Harrison 1963). As the only *Labeo* in the system, this constitutes a reliable occurrence record for sandfish. Furthermore, Keerom is a well-known pool on the upper Olifants River located at the confluence of the Dwars River with the mainstem and can be georeferenced with accuracy. In other cases, the site descriptions are less precise, such as a 1950 photograph of a leaping sandfish ascending "the Cascades on the Olifants River below Bulshoek" (Harrison 1963). The Cascades are a well-known 2 km reach located just below the Bulshoek Dam wall that has long been frequented by anglers. In these cases, a point within the described area was randomly assigned and its coordinates used as the geographic reference. Despite the lack of accuracy associated with some of these observations, they add much-needed temporal and spatial resolution, especially between 1930 and 1979, when only five records of sandfish could be downloaded from the FBIS. A total of 25 sandfish occurrence records were gathered from the grey literature (Figure 3.2).

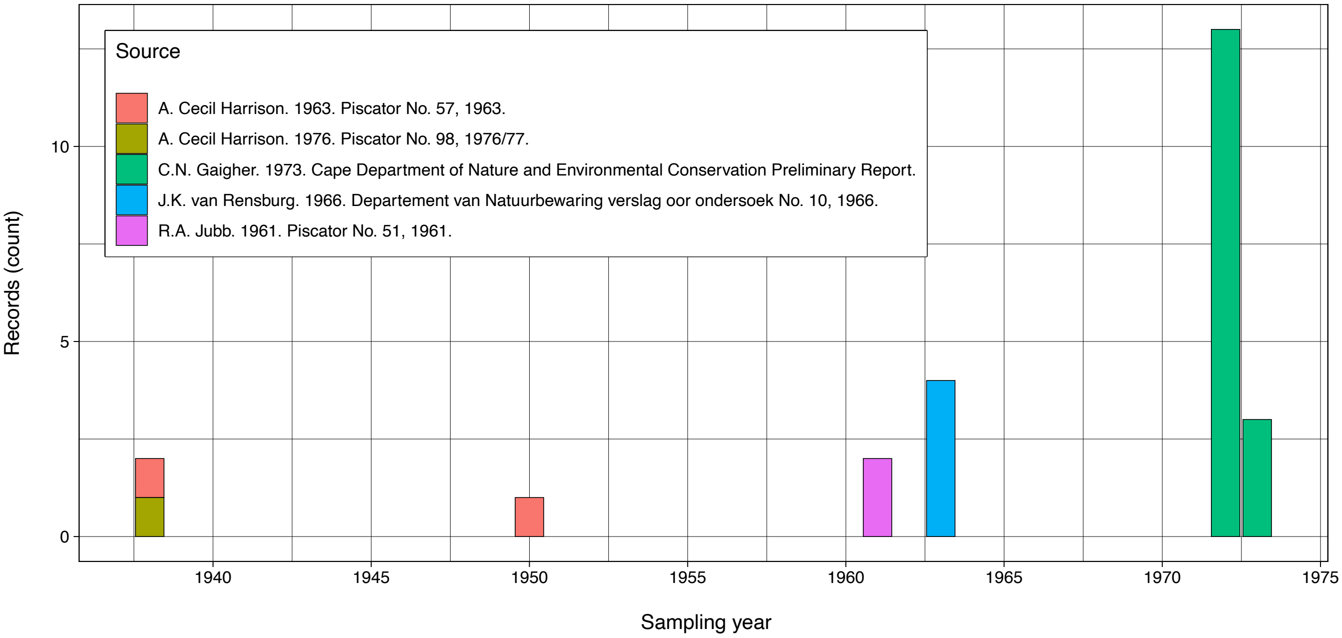

Figure 3.2. Dates and sources of sandfish records gathered from five sources in the grey literature between 1938 and 1973 (FBIS 2024).

Decadal maps were created using ArcGIS Pro (Version 3.2.2; ESRI 2024) mapping sandfish occurrence throughout the ODRS from the 1930s to the 2010s. Occurrence records downloaded from the FBIS were differentiated from those gathered from the grey literature using circles for the former and triangles for the latter.

3.2.1.2 Olifants-Doring Fish surveys

While occurrence records for sandfish started from 1936, semi-standardised and repeated surveys of the ODRS only began in 2001, with the first ODF survey. The ODF surveys collectively refer to the surveys undertaken in 2001, 2011, 2013, 2021, and 2022 focused on sampling the fish fauna of the mainstem and some tributary sites in the ODRS (Figure 3.3). Although the surveys included different sets of sites sampled with a variety of gear, they constitute the most complete recent record of indigenous fish distributions in the ODRS. Moreover, raw counts per site and sampling method are available, enabling some evaluation of population trends over the 20-year period. The sampling schedule for all surveys can be found in Appendix A 3.

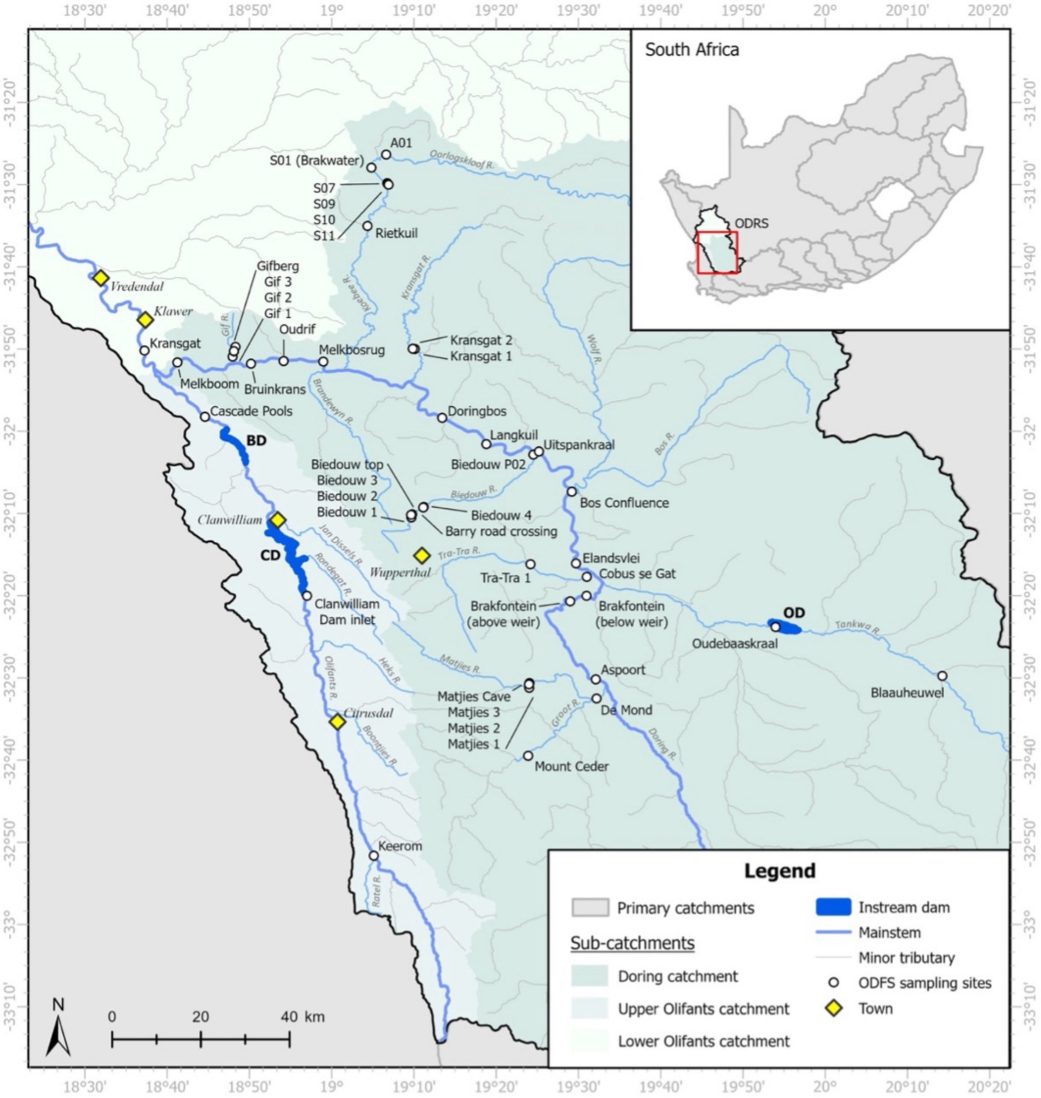

Figure 3.3. Site names of all mainstem and tributary sites sampled during at least one of the ODF surveys carried out in 2001, 2011, 2013, 2021, and 2022. Where more than one site occurs within a small area, the names have been listed from upstream-most site (top) to downstream-most site (bottom). BD = Bulshoek Dam, CD = Clanwilliam Dam, OD = Oudebaaskraal Dam, ODRS = Olifants-Doring River system. Note that there is a site called "Kransgat" on the Olifants mainstem just upstream of the town of Klawer; this is separate from the Kransgat River, a tributary of the Doring where sampling also took place.

In 2001, two surveys (in February and October) of the ODRS were undertaken by Southern Waters Ecological Research and Consulting on behalf of the Department of Water Affairs and Forestry and the Department of Agriculture (Paxton et al. 2002). The purpose of the surveys was to provide updated information on the distributions of the three large cyprinids indigenous to the ODRS (Clanwilliam yellowfish *Labeobarbus seeberi,* Clanwilliam sandfish *Labeo seeberi,* and sawfin *Cheilobarbus serra*) and of the non-native smallmouth bass (*Micropterus dolomieu*) and bluegill (*Lepomis macrochirus*). In February 2001, three sites on the Olifants River mainstem were sampled, as were eight on the Doring River mainstem, and one on each of the Koebee, Oorlogskloof, and Tra-Tra Rivers. In October of that year, four Olifants mainstem sites and seven Doring mainstem sites were sampled, as well as one on each of the Koebee, Oorlogskloof, Biedouw, Tra-Tra, and Matjies Rivers (Appendix A 3). A variety of methods was employed to target different species and size classes: gill netting, seine netting, hand netting, angling, spearfishing, electrofishing, dive transects, and visual observations (Appendix A 4). Although the results of these surveys were synthesized in a report (Paxton et al. 2002), Bruce Paxton (the lead author) provided the raw data to be used in the present analyses.

In February and March 2011, a third abdridged ODF survey was undertaken as part of the development of the Biodiversity Management Plan (BMP) for the Clanwilliam sandfish (Paxton et al. 2012). During this survey, two Doring mainstem sites were sampled, as were two on the Biedouw River, and one each on the Gif, Oorlogskloof and Tankwa Rivers (Appendix A 3). In November of that year, a fourth ODF survey took place as part of the Department of Water Affairs' River Health Programme. Six Doring sites were sampled, as was one Olifants mainstem site, and one Oorlogskloof River site. Electrofishing, seine netting, and fyke netting were employed at the different sites (Appendix A 4). These surveys constituted the first time that a large three-winged fyke net (opening: 2 m wide x 1.2 m high; 7 m trap; wings: 22 m long x 2.5 m deep with leadcore bottom and floats; mesh size: 18 mm) was used (Figure 3.4); this would become the standard sampling method for ODF surveys moving forward.

Figure 3.4. Photographs of the cod end of the large three-winged fyke net (left) and the net in the process of being set at Oudrif on the Doring River in October 2021 (right). Photographs by Jeremy Shelton.

In October and November 2013, the fifth ODF survey was carried out by the Endangered Wildlife Trust, during which seven Doring mainstem sites, one site on the Koebee River and another on the Tankwa River were sampled with the large three-winged fyke net (Appendix A 4). Bruce Paxton, who was involved in the data collection for the 2011 and 2013 surveys, shared the raw data from all three surveys for inclusion in this chapter. The 2021 and 2022 surveys of the Doring mainstem and tributaries carried out for the present study are described in detail below.

3.2.2 Current distribution

3.2.2.1 2021 ODF survey: Doring River mainstem

3.2.2.1.1 Study area

A 10-night survey of the Doring River mainstem took place in October 2021 during which 10 sites sampled during previous ODF surveys were re-visited. These sites were located along the length of the Doring River mainstem from its headwaters at Mount Ceder to its confluence with the Olifants River near Klawer (Table 3.1; Figure 3.5; Figure 3.6). Although the sites De Mond and Mount Ceder are technically on the Groot River, for the purposes of this study they were categorised as Doring mainstem sites, as the Groot is the largest upstream source of the Doring, and upstream of the confluence of these two rivers the Doring becomes a seasonally flowing floodplain delta, rather than a distinct river channel.

Table 3.1. Mainstem site locations, listed from upstream to downstream.

Site name	**Latitude**	**Longitude**	**Biome**	**Vegetation type** (Mucina et al. 2014)	**MAP (mm)** (Mucina et al. 2006; Rebelo et al. 2006)
Mount Ceder	-32.657890°	19.398310°	Fynbos	Cederberg Sandstone Fynbos	395
De Mond	-32.542070°	19.536860°	Succulent karoo	Swartruggens Quartzite Karoo, Tanqua Karoo	72-200
Brakfontein	-32.334510°	19.508980°	Succulent karoo	Swartruggens Quartzite Karoo	200
Bos Confluence	-32.122300°	19.486650°	Succulent karoo	Tanqua Karoo	72-112
Uitspankraal	-32.040940°	19.419830°	Succulent karoo	Tanqua Karoo	72-112
Langkuil	-32.025940°	19.313230°	Succulent karoo	Tanqua Karoo	72-112
Melkbosrug	-31.858970°	18.983310°	Succulent karoo	Doringrivier Quartzite Karoo	200
Oudrif	-31.857540°	18.902550°	Succulent karoo	Doringrivier Quartzite Karoo	200
Bruinkrans	-31.863010°	18.836330°	Succulent karoo	Doringrivier Quartzite Karoo	200
Melkboom	-31.860161°	18.687141°	Succulent karoo	Doringrivier Quartzite Karoo	200

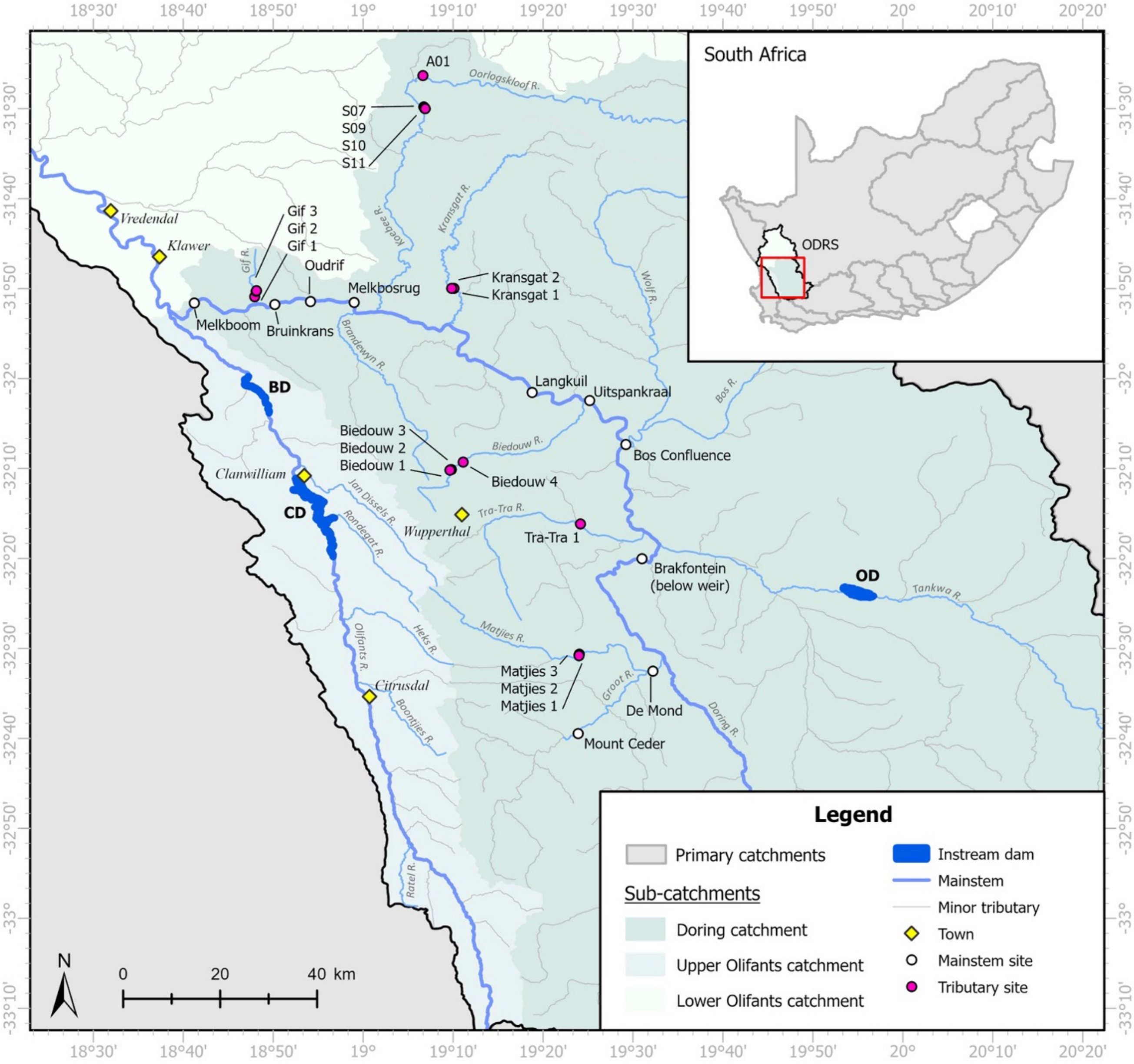

Figure 3.5. Study area showing sites sampled during the 2021 and 2022 ODF surveys. Where more than one site occurs within a small area, the names have been listed from upstream-most site (top) to downstream-most site (bottom). BD = Bulshoek Dam, CD = Clanwilliam Dam, OD = Oudebaaskraal Dam, ODRS = Olifants-Doring River system.

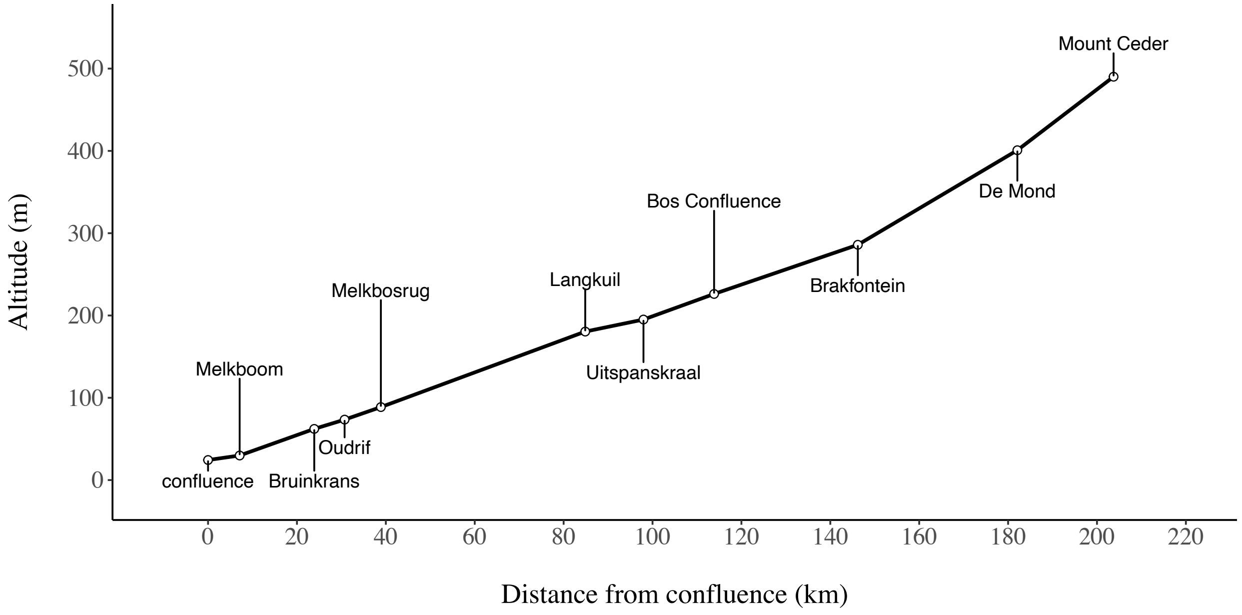

Figure 3.6. Distribution of mainstem sampling sites in relation to distance from the Doring-Olifants confluence and river gradient.

The Doring River catchment is located at the interface of two climatic zones, with most tributaries in the west falling within the Fynbos biome, and those in the east and north falling within the Karoo biome. Most of the mainstem sites fall in the latter biome (SANBI 2018; Table 3.1). All sites receive mostly winter rainfall, mostly between May and August, although that period can be shorter in the driest regions (Mucina et al. 2006). The high winter baseflows are mostly supplied by the western tributaries (largely located in the Fynbos biome), with flow ceasing for several months each year during the summer months. During this time, flows may resume for brief periods following summer thunderstorm activity over the Karoo (Paxton et al. 2002), delivering turbid water with high densities of suspended solids (DWAF 2005). All sites except Mount Ceder are characterised by a semidesert climate, receiving very low rainfall throughout the year.

3.2.2.1.2 Data collection

The large three-winged fyke net (Figure 3.4) was set overnight at each site for a duration of 12.5 to 16.0 hours. Although a single night of sampling per site may limit the detection of rare species, budget and personnel constraints, along with the need for sampling consistency with past ODF surveys, dictated this approach. In the morning, the net was retrieved, and the catch transferred to large containers filled with fresh river water. Each container was actively oxygenated using several battery-operated oxygen pumps.

Each fish present in the catch was identified to species, and measured (fork length FL and total length TL) to the nearest cm. Where there were more than 30 individuals of a single species, a random subsample of 30 was measured and the rest only counted. Prior to being measured, each fish was anaesthetised by being placed in a bucket containing a solution of water and MS-222 to minimise stress during processing. After being measured, fish were allowed to recover fully in an actively oxygenated bucket with fresh river water, then returned to the river.

3.2.2.2 2022 survey: Doring River tributaries

3.2.2.2.1 Study area

We prioritised the six tributaries where sandfish were known or suspected to persist according to Jordaan et al. (2020) during the 2022 tributaries survey. We sampled the Gif, Kransgat, Biedouw, Tra-Tra, and Matjies rivers in March and April 2022 (Appendix A 3; Figure 3.5). Staff from the Oorlogskloof Nature Reserve (OKNR) and the Northern Cape Department of Agriculture, Environmental Affairs, Rural Development and Land Reform (DAERL) sampled a sixth tributary, the Oorlogskloof River, in March 2022 as part of a regular sampling programme. These data were included as part of the 2022 tributaries survey dataset. Sampling took place at the end of the dry summer season to facilitate fyke sampling, as flow was either low (Matjies, Biedouw), or absent (Gif, Kransgat, Tra-Tra, Oorlogskloof) during this time, resulting in sampling from isolated pools in the latter four tributaries. Site coordinates and elevations can be found in Appendix A 5.

The Matjies River (Figure 3.7) flows into the Groot River, which in turn becomes the Doring River. As such, the Matjies is one of the source tributaries of the Doring River mainstem. It cuts through a gorge and flows year-round. The riparian vegetation around the Matjies River sites is classified as Swartruggens Quartzite Fynbos (SANBI 2018). It receives 300 mm mean annual precipitation (MAP), which falls mostly between May and August (Rebelo et al. 2006). The three sites sampled on the Matjies were chosen due to their proximity to the access point, as well as their size and depth, which were deemed sufficient to support sandfish, if present. Other nearby pools were smaller and shallower. Although non-native bluegill is present here in small numbers, the sites are located above a natural barrier that excludes the spotted bass and smallmouth bass found further downstream. The downstream-most site is located 17.4 river-kilometres from the confluence of the Matjies and the Groot Rivers.

Figure 3.7. Processing fishes at Matjies 2 in March 2022. Photograph by Jeremy Shelton.

The upper Biedouw River (Figure 3.8), which also cuts through a gorge, is perennial. The riparian vegetation in this area is classified as Cederberg Sandstone Fynbos (SANBI 2018). Mean annual precipitation is 395 mm, peaking between May and August (Rebelo et al. 2006). The river channel is overgrown by riparian vegetation (most of it native), making access to most pools difficult or impossible. The upstream-most sites (Biedouw 1, 2, and 3) are located in the gorge, and were selected due to their accessibility and depth. Biedouw 1 and 2 are located above the natural bass barrier, while Biedouw 3 is located below that barrier. Biedouw 4 is located further downstream, at the point where the gorge transitions into foothill morphology. It is one of the downstream-most pools located within the perennial reach of the Biedouw River. The vegetation type in the riparian zone surrounding this site is classified as a transition zone between Cederberg Sandstone Fynbos and Agter-Sederberg Shrubland (Mucina et al. 2014). The downstream-most site is located 29.9 river-kilometres from the confluence of the Biedouw and Doring Rivers.

Figure 3.8. Upstream-facing aerial views of the Biedouw River gorge, where Biedouw 1, 2, and 3 are located (left) and of the transition zone between foothill and gorge morphology, with the approximate location of Biedouw 4 marked by the yellow oval (right). Photographs by Jeremy Shelton.

The vegetation around the Tra-Tra River site (Figure 3.9) is classified as Tanqua Karoo (SANBI 2018). The area receives between 72 and 112 mm MAP, most of it falling between May and August (Mucina et al. 2006). The lower reach of the Tra-Tra River ceases to flow in the late summer, fragmenting into a series of isolated pools. The single Tra-Tra site was selected as it was the only accessible large pool holding water in the lower 15 km of the river at the time. It had also been identified by locals as a pool that has previously held sandfish. The site is located 13.8 river-kilometres from the confluence of the Tra-Tra and Doring rivers.

Figure 3.9. The Tra-Tra River site. At the time of sampling, the river had ceased to flow, resulting in remaining pools becoming isolated from one another. Cars in the foreground provide scale. Photograph by Jeremy Shelton.

The riparian zone of the Kransgat River sites (Figure 3.10) is classified as Doringrivier Quartzite Karoo (SANBI 2018). It receives 200 mm MAP, mostly falling between June and August (Mucina et al. 2006). The two sampling pools in the Kransgat River were selected as they are the only two pools in the tributary that hold sufficient water to support fish through the dry summer season, according to local farmers (Riaan van der Walt, pers. comm., 2025). The pools are suspected to be fed by groundwater, given that the remainder of the river desiccates completely each summer. They are located upstream of a natural bass barrier. Both pools are characterised by steep rock walls, and approximately 80% of Kransgat 2 was overgrown with bulrushes. The downstream-most site is located 11.6 river-kilometres from the confluence of the Kransgat and the Doring Rivers.

Figure 3.10. Photographs of Kransgat 1 (left) as seen from the upstream boundary facing downstream and Kransgat 2 (right) during net-setting facing downstream. Photographs by Jeremy Shelton (left) and Cecilia Cerrilla (right).

The riparian area around the Gif River sites (Figure 3.11) is also Doringrivier Quartzite Karoo (Mucina et al. 2014), receiving 200 mm MAP, mostly between June and August (Mucina et al. 2006). The Gif River sites were selected due to their proximity to the access point, as well as their depth, which resulted in their holding water through the dry summer season. In dry years they are the only pools known to have adequate summer habitat for fishes. Most other pools in the area were entirely dry at the time of sampling. The lowest Gif River site (Gif 1) is located below a natural bass barrier, while Gif 2 and 3 are located above that barrier. The downstream-most site is located 1.7 river-kilometres from the confluence of the Gif and Doring Rivers.

Figure 3.11. Gif 2 as seen from the downstream limit facing upstream (left) and from above (right). The image on the right illustrates the limited connectivity between pools during the sampling period. Photographs by Jeremy Shelton.

3.2.2.2.2 Data collection

Each of the Matjies, Kransgat, and Gif sites, and Biedouw 2, 3, and 4 were sampled with a single two-winged fyke net with a square opening (variably 60 x 60 cm, 67 x 67 cm, or 70 x 70 cm; all 4 mm mesh size). Biedouw 1 was sampled with a single-wing fyke net with a half-circle opening (67 cm wide x 60 cm high, 4 mm mesh size). The Tra-Tra site was sampled using four two-winged fyke nets due to its large size (approximately 107 m in length, or 1,500 m^2 in wetted area). As the Oorlogskloof River survey was part of a regular sampling programme led by staff at the OKNR, it was sampled according to their standardised procedure. Site A1 was sampled with a two-winged fyke net, while sites S07, S09, S10 and S11 were sampled with a seine net. No statistical comparisons in abundance were made between sites, as the difference in sampling gear and effort prevented meaningful comparison.

Where fyke nets were used, they were set overnight and retrieved the following morning, within two hours of light either side of sunset and sunrise, following standard protocol (Hardie et al. 2006). As with the mainstem survey, budget and personnel constraints limited sampling to a single night per tributary. The catch was transferred to a series of buckets containing fresh river water and actively oxygenated with battery-operated pumps. Fish were processed as in the Doring River mainstem survey (see 3.2.2.1.2) before being returned to the river. Where fish were caught using a seine net, they were immediately transferred to oxygenated containers and processed as above before being returned to the river.

3.2.2.3 Overlap of current distribution with protected areas

The current distribution of sandfish was mapped at a sub-quaternary catchment scale, based on the presence of occurrence records from 2000 onwards (either during the seven ODF surveys, or based on other historical records accessed from the FBIS). This includes all intervening sub-quaternary catchments which do not contain occurrence records, but which are bounded by sub-quaternary catchments which do contain records. A map was constructed which illustrates these occurrence records, highlighting the sub-quaternary catchments that comprise the current range. An additional layer was included in the map, which includes polygons delineating protected areas in the vicinity of the current range. The protected areas layer (South Africa Protected Areas Database, SAPAD) was downloaded from the website of South Africa's Department of Forestry, Fisheries and the Environment on 1 June 2024 (Department of Forestry, Fisheries and the Environment 2024). All mapping was carried out in ArcGIS Pro (version 3.2.2, ESRI 2024).

3.2.3 Data analysis

3.2.3.1 Change in sandfish distribution and catch rates in the Doring River system

Changes in sandfish distribution from the 1930s onwards were assessed visually after constructing presence/absence maps based on the collated data. The difference in sampling methods employed during the two 2001 surveys and the remaining surveys render statistical comparisons of sandfish catch rates between 2001 and subsequent years difficult. Data from the 2001 surveys were therefore excluded when comparing differences in catch across years. A Kruskal-Wallis test was used to test for differences in catch at mainstem sites between the four fyke net surveys (February/March 2011, November 2011, October 2013, and October 2021).

Average catch-per-unit-effort (CPUE) per tributary sampled in 2022 for all pools sampled with a single fyke net was calculated as the summed number of fish caught at all sites in that tributary, divided by the number of pools sampled.

3.2.3.2 Differences in species composition between surveys and sites

A series of 100% stacked bar charts were used to visualise and facilitate comparisons of species composition between mainstem surveys, between 2021 ODF survey sites, and between the tributaries sampled in 2022. Pearson's Chi-squared test was used to test for differences in frequencies of species composition at Doring mainstem sites during the 2021 ODF survey.

3.2.3.3 Differences in size distribution across ODF surveys

To inform the data analysis approach, potential biases in fish detectability – particularly those introduced by size-selective sampling gear – are first considered. Comparisons in size metrics of different populations of sampled fish (e.g., average size, size distribution) must be treated with caution, given biases in capture probability that may be introduced by differences in sampling gear, fish community composition, abiotic factors (temperature, discharge, water clarity, water depth), and fish size itself (Gwinn et al. 2016; Healy et al. 2022b). Capture probability can even vary depending on densities of non-native species present at the sampling location, despite the standardisation of sampling effort and gear type (Healy et al. 2022b). Moreover, differences in capture probability are not always consistent across species; some fishes may be less easily captured under higher discharge while others show no effect of discharge at all in the same sampling location (Healy et al. 2022b). Gear type is one of the most recognised factors that may impact detection probability, and it can interact with other factors in complex ways; for example, detection probability of carp gudgeon (*Hypseleotris* spp.) decreases as inundated wetland area increases when using fyke nets but increases under the same conditions when using seine nets (Beesley et al. 2014). Of special concern in studies comparing size distributions of fish populations over space or time is the size-selectivity of certain gear. In one study, boat electrofishing captured a higher proportion of large *L. macrochirus* in lake environments compared with other gear types (cloverleaf traps, mini-fyke nets, and beach seines), whereas cloverleaf traps captured smaller individuals (Sullivan et al. 2019).

The 2021 mainstem and 2022 tributaries surveys carried out as part of the present study standardised sampling methods within each survey, with mainstem sites being sampled with the large fyke net, and tributary sites with small fyke nets. These were supplemented with opportunistic angling (mainstem sites) and snorkel passes and visual observations (tributary sites) – though these were not part of the standard sampling protocol. The remaining surveys (including the 2022 Oorlogskloof survey) were carried out by different research groups, resulting in the use of eight different gear types and two observational techniques across surveys.

Given the differences in sampling methods across time and space, as well as uncontrollable differences between sites (discharge, temperature, water quality, depth, substrate type, community composition, etc.), statistical comparison of size structures across time and space would be misleading. However, differences in the spatial distribution of different size classes across the catchment remains an important research question, as this can reveal potential population source and sink areas. Therefore, in order to minimise bias and enable such a comparison, measured sandfish were categorised into six size classes, and their detection/non-detection during each survey compared: class 1 (< 20 mm TL), class 2 (21 – 100 mm TL), class 3 (101 – 200 mm TL), class 4 (201 – 300 mm TL), class 5 (301 – 400 mm TL), and class 6 (> 401 mm TL). The largest size class includes all large adults, while classes 3 to 5 are divided into 100 mm intervals. Fish under 100 mm TL were split into two classes to distinguish larvae from juveniles. It should still be borne in mind that even detection/non-detection data are susceptible to sampling biases (Gwinn et al. 2016) – so these comparisons are treated with appropriate caution.

Finally, nonmetric multidimensional scaling (nMDS) ordination applied to a Bray-Curtis similarity index was used to visualise differences in size distributions between sandfish populations sampled by fyke in 2021 (Doring mainstem) and 2022 (tributaries) following the methods described by Miranda (2024) and Miranda et al. (2024). Ordinations are not statistical in nature and serve as visual aids for assessing size structure (Miranda 2024). A similarity index is first calculated for each pairwise comparison of samples (in this case rivers); this index is then plotted onto a two-dimensional space such that the distances between points on the plot reflect their similarities (Miranda 2024). In other words – the closer the points, the more similar their size distributions. The Bray-Curtis similarity index was chosen because, unlike the Euclidean similarity index, it does not consider double zeros – in other words, it will avoid classifying two sites as more similar due to the shared absence of a particular size class (Miranda 2024). Unconstrained ordinations like nMDS ordinate data without regard to external factors like environmental conditions or fishing regulations, helping to visualise relationships between sites based solely on length-frequency distributions (Miranda 2024). Fish lengths were categorised into 50 mm TL bins to construct the ordination plot, and vectors corresponding to each of those bins were included to illustrate the contribution of different size classes to the observed patterns in sample dissimilarity. Length-frequency histograms of each sampled river are displayed alongside the nMDS ordination to facilitate interpretation of size similarities and dissimilarities between rivers. The Tra-Tra River sample was excluded due to the insufficient number of sandfish caught at that site (n = 2).

3.2.3.4 Overlap of current distribution with protected areas

The total area of overlap between sub-quaternary catchments in the sandfish's range and protected areas was calculated by clipping the catchment layer to the protected areas layer and summing the areas of the resulting polygons. Analysis was carried out in ArcGIS Pro (version 3.2.2, ESRI 2024).

The total area of occupancy (AOO) is defined by the IUCN as the area of suitable habitat currently occupied by the taxon, and is based on a 2x2 km^2 grid overlaid on all occurrences (IUCN Standards and Petitions Committee 2024). However, as noted by Jordaan et al. (2020a), this method tends to severely overestimate AOO for riverine species given that they are confined by the bounds of the river itself, which is seldom 2 km wide. As a result, the current potential AOO for sandfish was calculated here using a method modified from that used by Jordaan et al. 2020a. First, total river-length from the downstream-most occurrence record on the Doring River mainstem to the upstream-most record on each tributary was calculated. All sub-quaternary catchments which contain occurrence records, and all intervening catchments, were included in this calculation. Occupied mainstem river-length was multiplied by 0.03 km (the estimated average width of the Doring River) and occupied tributary river-length was multiplied by 0.01 km (the estimated average width of the occupied tributaries). These values were summed to provide a current potential AOO for the species. The term "potential" AOO is used here to differentiate it from the AOO as derived by Jordaan et al. (2020), as this study includes all intervening river-length between occurrence records to provide one continuous riverine distribution for the species. Only data from 2000 onwards were included.

All work was carried out with appropriate permits and ethics approval (Appendix B 1; Appendix B 2).

3.3 Results

3.3.1 Historical sandfish occurrence

The first available record of sandfish stored on the FBIS comes from a preserved specimen collected in 1936 in the upper Olifants River, just downstream of the Noordhoeks-Olifants confluence (FBIS 2024). In total, five records were collated from the FBIS and the grey literature before 1940, all from the Olifants River mainstem: one from the Cascade rapids below Bulshoek Dam, three from the same sampling location just downstream of the Noordhoeks River confluence, and the last from a well-known angling location in the upper Olifants called Keerom, located at the confluence of the Ratel and Olifants Rivers (Figure 3.12a). No records could be found from 1940-1949, and only seven records could be found within the grey literature between 1950 and 1969 (Figure 3.12b): five on the Olifants River mainstem, as far downstream as the Cascade rapids and as far upstream as Keerom; one on the Thee River (location unspecified) and the last on the Doring River mainstem just upstream of its confluence with the Olifants River. The number of records increases markedly from 1970 onwards, peaking after 2000 after the onset of the Olifants Doring Fish (ODF) surveys. During the 1970s, most records occurred on the Olifants River, with a single record from a tributary of the Groot River in the Doring River system (Figure 3.12c). In the 1980s, the bulk of records starts to shift from the Olifants to the Doring River system, with Olifants records isolated to the reach below Bulshoek Dam (Figure 3.12d). The last records from the Olifants River come from 1980-1984, all from the 20 km reach between Bulshoek Dam and the Doring River confluence. From 1990 onward, records from the Doring River system become more common; at the same time, records from the Olifants River disappear altogether (Figure 3.12e-g).

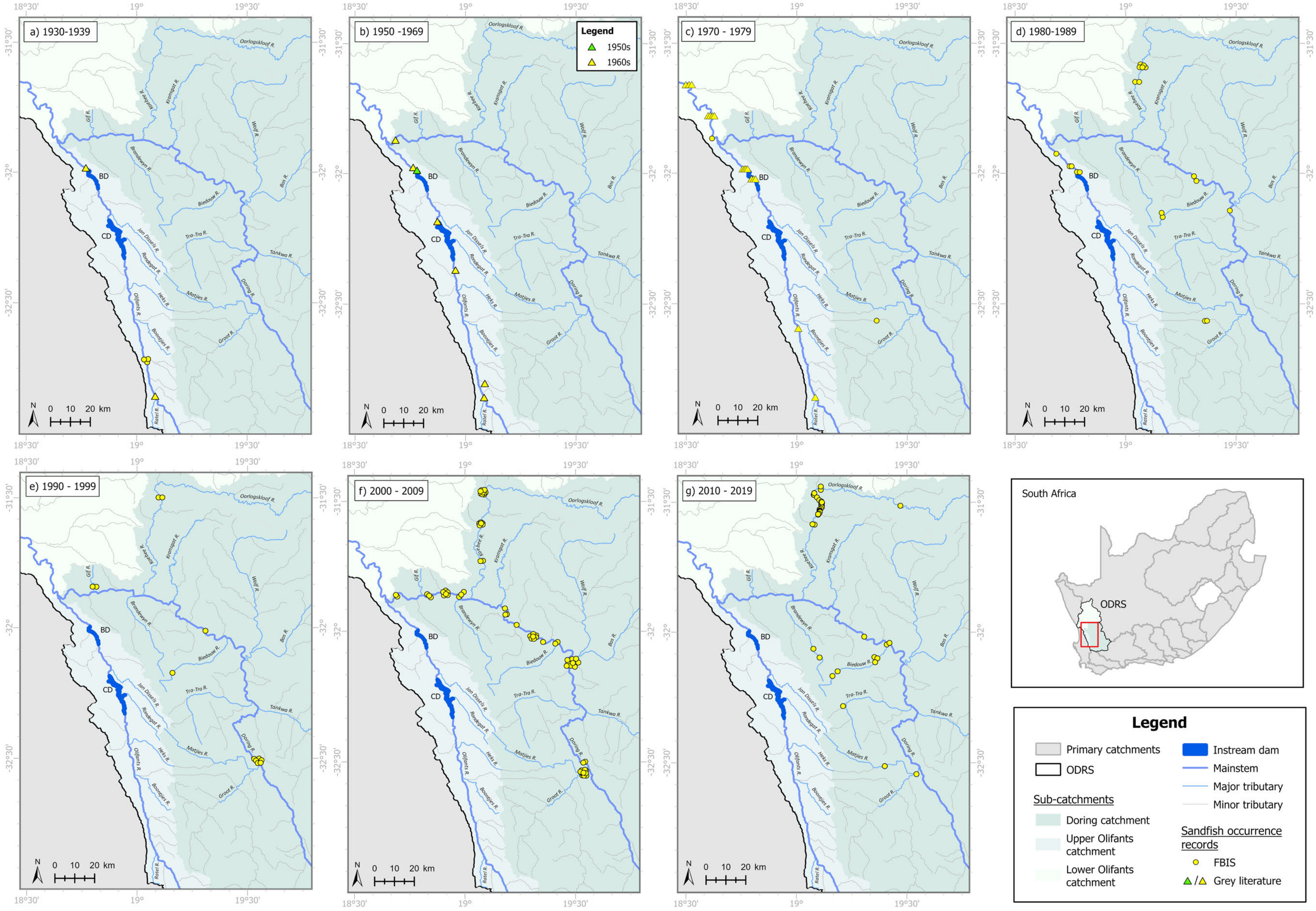

Figure 3.12. Sandfish occurrence records from 1930 to 2019. Records were downloaded from the Freshwater Biodiversity Information System (FBIS 2024; circles) and gathered from the grey literature (triangles). Each inset spans a single decade except for b), which covers two decades (1950 – 1969) because there was only one record from the 1950s. No records exist on FBIS, nor could be found in the grey literature, for the 1940s. Where several records exist at the same location within an inset, the points have been staggered. Displayed data do not include sandfish absence records.

3.3.2 Olifants Doring Fish surveys

3.3.2.1 2001 – 2021: Patterns across time

The ODF surveys, which began in 2001, were focused primarily on sites located within the Doring River system, although four Olifants River sites (Kransgat, Cascade Pools, Clanwilliam Dam inlet, and Keerom) were sampled in 2001 and one (Cascade Pools) in 2011 (Figure 3.13). Sandfish were not detected at the Olifants River sites over the course of these surveys. Gill net catches from the two 2001 surveys were concentrated in the middle to upper Doring mainstem, the Koebee River and the Oorlogskloof River gorge. A total of 333 and 52 sandfish were caught by gill net in February and October 2001, respectively (Figure 3.13a, b). In addition to the 55 sandfish sampled by gill net at Brakwater on the Oorlogskloof River in February 2001, 24 were caught using seine nets. In October 2001, 26 larval sandfish were also sampled by electrofisher in the lower Biedouw and 18 by hand net at the Bos-Wolf confluence.

During the February/March 2011 survey, only a small subset of Doring mainstem sites was sampled, although previously unsampled tributary sites on the Gif, Biedouw, and Tankwa were added (Figure 3.13c). No sandfish were caught at these tributary sites. During both 2011 surveys, mainstem catches remained low, although 53 sandfish were sampled by small fyke net at Brakwater on the Oorlogskloof River.

Sandfish were only present at three of the seven mainstem sites sampled in October 2013, these occurring in the middle Doring River at Langkuil, Bos Confluence, and Elandsvlei (Figure 3.13e). Rietkuil on the Oorlogskloof River, as in previous surveys where this site was included, yielded a relatively large catch of 38 fish.

During the most recent ODF survey in 2021, sandfish were caught at five of the 10 mainstem sites, with the catch again concentrated in the middle Doring River (Figure 3.13f). For the first time since February 2001, a small number (3) of sandfish was caught in the lower Doring River, at Bruinkrans.

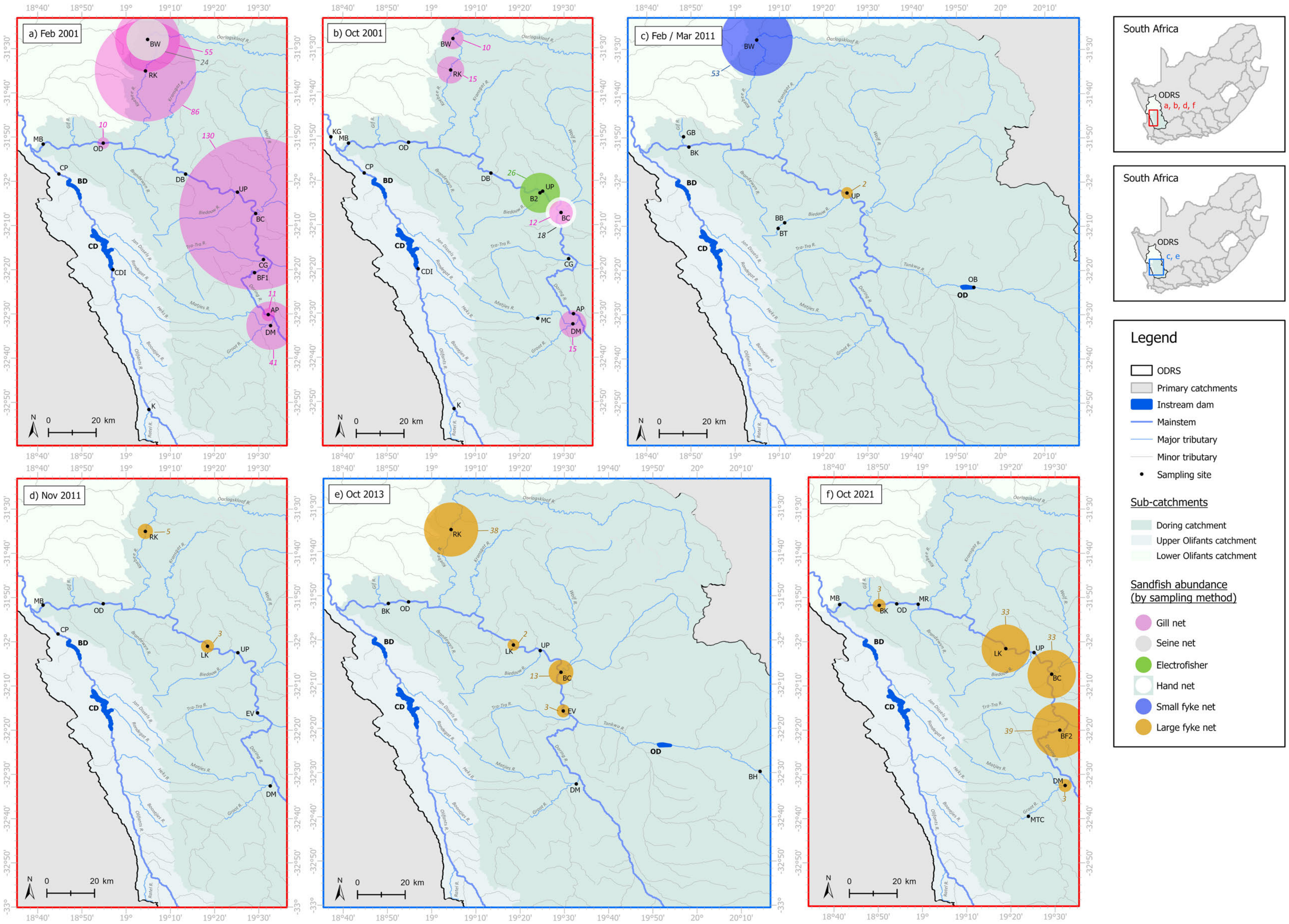

Figure 3.13. Sampling sites and sandfish catches recorded during the 2001-2021 ODF surveys. The size of each translucent bubble is proportional to the number of sandfish caught at each site (raw count), colour-coded by sampling method. Site name acronyms (regular font): AP = Aspoort, BB = Biedouw Barry's Road Crossing, BC = Bos Confluence, BF1 = Brakfontein (above weir), BF2 Brakfontein (below weir), BH = Blaauheuwel, BK = Bruinkrans, BT = Biedouw top site, BW = Brakwater (S01), B2 = Biedouw P02, CDI = Clanwilliam Dam inlet, CG = Cobus se Gat, CP = Cascade Pools, DB = Doringbos, EV = Elandsvlei, GB = Gifberg, DM = De Mond, K = Keerom, KG = Kransgat, LK = Langkuil, MB = Melkboom, MC = Matjies Cave, MR = Melkbosrug, MTC = Mount Ceder, OB = Oudebaaskraal, OD = Oudrif, RK = Rietkuil, UP = Uitspankraal. Dam name acronyms (bold font): BD = Bulshoek Dam, CD = Clanwilliam Dam, OD = Oudebaaskraal Dam. Data from 2001-2013 provided by Bruce Paxton.

A total of two sandfish were sampled at Doring mainstem sites in February/March 2011 (n = 2), three in November 2011 (n = 6), 18 in October/November 2013 (n = 7), and 111 in October 2021 (n = 10). As the large three-winged fyke net was only used for the first time in 2011, average catches can only be compared from then onward. Average sandfish catch in the Doring mainstem was 1.0 fish per pool in February/March 2011, 0.5 fish per pool in November 2011, 2.6 fish per pool in October/November 2013, and 11.1 fish per pool in October 2021 (Figure 3.14). Sandfish catches in the Doring River mainstem did not differ significantly between these surveys (Kruskal-Wallis chi-squared = 2.4239, df = 3, p = 0.49).

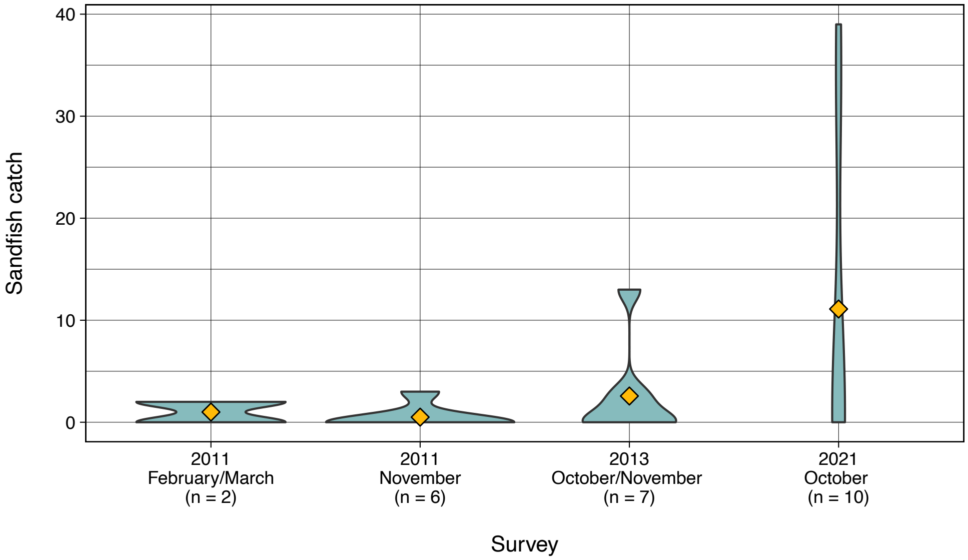

Figure 3.14. Violin plot showing the spread of sandfish catches (from the large 3-winged fyke) per site across surveys in the Doring mainstem. The sample sizes (n) indicated below each survey label on the x-axis refer to the number of sites sampled. Yellow diamonds represent the average catch per number of pools sampled.

No sandfish were recorded at the Olifants mainstem sites during the 2001 and 2011 surveys (Figure 3.15a). Of the native species, only *Labeobarbus seeberi* was found in the Olifants River during this time, with only one and three individuals sampled during the February 2001 and October 2001 surveys, respectively. Non-native species made up 88.2 – 100.0% of the catch in each survey, with *Micropterus* species dominating in 2001, and *L. macrochirus* making up the bulk of the catches in 2011 (90.2%). Eight non-native Mozambique tilapia (*Oreochromis mossambicus*) were sampled during the three surveys, and a single non-native common carp (*Cyprinus carpio*) in November 2011.

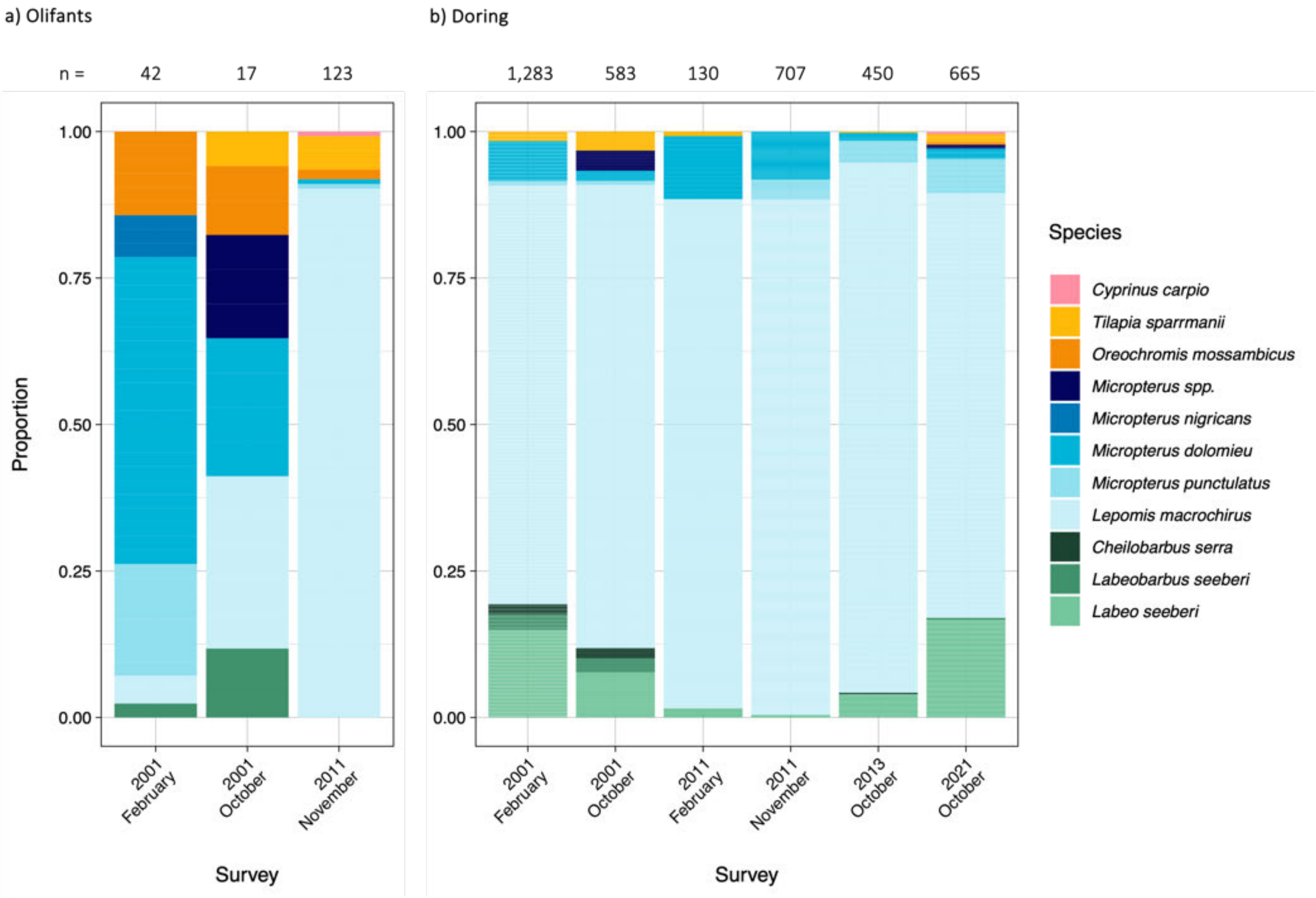

Figure 3.15. Proportional species catch during each ODF survey in the Olifants mainstem sites (a) and the Doring mainstem sites (b). All methods of sampling have been included. See Appendix A 4 for details on sampling methods. Sample size (number of fish caught per survey) is indicated above each bar.

Sandfish were present in the Doring mainstem during each ODF survey, although only two and three individuals were sampled in February and November 2011, respectively. Two other indigenous fish species were sampled over the course of the surveys, though in lower numbers than sandfish: *Labeobarbus seeberi* and *C. serra* (Figure 3.15b). Seven non-native species also occurred in varying proportions across years: four North American centrarchids (*Lepomis macrochirus, M. dolomieu, M. nigricans,* and spotted bass *Micropterus punctulatus*), one Eurasian cyprinid (*C. carpio*), and two extralimital cichlids (banded tilapia *Tilapia sparrmanii* and *O. mossambicus*). As on the Olifants River, non-native species dominated the total catch in each survey, making up 80.6 – 99.6% of the catch across surveys. *Lepomis macrochirus* alone accounted for 71.5-90.4% of the total catch, followed by sandfish (0.4-16.7%), and *M. dolomieu* (1.3-10.8%). The remaining seven species cumulatively comprised 0.8-11.5% of the catch in any given survey.

3.3.2.2 2021 – 2022: Patterns between sites

3.3.2.2.1 Doring mainstem

A total of 652 fish were sampled from the Doring mainstem in 2021 (Figure 3.16). In addition to sandfish, which was the only native species present in the fyke catches, seven species not native to the ODRS also occurred: *C. carpio, L. macrochirus*, *M. dolomieu, M. nigricans, M. punctulatus*, *O. mossambicus,* and *T. sparrmanii*. While most of these species have previously been sampled in the Doring River system, this is, to our knowledge, the first time that *C. carpio* and *O. mossambicus* have been recorded in the system. Although *M. nigricans* was not recorded in previous surveys, it is known from previous sampling and is not considered new to the catchment. Non-native species dominated the total catch, comprising 83% of all fish caught by fyke at the 10 mainstem sites. *Lepomis macrochirus* accounted for 74% of the total catch, followed by sandfish (17%), and *M. punctulatus* (6%). The remaining six species cumulatively comprised 3% of the catch. While there was a significant difference between observed and expected species frequencies per site ($X^2 = 372.52$, df = 72, $p < 0.001$), the fact that the standardized residuals are all small (absolute values < 0.55) suggests that these differences are minor. The groups driving these small differences in species frequencies are *O. mossambicus* and *M. punctulatus* at Bruinkrans (residual = 0.54 and 0.32, respectively) and *C. carpio* at Melkboom (residual = 0.41).

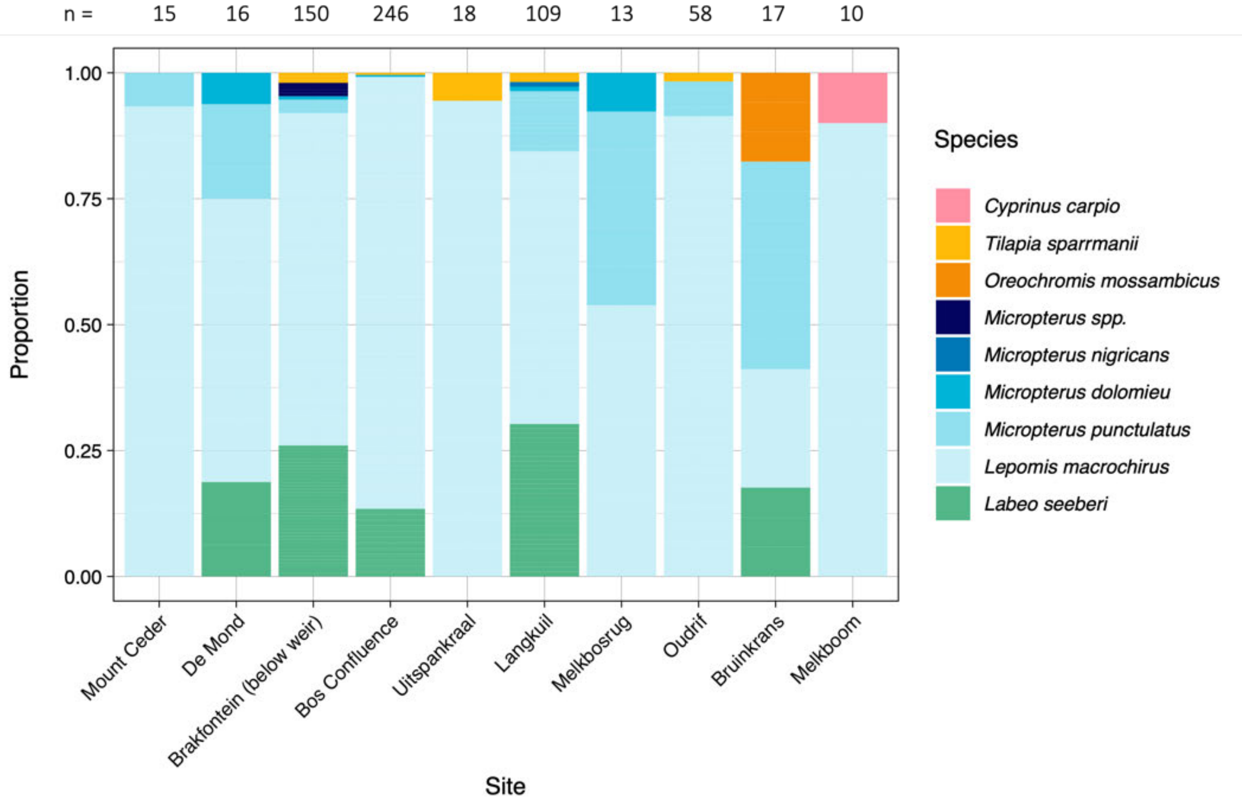

Figure 3.16. Proportional species catch at each of the ten Doring mainstem sites sampled by large fyke in 2021. Four bass from Brakfontein could not be identified to species (*Micropterus* species). Sample size (number of fish caught per site) is indicated above each bar.

3.3.2.2.2 Doring tributaries

Due to the large number of tributary sites sampled in March and April 2022, these have been mapped and analysed separately from the 2021 ODF survey mainstem sites. A total of 50 sandfish were sampled from the Gif River, 218 from the Oorlogskloof River, 276 from the Kransgat River, 11 from the Biedouw River, two from the Tra-Tra River, and 61 from the Matjies River. Sandfish were present at all sites sampled except for sites Gif 3 and Biedouw 4 (Figure 3.17).

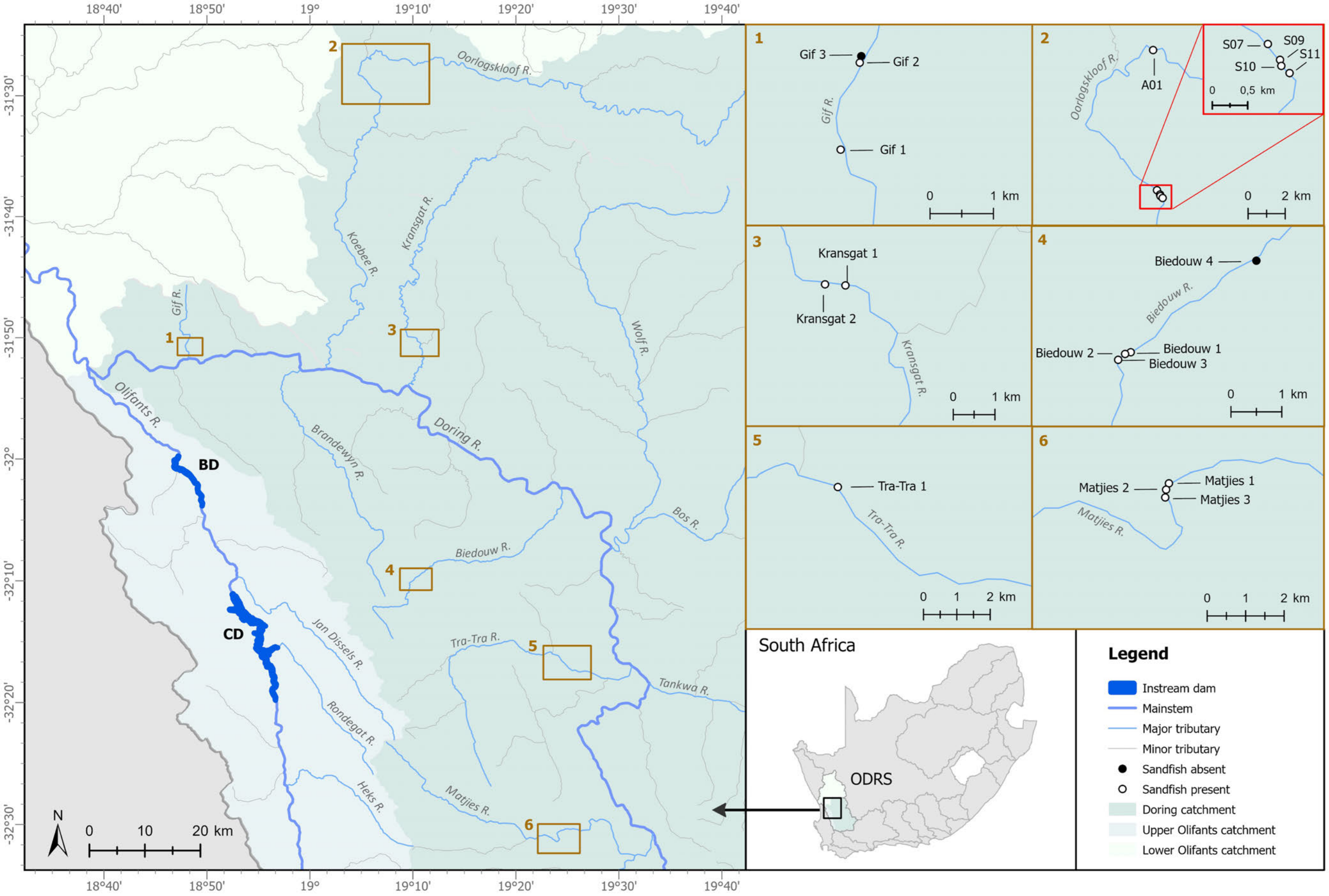

Figure 3.17. Doring tributaries survey area, with sampling sites (and sandfish presence/absence) indicated by the black and white circles in the inset maps. The insets (numbered 1-6) show the sampling sites per tributary. BD = Bulshoek Dam, CD = Clanwilliam Dam, ODRS = Olifants-Doring River system.

There was considerable variation in sandfish catches between tributaries and individual sites (Figure 3.18). Note that sampling method and effort varied between some of the sampling sites: all sites on the Biedouw, Gif, Matjies and Kransgat Rivers (as well as A01 on the Oorlogskloof) were sampled with a single fyke net, whereas the Tra-Tra was sampled with four fyke nets (due to its large size), and S07, S09, S10 and S11 on the Oorlogskloof were seined (due to standardised sampling methods established for the Oorlogskloof River surveys). The large spread in per-site catches among the Oorlogskloof sites can be separated by sampling method: the largest catch occurred at

A01 where a fyke net was used, whereas the smaller catches occurred at the remaining four sites where a seine net was used.

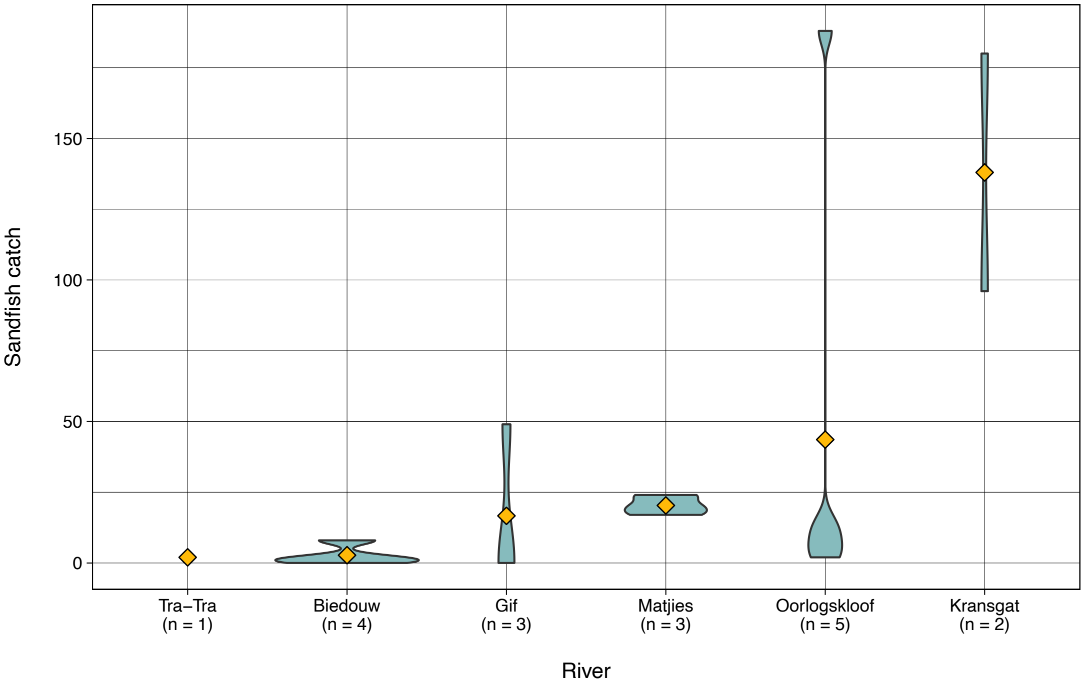

Figure 3.18. Violin plot showing the spread of sandfish catches per site across tributaries sampled in March/April 2022. The sample sizes (n) refer to the number of pools sampled on that tributary. Yellow diamonds represent the average catch per pool. Note that sampling method and effort varied between sites.

The largest single catch of sandfish occurred at A01 on the Oorlogskloof (188), followed by the two Kransgat sites (180 and 96 fish, respectively). The lowest catch occurred at the Tra-Tra site, where only two sandfish were caught, despite the use of four fyke nets. Catches at the Biedouw River were low across sites, ranging from zero at the downstream-most site (Biedouw 4) to eight at the site just downstream of the bass barrier (Biedouw 1). There was considerable spread among the Gif River sites, with one sandfish sampled at the downstream-most site (Gif 1), 49 at the middle site (Gif 2) and none at the upstream-most site (Gif 3). Catches on the Matjies River were most consistent, with 17-24 sandfish caught per site.

Average CPUE was lowest at the Tra-Tra (0.50 fish/pool), and highest at the Oorlogskloof (188.00 fish/pool), although it should be noted that only a single pool was sampled by fyke at each of these tributaries (Figure 3.19). Of the tributaries at which more than one pool was sampled, the Kransgat had the highest average CPUE (138.00 ± 42.00), followed by the Matjies (20.33 ± 2.03), the Gif (16.67 ± 16.17), and finally the Biedouw (2.75 ± 1.80). Due to the small sample sizes of the different tributaries (1-4 sites sampled by fyke per tributary), average CPUE between tributaries could not be statistically compared.

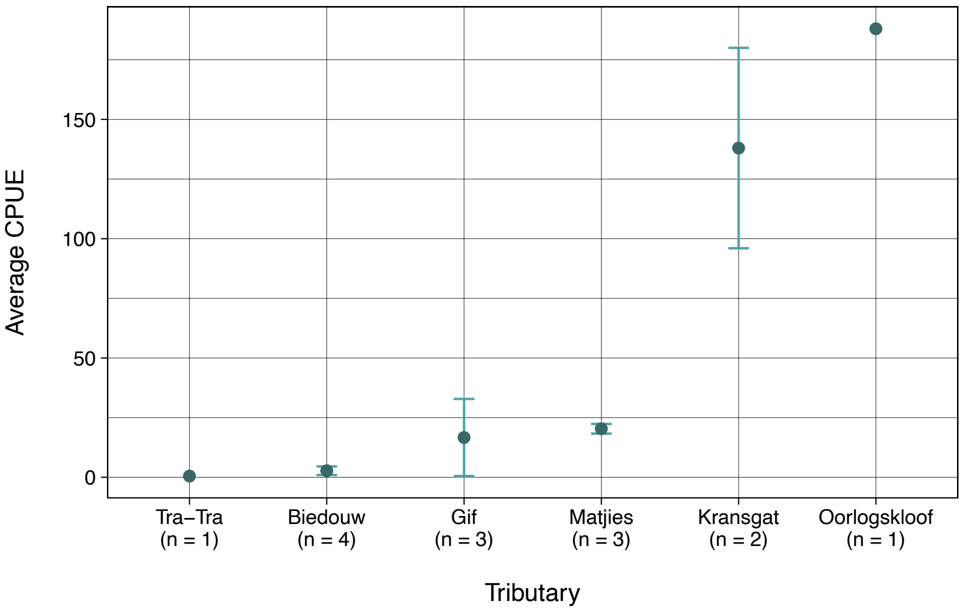

Figure 3.19. Average CPUE per tributary sampled by fyke in 2022. Average CPUE was defined as the sum of the CPUE of the pools sampled divided by the total number of pools sampled. Standard error bars are shown where n > 1.

Catches in the tributaries comprised predominantly native fishes in all cases except the Tra-Tra, where 99.7% of the catch consisted of non-native *L. macrochirus* (94.4%) and *M. punctulatus* (5.3%; Figure 3.20). Native fishes made up 91.1 – 99.7% of the catch at the Matjies, Biedouw, Kransgat, and Oorlogskloof rivers, and 72.6% of the catch at the Gif River. Non-native fishes were present in the Matjies (*L. macrochirus,* 0.3%) and Gif rivers (*M. dolomieu*, 0.3%), and extralimital *T. sparrmanii* occurred in the Kransgat (6.9%), Oorlogskloof (8.9%) and Gif rivers (27.1%). In the Biedouw River, *M. punctulatus* occurred at Biedouw 1, immediately below the known bass barrier, and at Biedouw 4 (along with *L. macrochirus*). In the Matjies River, *M. dolomieu* occurred only at Gif 1, along with one adult sandfish and three dead *C. serra.*

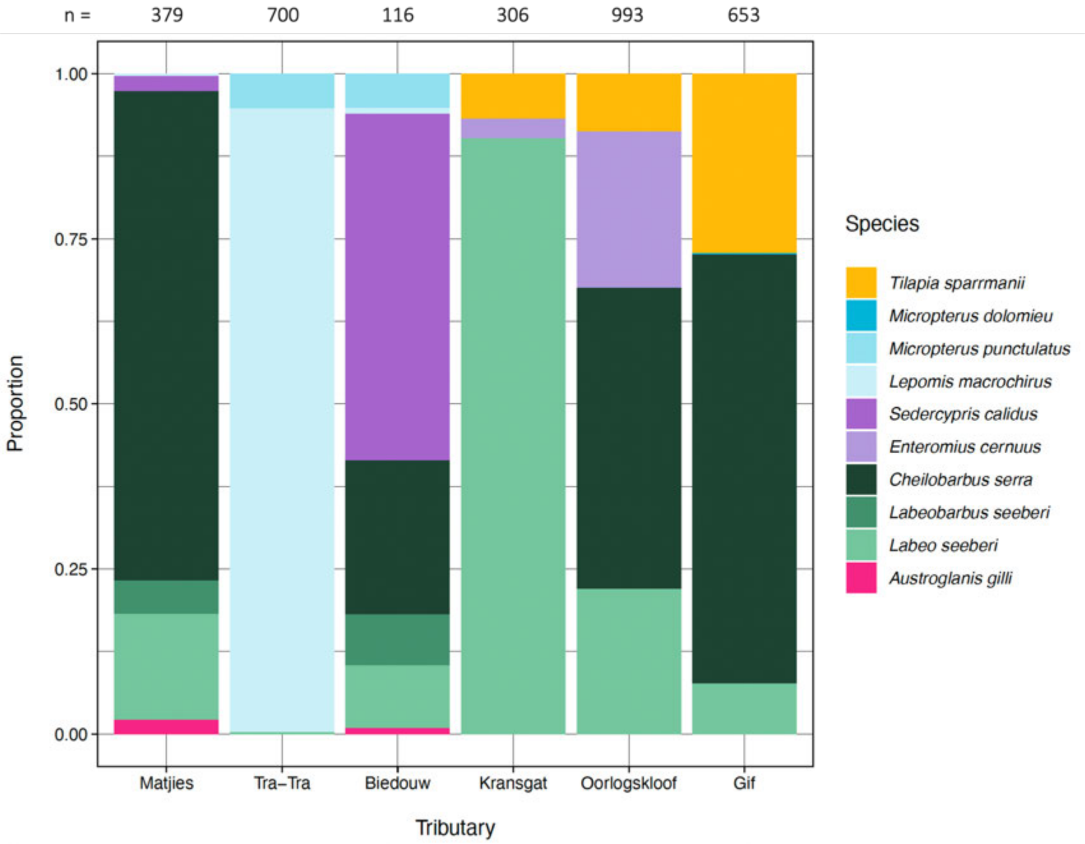

Figure 3.20. Proportional species catch at each of the tributaries sampled in 2022. For the Gif, two above-water visual observations of *M. dolomieu* were included and for the Oorlogskloof, 459 observations came from seine net samples and 534 came from fyke net samples. The remaining samples came from fyke nets. Sample size (number of fish caught per site) is indicated above each bar.

3.3.2.3 Size distributions

3.3.2.3.1 Doring mainstem

The number of sandfish sampled during the ODF surveys at Doring mainstem sites in 2001, 2011, 2013 and 2021 differed substantially between years (Table 3.2). Sampled sandfish measured 10 – 652 mm TL, with a mean of 470.92 ± 6.36 mm TL across years. Class 6 fish were sampled in the mainstem in all surveys excluding February 2011 (Table 3.3; Figure 3.21). They consistently made up the majority of sampled fish. Class 5 fish were sampled only in February 2001 and 2021. Although class 3 fish were detected in 2011 and classes 3 and 4 were detected in 2021, these consisted of 1-2 fish each. Class 2 fish were not detected in any mainstem survey. Eighteen class 1 sandfish measuring 10 – 17.5 mm TL were collected by hand net at the Bos-Wolf confluence in 2001.

Table 3.2. Summary of total length measurements taken from sandfish sampled at sites along the Doring River mainstem between 2001 and 2021. For details on sampling gear used during each survey, refer to Appendix A 4.

Year	N	Min TL (mm)	Max TL (mm)	Mean TL (mm) ± SE
2001	237	10	652	473.9 ± 9.0
2011	5	170	530	384.0 ± 84.4
2013	18	435	581	512.6 ± 8.1
2021	111	167	595	461.8 ± 7.9

Table 3.3. Counts of sandfish detected in each mainstem survey, categorised by size class. Cells with detections are shaded on a colour scale reflecting counts: yellow (1-50 fish), orange (51-100 fish), light red (101-150 fish), deep red (151-200 fish). These are raw counts that have not been calibrated by sampling effort. For details on sampling gear used during each survey, refer to Appendix A 4.

Survey	Class 1 0-20 mm	Class 2 21-100 mm	Class 3 101-200 mm	Class 4 201-300 mm	Class 5 301-400 mm	Class 6 > 401 mm	Total
2001 Feb	0	0	0	0	5	187	192
2001 Oct	18	0	0	0	0	27	45
2011 Feb	0	0	2	0	0	0	2
2011 Nov	0	0	0	0	0	3	3
2013 Oct	0	0	0	0	0	18	18
2021 Oct	0	0	1	2	28	80	111

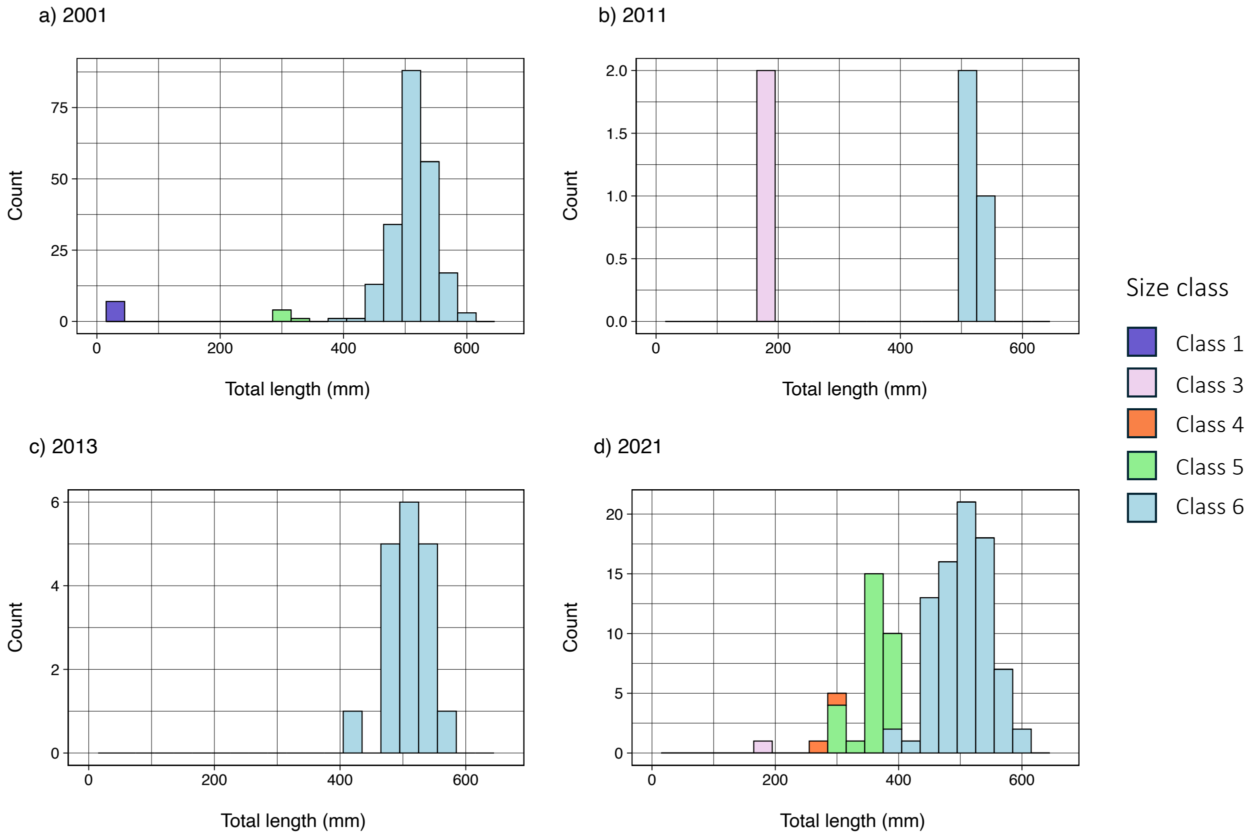

Figure 3.21. Size distributions of sandfish sampled at Doring mainstem sites in (a) February and October 2001, (b) February/March and November 2011, (c) October/November 2013, and (d) October 2021. Note that these data include sandfish sampled with different gear (see Appendix A 4 for specific gear used per survey). Size classes: class 1 (< 20 mm TL), class 2 (21 – 100 mm TL), class 3 (101 – 200 mm TL), class 4 (201 – 300 mm TL), class 5 (301 – 400 mm TL), and class 6 (> 401 mm TL). Note the different y-axis scales.

3.3.2.3.2 Doring tributaries

The range and spread of sizes sampled at tributary sites varied widely (Table 3.4). It should be noted that the Biedouw samples come from two distinct areas: 26 fish were sampled in 2001 from a pool located in the lower reach 1 km upstream of the Biedouw-Doring confluence, while 11 fish were sampled in 2022 from pools located 30 km upstream in the upper gorge. These have been designated “Biedouw (lower)” and “Biedouw (upper),” respectively.

Table 3.4. Summary of total length measurements taken from sandfish sampled at tributary sites between 2001 and 2022. For details on sampling gear used during each survey, refer to Appendix A 4.

Year	River	n	Min TL (mm)	Max TL (mm)	Mean TL (mm) ± SE
2001	Biedouw (lower)	26	11	22	16.48 ± 0.52
	Koebee	101	246	546	484.11 ± 3.93
	Oorlogskloof	89	140	352	248.98 ± 5.48
2011	Koebee	5	248	525	415.20 ± 51.88
	Oorlogskloof	53	71	350	167.02 ± 8.78
2013	Koebee	38	400	522	474.05 ± 4.62
2022	Biedouw (upper)	11	176	400	290.82 ± 24.87
	Gif	41	72	384	180.05 ± 7.53
	Kransgat	82	62	284	152.13 ± 5.29
	Matjies	61	147	395	241.38 ± 5.76
	Oorlogskloof	218	60	340	172.52 ± 3.47
	Tra-Tra	2	293	331	312.00 ± 19.00

Class 6 sandfish were only detected in the Koebee River across surveys (Table 3.5; Figure 3.22). Class 5 sandfish were detected in nearly every tributary across years, excluding the Koebee in February 2001, the lower Biedouw in October 2001 and the Kransgat in 2022. Class 4 sandfish were detected in nearly every tributary, with moderately high catches in the Oorlogskloof (February 2001 and 2022) and Matjies rivers (2022). Moderate numbers of class 3 sandfish were detected in the Oorlogskloof (February 2011), Gif (2022) and Kransgat (2022) rivers, with high numbers captured in the Oorlogskloof (2022). Class 2 sandfish were only sampled in moderate numbers from the Kransgat and Oorlogskloof Rivers in 2022. Only in the lower Biedouw site sampled in October 2001 were class 1 sandfish detected.

Table 3.5. Counts of sandfish detected in each tributary across surveys, categorised by size class. Cells with detections are shaded on a colour scale reflecting counts: yellow (1-50 fish), orange (51-100 fish), light red (101-150 fish), deep red (151-200 fish). These are raw counts that have not been calibrated by sampling effort. For details on sampling gear used during each survey, refer to Appendix A 4.

Survey	River	Class 1 0-20 mm	Class 2 21-100 mm	Class 3 101-200 mm	Class 4 201-300 mm	Class 5 301-400 mm	Class 6 > 401 mm	Total
2001 Feb	Oorlogskloof	0	0	20	55	4	0	79
	Koebee	0	0	0	1	0	85	86
2001 Oct	Oorlogskloof	0	0	0	7	3	0	10
	Koebee	0	0	0	1	1	13	15
	Biedouw (lower)	24	2	0	0	0	0	26
2011 Feb	Oorlogskloof	0	11	29	11	2	0	53
2011 Nov	Koebee	0	0	0	1	1	3	5
2013 Oct	Koebee	0	0	0	0	1	37	38
2022 Feb/Mar	Biedouw (upper)	0	0	3	1	7	0	11
	Gif	0	2	32	6	1	0	41
	Kransgat	0	19	48	15	0	0	82
	Matjies	0	0	9	47	5	0	61
	Oorlogskloof	0	21	129	67	1	0	218
	Tra-Tra	0	0	0	1	1	0	2

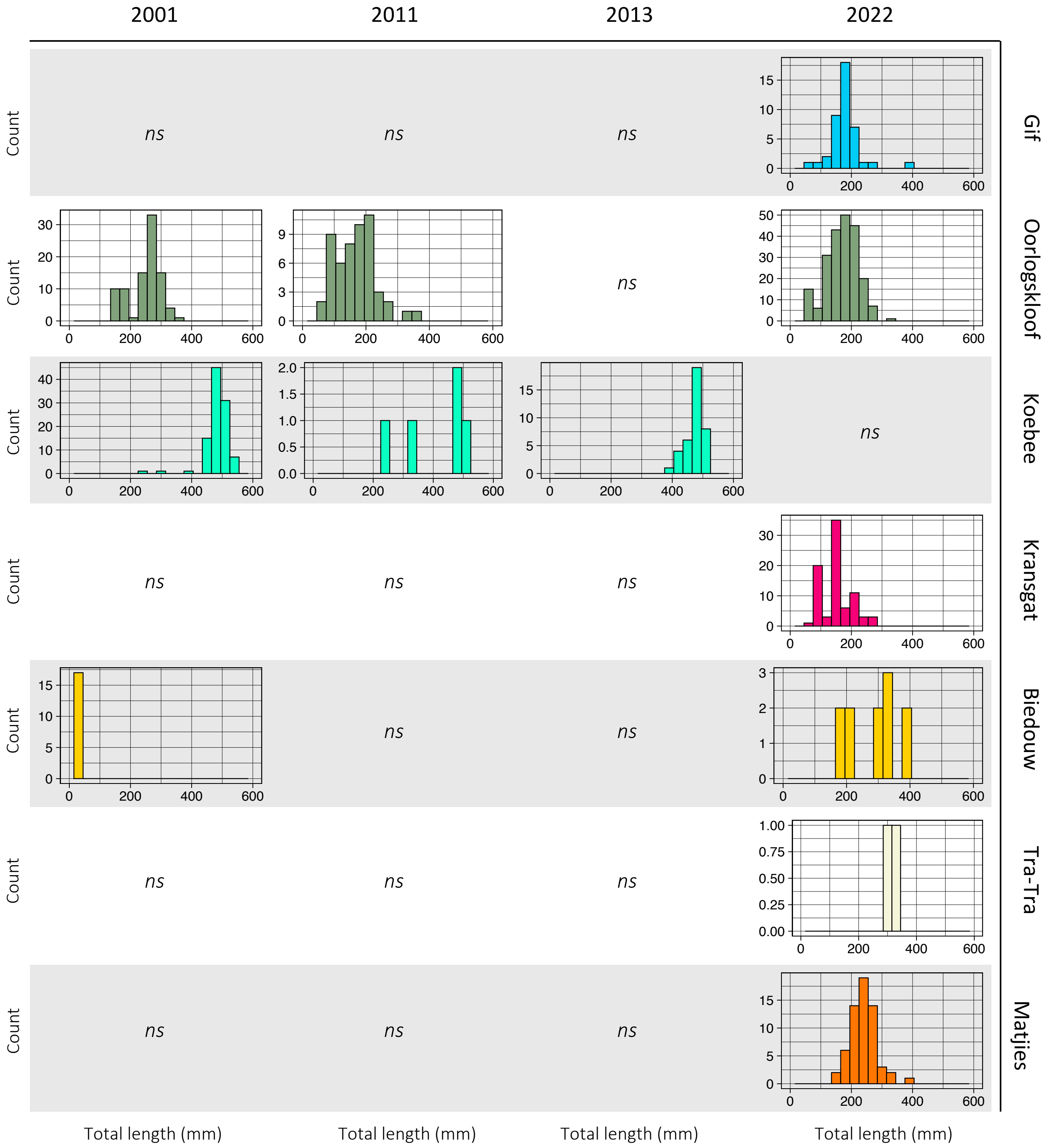

Figure 3.22. Size distribution of sandfish sampled at seven tributaries in 2001, 2011, 2013, and 2022. Note the different y-axis scales across panels. *"ns"* indicates that the tributary was not sampled in a given year.

The nMDS ordination (Figure 3.23b) grouped the Oorlogskloof, Gif, and Kransgat River samples closely along both axes. The directionality of the vectors in the ordination plot (Figure 3.23b) and the length-frequency histograms

(Figure 3.23a) indicate that these samples shared similar length distributions, characterised by a high frequency of smaller size classes. In contrast, the Doring River sample was the most isolated in ordination space, indicating the greatest dissimilarity in size structure. Its length-frequency histogram and vector positioning suggest a predominance of larger size classes. The Biedouw and Matjies Rivers are also isolated in ordination space, with the Matjies River sample closest to vectors representing lengths between 200 and 300 mm TL, and the Biedouw River sample closer to vectors for fish in the 300-350 and 400-450 mm TL ranges. The stress statistic, which measures the discrepancy between the observed and fitted distances in the ordination, was < 0.01. Values ≤ 0.2 are considered acceptable (Miranda 2024).

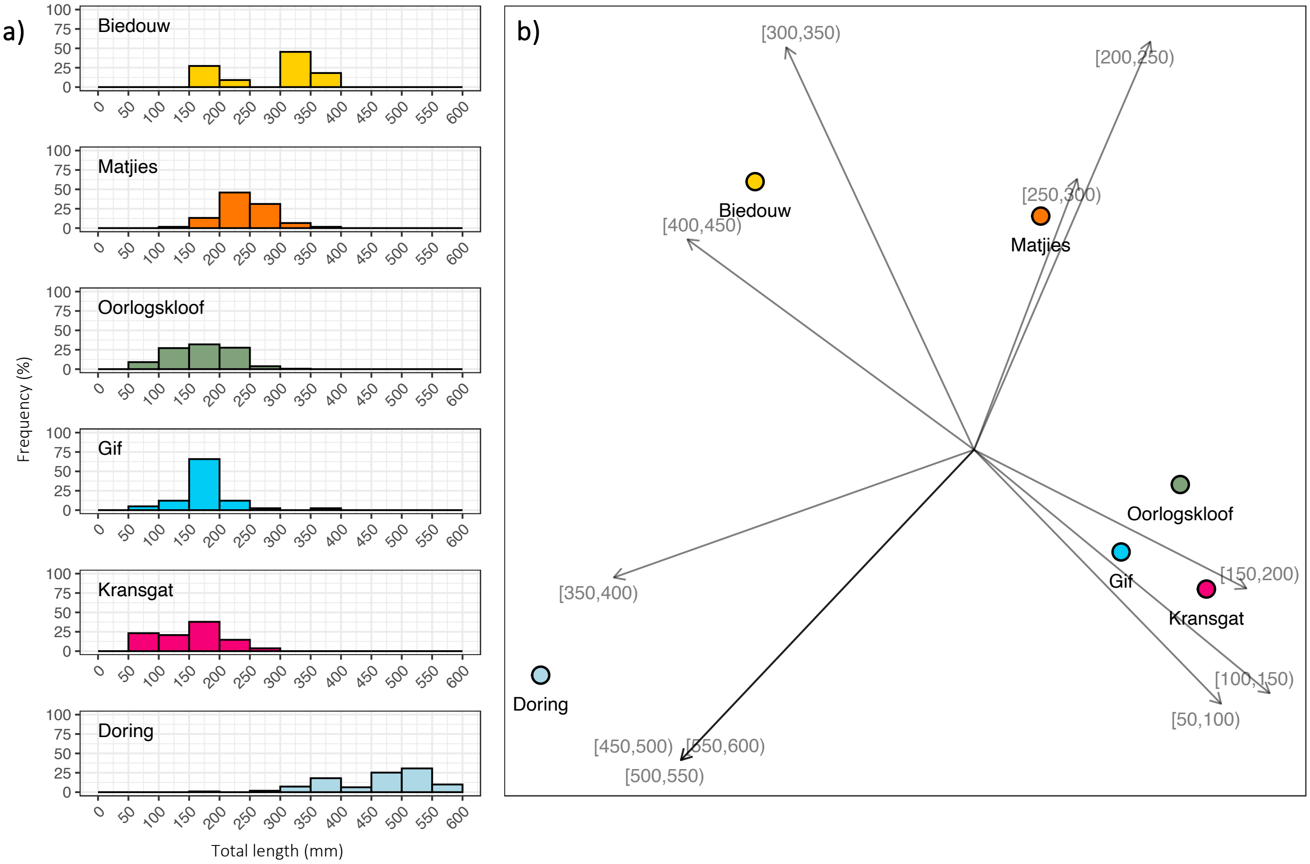

Figure 3.23. a) Length-frequency histograms and b) unconstrained nonmetric multidimensional scaling (nMDS) ordination applied to a Bray-Curtis similarity index of length-groups of Clanwilliam sandfish populations in the Doring mainstem and five of its tributaries. The Doring River was sampled in October 2021; the tributaries (upper Biedouw, Matjies, Oorlogskloof, Gif, and Kransgat) were sampled in March and April 2022. Although the Tra-Tra River was also sampled, it was excluded due to its low sample size (n = 2). Only data from fyke samples has been included. Both the histograms and ordination plot were constructed using total length (mm) data binned into 50-mm interval bins. The solid vectors correspond to each of those bins and illustrate the contribution of different size classes to the observed patterns of similarity/dissimilarity. Sample sizes (number of fish sampled per river): Biedouw (n = 11), Matjies (n = 61), Oorlogskloof (n = 188), Gif (n = 41), Kransgat (n = 82), Doring (n = 111). Because nMDS ordinations focus on the distance between points, axis units are unnecessary.

3.3.3 Overlap of current distribution with protected areas

The current distribution of sandfish at the sub-quaternary catchment scale, based on records from 2000 onwards (and including all intervening catchments) includes 36 sub-quaternary catchments in the Doring River system (Figure 3.24). This includes the Doring River mainstem from its confluence with the Olifants River to its confluence with the Groot River, as well as the following tributaries: Gif, Brandewyn, Koebee, Oorlogskloof, Kransgat, Biedouw, Tra-Tra, and Matjies Rivers. The summed area of the sub-quaternary catchments included in the current sandfish distribution is 4,161.0 km^2. This distribution spans 430.2 river-kilometres from the downstream-most site on the Doring River to the upstream-most site on each tributary. Using the same estimated river widths as those used in the 2016 IUCN Red List Assessment for the species (Jordaan et al. 2020a), this equates to a potential AOO of 7.86 km^2.

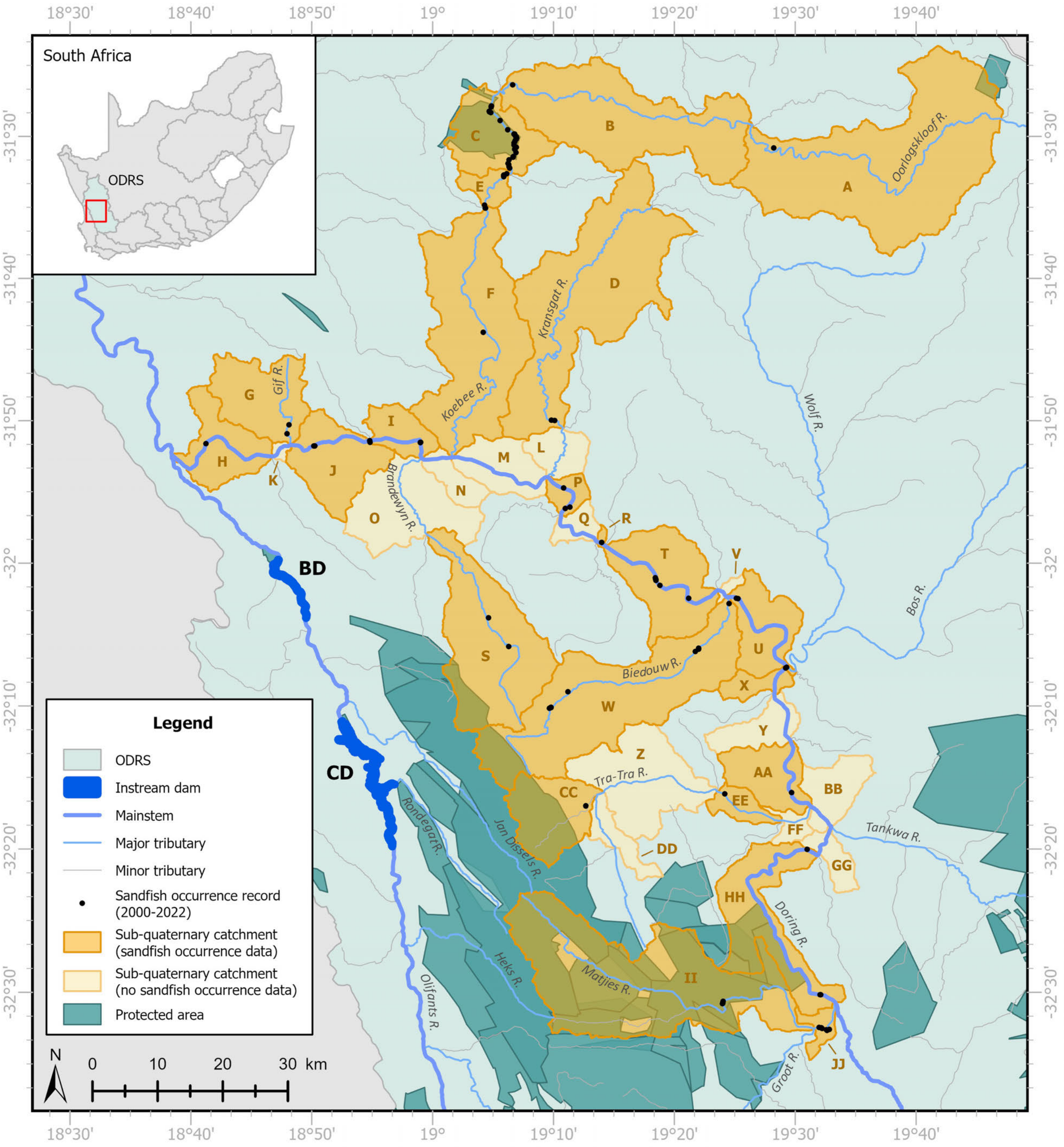

Figure 3.24. Current sandfish distribution in relation to surrounding protected areas (dark green polygons). Current distribution was defined as the sub-quaternary catchments containing post-1999 sandfish occurrence records (orange polygons), as well as all intervening sub-quaternary catchments (yellow polygons). Each sub-quaternary catchment was assigned a unique letter code. Occurrence records were obtained from FBIS and all ODF surveys. Protected areas layer (South Africa Protected Areas Database, SAPAD) downloaded from the website of South Africa's Department of Forestry, Fisheries & the Environment on 1 June 2024.

Only seven sub-quaternary catchments overlap with at least one protected area. Catchment A overlaps with the Akkerendam Nature Reserve; catchment C with the Oorlogskloof Nature Reserve; catchments S, W, and CC overlap with the Cederberg Wilderness Area (CWA) and the Cederberg Mountain Catchment Area (CMCA); catchment HH overlaps with the Matjiesrivier Nature Reserve and the Cederberg Private Nature Reserve; and catchment II overlaps with the CWA, the CMCA, and the Dwarsrivier, Kromrivierkloof, Nieuwe Gift, Hooggelegan, Taaiboschkraal, and Bakkrans Nature Reserves. A total of 531.0 km^2 (12.8%) of the area covered by the sub-quaternary catchments in the sandfish's range overlap with protected areas, encompassing approximately 70 river km – primarily in the Matjies River and its tributary, the Krom River. However, not all of these areas of overlap include important rivers. For example, Akkerendam Nature Reserve in the Northeast of the current range does not overlap directly with the Oorlogskloof River. Likewise, while catchments S, W and CC overlap with the CWA and CMCA, the rivers of interest (Brandewyn, Biedouw, and Tra-Tra) do not themselves overlap with a protected area. The Matjies River overlaps with protected areas over most of its length, and the Oorlogskloof Nature Reserve covers approximately 10 km of the Oorlogskloof River.

3.4 Discussion

This study compiles sandfish distribution records from the FBIS, the grey literature, and dedicated sandfish surveys undertaken since 2001 to produce a current distribution map for the species and evaluate distribution changes over time. These records indicate that sandfish were found throughout the Olifants River mainstem until the 1970s, when a notable range contraction first became apparent. During the 1980s, sandfish were only recorded in the Olifants River downstream of Bulshoek Dam and all subsequent surveys in the Olifants River system have failed to detect the species. Around the same time, the first surveys of the Doring River system began, with sandfish being recorded throughout the mainstem and in several tributaries – notably the Koebee-Oorlogskloof, Biedouw, Gif, Matjies, Tra-Tra and Brandewyn Rivers – through the 2010s. Doring River catches from 2001 onwards reveal a general lack of intermediate size classes (21 – 300 mm TL) in the mainstem, which may indicate a recruitment bottleneck, although size-selecting sampling equipment may also have underrepresented these size classes, if present. Furthermore, the prevalence of predatory non-native fishes in the mainstem poses a considerable threat to long-term persistence. Although there is evidence of spawning migrations in several tributaries[*], it appears many juveniles are either unable to survive past the juvenile stage (as in the lower Biedouw River) or face impassable predator "barriers" that prevent them from contributing to the mainstem population (as in the Oorlogskloof River). Whereas tributary populations may have once acted as sources to the mainstem sandfish population, it appears that these are now functionally acting as population refuges. Notably, the presence of small and intermediate size classes (< 300 mm TL) in most tributaries reveals a real potential for population recovery. In the species' remaining range, very little riverine habitat is formally protected. The data presented here indicate several key catchments where formal protection and/or other conservation interventions should be targeted, based on their potential contributions to population recovery.

3.4.1 Range contraction

In addition to documented records of sandfish throughout the Olifants River system from 1930 onwards, many narrative accounts attest to their former abundance and subsequent decline during this time. In 1937 and 1938, collections of indigenous fishes were made throughout the Olifants River system by the South African Museum, during which small sandfish were collected from Keerom in the upper reaches (Harrison 1963). During a 1960 collection of indigenous fishes in the system, it was noted that there was "no difficulty" in capturing sandfish specimens, and that the waters below Bulshoek Dam were "well-populated" with sandfish (as well as introduced *T. sparrmanii, M. dolomieu,* and *L. macrochirus*; Jubb 1961). Even as late as 1963, juvenile sandfish measuring less than 2 cm were still being sampled at Keerom and Kriedouwkrans, evidence that sandfish continued to spawn in the upper Olifants (van Rensburg 1966). During the early 1970s, subadult (12 – 20 cm) sandfish were sampled in the

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[*] Annual spawning has been observed by the project team in the Biedouw River from 2020-2023. An upstream migration comprising several dozen sandfish was also observed in the Tra-Tra in September 2023, although spawning itself was not observed. At the confluence of the Bos-Wolf tributary with the Doring River, larval sandfish were sampled in 2001, suggesting that spawning had taken place either in the tributary, or in the mainstem itself.

lower reaches below the Olifants-Doring confluence, and "reasonable numbers" of small sandfish were found in isolated pools in the Doring River close to its confluence with the Olifants (Gaigher 1973a).

At the same time, accounts of indigenous fish declines throughout the system were also common. A 1961 collection of indigenous fishes in the Olifants River system yielded no specimens of the small endemic species "which were so numerous in the 1930s, particularly the red-fin minnows" (Harrison 1963). Regarding the 1963 survey undertaken by van Rensburg and the numerous small (< 2 cm) sandfish sampled at Keerom and Kriedouwkrans at the time, Gaigher posits that although sandfish may have continued to breed in the mainstem, it was clear that this had not caused a population increase in a number of years, a classic example of recruitment failure (Gaigher 1973a). The conspicuous lack of sandfish smaller than 40 cm above the Bulshoek Dam during comprehensive surveys undertaken in 1972 and 1973 was seen as further evidence of this, deeming the sandfish sampled there a "secluded, unsuccessful breeding population," (Gaigher 1973a).

The primary reasons for the sandfish's decline and subsequent disappearance from the Olifants River system were well-recognised even in the mid-20th century. The first of these was the introduction and rapid proliferation of predatory North American sportfish throughout the ODRS. The ethos of recreational river users and custodians at the time centered around recreational angling, this being the primary reason for the intentional introduction of *Micropterus* species into the system in the 1930s and 1940s (Hargrove et al. 2017). In fact, it was the CDNR that facilitated the introduction of these fishes for sport-fishing, which "because of a lack of suitable indigenous species (except the yellowfish) has been based on excellent (in most cases) exotic species," (Gaigher 1973b). The potential impacts of bass on indigenous fishes were recognised as far back as 1937, only four years after the first stocking of *M. dolomieu* into the Olifants River system, when the South African Museum undertook a collection of indigenous fishes of the area due to the potential negative impacts of bass on indigenous fishes (Harrison 1963). Despite this, active stocking of sportfish into the ODRS by the CDNR continued until at least the 1970s (Dean Impson[*], pers. comm., August 2024)

It was well-known at the time of stocking that for the introduced sportfish to be successful, the indigenous fishes would be the ones to "pay the price" (Gaigher 1973b). The first to disappear from the Olifants mainstem were the small indigenous redfins: by the late 1950s, no *Sedercypris calidus* or *Pseudobarbus phlegethon* could be found in their type-localities (Gaigher 1973b), and the lack of small indigenous species during a 1960 survey of the Olifants was attributed to "the superior number of predatory fishes" (Jubb 1961). During this survey, *M. dolomieu*, *L. macrochirus* and *T. sparrmanii* were plentiful (Jubb 1961). Around 1950, as the small indigenous species were disappearing from the mainstem, the larger cyprinids, including yellowfish, could still be found (Harrison 1963). However, their decline was already becoming apparent, with populations of yellowfish, sawfin, and sandfish described as "seriously depleted" (Gaigher 1973b). Although sandfish were still "fairly abundant" in 1970, it was clear that predation by non-native fishes had already drastically affected their distribution and abundance (Gaigher 1978). These narrative accounts mirror the occurrence records compiled in this chapter, which show a wide distribution into the upper reaches of the Olifants River through the 1970s, followed by a range contraction to the area below the Bulshoek Dam in the 1980s, and finally extirpation from the system by the 1990s.

The second culprit implicated in the sandfish's decline in the Olifants River was habitat fragmentation caused by the construction of the Bulshoek Dam in 1919 and the Clanwilliam Dam in 1932 (Cambray et al. 1997). As far back as 1950, anglers and scientists documented large migrations of sandfish moving upstream to spawn. In the

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[*] Dean Impson is a research associate at the Freshwater Research Centre in Cape Town, South Africa and was a fish scientist with the Scientific Services Unit of CapeNature from 1994 to 2019.

1963 edition of the Cape Piscatorial Society's publication *Piscator*, a 1950 photograph was published of a leaping sandfish "ascending the Cascades on the Olifants River below Bulshoek" (Figure 3.25; Harrison 1963). The impacts of the two dam walls on indigenous fish migrations were apparent soon after the construction of the larger Clanwilliam Dam. In September 1938, thousands of sandfish, yellowfish and sawfin were seen massed below the barrages, which acted as "complete barriers to upstream movement of fish," during the spring spawning run (Harrison 1963). In 1950, shoals of yellowfish were observed travelling upstream, only to find "further passage stopped by the 75-foot barrage", referring here to the original height of the Clanwilliam Dam wall (Brooks 1950). In September 1972, the large catch of sandfish below Bulshoek Dam was attributed to the dam wall impeding further upstream migration (Gaigher 1973a). Furthermore, the 26 km reach between the two dam walls may have created a "trapped" population of sandfish, which would have been easily accessible by fishermen, further accelerating the species' decline (Gaigher 1973a).

Figure 3.25. The 1950 photograph of a sandfish on the Olifants River captioned "Leaping sandfish (*Labeo seeberi*) ascending the Cascades on the Olifants River below Bulshoek, during the spawning run in spring, 1950. (Photo: W. Harding)." (Harrison 1963).

That sandfish persisted in the Olifants River system until the 1980s, despite the two main disturbances (IAF introduction and dam wall construction) beginning in the 1930s may be symptomatic of an extinction debt – whereby species or populations experience a lag to extinction following the initial perturbation (Tilman et al. 1994; Kuussaari et al. 2009). The duration of this "extinction lag" (or time to extinction) for a particular population depends on the strength of the initial perturbation(s), the turnover rate of the species (i.e., generation time), and the availability of stable habitat patches (i.e, refuges) within the disturbed area (Kuussaari et al. 2009). Whether this is in fact what occurred for sandfish in the Olifants River system remains uncertain and would depend on several lines of evidence that are currently lacking, including sandfish generation time (as longer-lived species typically experience longer delays to extinction following perturbation; Kuussaari et al. 2009), and the rate and pattern of IAF spread across the system after introduction. Nevertheless, the observed decline in sandfish abundance across the system in the latter half of the 20th century does suggest a lagged population response to IAF introduction and dam construction. However, concluding that sandfish are in fact extirpated from the Olifants River system would require comprehensive, system-wide surveys, as the area has not been monitored closely since the 1990s.

The persistence of sandfish in the Doring River system, despite the widespread distribution of non-native centrarchids, is likely due to differences between the two branches of the ODRS and differences in certain stressors. On the Olifants River, the Bulshoek and Clanwilliam Dam walls act as complete barriers to upstream movement for all fishes. These barriers, located in the lower reach of the Olifants River mainstem, prevent adult sandfish from migrating upstream into the upper mainstem and major tributaries higher in the catchment, including the Jan Dissels, Rondegat, Heks, and Boontjies Rivers. Records indicate that at least one of these (the Jan Dissels) supported spawning migrations of fish sometime before 1960 (Jubb 1961).

In contrast, the Doring River is largely free-flowing, with only one major instream barrier – the Brakfontein Weir, approximately 10 m tall. Like the Olifants River dams, this weir is a complete barrier to upstream fish movement. However, unlike the Olifants barriers, Brakfontein Weir is located far upstream in the Doring River mainstem, beyond the confluences of the Koebee, Biedouw and Tra-Tra Rivers. These major tributaries are likely important historical spawning grounds for sandfish. Because Brakfontein Weir is situated upstream of these tributaries, it is unlikely to have impeded sandfish movement between the Doring River mainstem and these critical spawning habitats. It should be noted, however, that no mainstem sites (apart from De Mond) above Brakfontein Weir have been sampled since 2001, so the state of sandfish in the reach between Brakfontein and De Mond remains unknown.

Moreover, the naturally clear waters of the Olifants River, which result from the underlying quartzitic sandstone (DWAF 2005) may have increased juvenile cyprinids' susceptibility to predation by visual pursuit predators such as bass (Nyberg 1971; Sullivan & Atchison 1978). Water quality is more variable in the Doring River system, which is supplied both by fynbos mountain streams with low concentrations of total dissolved solids and by rivers originating in the Karoo, with elevated levels of suspended solids and consequently high turbidity (DWAF 2005). The more turbid waters of the Doring River system may therefore provide a seasonal predation refuge from non-native centrarchids (especially *M. dolomieu*, which cannot generally tolerate silted rivers; de Moor & Bruton 1988; Sweka & Hartman 2003), helping sandfish to persist in the Doring, when they have been extirpated from the Olifants.

The 7.86 km^2 potential AOO calculated here likely differs from the 3.11 km^2 AOO derived for the 2016 IUCN assessment for the species (Jordaan et al. 2020a) due to differences in derivation methods. Whereas the latter considered only the area around known occurrence records, this study includes all intervening river-kilometres between records (i.e., continuous Index of Area Occupancy). Moreover, the current analysis included additional records collected during the 2021/22 ODF surveys. It is likely that the current potential AOO substantially overestimates the actual AOO for the species, given the highly fragmented nature of the remaining populations. For example, although 114.6 km of the Oorlogskloof-Koebee River were included in the potential AOO calculation, sandfish only occur in large numbers within the 10 km reach bounded by the Oorlogskloof Nature Reserve. Similarly, migrating and juvenile sandfish only occupy the lower 15 km of the Biedouw River on a seasonal basis before it dries up each summer; they are found consistently only in the upper gorge. It is therefore recommended that the potential AOO presented here be treated as the conservative maximum estimate. These data will be made available during the next IUCN Red List assessment via the FBIS.

3.4.2 Olifants-Doring Fish surveys

3.4.2.1 Mainstem

The absence of sandfish from four Olifants River sites during the two 2001 surveys – including three historically occupied sites between 1930 and 1980 – and from Cascade Pools in November 2011, suggests that the species may have been extirpated from the Olifants system by that time. This possibility is further supported by the fact that sandfish were not detected during the 2001 and 2011 surveys despite the use of seven different sampling methods. In addition, there have been no recent anecdotal sightings of sandfish by the angling community in that system. Nevertheless, no comprehensive surveys of the Olifants system have been carried out since 2001, necessitating current sampling of key mainstem and tributary sites to provide an updated understanding of fish community dynamics in that system.

While the 20th century occurrence records offer a long-term view of sandfish distribution changes in the ODRS as a whole, data gathered from the ODF surveys since 2001 constitute a more detailed representation of the current state of the species in its remaining range across the Doring River system. While the different sampling practices employed during the 2001 surveys[*] complicate quantitative comparisons with subsequent surveys, clear patterns nonetheless emerge. The 60 km section between Langkuil and Brakfontein (the upper Doring reach) appears to be the stronghold for the species in the mainstem, with only three individuals sampled in the lower reach since 2001. Perhaps proximity to historical spawning tributaries such as the Biedouw River, differences in habitat quality, or exposure to anthropogenic stressors influence adult sandfish distribution in the mainstem, although more research is needed to determine the underlying mechanisms. Nevertheless, identifying both the highest concentrations of sandfish and areas where they are absent presents an opportunity to strategically direct conservation efforts —by enhancing protections for existing population strongholds and prioritising restoration and reintroduction in areas where sandfish have been lost. Although intermediate size classes were still largely absent from mainstem catches in 2021, the notably higher proportion of sandfish measuring less than 40 cm in 2021, compared with 2001, is promising and could signify some degree of population recovery. While caution is needed in interpreting these results due to differences in sampling methods across surveys, it is notable that the gill nets used in 2001 – which included four different mesh sizes designed to target a variety of size classes (Paxton et al. 2002) – would likely have captured a wider array of size classes, if present. Standardisation of sampling gear used in future ODF surveys is important to promote valid evaluations of population-level changes over time, though gill nets should be avoided due to the high levels of mortality. It is recommended that the large three-winged fyke net continue to be used on future ODF surveys; however, additional fyke nets should also be deployed along pool margins to improve detection of smaller size classes more likely to favour shallower habitats.

The overwhelming and persistent predominance of non-native fishes across surveys is cause for concern and appears to be the top threat to present-day persistence of sandfish in the system. The omnivorous *L. macrochirus*, by far the most numerous species caught across sites and surveys, is widely considered a pest due to its tendency to overpopulate waters where it has been introduced (Skelton 2024). This was found to be the case in surveys

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[*] The gill net sampling employed during the 2001 surveys resulted in about 10% mortality of captured fish, with many of those remaining released with capture-related injuries (B. Paxton, pers. comm., 2024) As a result, the authors recommended that though repeated sampling of the ODRS should be continued, less damaging methods must be employed in the future (Paxton et al. 2002). It is unclear to what extent the 2001 sampling impacted mainstem sandfish populations.

undertaken in the Oorlogskloof River between 2010 and 2018 where, together with *M. dolomieu,* they almost completely excluded small sandfish from the invaded reach (Cerrilla et al. 2022). As highly adaptable omnivores (Hossain et al. 2013), the impact of *L. macrochirus* on indigenous fishes is likely to consist of a combination of competition for resources (habitat and food) and predation on eggs and juveniles. Although *Micropterus* species made up a comparatively smaller proportion of the catch across surveys, they likely have the largest predatory impact on indigenous fishes in the system. Their destructive impacts on indigenous fish populations across the CFE have been extensively documented (see Chapter 1 Table 1.1), with small indigenous species and juveniles (< 20 cm) of larger species generally absent where bass occur. It is suspected that together with *L. macrochirus*, *Micropterus* species may be responsible for the non-detection of intermediate size classes in the mainstem.

The likely impacts of non-native fishes notwithstanding, it is important to consider the potential size-selective biases inherent to different sampling methods. Seine nets can select against large, agile fish which can easily evade capture (Bayley & Herendeen 2000), while fyke nets may trap small fish with larger predatory fish, resulting in a skewed catch toward the latter if small fish are consumed in the net (Breen & Ruetz 2006). While this last point is an important point to consider, the fact that small bluegill (< 100 mm TL) were abundant at sites also containing *Micropterus* species suggests that some small sandfish would also have remained in the fyke nets upon retrieval, if they were present. Nevertheless, future ODF surveys should consider including gut dissections of captured *Micropterus* species to assess whether this is a potential concern. It is also possible that a fyke net placed in the thalweg of a pool may miss smaller sandfish due to size-related behavioural differences. For instance, if younger sandfish prefer shallower areas, they may avoid the deeper sections where the net is set, resulting in a sampling bias toward larger individuals. Future ODF surveys could address this by placing smaller fyke nets near the banks to reduce size-selective bias.

The 2021 survey also marked the first known observations of the non-native *C. carpio* and extralimital *O. mossambicus* in the Doring River, although *O. mossambicus* has been present in the Olifants River since at least 2001, and *C. carpio* since November 2011. The widely introduced and highly successful invader *C. carpio* is extremely fecund and thrives in large, turbid rivers (Skelton 2024), suggesting it may fare particularly well in the Karoo-fed Doring River. It is also considered one of the world's worst 100 invasive species (Lowe et al. 2000). As an ecosystem engineer, *C. carpio* causes widespread ecological damage in novel ranges by increasing turbidity (King et al. 1997), destroying aquatic vegetation (Roberts et al. 1995), and altering invertebrate community composition (Robertson et al. 1997). While these impacts may have indirect consequences for the native fish species, resource competition with native fishes (Weber & Brown 2011) and predation of early life stages (Miller & Beckman 1996) could impact the native fish fauna more directly. On the other hand, introduced *C. carpio* have been shown to negatively affect the abundances of *L. macrochirus, M. dolomieu,* and *M. nigricans* in their native North American range due to resulting habitat degradation (Weber & Brown 2011), suggesting they may indirectly benefit the native fishes of the Doring River system by controlling the populations of the invasive centrarchids. The turbidity of the Doring mainstem is naturally variable; it typically receives isolated pulses of sediment-rich water from the eastern tributaries in the summer and clear water from the western tributaries during sustained rainfall periods in the winter – although aseasonal rain over the Karoo may also deliver pulses of turbid water in the winter (Figure 3.26; Paxton et al. 2002; DWAF 2005). While the indigenous fishes of the Doring River mainstem, sandfish included, are adapted to these variable sediment loads, it is unknown how they would respond to a shift towards consistently turbid conditions typical of carp-invaded systems. Close monitoring of its spread and ecological impacts in the coming years will be key to better understanding the ecosystem response to this novel invader.

Figure 3.26. A photograph of the clear waters of the Biedouw River (right) mixing with the turbid waters of the Doring mainstem. Photograph by Jeremy Shelton.

It is unclear what impacts *O. mossambicus* will have on the native fishes of the Doring River, although it, too, is among the world's worst invaders (Lowe et al. 2000). In the Oorlogskloof River, where the related cichlid *T. sparrmanii* (also extralimital, found naturally in the neighbouring Orange River system) was accidentally introduced in 2007, they were found to have no detectable impacts on the sandfish population (Cerrilla et al. 2022), perhaps due to phylogenetic relatedness between the invader and the native species. Indeed, high-impact invaders tend to be more phylogenetically dissimilar from recipient communities than low-impact invaders (Ricciardi & Atkinson 2004; Strauss et al. 2006), perhaps due to lack of enemies in the recipient system (Strong et al. 1984), or the tendency of less-related species to exhibit lower niche overlap (Darwin 1859, p. 490). The inverse condition – that more closely related species would exhibit higher niche overlap, thus limiting the invasibility of related taxa – may help explain the limited impact of *T. sparrmanii* in the Oorlogskloof River. Indeed, a meta-analysis of aquatic systems showed that high-impact invaders were more likely to belong to genera that were absent from the novel system (Ricciardi & Atkinson 2004), a conclusion supported by the invasive success of North American centrarchids across the ODRS, which was previously devoid of not only *Lepomis* and *Micropterus* species, but of any centrarchid. Given these considerations, it may be expected that *O. mossambicus*, which is naturally distributed across the lower Zambezi system and the Bushmans system in the Eastern Cape (Skelton 2024), and therefore is relatively closely related to the fishes of the ODRS, would have limited impacts on the indigenous fishes of the ODRS. However, as they attain a maximum size roughly twice that of *T. sparrmanii* (Skelton 2024), they may be expected to exert stronger predatory pressure on indigenous fishes. They should therefore be monitored closely in the future.

Additionally, since March 2023, the extralimital sharptooth catfish (*Clarias gariepinus*), indigenous to the Orange-Vaal and further north (Skelton 2024), has been observed below the Brakfontein Weir (Lelie Hough[*], pers.

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[*] Lelie Hough was a long-time resident of the lower Biedouw River valley close to the Doring River confluence.

comm., March 2023), in the lower and middle reaches of the Biedouw River (pers. obs., November 2023), in the Doring River mainstem at Doringbos (Mandy Schumann[*], pers. comm., December 2023), and in the Tra-Tra River (Niklaas Farmer[†], pers. comm., October 2024), indicating that it is now likely widespread throughout the Doring River system up to the weir at Brakfontein. This is the first time this species has been recorded in the Doring River System. This novel invader can reach 1.5 m SL and is a highly adaptable omnivore (Skelton 2024), known to consume fish and other large prey, including wetland birds, with its large gape (Anoop et al. 2009). Impacts on the indigenous fishes of the ODRS are difficult to predict, as *C. gariepinus* has been implicated in the decline of a small indigenous cyprinid in invaded pools of the Gamtoos River (Cambray 2003a), but it is able to co-exist with indigenous fishes (including the sandfish relative *L. umbratus*) without measurable negative impacts in the Sundays River system (Woodford et al. 2024). Furthermore, *C. gariepinus* is thought to have reduced the abundance of *Micropterus* species in several South African impoundments (Cambray 2003a), suggesting it may have a positive impact on the ODRS fish fauna during the initial stages of invasion. However, a decrease in indigenous fishes may very well follow a decline in invasive prey.

There are two significant concerns regarding the invasive and predatory potential of *C. gariepinus* that differentiate it from *Micropterus* spp. The first is its ability to surpass most obstacles that act as barriers to invasive centrarchids due to its ability to move overland (Cambray 2003b), which will likely enable the invasion of headwater native fish refugia inaccessible to *Micropterus* spp. Second, *C. gariepinus* is capable of consuming larger prey than *Micropterus* spp. owing to its greater maximum size (1500 mm SL compared to 600 mm SL; Skelton 2024) and disproportionately large gape (e.g., one published case recorded the gape of one individual measuring 20% of its total length; Anoop et al. 2009). While *Micropterus* spp. impacts on the native fish of the ODRS have so far largely been limited to individuals smaller than 200 mm TL (Weyl et al. 2013; van der Walt et al. 2016; Cerrilla et al. 2022), the predatory impacts of *C. gariepinus* are likely to extend to adults of large cyprinids like sandfish, with potentially severe consequences for population stability and long-term viability. It is therefore essential that the spread and impacts of *C. gariepinus* be carefully monitored and a management plan developed to mitigate its impacts in the Doring River system.

3.4.2.2 Tributaries

The function of tributaries in the life history of sandfish is not yet fully understood. Following the 2001 ODF surveys, Paxton et al. (2002) postulated three possible hypotheses to explain the natural spawning behaviour of the three large cyprinids in the system, including sandfish:

(1) Adults migrate from the mainstem or lower reaches of tributaries to the headwaters to spawn in spring, where offspring remain until they reach maturity, whereafter they migrate back to the mainstem and foothill reaches to feed and grow. This is otherwise known as a fluvial-adfluvial life history, *sensu* Ferguson et al. 2019.

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[*] Mandy Schumann of the Northern Cape Department of Agriculture, Environmental Affairs, Rural Development and Land Reform is a collaborator on the Saving Sandfish Project, and has been leading the semi-annual Oorlogskloof Nature Reserve fish surveys since 2010.

[†] Niklaas Farmer is a long-time resident of the middle Tra-Tra River valley whose lived experience offers an important perspective of historical changes in the landscape.

(2) Tributary populations are somewhat self-sustaining but are occasionally supplemented by migrations of adults from the mainstem. This would be considered a combination of a fluvial-adfluvial and river-resident life histories, *sensu* Ferguson et al. 2019.

(3) There is very limited or no migration by adult fish into the tributaries and limited downstream dispersal of juveniles and subadults. Tributary populations are sustained by stunted adults. This is otherwise known as a river-resident life history, *sensu* Ferguson et al. 2019.

Data and observations from the 2022 surveys of the various tributaries that are known to support sandfish suggest that the species may adopt a combination of these strategies. Annual spawning migrations of adult sandfish from the mainstems into the lower reaches of the Biedouw River have now been observed in four consecutive years (2020-2023). The highest observation of migrating sandfish occurred approximately 15 km upstream of the Doring confluence. In September 2023, dozens of adult (30 – 45 cm) sandfish were also observed moving up the Tra-Tra River in what appeared to be a spawning migration (J. Shelton and O. Whitehead, unpublished data, September 2023), approximately 15 km from its confluence with the Doring River. Farmer Willem van Zyl, now in his 70s, recalls seeing large numbers of sandfish migrating past his family farm at Rietkuil pool on the Koebee to spawn when he was a child (Willem van Zyl, pers. comm., 2021). These data show that sandfish do in fact undertake spawning migrations into the tributaries, as suggested by hypothesis (1); however, they appear to limit their movements to the lower foothill reaches of the tributaries, at least in the Biedouw River and potentially in the Tra-Tra River. Anecdotal evidence suggests that sandfish migrate further distances in the Biedouw River during years with higher sustained winter baseflows (pers. obs.). It is therefore expected that, under unmodified conditions (i.e., less water abstraction), sandfish would travel farther upstream towards the headwaters.

At the same time, there also appear to be some resident, self-sustaining tributary populations, as postulated by (2). The nMDS ordination revealed clustering between the Oorlogskloof, Gif and Kransgat samples; while the highest frequencies of fish measured between 100 and 200 mm TL, smaller individuals measuring less than 100 mm TL were also present, indicating recruitment in these tributaries. Long-term monitoring of the Oorlogskloof River sandfish population confirms that the reach located within the Oorlogskloof Nature Reserve hosts a self-sustaining refuge population with relatively high annual juvenile recruitment (Cerrilla et al. 2022). More frequent sampling of the Gif and Kransgat Rivers across different seasons is needed to determine whether those populations are also resident and self-sustaining. Intraspecies ecological plasticity in life-history strategies is not uncommon, and is well-illustrated by brown trout (*Salmo trutta*), which adopt a large diversity of both resident and migratory life histories depending on physiological condition, environmental factors, and genetic effects (Ferguson et al. 2019). Similarly, rainbow trout (*Oncorhynchus mykiss*) exhibit both freshwater resident and anadromous life history strategies, often in sympatry (Weigel et al. 2014).

The current collective knowledge on sandfish suggests that they exhibit at least two life histories: headwater residents and mainstem migrants. These occur mostly allopatrically, with the former confined to headwater reaches above complete or partial barriers and the latter residing in the mainstem or lower reaches of tributaries for most of the year. However, limited genetic differentiation between some of these populations suggests consistent historical gene flow, with evidence of recent fragmentation (Rhode et al. 2024), consistent with the data presented here. Further research on the genetic structuring of populations across the species' range is needed to better understand gene flow (and whether it is directional) and the impacts of recent isolation on key populations. The maintenance of two life histories within the Doring River system is likely due to fluctuating selection, which occurs when the costs and benefits of either strategy vary across time (Ferguson et al. 2019). This variation is common in stochastic environments such as the ODRS. Headwater river-residency may be beneficial during extended periods of drought, when flows become more intermittent in the foothills but persist in the headwaters, whereas periods of stable or abundant flows may favour a migratory strategy, as food abundance tends to be higher and resource competition lower in the larger, more productive foothills.

The degree to which the headwater residents would be isolated from mainstem migrants under natural conditions is unknown. In the Oorlogskloof River, a barrier to downstream dispersal of juvenile sandfish currently exists in the form of non-native centrarchids, which occupy 67 km of the lower reaches where the river is known as the Koebee River (van der Walt 2014). It is likely that there would have been a higher degree of downstream dispersal prior to the introduction of non-native fishes, with upstream dispersal into Oorlogskloof gorge limited to periods of flood that would allow sandfish to surpass the 1.6 m fall present during base flows (van der Walt 2014). The persistence of non-native centrarchids throughout the lower length of the Oorlogskloof-Koebee River therefore poses a substantial threat to gene flow and metapopulation connectivity.

Over the past two decades, Oorlogskloof River catches have consistently contained a wide range of size classes, including juveniles, indicative of a healthy, self-sustaining recruiting population. Although the 2022 survey of the Oorlogskloof included only six of the 38 repeat-sampling pools, the catch of 218 sandfish did include a range of size classes, including juveniles – an optimistic result given the recent 93% decline in abundance documented between 2013 and 2018 (Cerrilla et al. 2022). However, a detailed analysis of the most recent catch data must still be undertaken, and a full survey of the 38 repeat-sampling sites (which have not been fully re-sampled since 2018) should be carried out in 2025 to ascertain the current status of this critical population. Importantly, the area is under formal protection in the form of the Oorlogskloof Nature Reserve, which covers 5,755 hectares of land and 10 river-kilometres in the reach currently acting as a refuge. While this offers a degree of protection, the river is still vulnerable to upstream anthropogenic stressors, including flow reduction due to weir construction and non-native plants, and high silt loads caused by soil erosion due to overgrazing and land clearing for agriculture (Abrahams & Pretorius 2000).

The sandfish sampled from Rietkuil on the Koebee River, only 13 km downstream from S11 on the Oorlogskloof River, were, on average, larger than in all other tributary samples. At a mean of 479 mm TL across surveys, they match the mainstem fish more closely in size than they do other headwater populations. This is likely due to two important factors: (1) this site sits within a reach that is invaded by *M. dolomieu* and *L. macrochirus,* which almost entirely exclude young sandfish (Cerrilla et al. 2022); and (2) the Rietkuil pool is roughly 570 m long and more closely approximates the type of "foothill" habitat typical of the mainstem than the steep, narrow pools found in the headwaters of the sampled tributaries. It has been hypothesised previously that headwater populations may be growth-stunted (in partial support of hypothesis 3), perhaps as a phenotypic response due to competition or low productivity in the small, oligotrophic pools typical of these upper reaches (Paxton et al. 2002). That the large size classes most common in the Doring (> 450 mm TL) are entirely lacking in the tributaries (excluding the Koebee) lends some support to the growth-stunting hypothesis, although it could also be the result of slow growth or high mortality of adult fish (Miranda 2024). As length data alone cannot reveal the mechanism behind the lack of large adults in the tributaries, it is first necessary to understand ageing processes in sandfish across different habitats. Ageing a size-inclusive sample of sandfish across their current distribution is a natural first step. However, the number of individuals required to construct reliable length-age relationships must be given careful consideration as many ageing techniques often require lethal sampling (e.g., evaluation of growth rings in otoliths, vertebrae and opercular bones; Khan et al. 2015). Ageing using growth rings laid down on scales presents a non-lethal alternative (Skelton 2025). However, the accuracy and reliability of different methods for ageing sandfish must first be evaluated, as these can vary between species (Khan et al. 2015). Re-sampling and measuring known-age fish is another non-lethal and reliable method – this can be facilitated by re-capturing PIT-tagged individuals released as part of the head-start programme discussed in Chapter 4.

Importantly, the presence of large (>200 mm TL) sandfish in the Koebee suggests that limited downstream dispersal from the refuge population in the Oorlogskloof Nature reserve is still occurring – or that it occurred relatively recently. These fish are unlikely to be the result of successful recruitment within the bass-invaded reach, given the known predation pressure on juveniles, and are more likely to represent larger individuals that dispersed downstream. While more research (e.g., PVA and morphometric shape analysis) is needed to understand the differences between potentially self-sustaining headwater populations and migratory foothills populations, it is clear that prior to the arrival of bass and bluegill, there would have been much higher connectivity between the Oorlogskloof River gorge and the downstream reaches, with the former acting as a population source for the latter via downstream dispersal, and the reverse via upstream migration during floods. Genetic work carried out in 2013 provides evidence of fragmentation between above-barrier and below-barrier populations of sandfish on the Oorlogskloof (as well as a sampled population at the Bos-Doring confluence), and that the population is likely in the initial phases of genetic diversity decline (Rhode et al. 2024). However, the metapopulation was thought to retain sufficient genetic diversity to buffer the species from more deleterious effects in the short term (Rhode et al. 2024), indicating that conservation efforts aimed at increasing connectivity may prove successful if carried out soon[*].

That the ONR remains a sanctuary for a reproducing population of sandfish (as well as *E. anoplus* and *C. serra*), as well as the presence of several large pools downstream of the current barrier, offer opportunities to expand the river's role as an indigenous fish sanctuary and increase genetic exchange. The feasibility of AIF eradication downstream of the current bass and bluegill barrier must first be investigated and the cost of a barrier weir estimated. If the reach proves to be a candidate for IAF eradication, there would be a ready supply of native fish to repopulate the newly restored section immediately upstream.

The 2022 Matjies River length distribution is right-skewed relative to the three nMDS-clustered tributaries, with a notable lack of the smallest size classes at the time of sampling. This does not necessarily indicate a lack of recruitment – indeed, the tributary appears to host a healthy population of sandfish ranging in size from 147 to 395 mm TL. The lack of juveniles may be an artefact of the timing of reproduction which, if it mirrors that of the Doring mainstem population, would have occurred around September. More frequent sampling throughout the year – especially around October – would help determine whether the size structure is a result of seasonal sampling bias.

The Matjies River holds substantial potential as a sandfish refuge. As of 2014, just over half (54.9%) of the river was invaded by black bass, leaving 11 km of relatively pristine, bass-free habitat available for indigenous fishes (van der Walt 2014). The extent of bass-free habitat in the Matjies is second only to the Oorlogskloof, which hosts nearly 19 km of bass-free river length (van der Walt 2014). However, *M. nigricans* is present in the Driehoeks River

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[*] In this context, "increasing connectivity" refers not to the removal of physical barriers, but to the eradication of non-native fishes, thereby allowing sandfish metapopulations to move freely along extended stretches of river free from IAF. Paradoxically, this may involve constructing artificial weirs to prevent the re-invasion of IAF into newly restored reaches (Rahel 2013), a strategy termed *isolation management* (Novinger & Rahel 2003). It involves the construction of a barrier followed by the removal of unwated taxa upstream of the barrier and then by restocking (natural or assisted) of the area with native species (Rahel 2013). Such barriers can comprise relatively short vertical drops (> 1.1 m; van der Walt et al. 2016), which exploit differences in swimming and jumping ability between *Micropterus* spp. and sandfish: they effectively block upstream movement of *Microtperus* spp., while posing no barrier to sandfish migration during flood periods – thus enhancing metapopulation connectivity.

(Shelton et al. 2008; van der Walt 2014), which flows into the Matjies, and some have also been observed in the Matjies River gorge (Jeremy Shelton, pers. obs.) However, they have not established in high numbers. Although a single *L. macrochirus* was sampled at Matjies 3 in 2022, they do not appear to be widespread or abundant in the area. This presents an opportunity to mechanically remove these non-native fishes before they can establish. The Matjies River, like the Oorlogskloof, therefore plays a critical role as a sandfish refuge, and with five indigenous species present, can also act as a potential source of indigenous fishes following downstream bass eradication. This is especially important given the Matjies' location in the opposite end of the sandfish's range from the Oorlogskloof, and that it, too, has formal protection. In this case, the river overlaps with a series of private and provincial nature reserves along almost its entire length, including the headwaters of the Driehoeks River, rendering it the most well-protected catchment in the sandfish's current range.

Although juvenile survival is heavily compromised in the lower reach of the Biedouw River, the tributary remains extremely valuable from a conservation perspective for several reasons: 1) five indigenous fish species persist in the upper reach, 2) it is the only confirmed tributary where mainstem sandfish spawn annually, and 3) there are excellent relationships between landowners and the Saving Sandfish project team. Moreover, it has been the focus of a population augmentation programme since its inception in 2020 (see Chapter 4). Although the 2022 upper Biedouw River sample included only 11 sandfish, precluding any meaningful conclusions regarding its population structure, it would be valuable to know whether and to what extent recruitment takes place in this upper reach, as it is currently the target of an alien fish eradication project aimed at expanding the length of IAF-free habitat available to indigenous fish by ten-fold – from the current 900 m to a total of 9 km. A one-metre-high barrier weir has been constructed at the downstream limit of the treatment reach to prevent re-invasion after eradication. As the remaining lower 24 km dry up completely each summer, save for one or two pools near the upstream limit of the seasonal reach, non-native bass and bluegill could realistically be eradicated from the entire length of the tributary in future phases of the project. The removal of water-intensive non-native vegetation and collaborative efforts to manage the river's water resources more efficiently so that water remains in key pools throughout the river's length year-round, would then be needed to fully establish the Biedouw River as an indigenous fish sanctuary and a potential self-sustaining source for the Doring River sandfish population.[*]

The two adult sandfish caught in the Tra-Tra in March 2022 are suspected to have been trapped in the sampling pool by receding waters at the end of the spawning season. This, together with observation of dozens of migrating sandfish in the same area in September 2023, suggest that the lower reach of the Tra-Tra River is, like the Biedouw, being utilised by adult sandfish as spawning grounds. Unlike the Biedouw, the lower Tra-Tra is largely inaccessible, with no vehicle access in the lower 15 km, conferring some degree of protection from anthropogenic impacts. However, the upper catchment is impacted by agriculture, and all but the upper 500 m are invaded by *M. punctulatus* and *M. dolomieu* (van der Walt 2014), with *L. macrochirus* abundant in the lower reaches. Surveys of the lower reaches during and after the spawning season would reveal if sandfish spawn reliably in the lower Tra-Tra. However, the overwhelming predominance of *L. macrochirus* and *M. dolomieu* in the 2022 catch suggests that, like the Biedouw, any young sandfish born in the lower reaches are unlikely to survive past the juvenile stage. A survey would nonetheless help determine whether the tributary is a candidate for non-native fish eradiation or head-start efforts to supplement the Biedouw River programme. It would also inform whether any head-started fish released into the Biedouw River in previous years stray to neighbouring tributaries to spawn, shedding light on the species' philopatric behaviour and straying tendencies.

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[*] See Chapter 2 section 2.4.5 for further recommendations.

In 2014, the short 4.2 km reach in the Gif River inhabited by native fishes was found to be un-invaded by bass species, yet in the most recent survey, two *M. dolomieu* were observed in Gif 1, along with only one adult sandfish and three small, dead *C. serra*. This suggests that the current bass barrier is located somewhere in the 1.3 km reach between Gif 1 and Gif 2, resulting in 1.2-2.5 km of bass-free habitat and 2-3 km of bass-invaded habitat. The Gif River, like most other tributaries of the Doring, is seasonal, with only a handful of pools keeping water through summer (Riaan van der Walt[*], pers. comm., February 2022), including Gif 2 and Gif 3 which held healthy populations of both *C. serra* and sandfish. Although this presents an opportunity for mechanical bass eradication, as they would be trapped in a limited number of pools through summer, the high cost of weir construction to prevent re-invasion may not be worth the few river-kilometres reclaimed for indigenous fishes; bass eradication would likely yield better biodiversity returns elsewhere.

The catch from both sampled pools of the Kransgat River was dominated by sandfish, with a distribution of size classes from juvenile to small adult, although most fell into the subadult category. The two sampled pools constitute the only pools known to hold enough water to support fish through the summer in the lower reach of the Kransgat River and are located approximately 12 km from its confluence with the Doring River. The thick instream vegetation at Kransgat 2 caused highly hypoxic conditions to develop overnight, with the dissolved oxygen levels at the surface reading 0% during net retrieval. Hundreds of sandfish within and outside the fyke net were observed utilising aquatic surface respiration (ASR)[†], suggesting that while sandfish are capable of withstanding hypoxic conditions, the summer habitat in the Kransgat at present appears to be severely marginal. Nevertheless, the high sandfish density and absence of non-native species at the Kransgat sites (save for the extralimital *T. sparrmanii*) render the river a good candidate for efforts focused entirely on increasing flows. One approach would be the establishment of stewardship agreements with farmers in the catchment centered on invasive plant removal and efficient water management. Strategic water releases into key pools low in the catchment might also be necessary to sustain indigenous fishes through the summer. In the short term, removal of in-stream vegetation at Kransgat 2 could be considered to improve water quality there during the summer.

3.4.3 Conclusions and recommendations

This study utilised current and historical data to demonstrate that the sandfish has experienced a marked range contraction across its natural range, with the most apparent declines evident from the 1980s onwards. The earliest ODF surveys confirmed its likely extirpation from the Olifants River system, and the most recent ODF surveys in 2021 and 2022 show that the sandfish persists in the Doring River system, with the mainstem population concentrated in the middle to upper Doring River. In addition to the Biedouw River, the lower reach of the Tra-Tra River appears to also act as a spawning tributary, although recruitment is similarly expected to be limited due to predation by non-native centrarchids and stranding. Key refuge populations exist in the protected upper reaches of the Matjies and Oorlogskloof Rivers, which may prove to be essential source populations following future

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[*] Riaan van der Walt is a research associate at the Freshwater Research Centre, a founding trustee of the Fynbos Fish Trust, and director at Advanced Environmental Corporation. He served as a conservation manager at CapeNature for 25 years and has been involved in fynbos fish research and conservation since 2010.

[†] When exposed to severely hypoxic water, some fish skim the surface-atmosphere interface, which is better oxygenated than deeper waters, to obtain sufficient oxygen. This behaviour is known as aquatic surface respiration (ASR; Rantin et al. 1998).

rehabilitation efforts. The value of these refuge populations is underscored by the presence of large fish in the latest survey of the Koebee (2013) and the Doring mainstem itself – which suggests that some downstream dispersal from refuge populations into invaded reaches may still be occurring, though likely to a limited extent.

Despite the many challenges faced by sandfish in the Biedouw River, it is a key tributary where several interventions are underway, and it is likely to remain a critical focal point for sandfish conservation. While an isolated population of juvenile and subadult sandfish persists in the Gif River, the conservation potential of this tributary is constrained by the limited amount of habitat that could be reclaimed by alien eradication efforts. Rehabilitation efforts in the Kransgat River should focus on flow augmentation to ensure that sandfish and other indigenous fishes have sufficient water to survive the dry summer months. Genetic work should be carried out to determine if these populations represent unique operational taxonomic units, as their small distributions make them especially vulnerable to local extinction. It also presents an opportunity to carry out a population viability analysis (PVA) to gauge extinction risk.

While the Brandewyn and Bos/Wolf Rivers were not surveyed in detail during this study, they should be considered points of interest in future surveys. The observation of larval sandfish at the Bos-Doring confluence in October 2001 suggests that sandfish spawn in the area, either in the mainstem itself or in the Bos River directly upstream from the confluence (Paxton et al. 2002). Although the Brandewyn River has not been included in recent formal surveys, the observation of sandfish in the upper reaches during the 2010s warrants further investigation. Careful consideration must be given to efforts to carry out sandfish conservation in this area, however, as it was most likely naturally fishless due to a 5 m waterfall at its confluence with the Doring, and was actively stocked with sandfish by the CDNR in the 1980s (Riaan van der Walt, pers. comm.*, August 2024)

As far back as 1980 it was recommended that fish sanctuaries – entire catchment areas that are formally protected – be established to protect threatened indigenous fishes in the Western Cape (Gaigher et al. 1980). Where possible, formal protected areas including and upstream of key reaches should be established or expanded. However, much of the ODRS land not currently under formal protection is privately owned and used for agriculture and livestock farming. In these cases, stewardship agreements are a viable approach towards meeting freshwater biodiversity and sustainable water use goals. These should be prioritised in key tributaries such as the Biedouw River, where the return on investment is likely to be high. Implementing key interventions such as non-native fish removal throughout the river, alien plant clearing, responsible water management, and strategic water releases could transform the Biedouw River into a self-sustaining refuge and source population for the greater sandfish metapopulation.

Although the Doring mainstem remains the stronghold for large, reproducing sandfish, it is clear that the tributaries hold great potential for the persistence and recovery of the species. Compared with the mainstem, the tributaries tend to support much fewer proportions of non-native fishes, with the headwaters of many acting as native fish refugia. Moreover, only the tributaries have been shown to hold large numbers of young sandfish. Tributary habitats must therefore be prioritised as potential sources for the Doring, with interventions focused on expanding the size of native fish refugia, establishing additional protected areas and stewardship agreements, and increasing the downstream dispersal potential between tributaries and the mainstem. In a recent review, Bouska et al. (2023) highlight the value of focusing on tributaries for the enhancement of mainstem populations of large river

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fish via a portfolio of benefits or assets. Tributaries promote connectivity across a riverscape and offer a diverse set of habitats that large river fish often rely on to carry out their life cycles, and that become especially valuable when mainstems become homogenised (the 'connectivity' and 'habitat diversity' assets; Bouska et al. 2023). Different tributaries also provide temporally and spatially asynchronous conditions across a catchment that help buffer large fishes from localised disturbances (the 'asynchrony' asset; Bouska et al. 2023). The tributaries of the Doring River offer i) spawning habitat for migratory sandfish that – to our knowledge – is largely unavailable in the mainstem ('connectivity' and 'habitat diversity' assets); ii) perennial headwater refugia that likely buffer the species from the effects of drought ('habitat diversity'); iii) varied hydrological regimes distinct from the mainstem that play a key role in reproduction ('asynchrony') and iv) critical nursery habitat for juvenile sandfish born to mainstem migrants, historically providing a predation refuge from large omnivorous yellowfish (*Labeobarbus seeberi*) that inhabit the mainstem. Moreover, the tributaries of the Doring provide a more achievable target for IAF eradication than the mainstem. Given these considerations, it is apparent that tributary-focused management has the potential to improve the status of sandfish across the Doring River catchment.

The sampling methods employed in future ODF surveys need to be carefully considered to enable meaningful long-term comparisons. Standardisation of gear type and sampling effort across surveys is essential for detecting trends in population structure and community composition over time. Additionally, an appropriate array of sampling methods should be used to target different size classes. To this end, it is recommended that future ODF surveys deploy the large three-winged fyke net in the thalweg of each pool and several small fyke nets along pool edges. Standardised snorkel surveys would further enhance the accuracy of fish community assessments, though this may be impeded by low visibility conditions. Finally, eDNA sampling should be incorporated into future surveys to identify rare species that may not be detected by fyke or snorkel surveys. Without diversifying sampling beyond a single net type, it will remain difficult to determine whether observed size distributions reflect true ecological patterns or are artefacts of gear selectivity. Detailed monitoring recommendations for the Olifants and Doring mainstems, as well as a selection of Doring tributaries, can be found in Appendix A 6 and Appendix A 7. These include a list of sites, timing of surveys, sampling methods, and key metrics to record.

Several important research gaps exist that would positively inform future management decisions. The historical function of apparently self-sustaining, resident headwater populations needs to be better understood, as does the relationship between these populations and migratory populations in the foothills. To what extent did downstream dispersal from the headwaters contribute to foothill populations? Did upstream migrations by adults from the foothills historically extend to the headwaters, and if so, what was their contribution to those populations? Have deleterious effects of genetic isolation manifested in any key headwater refugia? Do spawning adults display high natal philopatry, and how does this impact population structuring? What is the population viability of the various isolated sandfish populations? Investigating these and other questions would help guide decisions to prioritise restoration of connectivity between key populations, such the Oorlogskloof gorge and the Koebee and Doring mainstem populations. Further work into the mechanisms driving the apparent stunting of tributary populations would also help researchers better understand the plasticity of the species' life history strategies, including differences in growth rates, size at sexual maturity, and migratory behaviour between populations. The species' genetics must also be better understood, especially as it relates to historical connectivity between populations, current patterns of isolation, and the genetic impacts of the Biedouw River head-start efforts on the greater metapopulation. An updated Doring River survey is urgently needed to track the dispersal and potential impacts of several novel invaders in the system, and to assess the outcomes of the sandfish head-start programme initiated in 2020 (see Chapter 4). These surveys should be repeated every five years if possible, and each site should be sampled with two small fyke nets at the banks in addition to the large fyke in the thalweg to target fish that may favour marginal habitats. Should budget constraints be an issue in future ODF surveys, several mainstem sites could be excluded, based on their close proximity to other sites (namely Uitspanskraal and one or two of the downstream-most sites). However, the inclusion of an additional site upstream of Brakfontein Weir should be considered to assess the potential impacts of the barrier on sandfish population dynamics. Lastly, a comprehensive survey of the Olifants River system is needed to evaluate the current state of that system and identify key intervention points to benefit native fish biodiversity. The use of environmental DNA (eDNA) is recommended as a complement to traditional sampling methods, due to its ability to detect species that might be missed by conventional approaches (Van Nynatten 2024).

This study collates historical and current distribution data to contextualise the current status of the Clanwilliam sandfish in the Doring River system. While the Doring mainstem continues to support a core population of adult sandfish, the tributaries play an important role in providing spawning and nursery habitat, refugia, and potential source populations for the greater sandfish metapopulation. Addressing threats such as non-native fishes and declining flows will help to support and expand these populations moving forward. To support future management efforts, continued monitoring, improved understanding of population dynamics and connectivity, and partnerships with landowners should be prioritised. The findings presented here provide a useful foundation to guide conservation actions for this threatened species in the future.

Chapter 4

4 Evaluating the effectiveness of a head-start conservation programme for Clanwilliam sandfish in the Biedouw River

4.1 Introduction

The Clanwilliam sandfish, the only large detritivorous fish in the Olifants-Doring River system (ODRS; Skelton 2024), has experienced a substantial range contraction since the 1930s (see Chapter 3). This has been driven primarily by habitat degradation, water abstraction and invasive alien fishes (IAF). Data from this study suggest that although healthy refuge populations persist in the upper reaches of some tributaries, there is evidence of recruitment failure in the mainstem, likely due to a lack of suitable nursery habitat and predation by IAF. If not addressed, persistent recruitment failure threatens the long-term viability of remaining sandfish populations, even where adult fish are present (Archdeacon et al. 2020). In these cases, population management actions, such as population augmentation, are essential for the recovery of fish populations suffering consecutive years of recruitment failure (Archdeacon et al. 2020).

4.1.1 Head-starting

Augmentation of declining populations is increasingly being used as a tool in the conservation of freshwater fishes in danger of extinction (George et al. 2009). Head-starting is one such method that involves the rearing of captive-bred or wild-collected individuals in captivity prior to their release into natural habitats (Alberts 2007). Head-starting is used in scenarios where juveniles in the wild have a low chance of survival: by being reared to a larger size before release, head-started individuals thus have a greater probability of surviving the neonatal period and recruiting into the adult population (Alberts 2007). The ultimate aim of this approach is to maximise survival of juveniles and therefore increase rates of recruitment into the breeding population (Alberts 2007). It is often applied in cases where juvenile recruitment is severely compromised by the predatory impacts of invasive species (Alberts & Phillips 2004) or by unsuitable habitat (Beebe et al. 2021). While population augmentation via head-starting does not directly address the underlying threats to the wild populations, in many cases it is a necessary emergency measure designed to prevent the extinction of imperilled fishes, while other more permanent interventions to secure the long-term future of the species are put into place (George et al. 2009).

Head-starting has long been a popular tool in the conservation of amphibians (Crane & Mathis 2011; Smith et al. 2020) and of reptiles such as tortoises, sea turtles, crocodiles, snakes and lizards (Sainsbury et al. 2021). It is now increasingly being used in the management of freshwater fishes. In the 1980s and 1990s it was recognized that the Critically Endangered razorback sucker (*Xyrauchen texanus*) was in imminent danger of extinction due to a sustained lack of recruitment among the remaining fragmented populations in the Colorado River basin (Mueller 1995). In response, a head-start programme was developed in which naturally spawned larval fish were reared in isolated nursery areas and subsequently released back into Lake Mohave, once they had reached a size large enough to evade most predators (> 25 cm; Mueller 1995). Unfortunately, as of the early 2000s, no noticeable improvement had been recorded and populations continued to dwindle (Schooley & Marsh 2007). Similar efforts in the same system were carried out for the Endangered humpback chub (*Gila cypha*), a large-bodied species which is threatened by predation and competition by invasive alien fishes (IAF) and by modified habitat conditions. Rescued juveniles were reared in hatcheries for up to 12 months before being released into a different part of the basin to establish population redundancy (Healy et al. 2020). Through these efforts, a reproducing population was successfully established, with promising survival and growth rates recorded in subsequent surveys (Healy et al. 2020).

In the 1990s, head-start programmes were also initiated for steelhead trout (*Oncorhynchus mykiss*) in California's Carmel River and for coho salmon (*Oncorhynchus kisutch*) in Washington's Columbia River, due to seasonal dewatering and fragmentation of critical habitats (Lopez Arriaza et al. 2017; Beebe et al. 2021). While the Coho Salmon programme increased the abundance of adults and lowered extinction risk when fish were held for a full year (Beebe et al. 2021), the steelhead trout programme was found to have detrimental effects on the wild population by decreasing juvenile smolt success (Lopez Arriaza et al. 2017). These variable outcomes highlight the importance of coupling comprehensive monitoring efforts with any head-start programme, especially given the relatively high costs of carrying out such an intervention.

4.1.2 The Saving Sandfish head-starting programme

The Saving Sandfish Project (SSP) is an effort led by the Freshwater Research Centre in Cape Town, in collaboration with the University of Cape Town, provincial conservation agencies (CapeNature and Northern Cape Department of Agriculture, Environmental Affairs, Rural Development and Land Reform), a non-governmental organisation (Fynbos Fish Trust), documentary filmmakers (Fishwater Films), and numerous landowners and interested individuals. As a key member of the implementation team, I was directly involved with the design and execution of the head-start programme throughout its duration. I also led the research and monitoring component, overseeing data collection to evaluate the programme's outcomes.

The evaluation of the past and present distribution of the Clanwilliam sandfish outlined in Chapter 3 demonstrated that the species has suffered a major range contraction in the past century, persisting in an ageing mainstem population in the Doring River and in some isolated self-sustaining tributary headwater populations above IAF barriers. It is clear from the most recent Doring River survey, carried out in October 2021, that there is no recruitment occurring in the mainstem population, as illustrated by the conspicuous lack of non-adult size classes in mainstem catches (see Chapter 3 section 3.3.2.3.1). Like *X. texanus*, adult sandfish continue to spawn, but unless the population is augmented with new generations of recruits, the species may face extinction in the wild within decades.

The 2013 discovery of the Biedouw River as a spawning tributary and the subsequent observation of zero juvenile survival created a unique opportunity to implement a head-start programme. The intervention was conceptualised as an emergency measure to prevent species extinction by augmenting the ageing wild population with subadult recruits. In other words, head-starting would bridge the sandfish life cycle, which is currently severed at the juvenile stage by IAF predation and seasonal dewatering.

In this intervention, juvenile sandfish are: 1) rescued from the lower reach of the Biedouw River in the months following spawning; 2) translocated to a series of off-stream IAF-free reservoirs (i.e. nurseries); 3) reared in these nurseries until they reach a size less vulnerable to IAF predation; and 4) subsequently returned to the wild after a minimum of one year (Figure 4.1)[*]. Previous work has shown that fish smaller than 200 mm are generally unable to co-exist with bass in invaded reaches of the ODRS (Weyl et al. 2013; van der Walt et al. 2016; Cerrilla et al. 2022). The minimum threshold for size at release was therefore originally set at 200 mm TL. The lower threshold was adjusted each release season if insufficient fish larger than 200 mm were recaptured from the reservoirs or to test specific hypotheses. This is discussed in more detail in Methods Section 4.2.2.4.

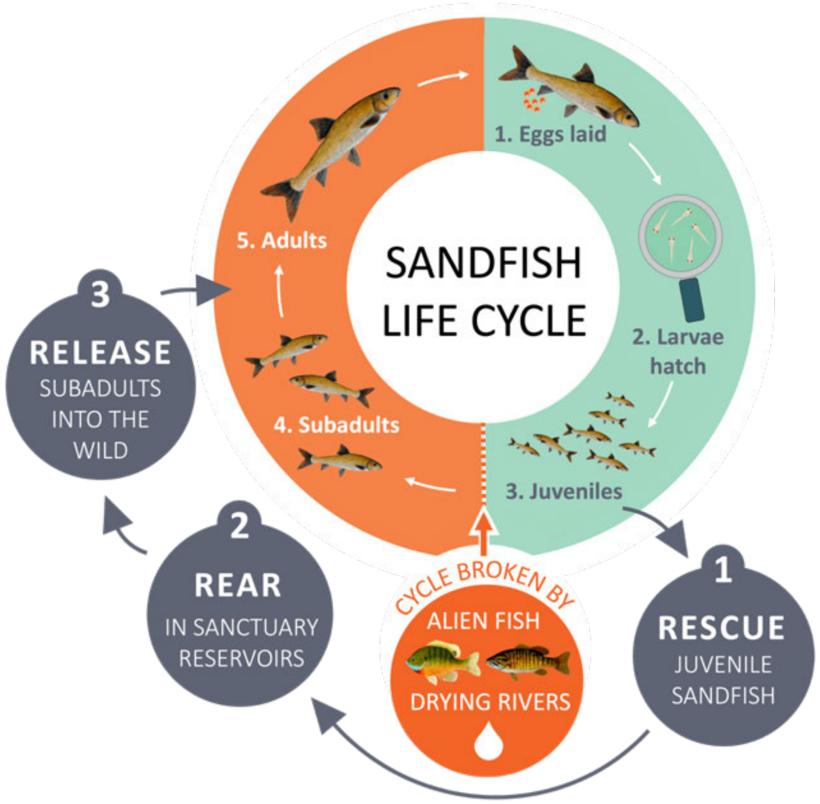

Figure 4.1. The current sandfish life cycle in the Biedouw River, given the impacts of IAF predation and seasonal dewatering of nursery habitats. Also illustrated is the SSP head-start intervention, which aims to bridge the gap in the species' broken life cycle by maximising juvenile recruitment into the breeding population. Illustration concept by Jamy Silver.

A critical feature of this intervention is that juvenile sandfish are naturally spawned and collected from the wild, ensuring that subadult sandfish produced under these conditions represent a greater genetic diversity than would result from more traditional hatchery methods. Additionally, the use of semi-natural sanctuary reservoirs precludes the need to spend considerable resources maintaining expensive and disease-prone hatchery facilities. Given that juvenile sandfish would historically have been confined to isolated, lentic pools in the lower Biedouw River during

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[*] The intervention, called the "Saving Sandfish Project" is led by the Freshwater Research Centre in partnership with CapeNature, the Northern Cape Department: Agriculture, Environmental Affairs, Rural Development and Land Reform, Fynbos Fish Trust, Endangered Wildlife Trust, World Fish Migration Foundation, Youth 4 Conservation, The Mission Flyfishing Magazine, Cederberg Conservancy, Driehoek, Alpha Excelsior, Gone., and Fishwater Films. Past and current funders include the National Geographic Society, IUCN SOS, the European Union, Ford Wildlife Foundation, Mohammed bin Zayed Species Conservation Fund, the Rufford Foundation, Bushmans Kloof Wilderness Reserve, Alu-Cab, FOSAF, Mout Ceder, Investec, and Caleo.

summer until winter rains restored river flow, the use of lentic reservoir environments as a nursery habitat during this life stage was considered appropriate.

4.1.3 PIT tags as a tool to monitor intervention outcomes

For the intervention to be considered successful, a large number of head-started fish must survive in the wild and spawn in subsequent years. Tagging is one method commonly used to assess the success of such efforts and can be used to evaluate survival, movement, or even reproductive success (George et al. 2009). Passive integrated transponder (PIT) tags are one of the most popular tools for the tracking of unique individuals of diverse taxa (e.g., snakes, small and large mammals, birds, tortoises, crabs, and fish, among others; Gibbons & Andrews 2004), and especially so within the context of head-starting.

A PIT tag is a small radio frequency identification (RFID) device that contains a unique identification code associated with an individual animal (Figure 4.2). The tag does not have its own power source, but is instead activated by a scanner. When the PIT tag comes within range of the scanner, the scanner sends a low frequency signal to the microchip in the PIT tag, providing the power needed to send its unique code back to the scanner (Biomark 2025). In other words, the PIT tag is energised by this signal and uses that energy to transmit its unique identification code back to the reader (Gibbons & Andrews 2004). PIT tags are implanted into the body of animals either intramuscularly (embedded within muscle), subcutaneously (under the skin), or intraperitoneally (within the peritoneal cavity). The tags serve as permanent coded markers equivalent to a barcode, and are as reliable as a fingerprint in the identification of an individual (Gibbons & Andrews 2004). During initial tagging, important information can be recorded, such as release location and body size, facilitating the assessment of growth rates and movement patterns upon recapture of tagged individuals (Gibbons & Andrews 2004). PIT tags can also be used with automatic detection systems which are placed along the suspected path of an individual or population of interest. When a tagged individual passes within a certain range of an automatic detection system, that unique PIT code is recorded along with temporal information. Automatic detection systems are especially useful in the study of migratory fishes with predictable migration routes.

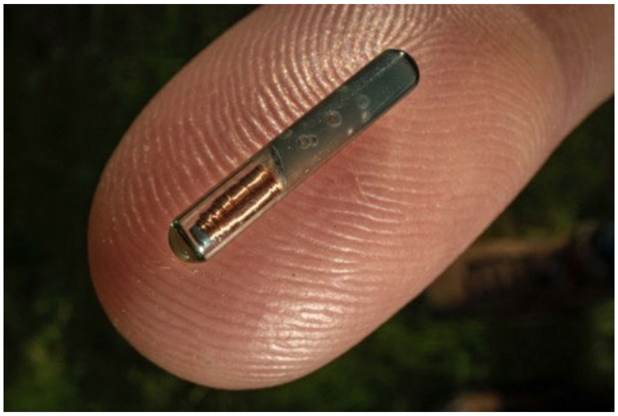

Figure 4.2. Biomark APT12 passive integrated transponder (PIT) tag. Photograph by Jeremy Shelton.

PIT tags hold several important advantages over external tags (e.g., spaghetti tags) and physical marks (e.g., fin clipping or scarring). External tags may be lost, result in injuries to the tagged animal, and affect the behaviour of the tagged animal and of its conspecifics, while external marks can fade, heal, or become illegible (Gibbons & Andrews 2004). One example comes from a 2001 study, in which a sample of the indigenous fishes of the Olifants-Doring River system (including 271 adult sandfish) were tagged with external anchor tags in order to better understand their current distribution and movement patterns (Paxton et al. 2002). Only two sandfish were recaptured during a follow-up survey eight months later; the tag came loose easily from the skin of the first fish as it was being retrieved from the net, and the second fish had a swollen lesion at the implanting site, where the tag looked to be in the process of being ejected. As a result, Paxton and colleagues (2002) recommended the use of more effective and less damaging tagging methods in the future.

PIT tags are internal and permanent and have been shown to have virtually no negative impact on the welfare or survival of tagged fish, provided they are inserted correctly (Gibbons & Andrews 2004, Hopko et al. 2010). The unique ID codes conferred by PIT tags are also unmistakeable, reducing detection error and increasing data validity. Tag detection is highly reliable and accurate, both measures having close to 100 percent efficiency (McCutcheon et al. 1994; Gibbons & Andrews 2004). Furthermore, because the tag itself does not require a battery, it can last for the duration of an animal's life, and beyond – for example, a tag belonging to a swamp snake was detected inside an eastern kingsnake, providing evidence of predation by the latter upon the former (Gibbons & Andrews 2004). While tag rejection is possible, it is rare, as long as the tag is implanted properly (Gibbons & Andrews 2004) – in these cases, tag retention can be 100 percent (Hopko et al. 2010).

While passive tracking with a fixed automated detection system has some disadvantages – namely that physiological information (e.g., size) and environmental parameters cannot be recorded at time of detection – it also has some important advantages. A major benefit is the ability to increase detection probability, when compared with manual scanning following the physical recapture of an individual. Hewitt and colleagues (2010) compared detection probabilities of PIT-tagged Lost River suckers (*Deltistes luxatus*) during spawning season in Upper Klamath Lake, Oregon, using both physical recaptures (via trammel netting) and remote recaptures (via a fixed antenna array). Over four years, the remote system detected nearly 10 times as many unique tags as the those detected after physically recapturing individuals. This allowed the authors to produce much more accurate estimates of encounter probabilities and survival. Another key benefit of remote PIT data capture is the collection of presence data without stressing the fish.

The main disadvantages of PIT tags are technical, the most important being detection range. Whereas telemetry tags can be detected from considerable distances (ranging from kilometres to essentially unlimited in the case of satellite telemetry tags), PIT tags can only be detected at close ranges of less than 50 cm, depending on the size of the PIT tag (Burnett et al. 2013). The orientation of the tag in relation to a fixed detection antenna system can also impact the read range, or whether the tag is read at all (Burnett et al. 2013). Furthermore, tagged fish swimming in large groups can sometimes pass undetected over a fixed antenna (Castro-Santos et al. 1996). Another concern is the potential transfer of pathogens between fish when PIT tagging large numbers of individuals in a short period of time (Cooke et al. 2013). This risk can be minimised by using tags pre-loaded into single-use needles (Biomark 2025). Despite these drawbacks, PIT tags have proved to be an invaluable tool in the monitoring of fish movement and survival.

One of the most common uses of PIT tags in freshwater systems is in the monitoring of hatchery-reared salmonids as they pass through fish passage structures to gauge survival and track movement patterns (e.g., Prentice et al. 1990; McCutcheon et al. 1994). Other studies have used PIT tags to investigate salmonid growth and survival

(Peterson et al. 1994), habitat use and activity patterns (Enders et al. 2007), behaviour (Teixeira & Cortes 2007), and response to drought conditions (Vander Vorste et al. 2020). Researchers working in the Colorado River Basin used PIT tags to monitor the outcomes of a large-scale population augmentation effort of two large-bodied endangered fish species, *X. texanus* and Colorado pikeminnow (*Ptychocheilus lucius*; Cathcart et al. 2018; Pennock et al. 2020). Wild-to-wild translocation and captive rearing head-starting efforts centered around *G. cypha* in the same system were also monitored using PIT tagging technology (Spurgeon et al. 2015; Healy et al. 2020, 2022). In the Mekong River basin, PIT tags were used to monitor the migratory patterns of a locally important fisheries species, demonstrating the need for integrating fish passage structures into planned hydropower developments (Robinson et al. 2024).

4.1.4 Objectives

This chapter documents the implementation of the Saving Sandfish head-start programme and evaluates: i) the effectiveness of fish rescues for increasing juvenile sandfish survival; ii) the suitability of off-stream reservoirs cleared of IAF as nurseries for rearing juvenile sandfish to release-ready size; iii) return rates of head-started sandfish and the factors influencing the probability of return; and iv) likely environmental cues to the timing of migration and spawning. The chapter concludes by reviewing lessons learned and giving recommendations on the way forward. This study was carried out concurrent to the head-start intervention to support responsive, informed decision-making in the management of the Clanwilliam sandfish.

4.2 Methods

4.2.1 Study area

All work was carried out with appropriate permits and ethics approval (Appendix B 1; Appendix B 2). The head-start programme was implemented in the Biedouw River, a tributary of the Doring River where adult sandfish undertake annual spawning migrations (Figure 4.3). Although fish exist along 33 km of river length, observations over a three-year period indicate that adult sandfish only travel up to 15 km from the river's confluence with the Doring River. The conservation intervention took place in this lower reach (Figure 4.4). For a full description of the Biedouw River, refer to Chapter 1 section 1.4.4.

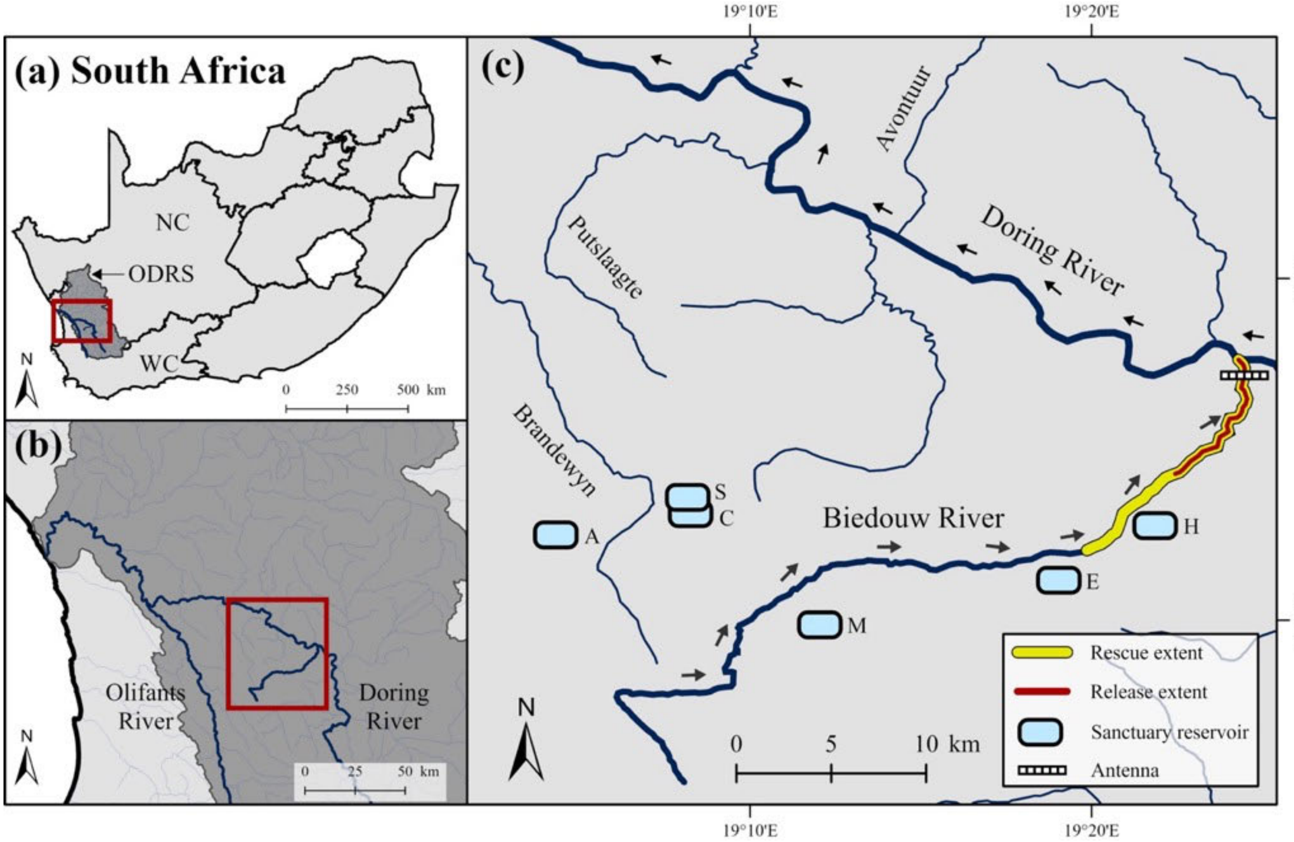

Figure 4.3. Map of study area: (a) South Africa with provinces outlined, (b) Olifants and Doring Rivers, and (c) Biedouw River and surrounding area. NC = Northern Cape, WC = Western Cape, ODRS = Olifants-Doring River System, A = Alpha Excelsior Reservoir, B = Syfer Reservoir, C = Clay Reservoir, D = Mertenhof Reservoir, E = Enjo Reservoir, F = Hartsbesluit Reservoir.

Figure 4.4. Aerial view of the Biedouw River facing upstream from P33 (located adjacent to the riverside structures) in September 2021 during the annual wildflower bloom, which coincides with the sandfish spawning run. Site P33 represents the uppermost limit of the rescue efforts. Photograph by Otto Whitehead.

4.2.2 Field methods and data analysis

4.2.2.1 Establishing sanctuary reservoirs

Six off-stream impoundments in the Biedouw and surrounding catchments were converted into sandfish sanctuary reservoirs between May 2019 and May 2022 (Table 4.1; Figure 4.3; Figure 4.5). Each reservoir was first surveyed using fyke nets (n = 4-7) to determine the fish species present. In all cases but two (Alpha Excelsior and Hartsbesluit Reservoirs), only non-native species were present. The only species present in Alpha Excelsior Reservoir were native Clanwilliam yellowfish (*Labeobarbus seeberi*); the reservoir was therefore excluded from eradication treatment. In addition to bluegill (*Lepomis macrochirus*), eight adult sandfish were sampled in Hartsbesluit Reservoir prior to treatment, having entered the reservoir from a furrow connecting the Biedouw River to the reservoir.

Table 4.1. Non-native fish eradication treatment conditions and sandfish stocking dates per sanctuary reservoir.

Reservoir name	Length (m)	Width (m)	Maximum depth (m)	Species present before treatment	Treatment date	Treatment type	First sandfish stocking date
Clay	174	72	3.5	*Lepomis macrochirus*	May 2019	Draining	Oct 2020
Mertenhof	125	60	4.0	*Lepomis macrochirus*	March 2020	Rotenone	Oct 2020
Enjo	135	31	2.0	*Lepomis macrochirus* *Cyprinus carpio*	March 2020	Draining + rotenone	Oct 2020
Syfer	184	84	2.5	*Lepomis macrochirus*	May 2021	Draining	Nov 2021
Alpha Excelsior	160	42	2.5	*Labeobarbus seeberi*	N/A. No non-native fishes present.	N/A	Dec 2021
Hartsbesluit	122	51	2.0	*Lepomis macrochirus* *Labeo seeberi*	May 2022	Rotenone	Nov 2022

Figure 4.5. Clockwise from top left: Enjo, Hartsbesluit, Mertenhof, and Clay Reservoirs. Photographs by Otto Whitehead (Enjo), Jeremy Shelton (Hartsbesluit and Mertenhof) and Cecilia Cerrilla (Clay).

To eradicate the non-native species, Clay, Mertenhof, Enjo, Syfer, and Hartsbesluit Reservoirs were either treated with the piscicide rotenone, drained, or both (Table 4.1). While rescue of the eight sandfish in Hartsbesluit Reservoir was attempted during treatment, an overabundance of aquatic vegetation prevented the use of a motorboat to rescue these individuals after they surfaced from initial rotenone exposure, and they succumbed to rotenone, along with the bluegill. Reservoirs were re-surveyed with fyke nets following treatment to confirm eradication success. Post-treatment fyke surveys revealed no fish remaining in any of the reservoirs.

4.2.2.2 Rescues

Following each spawning season in 2020, 2021, 2022, and 2023, young-of-the-year sandfish were rescued from the lower Biedouw River and relocated to various reservoirs (Table 4.2). The second rescue season (2021/22) followed an exceptionally wet winter, resulting in the river remaining wetted well into late summer. Rescues therefore continued through March 2022.

Table 4.2. Rescue dates and reservoirs to which rescued sandfish were translocated during the four rescue seasons.*

Rescue season	Rescue dates	Reservoirs stocked
2020	31 Oct – 5 Nov, 2020	Clay, Enjo, Mertenhof
2021/22	22 – 28 Nov, 2021 10 Dec, 2021 29 – 30 Jan, 2022 10 Feb, 2022 1 March, 2022	Alpha Excelsior, Clay, Enjo, Mertenhof, Syfer
2022	7 – 11 Nov, 2022 3 – 4 Dec, 2022	Alpha Excelsior, Hartsbesluit, Mertenhof, Syfer
2023	28 – 30 Nov, 2023	Enjo, Hartsbesluit, Mertenhof

Sandfish were rescued once flow in the Biedouw River had slowed or stopped entirely, when pools and runs were shallow enough to facilitate capture. Teams of rescuers pulled seine nets across pools and runs in the lower 15 km of the Biedouw River (Figure 4.6). Captured sandfish were placed into 25 L buckets, each containing a battery-powered oxygen pump, and transported by vehicle to the reservoirs. The fish were first acclimated for 5-15 minutes by slowly pouring water from the reservoir into each bucket until the temperature of the water in the bucket matched the temperature in the reservoir. Each bucket was then carefully inspected, and any non-native fish were removed and humanely euthanised using an overdose of MS-222 (500 mg/L).

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* Over the course of 2022, aquatic macrophytes overpopulated Enjo Reservoir to such an extent that it created highly hypoxic conditions and prevented tourism activities. It was therefore decided that the reservoir should be drained and the macrophytes dredged before refilling the reservoir. In February 2023, once the reservoir was shallow enough to allow for seine net passes, 234 sandfish measuring approximately 250-350 mm were rescued from Enjo Reservoir and relocated to Hartsbesluit Reservoir. Approximately 160 sandfish perished while the reservoir was draining, presumably from exposure to hypoxic conditions. Enjo Reservoir was subsequently dredged and filled over the course of 2022, and six sterile grass carp (*Ctenopharyngodon idella*) were introduced in August 2023 to help control macrophyte growth in the future. Young sandfish were again stocked into the reservoir in November 2023. Twenty-two sterile grass carp were also stocked into Hartsbesluit Reservoir in February 2023 to control aquatic macrophyte growth there.

Figure 4.6. Teams of rescuers pull seine nets through site P04 (March 2022; left) and through a run just downstream of site P31 (November 2021; right). Photographs by Jeremy Shelton.

During the 2021/22, 2022, and 2023 rescue seasons, a subsample of sandfish was measured prior to release into the reservoirs. Each subsample consisted of 30-55 sandfish from a variety of source pools that spanned the spatial range of rescue locations and rescue dates during each rescue season. Any dead sandfish were removed, and their numbers recorded. Remaining sandfish were counted, acclimated, and released into the reservoir by dipping the edge of the bucket below the surface of the water and allowing the fish to swim out into the surrounding environment. A linear regression was used to model the relationship between fork length and total length of sandfish rescued during all three rescue seasons to determine whether it was necessary to take both measurements. There was a very strong correlation ($R^2 = 0.995$; Appendix A 8), indicating that it is sufficient to take a single measurement and precluding the need for additional handling. While it would have been valuable to tag rescued juveniles at the time of translocation to gain insights into survival and growth within the sanctuary reservoirs, this was not feasible – most rescued fish were too small to tag safely, the large number of individuals made it logistically unmanageable, and limited funding for tags required us to prioritise fish being released back into the wild.

4.2.2.3 Rearing

4.2.2.3.1 Growth rates

Following relocation, rescued sandfish remained in the sanctuary reservoirs until the following spawning season (or longer), prior to being released. Fyke nets were used to monitor sandfish growth in the reservoirs at various times following stocking (Appendix A 9). Effort varied, based on availability of nets and sampling personnel. Sandfish were anaesthetised by placing them in a bucket with a solution of MS-222 (50 mg/L), then measured to the closest millimetre before being allowed to recover in an oxygenated bucket. They were then either returned to the reservoir (monitoring event) or released into the wild (release event). Due to budget constraints limiting the number of available tags, sandfish were only tagged at the time of their release into the wild.

Growth rates were assessed at Enjo, Mertenhof, Clay, and Hartsbesluit Reservoirs during various sampling events (Appendix A 9). Some monitoring events took place in the summer and spring months prior to the release season, during which sandfish were returned to their respective reservoirs after being measured. Size data from individuals released into the wild were also included in the assessment of growth rates.

Alpha Excelsior Reservoir has not been resampled since it was first stocked due to its distance from the core field site, and so was excluded from these analyses. Syfer Reservoir was stocked with rescued fish in November 2021 (n = 459) and February 2022 (n = 203). However, a subsample of rescued fish (n = 45) was only measured on the second occasion, and the reservoir was re-sampled one month later, in March 2022. Since none of the fish stocked in November 2021 were measured, an assessment on growth rate cannot be carried out with confidence. If, for example, a majority of the fish caught during the March 2022 sampling event comprised those stocked in November 2021 (rather than those stocked in February 2022), then the resulting growth rates (calculated for February – March 2022) would be misleading. Data from Syfer Reservoir has therefore been excluded from this analysis.

In some cases, a single reservoir was sampled several times over the course of two to five days – for example, during release events when nets were repeatedly set on several successive days for the release of head-started sandfish into the wild. This occurred at Enjo, Mertenhof, and Hartsbesluit Reservoirs. At both Mertenhof and Hartsbesluit Reservoirs, sandfish were stocked repeatedly over the course of several days, resulting in several instances of sampling dates occurring in closer proximity to one another. Where sampling dates occurred within five days of one another, they were grouped into a single sampling window, with corresponding data pooled into a single sample. For example, sandfish were captured from Enjo Reservoir and released into the wild on August 30, August 31, and September 3, 2021. As these dates fell within five days of one another, they were grouped into a single sampling window. At Hartsbesluit, rescued sandfish were stocked on 7, 8, and 9 November 2022, and so these dates were combined into one sampling window, and measurement data from these events were pooled into a single sample. Re-sampling of the same individuals over consecutive dates within a sampling window was not possible, as measured individuals were either released into the wild, or consisted of new rescues being stocked.

Despite the stocking of Enjo and Clay Reservoirs during two rescue seasons (2020 and 2021/22), continuous and sufficient size data were only available for the 2020 rescue cohort; this cohort was therefore the focus of growth rate assessments in these two reservoirs. Hartsbesluit Reservoir was stocked for the first time in November 2022, and so only data from the 2022 cohort form the basis of the growth assessments at that reservoir. At Mertenhof Reservoir, continuous and sufficient data exist for the 2020, 2021/22, and 2022 rescue cohorts. Clear size differences were apparent between rescue cohorts within a single sampling event, facilitating the assessment of growth rates by cohort.

Growth rates (mm/day) were calculated as the difference in total length (mm) divided by the number of days between two sampling dates (stocking, monitoring, or release dates). Where several dates were grouped into a single window, the first sampling date was used as the reference point.

Rescued sandfish were not measured during the 2020 rescue season. Growth rates of the 2020 rescue cohort therefore could not be calculated from time of stocking.

4.2.2.3.2 Survival

One of the factors influencing the outcome of the head-start programme is the suitability of the reservoir environment as rearing habitat for rescued sandfish. Post-stocking survival is a useful metric by which to gauge this. Population size in Enjo Reservoir was estimated one year after initial stocking (which occurred in October –

November 2020). One-year survival was then inferred by comparing the estimated population size to the total number of sandfish potentially available for capture at the time of sampling.

The population size of sandfish in Enjo Reservoir in November 2021 was first estimated using the Lincoln-Peterson index (LPI), a method commonly used to estimate population size under certain conditions. Use of the LPI requires two sampling periods: during the first sampling period, individuals are captured, tagged, and released back into the environment; during the second sampling period, the environment is re-sampled, and the total number of tagged and untagged individuals used to estimate population size. The LPI is defined as follows, where M is the estimated population size; n_1 is the number of animals caught, marked, and released in the first sample; n_2 is the total number of animals caught in the second sample; and m_2 is the number of marked animals present in the second sample (Table 4.3; Pine et al. 2003):

$$M = \frac{n_1 n_2}{m_2} \qquad \text{Equation 6}$$

The 95% confidence intervals around the abundance estimate were calculated with the *ciPetersen* function from the R package *recapr* (Tyers, 2021), using the normal approximation.

Table 4.3. Elements of mark-recapture study and survival estimate from Enjo Reservoir using the Lincoln-Peterson index ($M = \frac{n_1 n_2}{m_2}$; Pine et al. 2003) and estimated abundance in November 2021.

Time period	Variable name	Description	Value
Oct – Nov 2020	-	Initial stocking of rescued sandfish into Enjo Reservoir	1815 fish
Jun – Sep 2021	-	Removal of sandfish from Enjo Reservoir for release into the wild	- 588 fish
Oct 2021	-	Maximum number of fish potentially available for recapture in November 2021; that is, initial population size (stocking number: 1815) minus amount removed in Jun-Sep 2021 (588)	1227 fish
8 Oct and 6 Nov 2021	n_1	Number of sandfish caught, marked, and released at time 1	100 fish
28 Nov 2021	n_2	Number of sandfish in sample at time 2	150 fish
28 Nov 2021	m_2	Number of marked sandfish in sample at time 2	18 fish

On 8 October 2021, approximately one year post-stocking, 21 sandfish were captured and tagged with APT12 12.5 mm (0.1 g) PIT tags, recommended by Biomark for tagging fish larger than 55 mm (Biomark 2025). Fish were first anaesthetised by being placed into an MS-222 anaesthetic bath. A Biomark MK25 PIT Tag Implanter with a pre-loaded needle was used to inject the PIT tag into the peritoneal cavity, posterior to the pelvic fins and offset from the midventral line,* following the procedures outlined by the *PIT Tag Marking Procedures Manual* (CBFWA

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* There are several methods for PIT tagging fish, and your approach must consider the study location, species, and size of the individuals being tagged. Key considerations include the tagging procedure (surgical vs. injection) and the insertion site (intramuscular vs. intraperitoneal). In this study, we used single-use implant needles pre-loaded with tags to handling time and eliminate the need for equipment sterilisation between implants – an important advantage in field conditions. Tags were

1999). To increase confidence in the population estimate, a second tagging event took place on 6 November 2021, when a further 79 sandfish were tagged. Tagged fish measured 159-218 mm TL. Three weeks were allowed to pass to decrease the likelihood of trap-shy or trap-happy behaviour upon re-sampling. On 28 November 2021, two one-wing fyke nets were used to re-sample Enjo Reservoir. The LPI was thereafter used to calculate estimated population size (*M*).

The LPI assumes that the population is closed (i.e., that no immigration, emigration, births, or deaths occur between marking and recapture). The Enjo Reservoir population of sandfish was treated as a closed population, since immigration and emigration could not have occurred (the reservoir is land-locked and isolated from all water courses), and no births took place (verified by lack of spawning activity and larval fish). While some deaths may have occurred between tagging and sampling, they were likely minimal due to the short intervening time period. Nevertheless, the violation of this assumption may have biased the population estimate (*M*) and results should be interpreted with caution. Tagging of rescued juveniles upon initial stocking and subsequent monitoring under an open population model was not possible due to the small size of juveniles during stocking (23-143 mm, mean of 54 ± 1.5 SE), which precluded safe tagging.

The estimated population size in November 2021 (*M*) allows the estimation of a one-year survival rate if the number of fish potentially available for capture during the sampling event is also known. During October-November 2020, 1,815 juvenile sandfish were stocked into Enjo Reservoir (initial population size). During June-September 2021, 588 of these were recaptured from Enjo Reservoir and released into the Biedouw River. The remainder (1,277) is the maximum number of known stocked fish that could potentially be available for capture during the November 2021 sampling event. The estimated survival rate was inferred by dividing the estimated abundance derived from the LPI (*M*) by 1,227, while accounting for capture probability. The range of survival rates was calculated by dividing the upper and lower bounds of the 95% confidence intervals by this same value. All tagged fish captured during the re-sampling event were assessed to evaluate overall condition wound healing at the tag insertion site.

4.2.2.4 Releases

Reservoir-reared sandfish were released back into the Biedouw River during three release seasons: June – September 2021, August – October 2022, and August 2023 (Figure 4.7). Sandfish were released from Enjo and Mertenhof Reservoirs in 2021; from Clay, Enjo, and Mertenhof Reservoirs in 2022; and from Mertenhof and Hartsbesluit Reservoirs in 2023. As field time and personnel were limited, source reservoirs for releases were prioritised each year based on logistical considerations (e.g., distance to release sites), and to maximise catch (prioritising reservoirs stocked with the most sandfish). For these reasons, no sandfish were released from Clay Reservoir in 2021, or from Alpha Excelsior and Syfer Reservoirs in 2022 and 2023. Although efforts were made to capture fish from Clay and Enjo Reservoirs in 2022, catches were consistently poor (perhaps due to the lower stocking densities relative to Mertenhof), and so remaining efforts focused on Mertenhof Reservoir. Fish were not released from Enjo in 2023, as it was in the process of being refilled after draining.

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inserted intraperitoneally (with the tag sitting within the abdominal cavity), as subadult sandfish lack sufficient dorsal muscle mass for intramuscular insertion, and expert consultation indicated that this method was both appropriate and effective for the species.

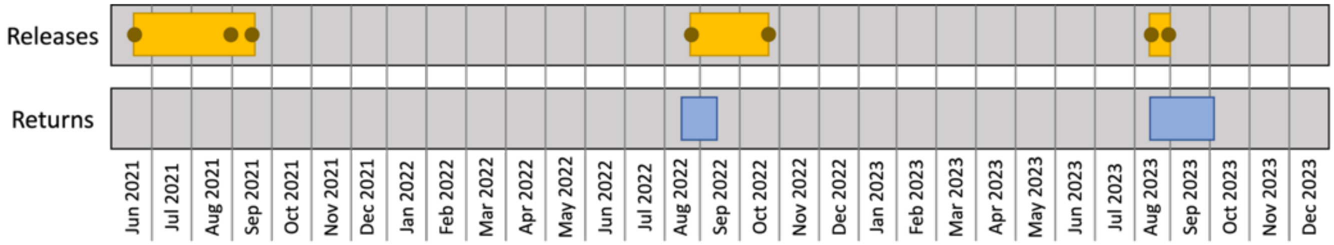

Figure 4.7. Timeline of release and return events between 2021 and 2023. Sandfish were released in 2021 (26 – 27 Jun, 30 Aug – 3 Sep, 12 Sep), 2022 (31 Aug – 3 Sep, 18 – 20 Oct), and 2023 (16 – 18 Aug, 30 – 31 Aug). Yellow rectangles represent the full range of release dates each year; brown dots represent release windows (dates within one week of one another are considered to belong to a single release window); and blue rectangles represent the full range of dates during which PIT tags belonging to returning sandfish were registered on the fixed antenna.

Releases during the annual spawning migration were prioritised to maximise the likelihood that head-started fish would follow migrating sandfish into the Doring River. However, logistical constraints necessitated releases in the weeks preceding and following the migration in 2021 and 2022. During each release event, fyke nets were placed in the reservoirs overnight and retrieved the following morning. Captured sandfish were placed into 25 L buckets fitted with battery-powered oxygen pumps (Figure 4.8) and transported by vehicle to various release sites along the lower 15 km of the Biedouw River (Figure 4.3). Release sites were chosen on the basis of adequate depth and cover available for released sandfish.

Figure 4.8. The release team places captured sandfish into buckets at Enjo Reservoir prior to their transport to the release site. Fyke net is visible in the foreground. Photograph by Otto Whitehead.

Once at the release sites, river water was slowly poured into each bucket until the temperature in the buckets matched the temperature in the river (5-15 min). Sandfish were then placed into large (3 m^3) holding containers filled with fresh river water and fitted with several battery-powered oxygen pumps. Prior to processing, sandfish were placed into an MS-222 anaesthetic bath. Once total loss of equilibrium was detected, fish were measured to the nearest millimetre and PIT tagged using the same procedure described in Section 4.2.2.3.2. If milt was expressed during this process, it was recorded. Following tag implantation, fish were allowed to recover in an actively oxygenated recovery bucket filled with fresh river water. Once they had recovered buoyancy control and regular swimming behaviour, they were released into the pool.

While our aim was to tag as many head-started fish as possible, a small percentage were released without tags. In 2021, 282 fish were released without a tag. Six were released without a tag due to shortage of tags during that tagging session, 21 escaped from holding containers and into the river prior to tagging, and 23 were released due to injuries or deformities (12 of these had pre-existing deformities such as missing fins or lacerations; six were tagged incorrectly so the tags were removed prior to release; two fell out of buckets during transport so were allowed to recover prior to being released without tags; and three were in a bucket where an oxygen pump failed, and so were released without a tag to minimise stress following possible hypoxia exposure). A total of 232 fish measuring less than 150 mm were released without tags to minimise handling stress to smaller fish, which are more vulnerable to predation. Five fish measuring 142-149 mm were tagged and released prior to the implementation of this size threshold.

In 2022, three fish were released without tags: two escaped prior to tagging, and one was injured due to incorrect tagging (the tag was removed, and the fish allowed to recover prior to release). In 2023, four fish were released without tags; all escaped prior to tagging. All tagged fish released between 2021 and 2023 were measured except for one in 2021, which escaped after tagging, but before measurements could be taken.

In 2022, enough fish over 150 mm TL were recaptured from reservoirs to justify excluding smaller individuals from release, in order to maximise the survival likelihood of released fish. Consequently, fish measuring less than 150 mm were returned to the reservoir prior to transporting the remainder to the release sites. However, five fish measuring less than 150 mm were missed during this process, and it was decided to tag them to track survival of smaller head-started fish. In 2023 the a priori decision was made to tag and release all fish larger than 95 mm to better address the question of survival and return rates among small head-started fish. A total of 433 fish measuring 98 – 149 mm were tagged and released in 2023.

One fish in 2021 and two fish in 2023 were euthanised humanely using an overdose of MS-222 after accidental ejection of the tagging needle into abdominal cavity during tagging.

Due to non-normality of the data, Mann Whitney U tests were used to assess whether size at release differed between source reservoirs during either 2021 or 2023 (as only two source reservoirs were being compared), while a Kruskal Wallis test was used to test for a difference in size at release between the three reservoirs in 2022.

4.2.2.5 Returns

Between 25 August and 17 September 2021, schools of large adult sandfish (~300-600 mm TL) migrated into the Biedouw River to spawn. During this time, three extensive visual surveys of the lower 15 km of the Biedouw River (the area occupied by migrating sandfish) were carried out to obtain an estimate of the size of the wild spawning migration. Pairs of researchers walked along the banks and counted the numbers of migrating sandfish in each pool. Sightings were cross-referenced with simultaneous sightings from other locations along the reach to ensure that each fish was counted only once. Surveys of this nature were possible because the pools were relatively shallow (< 1 m in most cases) and narrow (7-16 m wide), the water was relatively clear, and there were few in-stream visual obstructions (Figure 4.9). Although reservoir-reared sandfish (all < 220 mm TL) were released into the same area during this period, they were never observed schooling with the adults (> 300 mm TL) during the walking surveys – they were assumed to have left the Biedouw almost immediately after release.

Figure 4.9. View of migrating school of adult sandfish from the bank of a pool in the lower reach of the Biedouw River. Adult sandfish are easily spotted due to the water clarity, the shallow and relatively narrow nature of the pools, and a lack of in-stream visual obstructions. The fish in this photograph are estimated to measure between 300-600 mm TL. Photograph by Jeremy Shelton.

In August 2022 and July 2023, prior to each year's spawning migration, a 9 m-long solar-powered Biomark[*] Litz Cord pass-by antenna system – with an IS1001 data reader (scanner) – was installed across the width of the channel in the Biedouw River, 900 m upstream from its confluence with the Doring River (Figure 4.3). The antenna, which was anchored to the streambed, passively scans, detects and stores the unique identification number of any PIT-tagged sandfish moving across it to a depth of 50 cm (Figure 4.10). When a PIT tag comes within range of the antenna, the antenna emits a low frequency electromagnetic signal to the PIT tag that activates the tag. This powers the tag just long enough to transmit its unique identification code back to the data reader (scanner), where it is stored for future data retrieval (Biomark 2025). A PIT tag was used to test the system throughout the study period to confirm that fish swimming close to the surface at various flow rates and maximum depths would be recorded.

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[*] Biomark is a supplier of electronic identification and related monitoring systems to the fish and wildlife conservation communities and aquaculture industry. They can be contacted at biomarkservice@merck.com and found at www.biomark.com.

Additionally, fyke nets were set twice in September 2022 during the spawning migration, and each fish was scanned for PIT tags. In the first instance, one adult was captured by seine on the same morning as fyke net retrieval. Fish were briefly anaesthetised with MS-222, then sexed based on the expression of milt or eggs when massaged gently in the abdomen. Large fish with swollen abdomens were assumed to be female even if they did not express eggs. A subsample was weighed prior to being released.

Figure 4.10. The Biomark Litz Cord pass-by antenna was installed across a shallow riffle of the Biedouw River. Antenna is shown in low flow (left) and moderate flow (right) conditions. Photographs by Cecilia Cerrilla (left) and Jeremy Shelton (right).

A generalized linear mixed model (GLMM) was used to evaluate the effects of several conditions at the time of release on the probability of a fish returning to the Biedouw River in subsequent years. Antenna detections of individual PIT tags were used to determine whether fish released in 2021 and 2022 returned to the Biedouw River in 2022 and 2023. The response variable was the return status of the fish, a binary variable indicating whether a fish was detected in at least one of the two years post-release. Fish detected in either 2022 or 2023 were assigned a return status of 1, while those not detected were assigned a status of 0. The predictor variables included in the global model are defined in Table 4.4.

Table 4.4. Predictor variables included in the global GLMM.

Predictor variable	Variable type	Definition
Total length (cm)	Continuous	Total length (cm) of the fish at time of release.
Distance from the Doring River (km)	Continuous	Distance (km) of the release site from the Biedouw River's confluence with the Doring River.
Release time of year	Categorical (three levels)	Time of year fish were released relative to the annual spawning migration. "Early" releases took place prior to the start of the migration (June). "During migration" releases took place during the migration while adult sandfish were in the Biedouw River (August, September). "Late" releases took place after the migration had concluded, when no adult sandfish remained in the Biedouw River (October).
Time in reservoir	Categorical (two levels)	Time spent in sanctuary reservoir prior to release. Fish were not tagged when initially introduced into reservoirs during rescues, so the exact length of occupancy is unknown. Known rescue dates and stark differences in sizes at release made it possible to assign one of two levels to released fish. "Short" time in reservoir was defined as 6-11 months and was assigned to fish rescued in 2020 and released in 2021, and to those rescued in 2021 and released in 2022. "Long" time in reservoir was defined as 22-24 months and was assigned to fish rescued in 2020 and released in 2022.

A GLMM was fitted to the data using the "glmer()" function from the lme4 package in R (Bates et al. 2015), specifying a binomial distribution with a logit link function. Release year was included as a random effect to account for potential variations in return status attributable to the year in which a fish was released. Model selection was carried out using the "dredge()" function from the MuMIn package in R (Bartoń 2024) to evaluate all possible combinations of the fixed effects. The best-fitting model was identified based on Akaike Information Criterion (AIC) values. Non-significant predictors were removed from the model, leaving only fixed effects that contributed significantly to the prediction of return status.

4.2.2.5.1 Using simulations to guide tagging effort

Scaling up the head-start programme will require that a larger number of fish be released on an annual basis. To do so, one of the primary bottlenecks in this effort must be addressed – the time-consuming tagging process. If a smaller proportion of fish can be tagged while still generating enough detections to effectively monitor the programme, this would allow more head-started sandfish to be released into the wild. Custom simulations were carried out in base R to explore how different combinations of key parameters – survival probability, tagging proportion, and total number of fish released (Table 4.5) – might influence the likelihood of surpassing certain detection thresholds.

Table 4.5. Simulation parameters used to evaluate the likelihood of detecting a sufficient number of tagged fish under varying survival probabilities and tagging efforts.

Parameter	Description	Value / range	Justification
Release count	Hypothetical number of fish released in each simulated year	1,000 or 3,000	Between 2021 and 2023, an average of 942 fish were released annually. A rounded value of 1,000 was used to reflect typical annual releases. A higher value of 3,000 was included to explore a hypothetical scenario in which more fish could be released if tagging effort is reduced.
Detection probability	Probability that a tagged fish is detected when passing over the antenna	0.9	In 2023, 44 tagged fish released upstream in 2022 were detected. Of these, 3 (6.8%) lacked a prior outgoing detection, suggesting an efficiency of 93.2%. This was rounded down to a more conservative 90%.
Proportion tagged	Subset of hypothetical release cohort that is PIT tagged	0.1, 0.3, 0.5, 0.7, 0.9	These values reflect a range of realistic tagging intensities. Lower proportions (e.g., 10%) reduce tagging effort and costs, while higher proportions (e.g., 90%) reflect the upper limits of past tagging efforts. The range was chosen to explore the trade-off between tagging effort and probability of obtaining sufficient detections.
Survival probability	The real probability of survival after a fish is released	0-0.7	This range reflects a wide range of plausible survival probabilities, accommodating uncertainty and allowing exploration of different survival scenarios.
Detection thresholds	The minimum number of tagged fish detected in the year following release for the simulation to be considered successful; represents a practical threshold for obtaining sufficient data for future monitoring purposes.	30, 50, 70, 90	These values represent a practical range of detection totals that researchers might consider useful for drawing inferences about detection probability and survival. The appropriate threshold will ultimately depend on the specific analyses or models used in future studies. This range allows exploration of how tagging effort and survival probability influence the likelihood of reaching different potential data sufficiency targets.

In each simulation run, a hypothetical cohort of 1,000 or 3,000 fish is released. A subset of these fish—ranging from 10% to 90% of the release group—is assumed to be PIT tagged. Each tagged fish has an independent probability of surviving the year (the "survival probability"), which is varied systematically from 0 to 0.7 in 0.05 increments. Fish that survive have a fixed 90% probability of being detected if they pass the PIT antenna system, based on conservative estimates from detection data.

For each combination of survival probability, tagging proportion, and detection threshold, 1,000 simulation iterations were performed. In each iteration, the number of tagged fish that survived was determined using a binomial distribution using the "rbinom()" function. The number of surviving fish detected was then determined via another binomial draw. A simulation iteration was considered successful if the number of detected individuals equalled or exceeded a predefined threshold.

Four detection thresholds (30, 50, 70, and 90) were selected to explore how required tagging effort changes depending on what is considered a "sufficient" number of annual detections to support future monitoring efforts. For each parameter combination, the proportion of simulations in which the detection threshold was met was calculated to estimate the probability of success.

Results were plotted to visualise the trade-offs between tagging effort, expected survival, and the probability of collecting sufficient detection data. Survival probability was plotted on the x-axis and the probability of meeting the detection threshold on the y-axis. A dashed line at 80% was included as a heuristic benchmark for acceptable probability of success. This simulation framework provides empirical support for recommendations regarding tagging proportions in future years.

4.2.2.6 Migration and spawning cues

Water temperature and discharge in the Biedouw River were monitored throughout the project to investigate links between these factors and the timing of ecologically important events, such as the onset of annual migrations and spawning events.

A Solinst[*] Levelogger Edge Junior (Model 3001 LT F15/M5) was installed in the Biedouw River on 8 July 2020 at site P02 and data were downloaded opportunistically throughout the project duration. The logger was placed within a protective metal tube bolted onto a large boulder on the eastern bank of the pool. Every 30 minutes the logger records temperature and absolute pressure, which includes both barometric pressure and water pressure.

A Solinst Barologger (Model 3001 LT F5/M1.5), which measures air temperature and barometric pressure every 30 minutes, was installed at Enjo Nature Farm on 22 October 2021. The Barologger data were used to isolate water pressure from the Levelogger pressure readings. Because the Barologger was located 100 m higher in elevation than the Levelogger, all Barologger pressure readings were first corrected by adding 1 kPa to compensate for the difference in elevation (air pressure decreases at a rate of 1 kPa per 100 m increase in elevation close to the Earth's surface). Next, Barologger (air pressure) values were subtracted from the absolute pressure (air pressure plus water pressure) values recorded by the Levelogger, effectively isolating water pressure. The resulting water pressure readings represent the pressure (kPa) generated by the water column above the Levelogger when submerged, which give an indication of flow in the Biedouw River at 30-minute intervals.

While the Levelogger was installed in July 2020, the Barologger was only installed in October 2021, leaving approximately 15 months of Levelogger pressure readings with no corresponding Barologger readings with which to isolate water pressure. To correct for this, water pressure values were extrapolated to known absolute pressure values from the Levelogger for all readings prior to the Barologger's installation. First, a linear regression was generated from known absolute pressure and water pressure readings (Appendix A 10). The resulting linear equation (where X = absolute pressure and Y = water pressure) was then used to assign extrapolated water pressure values to records missing corresponding barometric data. All extrapolated water pressure readings are labelled as such.

Although water pressure readings give an indication of flow, with higher pressure corresponding to higher flows, river discharge (the volume of water flowing through a river at a given point in time) enables a more nuanced and environmentally meaningful understanding of seasonal hydrological dynamics. Discharge measurements were therefore used to calibrate the water pressure readings, converting kPa values to m^3/s of water moving through the channel (i.e. discharge).

Eighteen discharge transects were carried out between 20 August and 19 October 2022 at a run located 240 m upstream of the Levelogger (-32.050088°, 19.407079°) using a Hach FH950 Handheld Flow Meter equipped with a highly sensitive electromagnetic sensor (Figure 4.11). The flow meter records water depth and average current velocity (m/s) at multiple evenly spaced points across the river channel. By combining these measurements

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[*] Solinst Canada Ltd. designs and manufactures groundwater and surface water monitoring instruments. The company is based in Ontario, Canada, and can be contacted at instruments@solinst.com or www.solinst.com.

with the known width of the channel (measured on-site and entered into the meter) the device calculates the cross-sectional area (m^2) of the river at the transect location. It then uses the velocity and area data to automatically calculate the total discharge (m^3/s) for each transect.

Figure 4.11. Aerial view of a discharge transect in progress, facing upstream. Photograph by Jeremy Shelton.

The location for the transects was chosen due to its proximity to the Levelogger and the suitability of the riverbed, which was sandy, devoid of obstructions, and had a gentle gradient. Furthermore, there were no water abstraction points between the logger and the transect location. A total of 16-23 readings were taken during each transect, depending on the channel width at the time (8.28-14.55 m). The discharge transects were carried out across the widest possible range of discharges that could safely be measured. Beyond a discharge of 1.09 m^3/s, the current became too strong to sample safely. Total discharge across transects ranged from 0-1.087 m^3/s.

To convert water pressure values to discharge, a water pressure-discharge rating curve[*] was developed. First, each measured discharge transect was paired with the closest-in-time water pressure reading from the Levelogger. Because the Levelogger recorded water pressure every 30 minutes, these discharge-water pressure pairs always within 15 minutes of each other, minimizing potential discrepancies due to rapid changes in stream conditions. These paired values (discharge and corresponding water pressure) were then plotted, and an exponential function fitted to the resulting data (Figure 4.12). One discharge transect, with a value of 0 m^3/s, was excluded because log transformation is required to fit the curve, and log(0) is undefined. In addition, the second-to-last discharge transect

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[*] A "discharge rating curve" is a tool commonly used in hydrology to describe how river height relates to river flow. In this case, river height (called "stage") was measured using a Levelogger and converted to water pressure. By taking several measurements of both water pressure and the corresponding discharge (the volume of water flowing per second), a curve can be created that shows how one relates to the other. This curve can then be used to estimate river discharge at any point in time (within a specified range) based on water pressure readings. Paxton (2008) provides an example of this method.

(discharge = 0.023 m^3/s) was identified as an outlier and also excluded. This resulted in a total of 16 paired data points used to fit the rating curve.

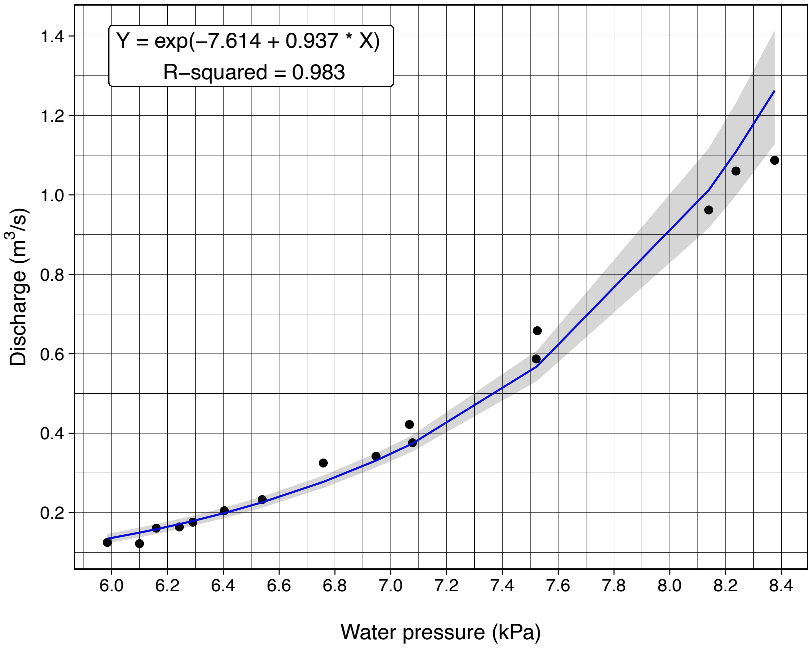


Figure 4.12. Water pressure-discharge rating curve developed for the Biedouw River at the location where discharge transects were carried out (-32.050088°, 19.407079°). The exponential regression line was fitted onto 16 water pressure-discharge data pairs. The shaded area around the line represents 95% confidence intervals.

The resulting equation from the fitted exponential curve was then used to extrapolate discharge from water pressures values across the study period. However, this extrapolation was limited to water pressure values up to 8.38 kPa, which corresponded to the highest discharge value measured directly with the flow meter. This upper limit was applied because the exponential relationship between water pressure above the Levelogger and discharge breaks down once the river overtops its banks. At this point, although total discharge may continue to increase as water spreads across the floodplain, the water pressure above the logger does not increase at the same rate. As a result, estimating discharge beyond this point using the same exponential curve would not be reliable. All analyses involving discharge were therefore limited to events occurring within the specified range (i.e., at kPa ≤ 8.38).

The Levelogger malfunctioned in April 2023, returning inaccurate data for the entirety of the 2023 flow season. Levelogger-derived water temperature and discharge data from the 2023 season were therefore excluded from analyses. To enable some evaluation of environmental conditions on migration and spawning cues in 2023, air temperature and accumulated daily precipitation data were used as proxies. These were downloaded from the Klein Pakhuis weather station, located 10.7 km West-Northwest of the upper fish limit on the Biedouw River (-32.141°, 19.055°; wunderground.com). Although located in a catchment neighbouring the Biedouw, it is the closest weather station to its headwaters, providing an indication of the likely magnitude of flow events and general patterns during the 2023 flow season. Anecdotal accounts from Biedouw Valley residents (verified with photographs and videos) were used to further understand local flow conditions at specific times.

4.3 Results

4.3.1 Rescues

4.3.1.1 Rescue counts

A total of 36,104 juvenile sandfish were rescued and relocated to sanctuary reservoirs between 2020 and 2023 (Table 4.6). Together, Mertenhof and Hartsbesluit Reservoirs received the majority (72.5%) of rescued sandfish, due to their large capacities and proximity to rescue locations. Clay, Syfer, and Alpha Excelsior were each stocked with less than 7% of all rescued sandfish, due to the larger distance (and therefore longer transport time) between rescue locations and these sanctuary reservoirs. Enjo Reservoir was smaller than both Mertenhof and Hartsbesluit Reservoirs, and so received fewer sandfish. In addition, it was in the process of being drained, dredged, and re-filled in 2022 and early 2023 to control the overabundant macrophyte population, and so was not stocked again until 2023.

Table 4.6. Numbers of sandfish relocated to the sanctuary reservoirs over the course of the four rescue seasons.

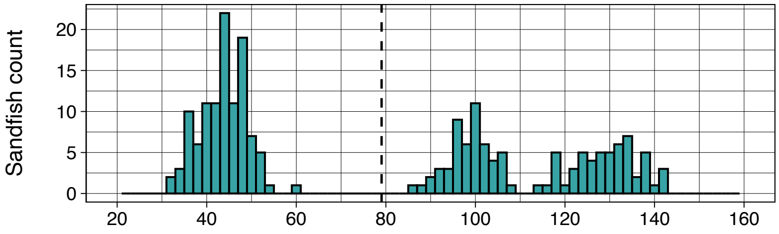
Reservoir	Rescue season				
	2020	2021/22	2022	2023	Total
Enjo	1,815	1,156	0	1,197	4,168
Mertenhof	4,999	3,464	4,947	1,166	14,576
Clay	885	1,398	0	0	2,283
Syfer	0	662	1,337	0	1,999
Alpha Excelsior	0	716	778	0	1,494
Hartsbesluit	0	0	11,234	350	11,584
Total	7,699	7,396	18,296	2,713	36,104

Mortality during the rescue process (seining and transport to sanctuary reservoirs) was 0.70% in 2021/22, 0.74% in 2022, and 0.37% in 2023, giving an overall mortality rate of 0.69%.

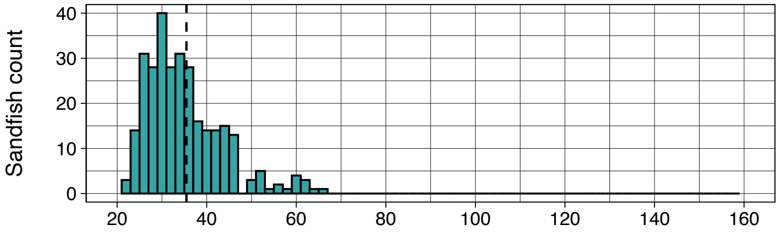
4.3.1.2 Size distributions

Sandfish rescued between 2021 and 2023 ranged in size between 23-143 mm TL (Figure 4.13; Appendix A 11). The 2022 and 2023 rescue seasons were short, spanning 2 – 26 days between November and December, whereas the 2021/22 rescue season lasted 96 days and extended into March. As a result, the size at rescue was strongly influenced by the timing of collection, with sandfish rescued later in the season being larger. This accounts for the larger maximum and mean sizes of sandfish rescued in the 2021/22 season. All juvenile sandfish rescued during the 2021/22 season (Figure 4.12a) originated from the same spawning period. The difference in size distributions reflects the different rescue dates, with smaller fish (< 80 mm TL) collected in November 2021 and larger individuals (> 80 mm TL) collected in February-March 2022.

a) Rescue season 2021/22 (n = 215)



b) Rescue season 2022 (n = 296)



c) Rescue season 2023 (n = 30)

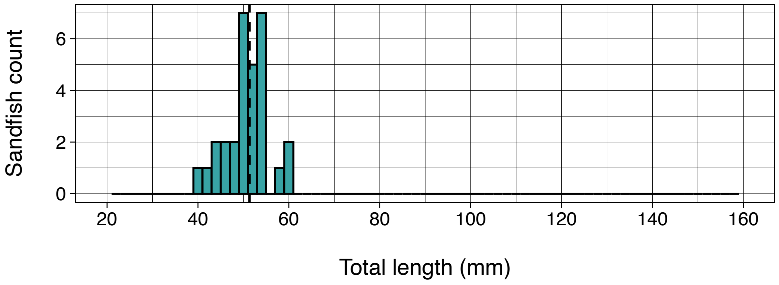


Figure 4.13. Size distributions of a subset of fish rescued during the a) 2021/22; b) 2022; and c) 2023 rescue seasons. Dashed lines represent mean size at rescue (mm TL) during each rescue season. The sample sizes (n) refer to the number of fish measured during a given rescue season.

4.3.1.3 Growth rate in the wild

The extended rescue period during the 2021/22 rescue season allows for the estimation of growth rate in the wild, by comparing mean size at rescue in the beginning of the rescue season to mean size at the end. The mean size of sandfish rescued in November 2021 and March 2022 was 44.29 mm TL (± 0.50 SE) and 131.60 mm TL (± 2.32 SE), respectively, a growth rate of 6.36 mm/week (Appendix A 12). However, as these estimated growth rates are based on population averages rather than the tracking of individuals over time, this result should be interpreted with caution. This is especially pertinent given that smaller juveniles would have been more likely to be predated by non-native fishes in the intervening period, thereby inflating the true growth rate.

4.3.2 Rearing in sanctuary reservoirs

4.3.2.1 Growth rate in reservoir environment

4.3.2.1.1 Enjo Reservoir

The 2020 cohort in Enjo Reservoir measured, on average, 185.50 (± 1.34 SE) mm TL in June 2021 (Figure 4.14; Appendix A 13). Between 26 June and 12 September 2021 (78 days), negligible growth was observed. Between 12 September and 6 November 2021, sandfish experienced an average growth rate of 1.96 mm/week. The most rapid growth occurred between November 2021 and March 2022, and over these 116 days, the mean growth rate was 3.57 mm/week. An overall growth in mean TL of 73.45 mm occurred between the first and last sampling windows (249 days), translating to a growth rate of 2.06 mm/week between 26 June 2021 and 2 March 2022.

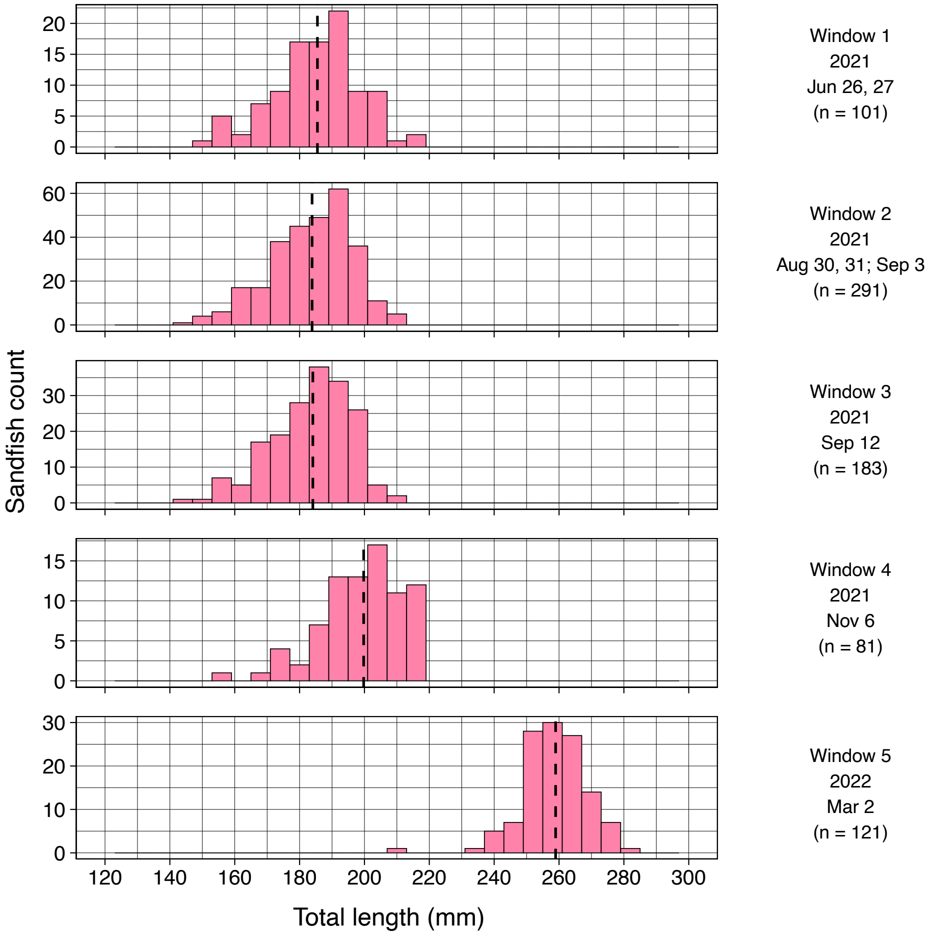


Figure 4.14. Size distribution of sampled sandfish (all belonging to the 2020 rescue cohort) in Enjo Reservoir over time. Dashed lines represent mean total length.

4.3.2.1.2 Mertenhof Reservoir

Growth rates for three rescue cohorts (2020, 2021/22, and 2022) at Mertenhof Reservoir were assessed (Figure 4.15; Appendix A 14). The 2020 rescue cohort grew by an average of 1.19 mm/week between 21 March and 1 September 2021 (164 days). During the corresponding period the following year (1 March to 31 August 2022; 183 days), the cohort's growth rate was 0.47 mm/week. In September 2021, one-year-old fish measured an average of 156 mm TL. This contrasts with the 2021 rescue cohort, which in September 2022 measured an average of 106 mm TL. Both the 2020 and 2021/22 rescue cohorts experienced minimal growth between March and October 2022: an average increase of 13.53 mm and 16.10 mm TL, respectively, in the intervening 231 days (0.41 and 0.49 mm/week).

Sampling window 2 (1 September 2021), window 6 (31 August 2022), and window 9 (30 August 2023) are almost exactly one year apart, facilitating the calculation of two annual growth rates for the 2020 cohort, and one annual growth rate for the 2021/22 cohort. Mean total length for the 2020 cohort increased by 52.45 mm between 2021 and 2022 (1.00 mm/week) and by 45.13 mm between 2022 and 2023 (0.87 mm/week). The 2021/22 rescue cohort experienced an increase of 83.86 mm mean TL between 2022 and 2023 (1.61 mm/week).

Windows 3 (25 November 2021) and 8 (8 November 2022) provide size data from the 2021/22 and 2022 rescue cohorts, respectively, upon initial rescue and stocking. When they were stocked into Mertenhof Reservoir, the 2021/22 and 2022 rescue cohorts measured, on average, 43.76 mm and 30.61 mm TL, respectively. The newly stocked 2021/22 cohort experienced a growth rate of 3.57 mm/week between stocking and the next sampling period in March 2022. Their average growth rate between stocking and the start of the following release season in August 2022 was 1.55 mm/week. The 2022 cohort was not resampled again until August 2023, during which time they experienced a growth rate of 2.10 mm/week.

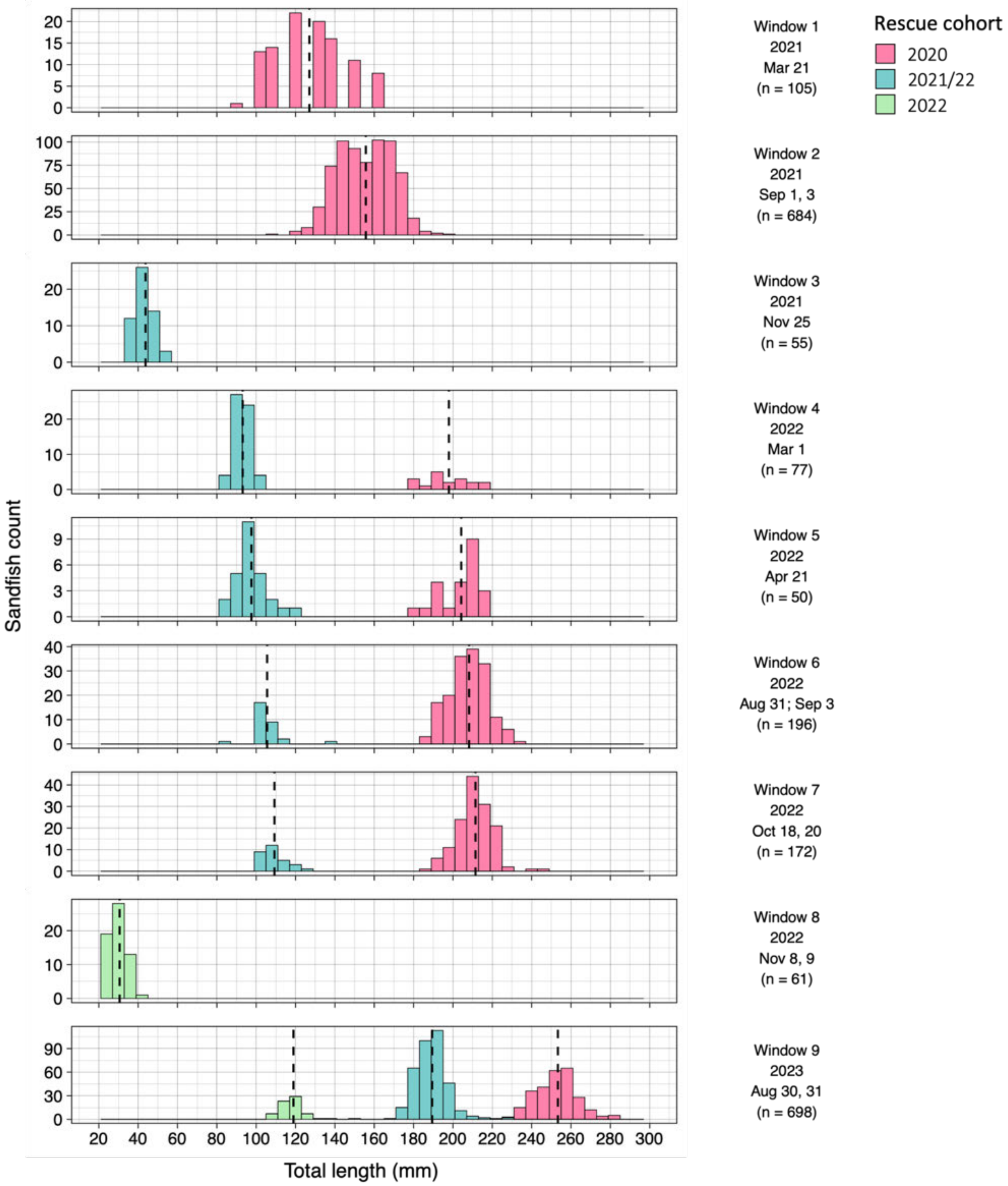


Figure 4.15. Size distribution of sampled sandfish in Mertenhof Reservoir over time. Dashed lines represent mean total length of each cohort per sampling event.

4.3.2.1.3 Clay Reservoir

Clay Reservoir was sampled less frequently than Enjo and Mertenhof Reservoirs, due to its distance from the main field site. Catches were also consistently lower; caution is therefore necessary when interpreting these results. Windows 1 (22 March 2021) and 2 (10 March 2022) occurred nearly one year apart, allowing for the calculation of an annual growth rate for the 2020 cohort. During this time, sandfish increased in mean total length by 116.69 mm, or 2.24 mm/week (Figure 4.16; Appendix A 15). Growth during the winter months (between March and September 2022) was relatively slow, averaging to 0.77 mm/week between March and September 2022, although results should be interpreted with caution given the small sample sizes (n = 4 and n = 12, respectively).

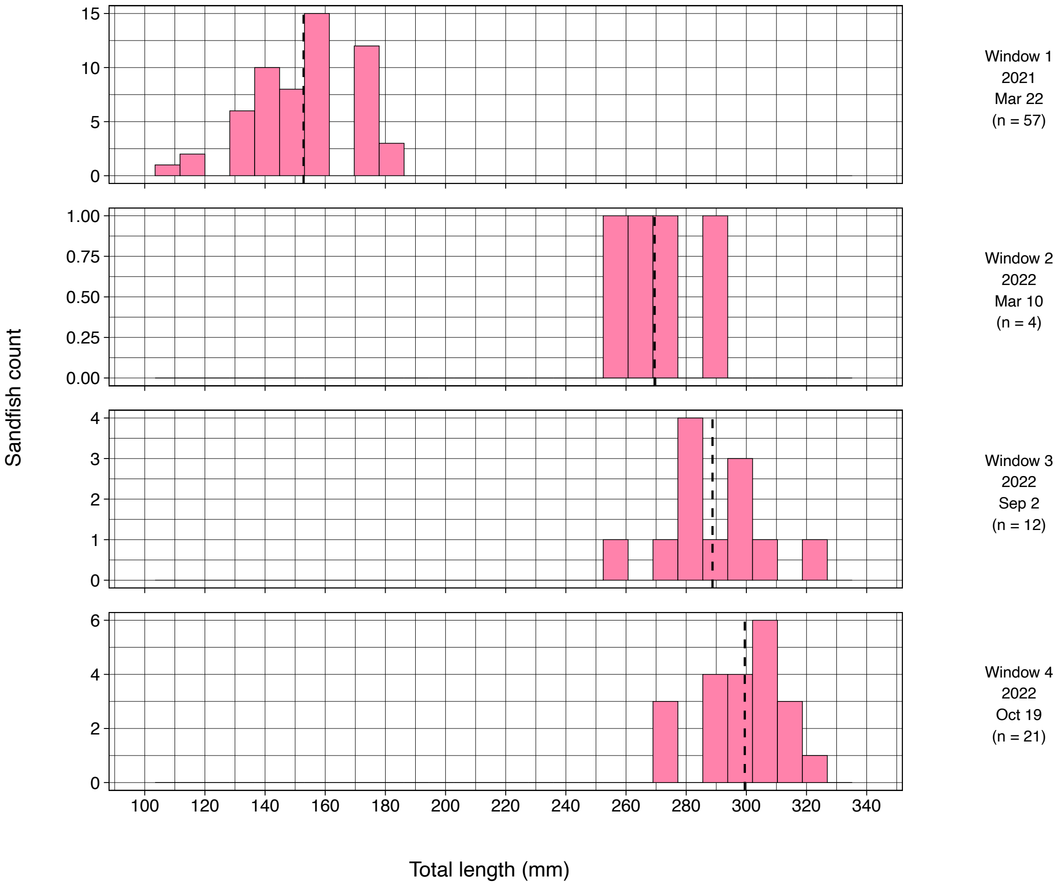


Figure 4.16. Size distribution of sampled sandfish in Clay Reservoir over time. Only 2020 rescue cohort data were included. Dashed lines represent mean total length.

4.3.2.1.4 Hartsbesluit Reservoir

Hartsbesluit Reservoir was stocked on two occasions: 7-9 November and 3 December 2022. The change in total length from Window 1 to Window 2 therefore cannot be said to represent growth rate in Hartsbesluit Reservoir, but rather reflects growth rate in the wild between the two dates. The reservoir was sampled for monitoring purposes on 12 July 2023, and for release during 16 – 18 August 2023 (Figure 4.17; Appendix A 16).

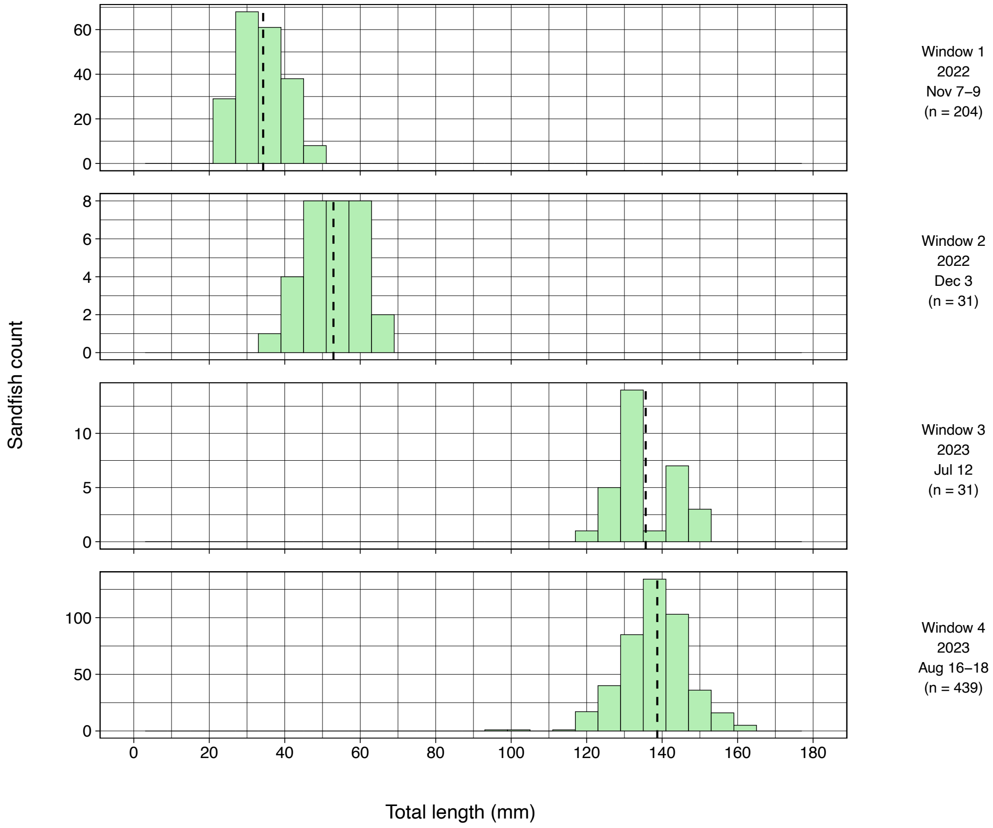


Figure 4.17. Size distribution of sampled sandfish in Hartsbesluit Reservoir. Dashed lines represent mean total length.

Sandfish stocked in November 2022 grew by 2.87 mm/week between stocking and July 2023. A total of 439 fish stocked in November and December 2022 were released nine and eight months later, respectively, in August 2023. Between stocking and release, they increased in mean total length by 104.43 mm (November 2022 rescues) and 95.81 mm (December 2022 rescues), equating to growth rates of 2.59 mm/week and 2.35 mm/week, respectively. Growth was relatively slow between July and August 2023, at 0.61 mm/week.

4.3.2.2 Survival rate in reservoir environment

Capture probabilities during the first and second tagging events (8 October and 6 November 2021) at Enjo Reservoir were as follows: both events (0.022), first event but not second (0.098), second event but not first (0.158), and neither event (0.722). The LPI yielded an estimated population size (M) of 833 (95% CI: 472, 1194) sandfish at Enjo Reservoir in November 2021, during the resampling event on November 28, 2021. Given the maximum number of fish available for capture (1,227), this corresponds to an estimated survival rate of 0.679 (95% CI: 0.385 to 0.973) one year after initial stocking. All tagged fish captured during the re-sampling event appeared to be in good condition, with tag insertion sites fully healed and showing no signs of adverse effects.

4.3.3 Releases

A total of 2,826 head-started sandfish were recaptured from sanctuary reservoirs and released to the Biedouw River over the course of several sessions per year between 2021 and 2023 (Figure 4.3). Of these, 2,537 (89.8%) were tagged prior to release (Figure 4.18).

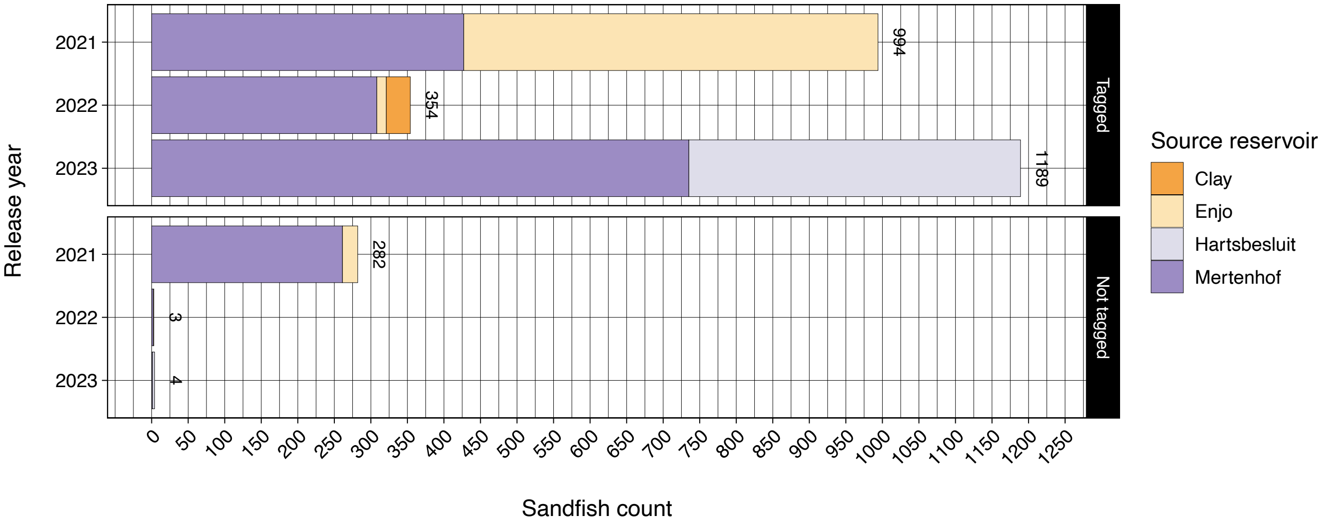


Figure 4.18. Number of sandfish released each year, grouped by tagged status.

The size distributions of tagged head-started sandfish differed between years (Figure 4.19). Mean size at release was 175.70 (± 0.47 SE) mm TL in 2021, 217.27 (± 1.57 SE) mm TL in 2022, and 185.30 (± 1.38 SE) mm TL in 2023 (Appendix A 17). Releases from 2021 comprised a single rescue cohort (2020) and consequently resulted in the narrowest range of sizes released. While the 2022 releases from Mertenhof and Clay Reservoirs all belonged to the 2020 rescue cohort, the 2022 Enjo releases comprised two cohorts: 2020 (n = 9) and 2021/22 (n = 5). Compared with 2021, the overall mean size at release in 2022 was 41.57 mm TL higher. The 2023 releases are clearly differentiated by rescue cohort. Fish from all three cohorts were released from Mertenhof in 2023, with the smallest measuring 106 mm TL. Most of the fish released from Hartsbesluit in 2023 belonged to the 2022 rescue cohort, measuring 98 – 165 mm TL (n = 510). However, 15 fish released from Hartsbesluit measured between 186 – 296 mm TL; these were rescued from Enjo Reservoir in 2021 while it was being drained in February 2023 and relocated to Hartsbesluit. They comprise two rescue cohorts: 2020 (263 – 296 mm TL; n = 7) and 2021 (186 – 217 mm TL; n = 8).

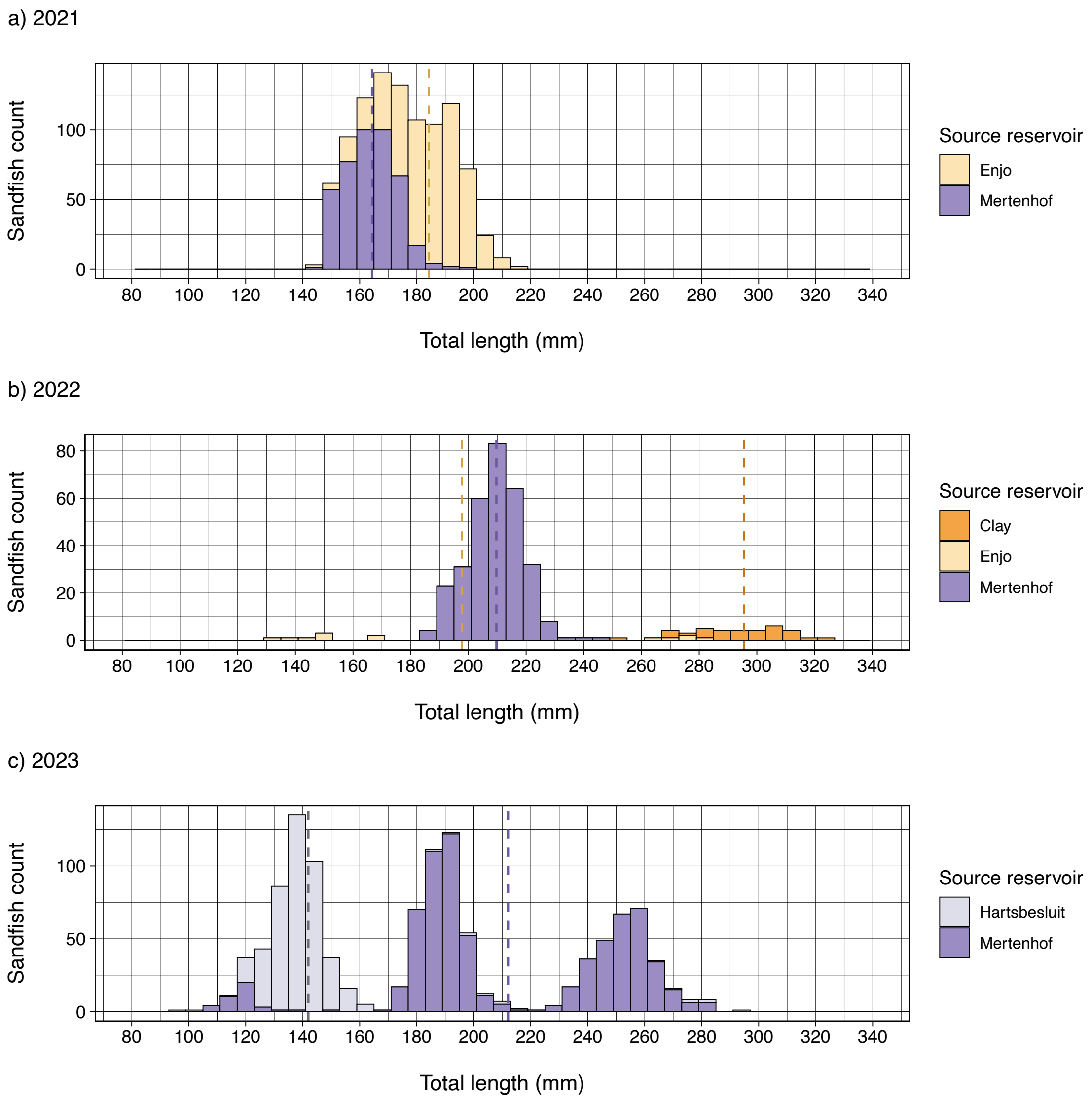


Figure 4.19. Size distributions of reservoir-reared sandfish at time of release in a) 2021; b) 2022; and c) 2023. Data from non-tagged fish are excluded. The dashed lines represent the mean size at release per reservoir.

In 2021, fish released from Enjo Reservoir were significantly larger than those released from Mertenhof Reservoir (by, on average, 19.96 mm; W = 215715, $p < 0.001$; Figure 4.20). In 2022, there was a significant difference in the distribution of size at release across the three source reservoirs (chi-squared = 454.04, df = 1, $p < 0.001$). Dunn's Test of Multiple Comparisons showed that fish released from Clay Reservoir were significantly larger than those released from both Enjo Reservoir (by, on average, 97.81 mm; $p < 0.001$) and Mertenhof Reservoir (by, on average, 85.88 mm; $p < 0.001$). Fish released from Enjo and Mertenhof Reservoirs in 2022 did not differ significantly from one another in size at release ($p = 0.329$). In 2023, fish released from Mertenhof Reservoir were significantly larger than those released from Hartsbesluit Reservoir (by, on average, 70.02 mm; W = 24542, $p < 0.001$).

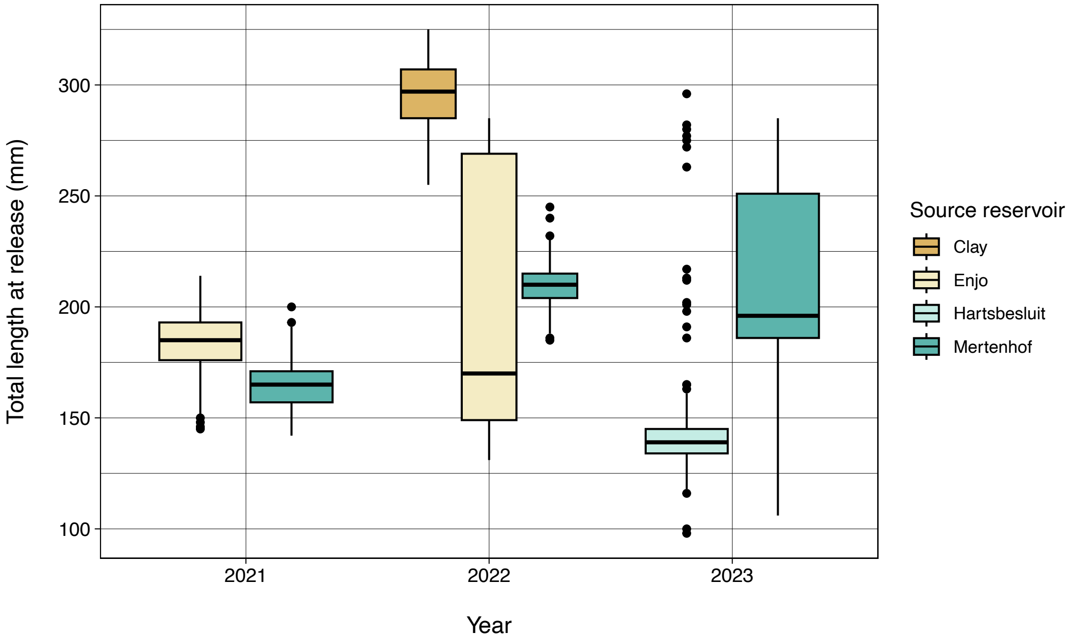


Figure 4.20. Size at release of tagged head-started fish, grouped by release year and source reservoir.

A total of 232 fish released from reservoirs between 2021 and 2023 expressed milt during the tagging process. Those that expressed milt measured 150—307 mm TL (mean 200 ± 1.73 SE).

4.3.4 Returns

4.3.4.1 Wild run size and recaptures

The 2021 visual surveys yielded an estimated wild run size of 180 adults. On 2 September 2022, during the migration, seven adult males and one female were captured by fyke and seine net, respectively. On 9 September 2022, 20 males, one female, and two fish of unknown sex were captured by fyke net. Seventeen of the fish were measured prior to release, ranging in size from 281 – 525 mm TL (mean 403 ± 17.9 SE; Figure 4.21). The two females were some of the largest specimens at 495 and 525 mm TL. The largest female weighed 1.38 kg, and a male measuring 431 mm TL weighed 0.88 kg. A 309 mm TL male had a PIT tag (3DD.003E0F4784) identifying it as a male released in September 2021 at 194 mm TL (one-year growth: 115 mm). The percentage increase in size (59%) was higher than the percentage increase in mean size of the 2020 rescue cohort reared in Mertenhof over the same period (34%).

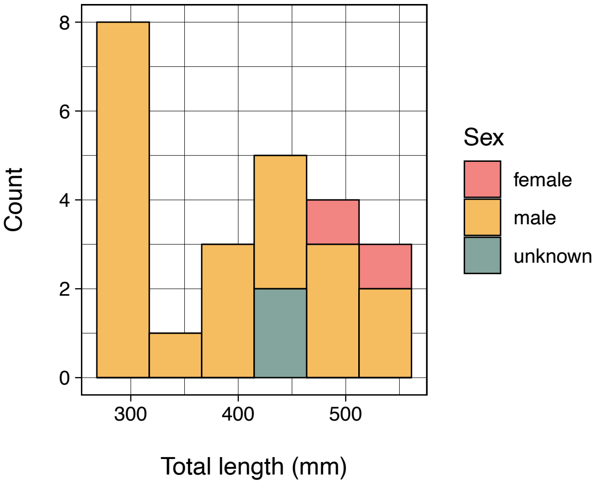


Figure 4.21. Size distribution of 17 fish captured by fyke or seine on 2 September and 9 September 2022 during the annual spawning migration.

During a rescue session on 4 December 2022 in the lower Biedouw River, two PIT-tagged sandfish were captured by seine net at site P04. They had both been released on 31 August 2022 and had apparently become stranded in an isolated pool as flows receded. The first (3DD.003E0F468A) had grown from 206 mm TL at release to 213 mm TL upon recapture (a growth rate of 0.07 mm/day) and the second (3DD.003E0F4675) had grown from 212 mm TL at release to 233 mm TL upon recapture (a growth rate of 0.22 mm/day). As they both had a heavy parasite load, they were moved to a perennial pool near the upstream limit of the seasonal reach of the Biedouw (23 km upstream of the Biedouw-Doring confluence), rather than being released into the Doring population. Antenna detection data from the 2023 season showed that the smaller fish was detected several times between 12 September and 1 October 2023, with a river occupancy of 20 days. The larger fish was not detected in 2023.

4.3.4.2 Antenna detections and river occupancy

Of the 994 head-started sandfish released with a PIT tag in 2021, 77 were detected by the fixed antenna detection system in 2022, a return rate of 7.7% (Figure 4.22). Occupancy in the Biedouw River, defined here as the number of days between the first and last detections of a unique tag, ranged from one to 27 days. Four fish were detected on only one day, resulting in unknown occupancies. These fish were excluded from the calculation of mean occupancy (9.4 ± 1.0 days) during the 2022 season (Table 4.7). Of the 77 fish that were detected in 2022, 70 (90.9%) were detected again in 2023, including three of the four fish that were detected only once in 2022.

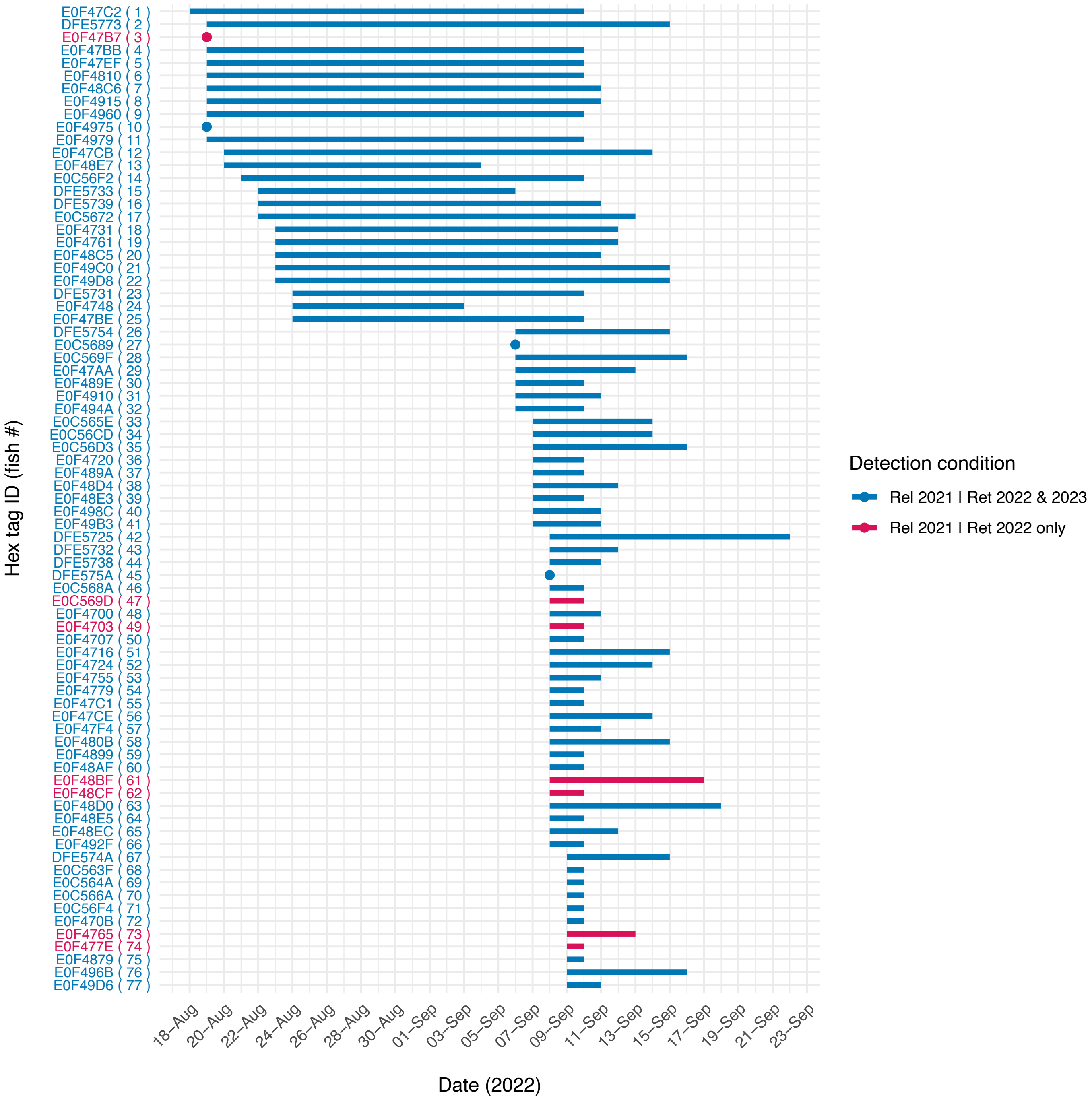


Figure 4.22. Occupancy of the 77 tagged sandfish released in 2021 that were detected in 2022, color-coded by detection condition. The blue-coded tags represent fish that were detected in 2022 and again in 2023, whereas the pink-coded tags represent fish that were detected in 2022 but not in 2023. Dots represent fish that were detected on only one day during the 2022 detection season, whereas bars span the occupancy (first detection date to last detection date) of that individual in the Biedouw. All hex tag IDs begin with "3DD.003", followed by a unique six-digit identifier; for aesthetic reasons, only the latter identifier has been displayed on the y-axis labels. The parenthetical numbers are unique identifiers assigned to each ID detected by the antenna; they have been included to facilitate comparisons between specific individuals in this figure and Figure 4.23.

Table 4.7. Biedouw River occupancy (days spent in river) by return season and migration wave. Fish that were detected only once were excluded from the reporting of the minimum, maximum, and mean calculations.

	2022			2023
	Wave 1	Wave 2	All	All
Min (days)	10	1	1	1
Max (days)	27	14	27	63
Mean ± SE (days)	20.5 ± 0.8	4.2 ± 0.4	9.4 ± 1.0	22.2 ± 1.2
# fish detected only once	2	2	4	9

A total of 221 unique PIT tags were recorded in 2023 (Figure 4.23), comprising 16.4% of the 1,348 sandfish released with a tag in either 2021 or 2022. Fifty-three (15.3%) of the 354 tagged fish released in 2022 were detected in 2023, as were 168 (16.9%) of the 2021 tagged releases. Ninety-eight (9.9%) of the tagged fish released in 2021 were recorded for the first time in 2023. During the 2023 season, nine fish were detected on only one day. The remaining 212 had a mean occupancy of 22.2 (± 1.2 SE) days, although this varied between two and 63 days (Table 4.7). A total of 228 unique PIT tags were detected between 2022 and 2023.

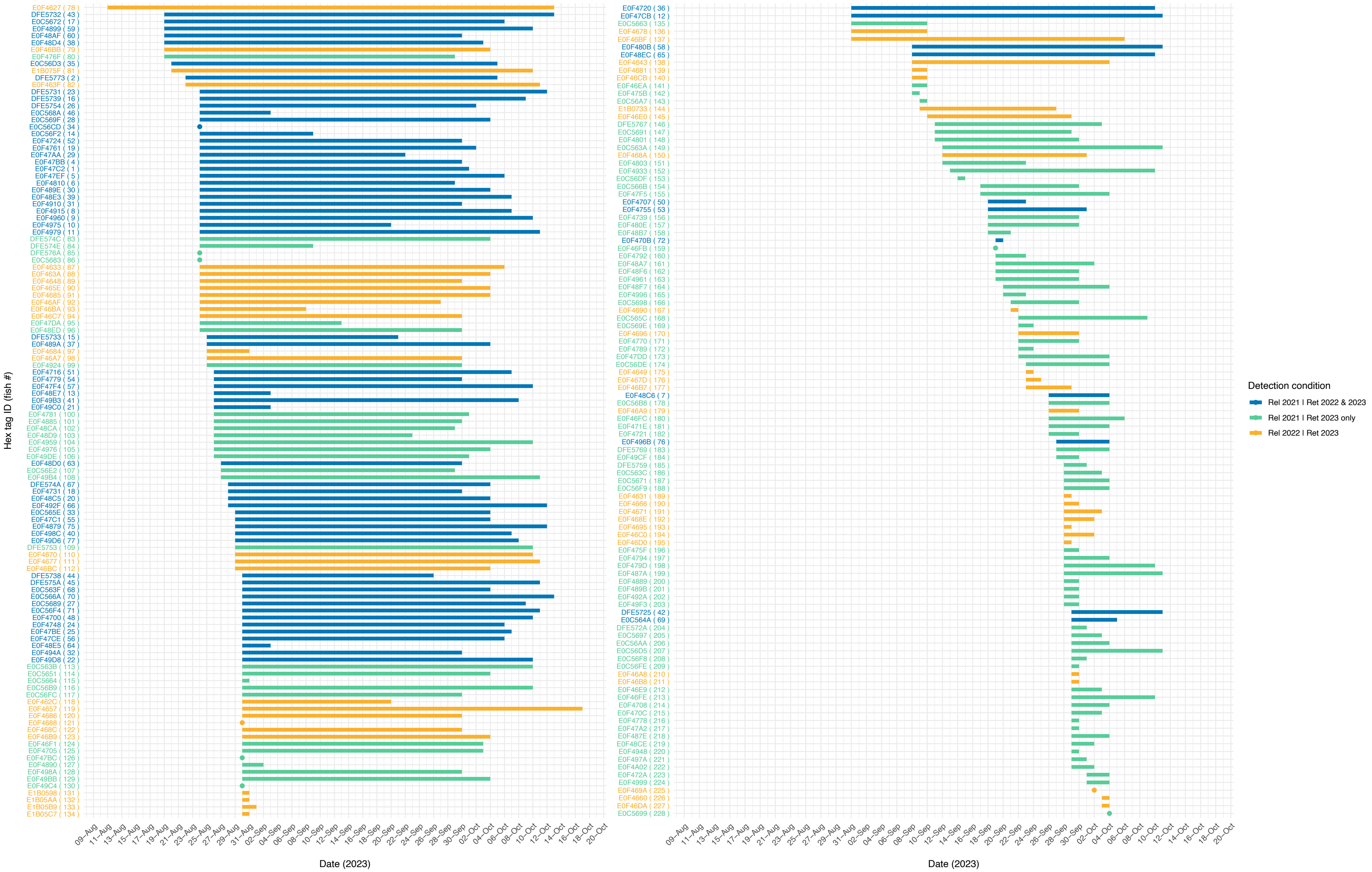


Figure 4.23. The 221 tagged sandfish released in 2021 and 2022 that were detected in 2023, color-coded by detection condition. The blue-coded tags represent fish released in 2021 that were detected in 2022 and again in 2023, the green-coded tags are those that were released in 2021 and were detected for the first time in 2023 (i.e., they “skipped” a year post-release), and the yellow-coded tags are those that were released in 2022 and were detected in 2023. Dots represent fish that were detected on only one day during the 2023 detection season, whereas bars span the total occupancy of that individual in the Biedouw. All hex tag IDs begin with “3DD.003”, followed by a unique six-digit identifier; for aesthetic reasons, only the latter identifier has been displayed on the y-axis labels. The parenthetical numbers are unique identifiers assigned to each hex tag ID detected by the antenna; they have been included to facilitate comparisons between specific individuals in this figure and Figure 4.22.

4.3.4.3 Patterns of upstream migration and spawning in relation to hydrological conditions

During periods of sustained flow (excluding brief periods of flow lasting ≤ three days), water temperature varied from 7.2-33.6 °C (mean 17.1 ± 0.03 SE), while water pressure above the Levelogger (barometrically compensated) fluctuated from 3.0-17.7 (mean 5.6 ± 0.01 SE) kPa (Figure 4.24).

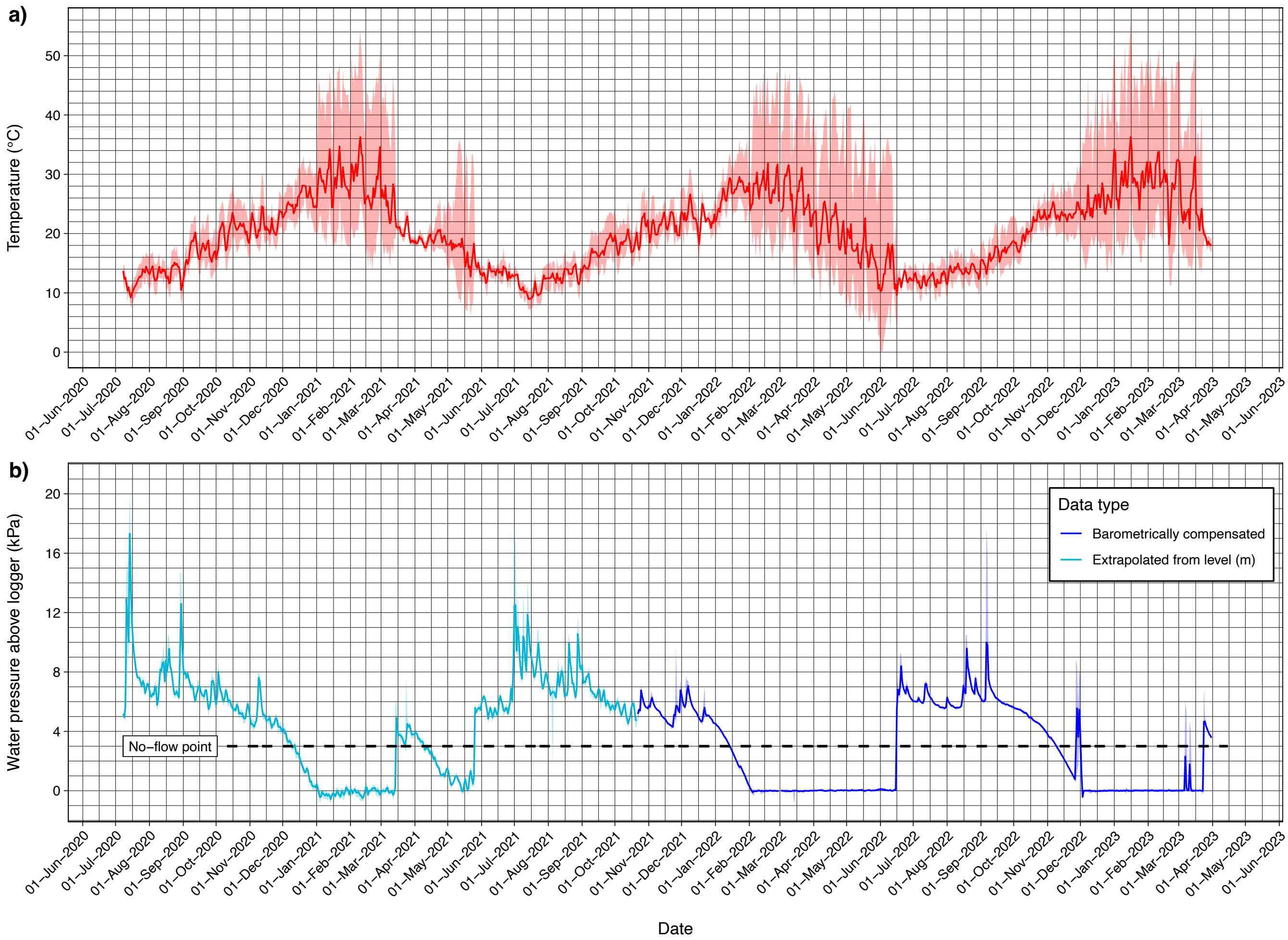


Figure 4.24. Water temperature (a) and water pressure above Levelogger (b) throughout study period. The solid lines represent daily means, while the translucent red and blue shading represent daily minima and maxima. The Barologger was installed on 22 October 2021, enabling the barometric compensation of Levelogger pressure readings thereafter. Levelogger water column level (m) values prior to that date were extrapolated to water pressure (kPa) using a linear relationship between level and water pressure. Periods of extreme temperature fluctuations (e.g., January to mid-March 2021) correspond to periods during which the Levelogger was exposed to the air.

During the 2021/22 flow season (27 May 2021- 14 January 2022; 233 days), water temperature varied between 7.2- 33.6 °C (mean 17.2 ± 0.05 SE) and water pressure above the Levelogger (barometrically compensated) varied between 3.0- 9.7 kPa (mean 5.2 ± 0.01 SE; Figure 4.25). Although no PIT tag data were yet available in 2021, adult sandfish were visually observed in the Biedouw River between 24 August - 17 September (likely an underestimate of their actual occupancy), during which water temperature varied between 9.0- 20.7 °C (mean 14.8 ± 0.07 SE) and water pressure above the Levelogger (extrapolated) varied between 6.0- 11.9 kPa (mean 7.4 ± 0.03 SE). Spawning was observed on the evenings of September 8, 9 and 10 during a period of subsiding flows and rising temperatures.

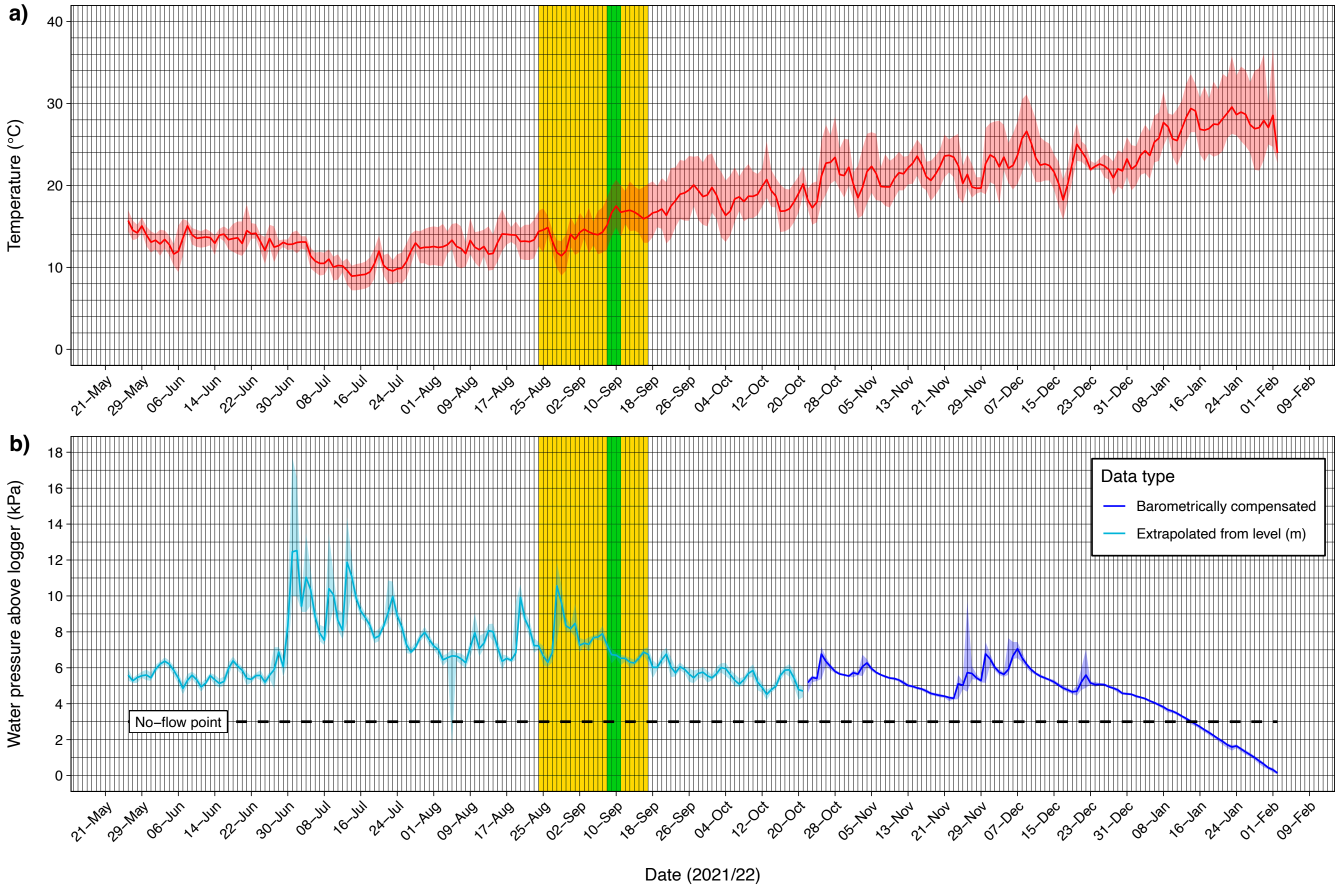


Figure 4.25. Water temperature (a) and water pressure above Levelogger (b) during the extended 2021/22 flow season. The solid lines represent daily means, while the translucent red and blue shading represent daily minima and maxima. The translucent yellow shaded area covers the period of known adult sandfish occupancy in the Biedouw River, based on visual observations. The green bar represents the period during which spawning was observed (8, 9, and 10 September 2021).

The 2022 flow season lasted from 16 June- 9 November 2022, a period of 147 days. During this time, water temperature varied from 8.4- 28.7 °C (mean 15.7 ± 0.05 SE) and water pressure above the Levelogger varied from 3.0- 17.7 kPa (mean 5.9 ± 0.01 SE; Figure 4.26). PIT tag detections indicated that adult sandfish first arrived in the Biedouw River on 18 August, with the last detection occurring on 22 September (an occupancy period of 36 days; Figure 4.22; Figure 4.26). There appeared to be two distinct time periods during which unique PIT tags first registered on the antenna (i.e., “migration waves”). Twenty-five unique tags were detected during a seven-day period (August 18 – 24), which was followed by a 13-day period during which no new tags were detected, and then by a four-day period (September 6 – 9) during which 52 new tags were detected. A maximum number of 25 unique tags were detected in a single day during the 2022 season. During the period of adult sandfish occupancy, water temperature ranged from 9.5- 22 °C (mean 15.1 ± 0.06 SE) and water pressure above the Levelogger ranged from 5.8- 17.7 kPa (mean 6.9 ± 0.03 SE). Spawning was observed on the evenings of 1 and 9 September.

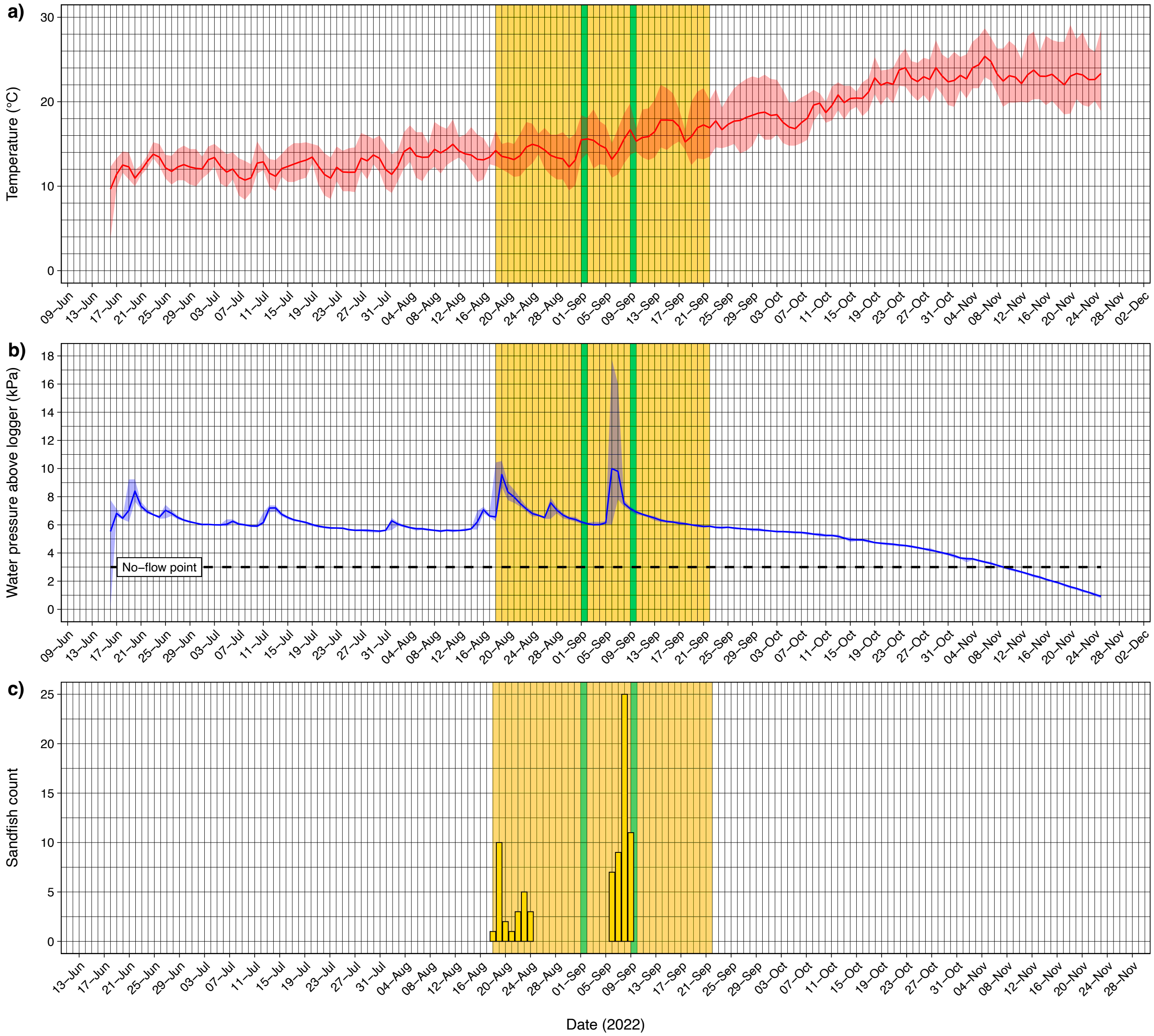


Figure 4.26. Water temperature (a), water pressure above Levelogger (b), and timing of sandfish arrival detections (c) during the 2022 wet season. The solid lines in (a) and (b) represent daily means, while the translucent red and blue shading represent daily minima and maxima. Each yellow bar in (c) represents the total number of PIT tags that were detected for the first time during a given day (i.e., number of arrivals in each day). The translucent yellow shaded area covers the period of adult sandfish occupancy in the Biedouw River, based on PIT tag detections. The green bars represent the evenings during which spawning was observed (1 and 9 September 2022).

In 2021, spawning was observed during three continuous evenings. During these evenings (16:00 on the indicated day to 4:00 on the following morning), water temperature varied from 14.8- 20.7 °C (mean 17.4 ± 0.18 SE) and water pressure above the Levelogger (extrapolated) varied from 6.2-7.1 kPa (mean 6.7 ± 0.03 SE; Figure

4.27). This equates to a discharge of 0.17- 0.38 m^3/s (mean 0.28 ± 0.01 SE). During the two known spawning evenings in 2022, the water temperature range was 14.3- 19.8 °C (mean 16.9 ± 0.21 SE) and water pressure above the Levelogger (barometrically compensated) varied from 6.1-7.1 kPa (mean 6.6 ± 0.06 SE). This equates to a discharge of 0.14- 0.38 m^3/s (mean 0.25 ± 0.01 SE).

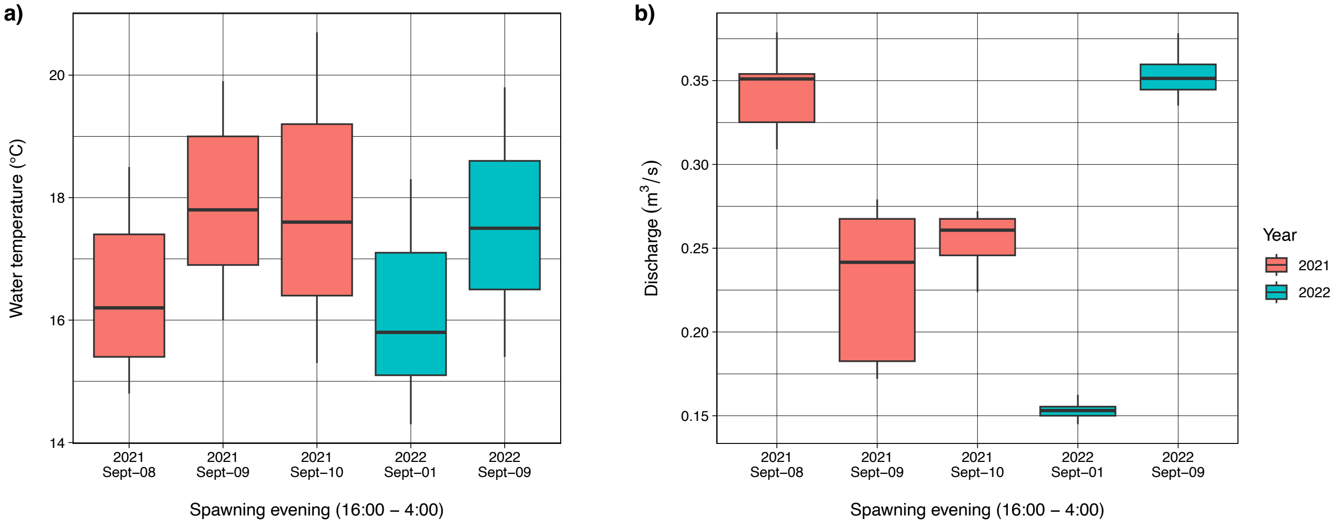


Figure 4.27. Box-and-whisker plots displaying the distribution of water temperature (a) and discharge (b) on known spawning evenings in 2021 and 2022.

Due to the Levelogger's malfunction in 2023, a combination of weather station data and accounts from Biedouw Valley residents was used to relate sandfish migration and spawning to environmental conditions. The Biedouw River experienced two extreme floods in 2023: the first between 14-17 June and the second on 25 September (Guy Bradley[*], pers. comm., 2023). Patterns of accumulated daily precipitation in the Biedouw headwaters align with these observations (Figure 4.28). Flows remained abnormally high through August and September (Guy Bradley, pers. comm., 2023).

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[*] Guy Bradely is the owner of Enjo Nature Farm, a guest accommodation on the banks of the Biedouw River. He lives there year-round and has been a key collaborator of the Saving Sandfish Project since its inception in 2019. Enjo Reservoir, one of the reservoirs used for rearing rescued sandfish, is located on Enjo Nature Farm.

Figure 4.28. Air temperature (a) and accumulated daily precipitation (b) measured at Klein Pakhuis weather station (wunderground.com), and timing of sandfish arrival detections (c) during the 2023 wet season. The solid line in (a) represents daily means, while the translucent red shading represents daily minima and maxima. Each yellow bar represents the total number of PIT tags that were detected for the first time during a given day (i.e., number of arrivals in each day). The translucent yellow shaded area covers the period of adult sandfish occupancy in the Biedouw River, based on PIT tag detections. The green bars represent the evenings during which spawning was observed (30 August and 8 September 2023) and the blue bars represent the known dates of large floods as verified by Biedouw Valley residents.

Adult sandfish occupancy in the Biedouw River lasted 68 days in 2023 (11 August- 17 October; Figure 4.23; Figure 4.28), nearly double the length of occupancy in 2022. The initial migration period lasted 13 days, during which 121 individuals were first detected. A period of seven days followed during which no new detections occurred. Between 8 September and 4 October (27 days), 100 new individuals were detected. The maximum number of individuals detected for the first time in one day was 35 fish. The longest period of occupancy by a single fish was 63 days (Table 4.7).

4.3.4.4 Modelling return probability

The top-ranked GLMM excluded time in reservoir and distance from Doring, retaining total length and release time of year (Table 4.8). Models within ΔAIC < 2 of the top-ranked model were considered to have substantial support, but only if they improved model fit and did not contain uninformative parameters (Arnold 2010). Although two models had AIC scores within 2 units of the top-ranked model, each of these increased model complexity by adding an additional parameter without substantially improving model fit. The top-ranked model, which retained only two parameters, was therefore selected as the most parsimonious and biologically meaningful.

Table 4.8. Top five ranked models predicting the probability of return for PIT-tagged fish, based on Akaike's Information Criterion (AIC). Models are ranked by increasing AIC. k represents the number of fixed-effect predictor variables included in each model. ΔAIC indicates the difference in AIC from the top-ranked model (ΔAIC = 0).

Model rank	Parameters included	k	AIC	ΔAIC
1	Total length Release time of year	2	1186.17	0
2	Total length Release time of year Distance from Doring	3	1187.94	+ 1.77
3	Total length Distance from Doring Time in reservoir	3	1187.95	+ 1.78
4	Total length Release time of year Distance from Doring Time in reservoir	4	1189.71	+ 3.54
5	Release time of year Time in reservoir	2	1191.17	+ 5.00

The odds ratio estimates predict that for every centimetre increase in size at release, the odds of return increase by 11.0% ($p < 0.001$; 95% CI 4.7 – 17.7%; Table 4.9). Compared with releasing during the migration (the reference category), fish released after the migration (late) have 90.4% lower odds of returning ($p < 0.001$, 95% CI 75.0 – 95.7%). The odds of return for fish released before the migration (early) did not differ significantly from those of fish released during the migration ($p > 0.05$). Release year did not contribute any meaningful variance to the model, suggesting that return probability is not significantly different between fish released in different years.

Table 4.9. Results of generalized linear mixed model selection examining predictors of return status. All fixed effects included in the global model are listed. Those that were not retained in the final model are right justified and greyed out; statistics for these come from the global model. Statistics for the retained predictors come from the final model. The reference category for each categorical variable is reported in parentheses.

Response variable	Fixed effects	Odds ratio estimate	Standard error	Coefficient	p-value	Confidence intervals
Return status (no, yes)	Intercept	0.034	0.019	-6.061	< 0.001***	0.011, 0.101
	Total length (cm)	1.110	0.033	3.490	< 0.001***	1.047, 1.177
	Release time of year (ref: migration)					
	Early	0.904	0.252	-0.362	0.718	0.523, 1.562
	Late	0.104	0.047	-5.034	< 0.001***	0.043, 0.250
	Time in reservoir: long (ref: short)	1.136	0.288	0.503	0.615	0.691, 1.868
	Distance from Doring (km)	1.026	0.051	0.509	0.611	0.931, 1.130

Random effect	Variance	SD
Release year	0	0

Although releasing larger fish increases the probability of return across release periods, this effect is strongest when fish are released before or during the annual migration, and weakest when released after the annual migration (Figure 4.29). Overall probability of return is significantly higher if released before or during the migration (compared with after the migration) across release sizes.

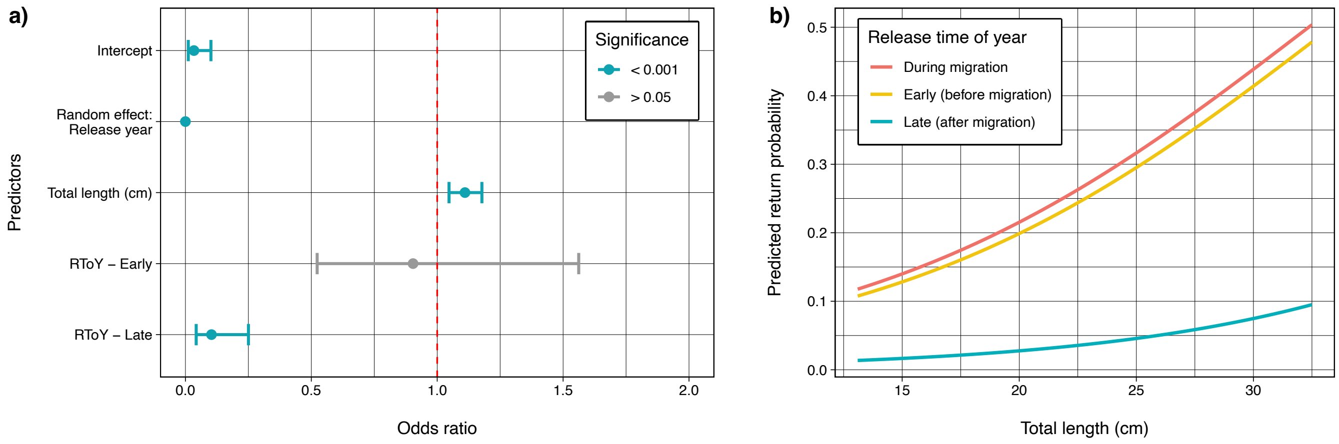
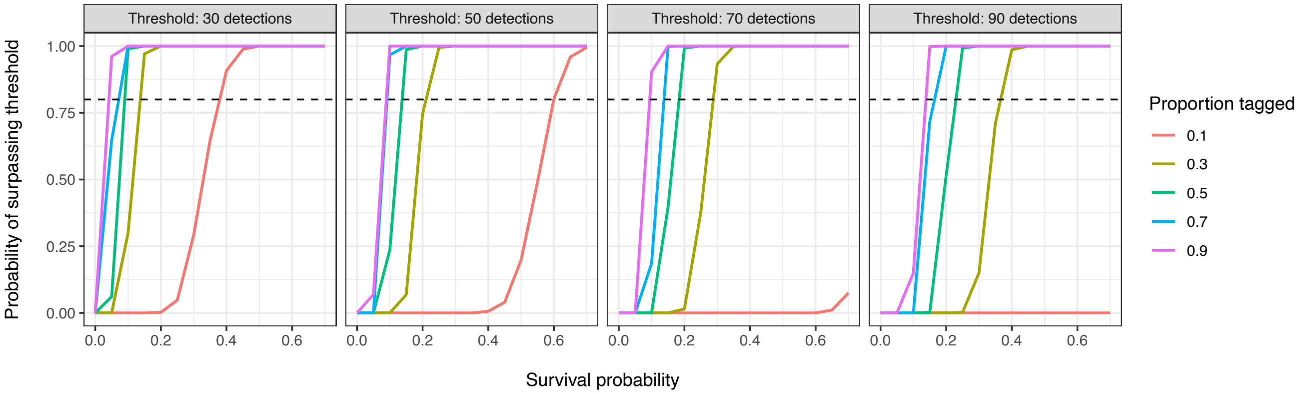


Figure 4.29. Analysis of fish return status based on model results. (a) Odds ratios for the fixed effects and the random effect (release year), illustrating the influence of predictor variables on the likelihood of fish returning. Each point represents the odds ratio estimate, with error bars indicating 95% confidence intervals. The dashed red line at odds ratio = 1 separates variables that decrease likelihood (to the left) from those that increase likelihood (to the right). The reference category for release time of year is "during migration". (b) Predicted probabilities of fish return based on total length at release, differentiated by release time of year.

4.3.4.5 Using simulations to guide tagging effort

The probability of detecting a number of tagged fish above various detection thresholds increased with both the proportion of fish tagged and the survival probability (Figure 4.30). When 1,000 fish were released (Figure 4.30a), achieving an 80% probability of detecting at least 30 tagged fish required a survival probability of at least 0.2 if 30% of the cohort was tagged, or 0.1 if 50% or more were tagged. For higher thresholds (70 and 90 detections), survival probabilities of 0.3–0.5 were needed even at high tagging proportions (≥0.5). Detection probabilities dropped sharply at low survival values, particularly when tagging effort was low.

(a) Probability of detecting fish above threshold if 1,000 fish are released



(b) Probability of detecting fish above threshold if 3,000 fish are released

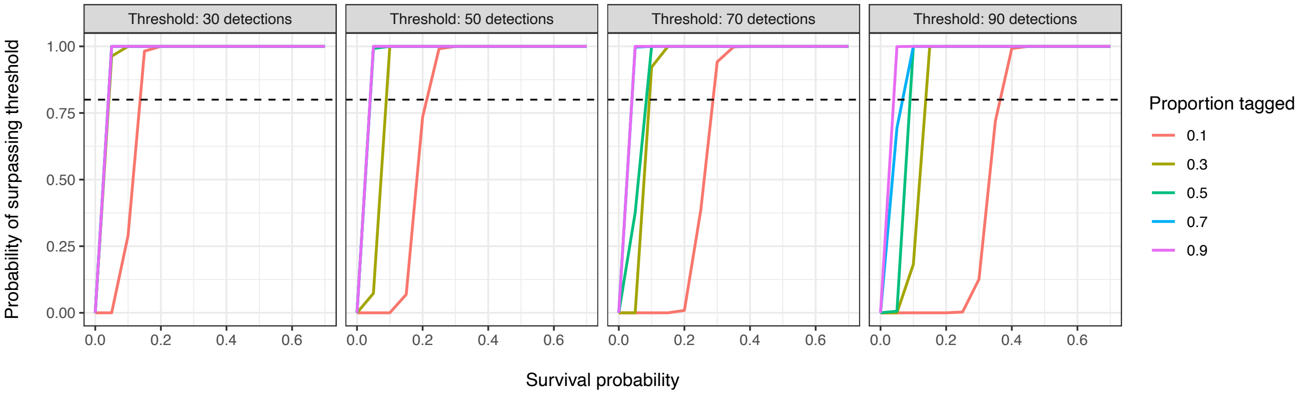


Figure 4.30. Probability of detecting tagged fish above specified thresholds one year after release, based on simulations varying survival probability and proportion tagged. Panels show results for two release scenarios: (a) 1,000 fish and (b) 3,000 fish. Each panel represents a different detection threshold (30, 50, 70, or 90 tagged fish detected). Coloured lines represent different proportions of the released cohort that were PIT tagged. The dashed horizontal line indicates an 80% probability of surpassing the threshold.

In contrast, when 3,000 fish were released (Figure 4.30b), detection probabilities were substantially higher across all thresholds and tagging proportions. Even with only 10% of fish tagged, the probability of surpassing a 90-detection threshold exceeded 80% at survival probabilities as low as 0.3. At higher tagging proportions (≥0.3), the 80% probability target was met across all thresholds at survival probabilities of 0.2 or higher. These results suggest that increasing either the release number or the tagging proportion improves the likelihood of surpassing detection thresholds, especially when post-release survival is uncertain or low.

4.4 Discussion

This head-start intervention is the first of its kind in South Africa (Appendix A 18), and, to our knowledge, the largest ever documented in Africa. With more than 36,000 juvenile sandfish rescued and 2,829 released to date, it has the potential to be among the largest freshwater fish head-start programs ever documented for a non-salmonid. It resulted in a more than doubling of the depleted sandfish migration two years after the first releases. The data presented here provide insights into i) fish rescues as a tool to enhance the survival of juvenile sandfish; ii) the viability of using off-stream reservoirs as nurseries to rear juvenile sandfish to a size suitable for release; iii) the variables that influence return likelihood; and iv) environmental signals that trigger migration and spawning behaviour.

4.4.1 Fish rescues as a means of boosting juvenile sandfish survival

To our knowledge, no other freshwater fish head-start programmes in Africa have used the approach described here: capturing wild juveniles, rearing them in semi-natural environments, and releasing them at a larger size to improve survival. However, there have been a slew of conservation initiatives under the “rescue” umbrella that have taken place across South Africa and Lesotho since at least the 1970s. These have included assisted translocations, re-introductions, and efforts to establish refuge populations of threatened indigenous species (Appendix A 18). Unfortunately, capture methods and mortality rates are rarely reported, making it difficult to contextualise the efficacy of our efforts. Nonetheless, the exceedingly low mortality rates recorded during the 2021-2023 sandfish rescues indicate that our capture and transportation methods were largely appropriate for individuals of the rescued size classes (> 23 mm TL). The rescues carried out during the 2020 rescue season do, however, bear mentioning. Sandfish were rescued early in the season during the first week of November. Although a subsample was not measured and mortality was not specifically documented, field notes indicate that fish were 20-30 mm in length and that approximately 30% perished during the process. The high rates of mortality are thought to have been caused by injuries sustained to small fish during seining combined with the stress of relatively high water temperatures (Riaan van der Walt*, pers. comm., 2020) This level of mortality was not observed again in later years, when rescues were carried out later in the season, once fish had grown larger; the one exception was in 2022 when 25% of rescued fish measured less than 30 mm TL. The reason for the discrepancy in mortality is unknown, but it does highlight the value of tracking mortality rates and causes during each rescue season. It also underscores the delicate balance that must be struck in terms of the timing of future rescues. Rescues conducted later in the season, when flows are low or absent, allow for more efficient seining and ensures that rescued fish are larger. However, this has trade-offs, including warmer water temperatures, which may increase stress for captured fish and prolonged exposure to IAF predation, which may deplete populations available for translocation.

Considerably more sandfish were rescued in 2022 compared with 2020 and 2021/22. While environmental factors could have contributed to this increase, there is no clear evidence of markedly improved conditions in 2022. A likely explanation is a higher overall reproductive output resulting from the addition of at least 77 head-started fish to the migration – and potentially the spawning population – in 2022. The considerably fewer sandfish rescued

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the following year, despite further population augmentation, is suspected to be due to the unusually large flood of September 2023, which could have covered eggs and larvae with silt, or flushed them out of the Biedouw entirely. A similar hypothesis was posited for the precipitous decline in young-of-the-year sandfish in the Oorlogskloof River between 2013 and 2014 (Cerrilla et al. 2022).

The rescue process also enabled the capture of important biological information, including the growth rate of young-of-the-year sandfish in the wild, which was measured as 6.36 mm/week between late November 2021 and early March 2022. Furthermore, the capture of both fork length and total length from a subsample of rescued sandfish allowed the establishment of a strong linear relationship between these two measures ($R^2 = 0.995$), obviating the need to continue to measure fork length during future rescues. Although young sandfish have proved to be resilient, a decrease in handling time minimises stress and reduces the potential for injury or mortality during rescues.

4.4.2 Off-stream reservoirs as effective nurseries for rearing rescued sandfish

The rearing strategy used in this study differs markedly from many similar population augmentation programmes involving a holding period for the target species. Many programmes rely on the mass-production of hatchery-reared fish for population augmentation, most notably those aimed at maintaining salmonid populations (e.g., Aho et al. 2006; Christie et al. 2012). Post-release survival of fish produced in hatcheries is typically low due to behavioural deficits resulting from relatively benign captive conditions (Brown & Day 2002), and genetic diversity tends to be lower than desired because of bottlenecks resulting from limited broodstock (Aho et al. 2006; Christie et al. 2012). Other programmes, the sandfish head-start intervention among them, collect wild gametes or juveniles and hold them temporarily in captivity or semi-captivity before releasing them back into the wild. For example, as part of a long-running management programme for the Critically Endangered razorback sucker (*Xyrauchen texanus*), wild-spawned larvae were collected from Lake Mohave and reared in laboratory tanks to 2 cm, then distributed to isolated backwaters and released into Lake Mohave once they were large enough to avoid IAF predation (Mueller 1995). Survival rates of juveniles reared in the backwater habitats used as nurseries varied between 12-76% (Mueller 1995). Low survival in some of the backwaters was attributed to poor water quality and berm failure. Several other methods of producing viable juveniles were attempted, including stocking backwaters with artificially collected gametes, stocking backwaters with spawning adults, and using hatchery-produced fish (which experienced much slower growth compared with those reared in natural nursery areas). Ultimately, the collection of wild-spawned larvae and subsequent rearing in semi-natural environments was recognised as the recommended method, due to higher relative rates of survival and greater genetic diversity of surviving juveniles (Mueller 1995). These factors, along with the prohibitive cost and complex logistics of hatchery rearing, were also considered when evaluating rearing options for rescued sandfish.

Another important advantage of using local off-stream reservoirs as rearing facilities is the involvement of local landowners in the project, which consequently increases local support and promotes a sense of responsibility for the fate of the species. As these landowners comprise important land- and water-users in the catchment, this benefit cannot be overstated. Additionally, in creating sanctuary reservoirs, five potential future sources of non-native fish invasion were removed from the Biedouw catchment and the greater ODRS. While *Micropterus* spp. were intentionally introduced into the system between 1930 and 1950 to promote angling opportunities (Skelton 2024), bluegill invaded after escaping from stocked off-stream reservoirs in the catchment (Harrison 1963). The removal of non-native species from such impoundments must therefore be considered a conservation priority, especially in catchments where these species are not yet present in rivers, or where they have already been eradicated from these rivers.

While there was a wide range of one-year survival estimates at Enjo Reservoir based on the bounds of the calculated confidence intervals, the survival estimate based on the LPI estimate was relatively high (0.679). This may be attributed to the species' natural ability to survive in isolated lentic habitats during the summer months and its adaptation to extreme seasonal environments. The closely related *Labeo umbratus* and *Labeo capensis* are also known to thrive in impoundments (Gaigher 1984; Potts et al. 2005; Skelton 2024). It is important to note that the survival rate mentioned here was derived from a single reservoir; survival rates could vary between reservoirs due to differences in several factors, including presence and abundance of predators such as birds and terrapins, vulnerability to predation due to water clarity, availability of food, and competition for resources owing to different stocking densities. Indeed, there are key differences between the reservoirs: Enjo and Hartsbesluit Reservoirs both had high densities of aquatic macrophytes and relatively clear water, while Mertenhof was largely devoid of macrophyte growth and highly turbid. The reservoirs also vary in size, with the smallest (Enjo) comprising roughly 30% of the footprint of the largest (Syfer). Given these differences, similar mark–recapture studies should be carried out at the different reservoirs over the course of several years to assess factors impacting sandfish survival in reservoir environments in the long term.

Growth rates of reservoir-reared fish varied between reservoirs and across years, likely due to the interaction of several factors, including available resources, temperature, and differences in stocking densities. For example, although sandfish released from Mertenhof and Clay Reservoirs in 2022 all belonged to the 2020 rescue cohort, Clay releases were 41% larger than those from Mertenhof. Although resource availability and water temperature in the different reservoirs were not monitored over the course of the study, it is likely that in this case the large disparity in stocking densities between the two reservoirs was responsible for the substantial differences in size, since Mertenhof, about 40% smaller than Clay, received nearly four times as many fish during the first two rescue seasons, due to its proximity to the rescue sites. Survival rates were not directly assessed for each reservoir, but it is possible that the higher stocking densities in Mertenhof may have negatively affected rates of survival, in addition to growth. Future studies could investigate the interactions between abiotic factors (namely temperature), resource availability, stocking density, growth, and survival in more detail, with future stockings carried out accordingly.

Despite notable environmental differences between reservoirs, those where growth was monitored showed similar seasonal growth patterns, with most of the growth occurring during the warm summer months (November – March), and very little growth recorded during the colder winter months (April – October). While fish growth is regulated by several factors, including food availability and feeding rate, water temperature has repeatedly been shown to be one of the most important drivers (McCullough et al. 2009; Thompson & Beauchamp 2016). The consistent patterns observed across reservoirs suggest that while rearing environments may differ, temperature may play a dominant role in regulating the growth rates of rescued fish, although this should be tested explicitly. It may also be worth considering providing supplemental feeding to reservoir-reared sandfish, particularly if food availability appears to be a limiting factor to growth. The rapid growth rates of juveniles in the Biedouw River between November 2021 and March 2022 suggest that temperature may also regulate growth of wild populations, as is the case with other large-bodied desert-dwelling cyprinids, such as *G. cypha* (Spurgeon et al. 2015).

Notably, the growth rate of wild fish was higher among young-of-the-year fish and for one head-started subadult compared with the reservoir environment. Wild juveniles experienced a growth rate of 6.36 mm/week between late November 2021 and early March 2022, while juveniles relocated to Mertenhof grew 3.57 mm/week over the same period. While the reasons for this can only be hypothesised, the faster growth of wild juvenile fish may be due to higher relative densities of fish stocked into the reservoirs compared to wild fish in river pools, and increased temperatures in the river following seasonal intermittency, which likely accelerates growth. Similarly, the single tagged recapture (released in September 2021 and recaptured one year later), experienced a 59% increase in size, whereas fish of the same rescue cohort but reared in Mertenhof over the same period experienced a mean increase of only 34%. The slower somatic growth observed in the reservoirs may reflect stressful environmental conditions that may reduce the conservation value of these habitats. In addition to improving survival rates relative to non-rescued juveniles, a primary objective of rearing rescued fish in reservoirs is to accelerate growth beyond the gape limits of predators. If this goal is being hindered by over-stocking or other suboptimal environmental conditions, then the effectiveness of the head-starting strategy may be compromised. Moving forward, relevant environmental conditions including dissolved oxygen, water temperature, food availability and turbidity should be monitored in each reservoir and related to fish growth and survival. Their effects on sandfish growth rates should be explicitly modelled, along with the impact of stocking density and its potential interactions with these environmental factors. Additionally, to better assess the body condition of reservoir-reared fish in comparison to wild individuals, the sampling protocol should include weighing a subsample of fish prior to release, as well as weighing sandfish captured in the wild.

4.4.3 Contextualising return rates of head-started sandfish

PIT tags are widely used in developed countries to monitor freshwater fish movement and survival (e.g., Teixeira & Cortes 2007; Hewitt et al. 2010; Dzul et al. 2021). The successful translocation of PIT-tagged *G. cypha* (Healy et al. 2022a) and the large-scale tagging and stocking of captive-reared razorback suckers (*Xyrauchen texanus;* Zelasko et al. 2010; Dowling et al. 2013; Cathcart et al. 2018) within the Colorado River Basin provide some examples of the application of this technology in monitoring freshwater fish conservation efforts in the United States. However, few comparable studies have taken place in developing countries, and none have documented the use of PIT tags in monitoring freshwater fishes in Africa (Burnett et al. 2021). To our knowledge, this is the first study to use PIT tags to monitor a freshwater fish conservation intervention in the continent. It thus serves as an important proof of concept to catalyse the use of this valuable and cost-effective technology for conservation purposes in low- and middle-income countries.[*]

The return of 228 PIT-tagged head-started sandfish to the Biedouw River over the course of two years suggests that this intervention can increase the survival of young sandfish into adulthood, effectively bypassing the recruitment bottleneck resulting from non-native fish predation and excessive water abstraction. Because 286 fish were released without a tag, the detections are likely an underestimate of the true number of returning head-started fish. The 221 detections in 2023 therefore represent a minimum 123% increase in run size, when compared with the 180 wild sandfish observed migrating in 2021. This comparison, however, should be interpreted with

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[*] The start-up costs of setting up a passive detection system can be moderately high – in this case the antenna (cord and exciter table) cost approximately USD $3,300, the reader cost approximately USD $2,200, and the independent energy system (solar panels and batteries) cost approximately $1,000. However, the PIT tags themselves represent the only ongoing cost (apart from battery replacements every 5-7 years) and are relatively low-cost when compared to competing technologies like satellite tags, at USD $2.50 per PIT tag. All equipment (excluding the batteries and solar panels) was purchased from Biomark.

caution, given that detection probability likely varies between visual surveys and passive PIT tag detection. Despite this, the magnitude of the potential increase in run size is meaningful.

Although only 7.7% (77 fish) of tagged 2021 releases were detected in 2022, it is notable that of those, 91% (70 fish) were subsequently detected in 2023. This indicates that subsequent-year return rates are substantially higher than first-year return rates among head-started sandfish.[*] Similarly, among PIT tagged *X. texanus* stocked into Colorado River basin streams, first-year survival of average-size fish was 5%, yet subsequent-year survival was 75% (Zelasko et al. 2010). In the case of *X. texanus*, high post-release mortality typical of hatchery-reared fish was implicated in the low first-year survival estimate, perhaps due to predation by non-native fishes and the behavioural deficits caused by typically benign hatchery conditions (e.g., inadequate prey selection and predator avoidance; Brown & Day 2002). While head-started sandfish were wild-spawned and reared in semi-natural reservoir conditions, they may also suffer high post-release mortality due to similar considerations, especially given that rescued fish would not have encountered predatory fishes while in the reservoir environment. It is worth noting, however, that the first-year return rate of the 2022 release cohort was substantially higher than that exhibited by the 2021 cohort at 15.0%. This figure is encouraging, as it indicates that low first-year return rates may not necessarily reflect low survival in the wild. Ten percent of the 2021 release cohort was detected for the first time in 2023, suggesting they either "skipped" migration in the year following release or migrated in 2022 but were not detected due to tag collisions or other detection errors. These data emphasize the importance of ongoing monitoring for our understanding of post-release survival. Indeed, the 2023 detections effectively increased the known minimum survival of 2021 releases from 7.7% to 16.9%. Future monitoring will continue to shed light on how return rates relate to survival in the wild, and how first-year survival compares to subsequent-year survival for different release cohorts.

The first-year and subsequent-year return rates observed thus far among head-started sandfish are notably higher than that observed for *X. texanus*, perhaps due to the fact that head-started sandfish were wild-spawned and reared in reservoirs, while *X. texanus* were hatched and reared in hatcheries. It is worth noting, however, that *X. texanus* were physically recaptured using electrofishing, fyke nets, and trammel nets, whereas head-started sandfish were passively detected using a PIT tag antenna array. Differences in sampling techniques in this and other similar studies make it difficult to draw any direct comparisons regarding the relative efficacy of population augmentation programmes.

The reason for the higher overall return rate in 2023 (16.4%) compared with 2022 (7.7%) is suspected to be due to more favourable environmental conditions in 2023. Although specific river discharge and temperature data for 2023 are unavailable, team observations and accumulated daily precipitation data confirm noticeably higher sustained flows in the Biedouw River during the 2023 migration season. As rheophilic spawners, higher flows may trigger increased migratory behaviour in sandfish, possibly explaining why so many 2021 releases deferred first migration to 2023. A second hypothesis – that sandfish defer migration until they are sexually mature – could also explain the observed return rates. Investigations into growth rates and size at sexual maturity would help test this hypothesis.

As remote PIT tag detections do not provide length data, returning fish must be recaptured to obtain these measurements. The head-started male recaptured in September 2022 had grown 115 mm to a total length of 309

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[*] The use of the term "return rates" as opposed to "survival rates" is deliberate, as PIT tag detections in the Biedouw River do not necessarily reflect annual survival rates. However, as the fate of the un-detected fish is unknown, annual return rates are assumed to represent a minimum annual survival rate.

mm since its release one year prior. Females of *L. umbratus* and *L. capensis* reach sexual maturity at 200 – 380 mm and 240 – 400 mm SL, respectively (Gaigher 1984; Tómasson et al. 1984; Skelton 2024). If female sandfish experience similar growth rates to males post-release, and patterns of sexual maturity in sandfish mirror those of *L. umbratus* and *L. capensis*, then it is likely that most head-started females would have been sexually mature one to two years post-release. Notably, reservoir-reared males as small as 150 mm TL expressed milt when tagged, suggesting males mature at a smaller size – though patterns of sexual maturity could differ in wild populations. Male *Labeo umbratus* and *L. capensis* mature at 150 mm SL and 220 mm SL, respectively (Skelton 2024). Additional net sampling of returning individuals should be carried out to assess growth rates in the wild, as well as size at sexual maturity, which remains poorly studied.

The fate of the 1,120 tagged head-started sandfish released in 2021 and 2022 that were not detected in 2022 or 2023 is unknown. It is likely that natural predation accounts for some of these absences. Although PIT tag retention is typically high in fishes (Hopko et al. 2010), tag loss may also account for some of the absent releases. Furthermore, imperfect detection probabilities, which are influenced by the orientation of the passing tag, electromagnetic interference from outside sources, and other factors (Burnett et al. 2013), may have resulted in an underestimation of returning tagged sandfish. Although the detection error rate (proportion of tagged fish crossing the antenna without being detected) cannot be precisely determined, it can be estimated from the number of fish that were not detected during their post-release downstream journey in 2022, yet were subsequently detected in 2023. Forty-four tagged fish released upstream of the antenna in 2022 were detected in 2023. Of these, three (6.8%) lacked an outgoing tag, indicating that detection errors are relatively rare, but present. It is worth noting that error rates likely vary over time based on environmental conditions such as flood level and external interference.

Furthermore, as a migratory species, olfactory imprinting cues may be especially important in determining homing probability in subsequent years (George et al. 2009). While rescued sandfish spent their first two to five months in their natal river and most were returned there upon release, little is known about sandfish natal philopatry and natural stray rates. While it is possible that captive rearing may lead to increased straying in migratory fishes (Keefer & Caudill 2014), more research into sandfish imprinting mechanisms and natural stray rates is needed to better contextualize the return rates. Future fyke and antenna-assisted monitoring of spawning migrations in the Biedouw River, neighbouring tributaries, and in the mainstem will be especially telling in this regard.

4.4.4 Factors influencing return probability

Model outputs suggest that size of fish at release significantly impacts return probability, consistent with outcomes from similar conservation programmes in the United States. In the Colorado River Basin, first-year survival of hatchery-reared *X. texanus* was positively correlated with total length at stocking (Zelasko et al. 2010), and apparent annual survival of translocated juvenile *G. cypha* appeared to be dependent on length (Spurgeon et al. 2015; Healy et al. 2020). Given the known size-selective impacts of *Micropterus* spp. on indigenous fishes in the ODRS (e.g., Woodford et al. 2005; Weyl et al. 2013; van der Walt et al. 2016; Cerrilla et al. 2022), it is likely that predation of smaller sandfish post-release accounts for their lower predicted likelihood of return. The model's effect size (11% higher odds of return for every additional centimetre at release), while significant, is smaller than that predicted by the earlier model generated by Cerrilla et al. 2024 (23% higher odds). The latter included only data from 2021 releases and 2022 detections, while the current model includes two years each of release and return data. Although neither model explicitly accounted for environmental variables, it is likely that a fish's odds of return in years after its release change depending on the complex interaction of ecological factors present during each migration season. River discharge and water temperature data were not available for the 2023 return year due to equipment malfunction; these factors are, however, suspected to play a direct role in a sandfish's decision to migrate, and should be incorporated into future models predicting return probability. Nevertheless, the results of both models highlight the importance of prioritising larger fish in future releases. Future models incorporating the detection status of the small (< 150 mm TL) fish released in 2023 will further refine these predictions. Information on the gape size of *Micropterus* spp. inhabiting the mainstem Doring River, as well as of the novel invader *C. gariepinus*, could also help determine the minimum size at which sandfish should be released in future.

The time of year during which a fish is released also appears to significantly impact its likelihood of return. Similarly, season of stocking had a significant effect on first-interval survival of hatchery-reared *X. texanus,* with summer-stocked fish exhibiting much lower survival rates than those stocked in spring, autumn, and winter (Zelasko et al. 2010). While sandfish released before or during the migration have similar return odds, post-migration releases have significantly lower (90%) odds of return. Although larger release sizes do enhance the probability of return for post-migration releases, this effect is much weaker compared with fish released prior to and during the migratory period. Environmental conditions may be less favourable later in the year, resulting in decreased survival rates for fish released in October. Apart from lower flows, water temperature in the Biedouw River during the October 2022 release period was substantially higher than during releases earlier in the year (22 °C versus 13-17 °C, respectively[*]). Increased water temperature, which may further weaken fish already stressed from transport and handling and is associated with higher rates of activity in predatory centrarchids, was posited as a potential mechanism explaining lower survival of summer-stocked *X. texanus* (Zelasko et al. 2010).

Alternatively, fish released post-migration may lack the opportunity to learn migratory behaviour from their wild conspecifics, potentially decreasing the likelihood of future migration up the Biedouw River, though not necessarily affecting survival. The adopted migrant hypothesis posits that iteroparous fish species pass down migratory knowledge through social learning (McQuinn 1997; Rogers et al. 2018). Although this research has largely been limited to marine species, it raises the possibility that social transmission of migratory information may play a role in the homing behaviour of potamodromous fishes.

Importantly, all fish released prior to or during the migration were released into the Biedouw River, while those released after the migration were released directly into the Doring River, due to stranding concerns stemming from declining flows in the Biedouw at that time. The significant difference in return likelihood may thus be due to release location, rather than the time of year in which it occurred. It is possible that exposure to the physicochemical characteristics of the Biedouw River positively influences the strength of olfactory imprinting to the natal river, thus increasing the likelihood of philopatric behaviour in subsequent years. An alternative mechanism could be that fish that spent more time in the smaller and relatively safer Biedouw River prior to entering the Doring River were able to acclimate to wild conditions more gradually, thus increasing their likelihood of survival upon entering the mainstem. Importantly, distance from Doring at the time of release was retained in the model published by Cerrilla et al. (2024), but was excluded from the current model. It was previously assumed that fish released further from the Doring River would have spent a longer time in the Biedouw River prior to entering the mainstem, perhaps strengthening the olfactory imprinting mechanisms responsible for homing behaviour. The exclusion of this variable

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[*] Note that fish released in October 2022 were released directly into the Doring River due to low flows in the Biedouw River raising stranding concerns. Water temperature in the Doring River was likely lower than that measured in the Biedouw River, given comparatively higher flows.

from the current model, and the retention of release time of year (which strongly correlates with release river) suggests that exposure to the Biedouw River itself, rather than the duration of exposure, influences return likelihood. Research on the nature of imprinting mechanisms among sandfish and continued monitoring of return rates are necessary to ascertain the mechanisms behind the observed patterns.

It should be noted that in 2021 and 2022, 2,242 small fish (< 150 mm TL) captured from sanctuary reservoirs were intentionally returned to their respective reservoirs (not released) to mitigate potential predation by bass in the wild. Additionally, small fish that were released to the Biedouw River in 2021 (n = 240) were set free without a tag to avoid potential adverse impacts of tagging on smaller fish. While the rationale for these decisions was informed by legitimate survival concerns, the absence of small tagged releases precluded the empirical assessment of their survival and return rates, and prevents their inclusion in the predictive model. Based on data from 2022, Cerrilla et al. (2024) recommended that several hundred fish measuring less than 150 mm TL should be tagged and released to enable the establishment of a minimum threshold of size at release, potentially allowing for the release of a much larger number of fish in subsequent years. In 2023, therefore, 433 fish under 150 mm TL were tagged and released. Although their return rates were not assessed as part of this chapter (due to the ongoing nature of the sandfish migration at the time of writing), their inclusion as part of the 2023 release cohort will enable future evaluation of survival and return rates of small head-started sandfish, and will help refine model outputs of factors affecting return probability.

4.4.5 Migration and spawning cues

In 2021, although no PIT tag data were yet available and water pressure has been extrapolated from level, it appears that sandfish arrived during a strong pulse in flow. It is likely that sandfish began to migrate several days before they were first observed. Field notes indicate that the elevated flows on 19 and 20 August substantially decreased water clarity, leaving deeper pools turbid through 23 August. Water clarity had markedly improved by 24 August, allowing the first visual observation of sandfish approximately 5.5 km from the Biedouw-Doring confluence.

PIT tagging head-started fish has not only facilitated the assessment of the intervention's effectiveness, but also provided valuable insights into the spawning patterns and migratory behaviour of sandfish. In 2022, both Levelogger and PIT tag data were available throughout the migration period. Using PIT tag detections as a proxy for the migratory patterns of the spawning run as a whole, it appears that migration was triggered by high pulses in flow, with the two recorded high-flow events coinciding with the two migration waves in 2022. It is important to note that beyond a water pressure of about 8.4 kPa, discharge estimates become inaccurate, because the river will have broken its banks and spread across the floodplain. The magnitude of the rise in discharge beyond this point is therefore substantially higher than is implied by the water pressure data. Nevertheless, it is clear that large floods in August and September triggered migratory behaviour of sandfish in the Biedouw River.

Although Levelogger data were unavailable for the duration of the 2023 spawning migration, the accumulated daily precipitation readings recorded near the Biedouw’s headwaters at Klein Pakhuis give some indication of likely flow conditions downstream. However, it is worth noting that the single weather station cannot capture the complexity of Biedouw River flow conditions, which are influenced by catchment-wide rainfall patterns, groundwater saturation, and abstraction. Nevertheless, the pattern of migratory sandfish arrivals appears to correspond with what would likely be pulses in flow, which is consistent with the patterns observed in the preceding two years. Many African labeos undertake mass spawning migrations during floods (Skelton 2024), including *Labeo altivelis, L. cylindricus, L. lunatus, and L. congoro* in Lake Kariba's Mwenda River (Bowmaker 1973); *L. cylindricus* in the rivers of Lake Chicamba, Mozambique (Weyl & Booth 1999); and *L. umbratus* in South Africa's Orange River (Tómasson et al. 1984).

In both 2021 and 2022, sandfish spawned during periods of declining flows and rising water temperature during evenings with a mean of about 17 °C. In 2023 the two spawning events coincided with a noticeable rise in air temperature, which would be reflected to some extent in water temperature. Discharge during spawning evenings in 2021 and 2022 varied widely from one event to the next, with discharges as high as 0.38 m^3/s and as low as 0.14 m^3/s. It should be highlighted that the three known spawning sites are located 4.4, 7.0, and 10.5 km upstream of the Levelogger, with several known water abstraction points in between. Discharge measurements derived from Levelogger readings should therefore be considered estimates rather than precise values.

The spawning habits of related labeos and other cyprinids suggest that temperature may influence reproductive timing. Among *L. umbratus* in small South African reservoirs, gonadosomatic index (GSI) was found to be positively correlated with water temperature, indicating that this plays an important role in the timing of the species' reproductive processes (Potts et al. 2005). In the Olifants River, the co-occurring Clanwilliam yellowfish (*Labeobarbus seeberi*) was observed spawning in water temperatures of 20 °C, although spawning success was linked primarily with pulses in flow rather than temperature (Cambray et al. 1997). Among common carp (*Cyprinus carpio*), temperature is the primary stimulus to gonadal development, with spawning occurring under rising water levels and calm conditions (Shields 1958).

A study conducted between 2004 and 2006 investigated the spawning requirements of the Clanwilliam sawfin (*Cheilobarbus serra*), a related cyprinid, in the Driehoeks River, a tributary of the Matjies River in the upper Doring River system. Adult sawfin began spawning at water temperatures of 12-14°C, with peak spawning occurring at 19.5-22°C. Notably, the onset of spawning appeared to depend on an increase in water temperature over approximately seven days (Paxton 2008). Similarly, sandfish spawning was linked to rising temperatures and occurred within a comparable thermal range. While both species spawned at the end of the high-flow season during the recession limbs of floods, sawfin exhibited a much longer spawning season of around 100 days (Paxton 2008). In terms of discharge rates, sawfin spawned across a range of 0.2–0.3 m^3/s (Paxton 2008), similar to sandfish spawning discharges of 0.1–0.4 m^3/s.

The role of temperature in the timing of spawning among sandfish may also be related to the eggs' incubation requirements. Environmentally cued hatching, or variation in the timing of hatching based on environmental stimuli (e.g., temperature or oxygen supply), is commonplace across taxa, including fish, crabs, reptiles, invertebrates, frogs and birds (Warkentin 2011). Shortened embryonic periods at higher temperatures have been documented in many freshwater fish species (Korwin-Kossakowski 2008). In one experimental study, the acceleration in time to hatching between *C. carpio* and *C. idella* eggs reared in 20°C and those reared at 32°C was 3- and 2.5-fold, respectively (Korwin-Kossakowski 2008). Among sandfish, it may be advantageous for spawning to take place during periods of stable or rising temperatures, ensuring eggs develop at higher temperatures. In both 2021 and 2022, spawning occurred during such periods: in 2021, water temperature remained relatively constant for about one week before rising further; in 2022, temperatures fell briefly after the first spawning event but rose again after the second. Spawning during these windows may allow eggs to develop more quickly, reducing the time they spend in this vulnerable life stage. Elevated and stable temperatures, hypothesised to promote the growth and development of young fish, were also postulated as factors contributing to the increased survival of young *C. serra* in the Driehoeks River after hatching (Paxton 2008). More research is needed to understand the ultimate cues for sandfish spawning, especially as it relates to the physiological thresholds for embryonic development and hatching.

The relatively short migration period in 2022 consisted of two discrete waves of migrants with very different mean occupancies. The first cohort of 25 migrants arrived over the course of seven days and remained in the Biedouw for an average of 21 days. In contrast, the second cohort of 52 migrants arrived over the course of four days just prior to and including the spawning period, staying for an average of only four days. Based on the known spawning dates, it appears that the second cohort arrived just in time for spawning, whereas the first cohort arrived early, perhaps waiting for ideal spawning conditions, or to reduce the rate of travel to their spawning grounds. The 2023 migratory season was characterised by substantially higher base and maximum flows compared to 2022, which likely accounted for the more uniform spread of arrivals over 55 days, rather than occurring in discrete waves. Although mean occupancy in 2023 was 22 days, there was a much larger spread than in 2022, with 28% of detected fish remaining in the river for five days or fewer, but 43% staying for over 30 days.

Patterns of upstream migration among freshwater fishes vary by species. In the late 1960s, a study of migratory fishes in the Mwenda River of Lake Kariba found the timing and duration of the upstream spawning runs to vary between species, with *Clarias* species undertaking a single massive run and others, including labeos, exhibiting more fragmentary migrations dependent on flow volume (Bowmaker 1973). Tag detections from 2022 and 2023 suggest sandfish exhibit similarly fragmentary migrations. Although date of arrival varied widely across the migration season, sandfish tended to exit the Biedouw over a shorter period of time. This is most apparent in 2022, when 96% of outgoing detections occurred over a seven-day period immediately following the 9 September spawning event as flood conditions were declining. Indeed, spent Lake Kariba labeos appeared to return downstream “as soon as flow allowed” (Bowmaker 1973), concurrent with the 2022 observations. In 2023, outgoing detections also occurred over a shorter period than during arrival, with 77% of fish exiting the Biedouw River over the course of 15 days between 29 September and 13 October. This, too, appeared to follow the last large flood of the season.

These data provide evidence to support that sandfish are highly dependent on flow and temperature conditions in the catchment, relying on these cues to successfully migrate and spawn. Conservation efforts must therefore prioritise the maintenance of adequate flows throughout the migratory period to support the timing and success of these events, as discussed at length in Chapter 2. Without proper management, disruptions to these natural cues may jeopardise the long-term viability of sandfish populations.

4.4.6 Using simulations to guide tagging effort

The detection probability simulations were developed to help managers determine the proportion of head-started sandfish to tag over the course of future release efforts. One of the primary bottlenecks in scaling up the sandfish releases is the time and effort involved in tagging – tagging more fish often means fewer fish can be released overall. The simulations allow managers to weigh trade-offs between tagging proportion and release numbers, given likely survival probabilities in the wild following release. An additional parameter – number of future detections – was also considered, as different analyses will require different detection thresholds to yield meaningful results.

The results of these simulations must be interpreted in light of the uncertainty around one key parameter: the true survival probability of released fish. Past detection data offer some limited insights: first-year detection rates for fish released in 2021 and 2022 were 7.7% and 15.3%, respectively, while the second-year detection rate for the 2021 release cohort was 16.9%. These values are unlikely to represent true annual survival rates, especially given the difference in detection rates of the 2021 cohort between year 1 and year 2. Additionally, while PIT tag detection efficiency with a stationary antenna is typically high, it is imperfect, with detection errors generally attributed to environmental conditions, fish position, and swimming speed (Castro-Santos et al. 1996; Aymes and Rives 2009; Burnett et al. 2013). In 2023, 6.8% of returning sandfish released upstream of the antenna in 2022 lacked an outgoing tag upon release, implying a detection efficiency of approximately 93% – similar to the 94.7—98.4% downstream detection efficiency recorded by Aymes and Rives (2009) in France. Some surviving fish may have also migrated up other tributaries or skipped migration altogether. These detection rates, therefore, offer a tentative lower threshold of possible survival rates of head-started sandfish.

To account for this uncertainty, the simulations explored a broad range of survival probabilities (from 0 to 0.7), allowing for conservation planning under a range of assumptions. For example, based on the available detection data and accounting for imperfect detections, a 20% survival rate may be a reasonable – if cautious – starting point for planning purposes. Based off this survival rate, if 3,000 fish are released, tagging 30% would likely yield sufficient detections for future analyses. However, if only 1,000 fish are released, it may be necessary to tag up to 70%. While the detection thresholds used in this analysis do not correspond to specific statistical requirements or model assumptions, they serve as practical benchmarks adaptable to different monitoring or research goals.

4.4.7 Conclusions, lessons learned and recommendations

The sandfish head-start programme increased the size of the Biedouw River spawning sandfish population, more than doubling numbers of migrating fish in two years. Moreover, the high numbers of rescued sandfish and low associated mortality rates have resulted in the establishment of six refuge populations, providing biological insurance via population redundancy and sources for future augmentation efforts. Although growth rates in reservoirs vary and are lower than in the wild, the presence of several generations of rescue cohorts ensures that release-ready sandfish are available for annual release efforts. The relatively high survival rate of sandfish in the reservoir environment further indicates that these habitats are suitable for rearing rescued fish. Modelling the factors influencing return probability has enabled the optimisation of future release efforts to maximise post-release survival. Lastly, the considerable PIT-tagging effort and subsequent remote monitoring of tagged fish have allowed for the identification of the environmental cues for migration and spawning.

While these outcomes demonstrate encouraging progress, a structured framework is needed to evaluate whether the programme is achieving its long-term conservation goal. Monitoring plays a critical role in ecosystem management, but to assess the effectiveness of management actions, they must be evaluated against discrete, quantifiable objectives (Lyons et al. 2008). The fundamental objective of the sandfish head-start programme is to augment the Doring River sandfish population to decrease extinction risk. However, specific *means objectives* – defined as specific steps required to achieve a fundamental objective (Lyons et al. 2008) – must be established to evaluate whether the overarching goal is being met. Without such benchmarks, it is difficult to judge whether the programme is working as intended or should be adapted. To this end, it is recommended that a dedicated workshop be convened, bringing together the Saving Sandfish Project team along with external experts to develop a set of concrete conservation objectives and performance measures to guide and evaluate the programme moving forward.

Two means objectives are proposed here as a starting point. The programme may be considered successful if 1) the survival rates of head-started fish released into the wild exceed that of non-translocated juveniles in the Biedouw River, and 2) augmentation with head-started sandfish reduces the probability of extinction of the Doring River population over a 50-year period. Although the survival rate of juveniles spawned in the Biedouw River has thus far been assumed to be close to zero due to the seasonal dewatering of the lower reach, this assumption remains untested. Marking methods suitable for small (< 6 cm) fish, such as visible implant elastomer (VIE) tags, could be used as part of a mark-recapture study to assess annual survival rates of non-translocated juveniles. These data could then be compared with survival estimates of head-started fish, obtained through mark-recapture modelling of PIT tagged individuals, to evaluate the effect of head-starting on survival outcomes.

One way to test the second objective is to conduct population viability analyses (PVAs) that incorporate different levels of population augmentation and assess their effects on key parameters – such as survival rates by life stage, population size, and structure – to estimate extinction risk. Establishing a mark-recapture programme for wild adults in the Doring mainstem would support this effort by providing essential data on population dynamics. Importantly, foundational demographic data must first be collected before reliable PVAs can be conducted, including fecundity, age and size at maturity, sex ratio, generation time, and reproductive frequency. These performance measures will enable an evidence-based assessment of whether the head-start programme is meeting its fundamental objective. They also provide a framework for evaluating outcomes and ensuring that conservation resources are used efficiently – an important consideration given the logistical complexity and cost of the head-start programme.

Several important lessons can be drawn from this work, which should guide future management actions. Rescue success is contingent on its timing, and a careful balance between ensuring high survival and limited exposure to predators must be struck. Future rescues must be planned on a case-by-case basis, informed by hydraulic conditions, the timing of spawning events (which will have a direct bearing on the size of fish), and length of exposure to IAF predation. Overstocking of reservoirs negatively affects growth rates and thus decreases the releasability of reservoir-reared fish. Future stocking efforts should be distributed strategically across reservoirs based on their relative sizes. Collaboration with key partners who can aid in the transport of rescued fish to the more distant reservoirs (Clay, Syfer, and Alpha Excelsior) will be essential. Bushmans Kloof Nature Reserve have assisted in this capacity before. Releases from Hartsbesluit and Mertenhof, the most heavily stocked reservoirs, should be prioritised in the near future. The introduction of sterile grass carp to control overabundant macrophyte growth in Enjo and Hartsbesluit following the development of hypoxic conditions in Enjo Reservoir highlights the benefit of continuous monitoring in enabling timely management responses to emerging challenges. Careful monitoring of aquatic vegetation is recommended, and the further stocking of grass carp should be pursued if necessary. To reduce the risk of data loss, as occurred in 2023 due to flow logger malfunction, it is recommended that data be downloaded at more regular intervals to address potential issues as they arise. This is especially important given that sandfish migration patterns and their environmental cues remain a key focus for future research.

The primary bottleneck in the head-start programme is the number of fish that can be released each season, which is constrained by low reservoir catch rates, limited personnel, and labour-intensive tagging requirements.

Catch rates in reservoirs are often low because releases take place in winter when fish activity is low, while fyke nets are most effective when fish are active. To address this, the use of large seine nets should be trialled at Mertenhof Reservoir, where the lack of macrophytes and a uniform bottom would facilitate their use. Limited release personnel could be supplemented by recruiting landowners who have previously shown interest in becoming more involved. Release numbers could also be increased by reducing the relative tagging effort. The simulations provided here offer a useful framework for identifying appropriate tagging proportions given known or estimated parameters – total release count and detection probability – across a range of survival probabilities. Given current uncertainties, a conservative survival probability of 20% is recommended for planning purposes. Moving forward, survival estimates for head-started sandfish should be refined using mark-recapture approaches, such as Cormack-Jolly-Seber models (e.g., Hewitt et al. 2010).

To increase return rates beyond 20%, future releases should prioritise fish measuring at least 20 cm, based on model predictions. The evaluation of data from the 2023 release of small fish (<15 cm) will help refine this minimum size threshold. The model also indicates that releases should coincide with or precede the wild migration, but this variable is fully confounded with release location (Biedouw versus Doring River). To better understand the effects of release timing and location, return rates should be explicitly tested under each combination of release conditions (early or late timing and Biedouw or Doring location).

The involvement of the local community (via the transformation of farm reservoirs into sandfish sanctuaries and direct employment as rescuers) has been crucial in building support for, and active participation in, sandfish conservation. This should continue and expand to include the active inclusion of local youth by collaborating with schools to offer opportunities to assist in sandfish rescues and releases. Importantly, any water management plans for the Biedouw Valley must be preceded by efforts to understand the specific water needs of the local community. Collaboration with local stakeholders, hydrologists, and social scientists will be essential for developing mutually beneficial strategies to manage the catchment's limited water resources.

While head-starting is designed to maintain population viability in the short-term, future conservation efforts must also address the underlying threats that inhibit natural recruitment (Beebe et al. 2021). Such efforts in the ODRS should focus on (1) increasing habitat availability for threatened endemic fishes by eradicating IAF from critical riverine habitat and (2) increasing flows and reversing summer dewatering by removing thirsty non-native plants and improving water management practices in critical catchments. The latter is especially important, given the substantial predicted future increase in frequency and intensity of droughts in the Western Cape as a consequence of climate change (Naik & Abiodun 2020).

Future research avenues and monitoring efforts should include:

(1) Collection of key demographic data of the larger sandfish population to enhance our understanding of the species and enable population viability analysis (PVA). Key parameters of interest include survival rates, growth rates, fecundity, sex ratio, size at reproductive maturity, and reproductive frequency.
(2) Continued use of the antenna array to monitor the Biedouw River annual spawning migration, which will yield further insights on the factors influencing survival and return rates of reservoir-reared sandfish and help refine our understanding of environmental cues for migration and spawning.
(3) Fyke net-assisted recapture of reservoir-reared sandfish during subsequent migrations to monitor wild growth rates, assess size at reproductive maturity, and gain insights into sandfish longevity.
(4) Antenna-assisted monitoring in neighbouring tributaries to investigate stray rates and natal philopatry.

(5) Targeted use of otolith microchemical analysis, once the population has recovered sufficiently to allow limited lethal sampling. This could help determine the natal origins of wild-caught sandfish and identify additional spawning tributaries beyond the Biedouw River.

(6) Development of a predictive phenology model using temperature and flow to better understand the roles of these environmental cues as cues to migration and spawning.

(7) Comparison of the genetic diversity of head-started sandfish with that of the greater Doring metapopulation to assess genetic impacts of intervention.

(8) A Doring River survey to assess the impacts of the head-start intervention on catchment-wide population trends. These data will allow managers to better assess the wider impacts of conservation efforts and optimize the intervention toward the strategies that will most likely result in population augmentation in the long term.

(9) Evaluation of the suitability of head-starting for other large cyprinids in the ODRS with declining populations, such as *C. serra* and *Labeobarbus seeberi.*

The sandfish head-start programme has yielded clear conservation benefits by increasing the number of spawning individuals, establishing secure refuge populations, and generating insights into factors influencing post-release detections. While these outcomes are promising, the long-term success of the programme will depend on the continued refinement of rearing and release strategies, the development of measurable objectives, and the collection of key demographic data to inform viability assessments. This chapter has outlined practical recommendations and priority research directions to strengthen the programme's impact. Importantly, this programme offers a valuable model for the conservation of threatened freshwater fishes elsewhere in Africa, particularly in its use as an emergency measure where imminent extinction is a distinct possibility. This initiative not only offers a promising path forward for the recovery of the Clanwilliam sandfish, but also provides a broader framework for species recovery efforts in the face of accelerating freshwater biodiversity loss.

Chapter 5

5 Synthesis and conclusions

Freshwater fish populations in South Africa are largely tracking the global trend of widespread population declines and localised extinctions, driven primarily by invasive alien fishes, habitat fragmentation, and decreasing water availability. The added pressures of climate change and other emerging threats are predicted to further exacerbate these impacts. As a migratory species dependent on a large network of high-quality, free-flowing rivers, sandfish act as ambassadors of healthy, connected river systems. Their complex life history also makes them particularly vulnerable to these interacting anthropogenic stressors and renders them valuable indicators to highlight the most pressing threats to freshwater fishes in the Cape Fold Ecoregion. Furthermore, the precarious state of the species presents an opportunity to trial a population augmentation strategy, which has primarily been limited to North American fishes until now. This research investigated Clanwilliam sandfish habitat requirements, mapped historical distribution shifts, and evaluated an ongoing conservation intervention with the aim of informing and guiding future conservation strategies and management decisions.

The research presented in Chapter 2 demonstrated clear ontogenetic niche differentiation among three critical sandfish life stages. Anecdotal observations prior to this study suggested that sandfish selected specific habitat attributes by life stage. Spawning sandfish, for example, had previously been observed spawning over shallow riffles in the Biedouw River. The repeated sampling of three reaches within the Biedouw, however, enabled the characterisation of each developmental stage's specific habitat preferences, with the identification of specific preferences for depth, water velocity, substrate type, and, to a limited extent, physical cover. Adult sandfish selected relatively shallow, fast-flowing riffles while spawning, preferentially spawning over pebbles. Larvae and juveniles, on the other hand, preferred mostly sandy, still waters, with larvae selecting shallow backwaters and pool edges and juveniles favouring intermediate depths further from the banks.

That sandfish utilise a wide breadth of different habitats to complete their life cycle underscores the importance of maintaining natural spatio-temporal heterogeneity across the riverscapes on which they rely. Especially important is the identification of potential vulnerabilities per life stage that enable the targeting of management actions at the appropriate scale. This study, for example, identified the removal of non-native plants and strategic water releases into nursery pools during the summer as potential interventions to provide sufficient habitat to juveniles which would otherwise become stranded. Furthermore, the co-management of water abstraction practices with water users in the Biedouw Valley will be necessary for the maintenance of adequate flows to support spawning on an annual basis. These findings provide some of the first empirical evidence of fine-scale, ontogenetic habitat differentiation in a threatened cyprinid in South Africa. By documenting distinct habitat preferences across larval, juvenile, and adult life stages, it highlights how life-stage-specific vulnerabilities can inform targeted conservation interventions. This study further contributes to a broader understanding of freshwater fish ecology in the southern hemisphere, which remains underrepresented in the life history literature. Finally, it underscores the importance of managing rivers for spatio-temporal habitat heterogeneity where they play key roles in the life cycles of migratory fishes.

Together with the 2021/22 Doring mainstem and tributaries surveys, the collation of all known historical records of sandfish in Chapter 3 showed how sandfish distribution has changed over the past century. Once found across the Olifants River, the study confirmed that sandfish had suffered a substantial range contraction, culminating in their likely extirpation from the Olifants River system by the 1990s. Although sandfish persist in the Doring River system, they consist of an ageing mainstem population separated from several headwater 'refuge' populations by kilometres of bass-invaded waters and the unnatural seasonal dewatering of lower tributary reaches. The scarcity of mainstem subadults in the latest ODF survey confirms that although the upper reach of the Doring River appears to be a mainstem stronghold, recruitment is minimal, placing the species on a precarious trajectory towards extinction. Indeed, the predominance of non-native fishes in the mainstem across all ODF surveys confirms IAF as a top threat to long-term species persistence. Moreover, the identification of two novel invaders in 2021 (*Cyprinus carpio* and *Oreochromis mossambicus*), and the subsequent discovery of *Clarias gariepinus* in 2023 across the catchment, highlights the value of routine monitoring as a conservation and management tool – providing early warning of new threats and enabling timely intervention.

The 2022 tributary surveys indicated that the refuge populations persisting in the headwaters of certain rivers hold considerable recovery potential for the species. This study used population size structure, fish community composition, and important characteristics of headwater reaches (such as overlap with protected areas) to highlight opportunities for intervention likely to yield the largest returns. Certain tributaries, such as the Oorlogskloof and Matjies Rivers, are better candidates for IAF eradication, whereas others (the Kransgat) would benefit from concerted water management programmes to augment flows during critical periods. More broadly, this study underscores the critical role of tributaries in sustaining healthy mainstem populations of large river fish, reinforcing their value as strategic focal points for conservation – especially for migratory species. These findings also emphasise the value of historical distribution data in contextualising current population trends and informing targeted conservation strategies to address long-term declines.

Although head start programmes are commonly used in North America, this study represents an early application of this approach to freshwater fish conservation in Africa. Moreover, to our knowledge, PIT tags have never before been used as a monitoring tool for freshwater fishes on the continent. As such, this study constitutes a pioneering trial of the method for the augmentation of endangered freshwater fish species in Africa. The use of PIT tags, paired with a passive detection system, enabled the assessment of the intervention's effectiveness, ultimately demonstrating that head-starting is a viable method for increasing the size of the Biedouw River spawning run. Two conditions at release – size of the fish and the timing of release – were identified as significant predictors of return likelihood in the two years post-release, whereas a third factor (distance from the Doring) was not. These insights will allow managers to plan future releases to optimise the likelihood of return in subsequent years. Moreover, this study serves as a proof of concept for the use of head-starting to augment the mainstem sandfish population. There is now a need to scale up the intervention to maximise recruits into the adult size classes over the coming years to secure the species in the medium term. Strategic habitat restoration programmes must follow to ensure the sustainability of conservation efforts in the long term.

The passive PIT tag detections served a second important purpose: together with flow and water temperature readings derived from the Levelogger, PIT tag detections facilitated the analysis of the influence of these variables on the onset and conclusion of the spawning migration, and the timing of spawning events. Although the centrality of these environmental cues was previously suspected, based on the behaviour of related Labeos and informal observations, the results from this study have greatly enhanced our understanding of the conditions necessary for successful sandfish reproduction. This study also evaluated the first phases of the head-start intervention, allowing us to derive important information, such as growth rates of juveniles in the reservoir environment and in the wild, and the suitability of the reservoir environment for the rearing of rescued fish. Overall, the work presented in Chapter 4 provides a useful framework for applying a similar head-starting approach in systems where 1) juvenile mortality limits population recovery, 2) juveniles can be easily rescued, and 3) farm reservoirs or similar ponds are available as a resource-efficient alternative to conventional hatcheries for rearing.

The research presented in this thesis opens several avenues for future work. To date, most survey efforts have focused on mainstem and tributary headwater reaches. Moving forward, the lower reaches of key tributaries, especially the neighbouring Tra-Tra River, should be surveyed during the migration and juvenile periods to identify additional locations where interventions similar to the Biedouw River head-start programme could be implemented. The habitat preferences gleaned from this study will be instrumental in this regard, helping to identify likely spawning and nursery grounds based on established preference ranges. Additionally, given the natural environmental variability of the study system and predicted climatic changes, these results should be incorporated into hydraulic models to predict how the availability of suitable habitats might vary with changes in flow. Future work should also track the impact of flow management strategies, including alien tree removal and strategic water releases, to assess their effectiveness in supporting sandfish at different points in their life cycle.

While sandfish have not been observed in the Olifants River since the 1980s, no comprehensive surveys have taken place in decades. An updated survey of the Olifants River mainstem is urgently needed to evaluate the state of the fish community, determine habitat suitability for sandfish, and identify candidate tributaries for IAF eradication and water management schemes. The Doring River should be surveyed every five years to track the dispersal and impacts of novel invaders, monitor indigenous fish population trends, and assess the impact of the head-start intervention at the catchment scale. The differences in life histories between mainstem migrants and headwater residents must also be better understood to target conservation interventions appropriately. Research investigating the potential deleterious effects of genetic isolation between mainstem and headwater populations should be prioritised, as should those assessing the genetic impacts of the current head-start programme on the greater metapopulation.

The release of over 2,500 PIT-tagged sandfish creates several research opportunities. The deployment of passive detection systems to neighbouring tributaries has the potential to not only provide a more accurate assessment of the intervention's efficacy, but also to yield valuable insights into sandfish homing behaviour and stray rates. A more nuanced understanding of adult sandfish movement patterns will enhance our ability to protect the species across the Doring River system. Monitoring of the Biedouw spawning run should continue in the coming years, especially since annual return rates may depend on inter-annual environmental variability. This is particularly important given the release of a large number of small sandfish in 2023. Assessing their survival and return rates may enable the release of larger cohorts in future years. Additionally, continued passive monitoring will offer insights on sandfish longevity, which would: 1) help measure the intervention's long-term impact on reproductive output (as sandfish are iteroparous), and 2) contribute to a more nuanced understanding of population dynamics and stability.

Furthermore, the timing of future detections, combined with Levelogger readings, will allow us to better model the flow and temperature conditions that trigger migration and spawning. As more data become available, modelled return probabilities should continue to be refined, helping to improve release methods to optimise survival and reproductive contribution in the wild. Lastly, physically recapturing tagged sandfish during the annual migration would offer valuable opportunities to assess growth rates in the wild, as well as size and age at sexual maturity.

Although the work presented in this thesis has increased our understanding of Clanwilliam sandfish ecology and behaviour, it is critical to place this new knowledge within the context of the wider sandfish population. To properly evaluate the impact of the head-start programme and other potential interventions, it is essential to compare key demographic rates between head-started individuals and wild fish not subjected to the programme. A concerted, long-term effort should be made to collect baseline demographic data for non-head-started fish, including natural survival rates, migration patterns, somatic growth rates, fecundity, age and size at reproductive maturity, sex ratios, and homing behaviour. These data should form part of an ongoing monitoring programme designed to track demographic rates across a range of environmental conditions. This will help elucidate how factors such as water availability and predator density affect population dynamics. For example, the somatic growth and survival rates of non-translocated juveniles in the Biedouw River could be monitored across multiple summers to assess the impacts of seasonal water retention variation. These data would be especially valuable if management interventions, like strategic water releases, are implemented in the lower Biedouw. Another priority is to establish a mark-recapture programme for non-head-started residents of the Doring mainstem, which would provide critical insights into adult survival, movement patterns and population dynamics.

Overall, this thesis makes a substantial contribution to addressing key knowledge gaps in Clanwilliam sandfish ecology and conservation. By examining ontogenetic differences in habitat preferences, mapping historical distribution shifts, and assessing the efficacy of a head-start conservation programme, this work directly responds to long-standing calls for a deeper understanding of the species' ecological needs and the specific threats posed by habitat fragmentation, flow modification, and IAF predation. The integration of PIT tags and a passive detection system has provided valuable insights into head-started individuals' survival and return rates, allowing a nuanced assessment of the programme's efficacy and enhancing our understanding of the species' behaviour in the Doring River system.

Collectively, this research advances the conservation of the Clanwilliam sandfish while also benefiting co-occurring indigenous fishes and the broader aquatic community. As a large, migratory species reliant on expansive, connected riverscapes to complete its life cycle, conserving sandfish and its habitats delivers broader ecological benefits by maintaining connectivity, safeguarding critical habitats, and sustaining the integrity of riverine food webs. This research further contributes to the broader field of freshwater fish conservation by highlighting the value of integrating ecological research and comprehensive monitoring programmes with conservation action to support evidence-based management. The findings and recommendations presented here lay the groundwork for future studies aimed at improving conservation outcomes for sandfish and other freshwater fishes, especially in regions facing similar challenges.

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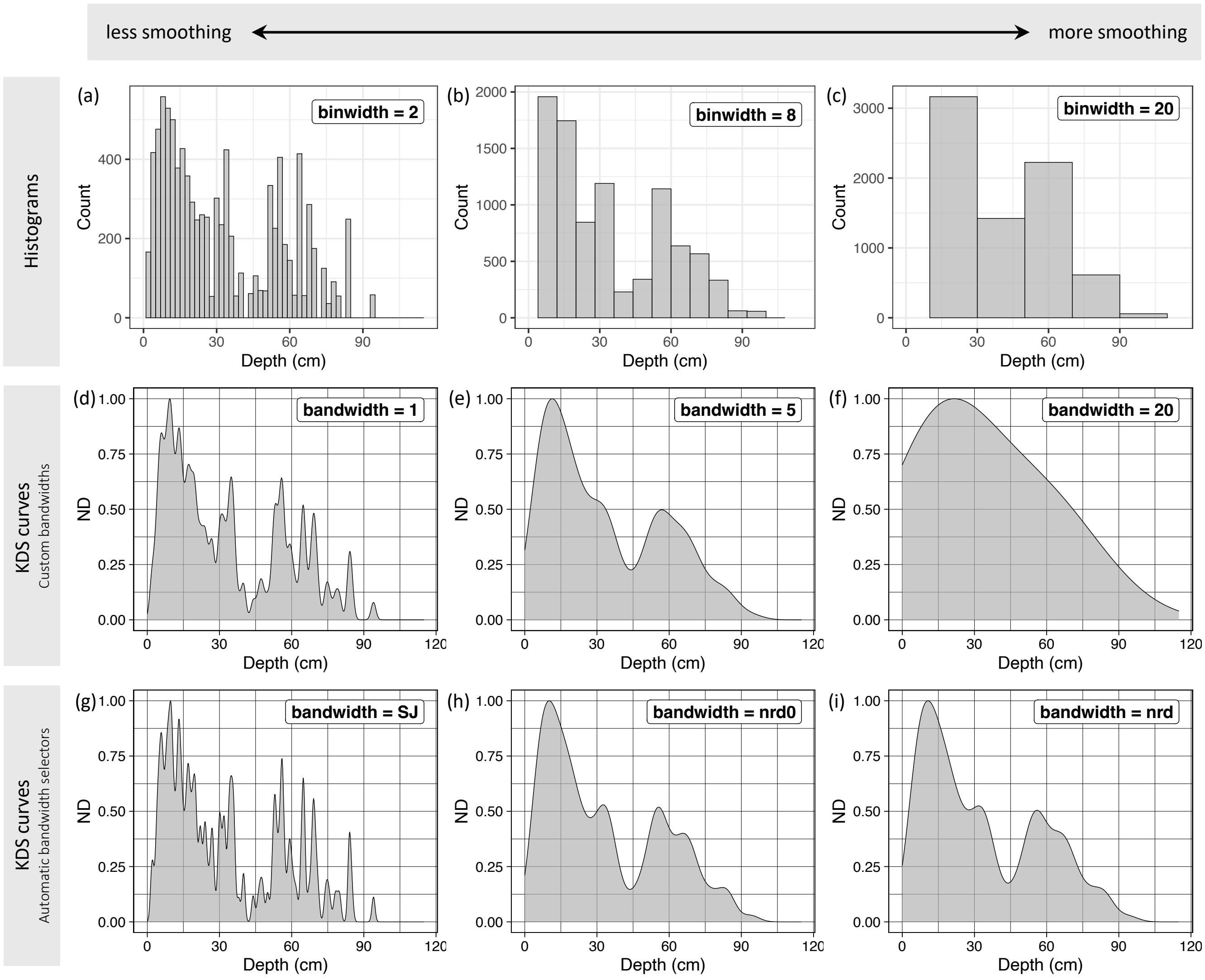
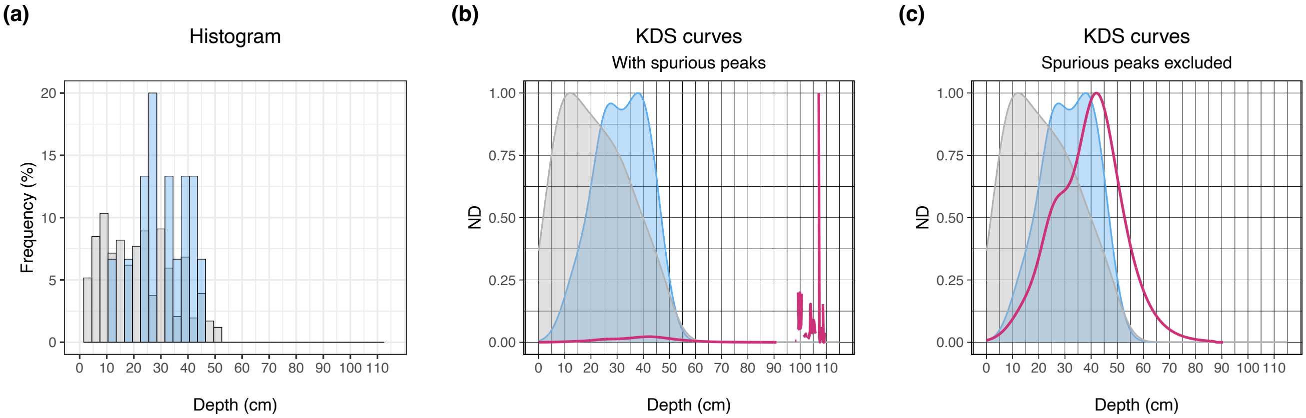
APPENDIX

Appendix A 1. Comparison of histogram bin widths (a, b, c) and Kernel Density Smoothing (KDS) bandwidths using both custom (d, e, f) and automatic (g, h, i) bandwidth selection. Histograms with smaller bin widths (a) reveal more detail but can appear noisy and prevent meaningful interpretation, while larger bin widths (c) smooth over variation. Similarly, KDS curves with small bandwidths (d, g) retain fine-scale structure, whereas large bandwidths (f) produce smoother, more generalised curves. Automatic selectors (g-i) produce varying results depending on data distribution. Selecting a KDS bandwidth is conceptually similar to choosing a bin width for histograms: both influence the degree of smoothing and the level of detail visualised. All KDS curves were produced with the geom_density function (package 'ggplot2'; Wickam 2016). Data shown are habitat availability data from Survey 2, Leonard's Reach.



Appendix A 2. Illustration of how spurious peaks in Habitat Preference Curves (HPCs) can result from Kernel Density Smoothing (KDS). The raw data are shown in (a) as a histogram of habitat utilisation (blue) overlaid on habitat availability (grey), indicating no observations beyond 55 cm. Panel (b) displays KDS-derived curves for habitat utilisation (HUC; blue), availability (HAC; grey), and preference (HPC; pink). Despite the absence of observations at the right extreme, the HPC shows an exaggerated peak due to the division of near-zero density values. Panel (c) shows the corrected HPC after excluding points with extremely low HAC values, removing the spurious peak and more accurately reflecting true habitat preference. Data shown are from Survey 3, Jensen's Reach.



Appendix A 3. Sampling schedule for Olifants-Doring Fish Surveys from 2001 to 2022. Cells containing “1” indicate that a given site was sampled during the indicated survey. Sites are sorted first by river class, then from upstream to downstream. Data origins: February and October 2001, February and November 2011, and October 2013 data were provided by Bruce Paxton; all 2021 and 2022 data were collected by me and a sampling team except for the 2022 Oorlogskloof data, which were collected by Oorlogskloof Nature Reserve staff and volunteers. The table continues on the next page.

River name	River class	Site name	Coordinates	Survey						
				2001 Feb	**2001 Oct**	**2011 Feb**	**2011 Nov**	**2013 Oct**	**2021 Oct**	**2022 Mar**
Groot	Mainstem	Mount Ceder	-32.65789, 19.39831	0	0	0	0	0	1	0
Doring	Mainstem	De Mond	-32.54207, 19.53686	1	1	0	1	1	1	0
Doring	Mainstem	Aspoort	-32.50270, 19.53516	1	1	0	0	0	0	0
Doring	Mainstem	Brakfontein (above weir)	-32.34444, 19.48333	1	0	0	0	0	0	0
Doring	Mainstem	Brakfontein (below weir)	-32.33360, 19.51657	0	0	0	0	0	1	0
Doring	Mainstem	Elandsvlei	-32.26796, 19.49509	0	0	0	1	1	0	0
Doring	Mainstem	Bos Confluence	-32.12230, 19.48665	1	1	0	0	1	1	0
Doring	Mainstem	Uitspankraal	-32.04094, 19.41983	1	1	1	1	1	1	0
Doring	Mainstem	Langkuil	-32.01915, 19.30753	0	0	0	1	1	1	0
Doring	Mainstem	Doringbos	-31.97278, 19.22389	1	1	0	0	0	0	0
Doring	Mainstem	Melkbosrug	-31.85897, 18.98331	0	0	0	0	0	1	0
Doring	Mainstem	Oudrif	-31.85679, 18.91245	1	1	0	1	1	1	0
Doring	Mainstem	Bruinkrans	-31.86301, 18.83633	0	0	1	0	1	1	0
Doring	Mainstem	Melkboom	-31.86016, 18.68714	1	1	0	1	0	1	0
Olifants	Mainstem	Keerom	-32.86056, 19.08528	1	1	0	0	0	0	0
Olifants	Mainstem	Clanwilliam Dam inlet	-32.33333, 18.95000	1	1	0	0	0	0	0
Olifants	Mainstem	Cascade Pools	-31.97060, 18.74336	1	1	0	1	0	0	0
Olifants	Mainstem	Kransgat	-31.83619, 18.62090	0	1	0	0	0	0	0
Matjies	Tributary	Matjies Cave	-32.52000, 19.40000	0	1	0	0	0	0	0
Matjies	Tributary	Matjies 3	-32.51323, 19.39985	0	0	0	0	0	0	1
Matjies	Tributary	Matjies 2	-32.51172, 19.40004	0	0	0	0	0	0	1
Matjies	Tributary	Matjies 1	-32.51047, 19.40068	0	0	0	0	0	0	1
Tankwa	Tributary	Blaauheuwel	-32.49603, 20.23697	0	0	0	0	1	0	0
Tankwa	Tributary	Oudebaaskraal	-32.39681, 19.89952	0	0	1	0	0	0	0
Tra-Tra	Tributary	Tra-Tra 1	-32.26933, 19.40262	0	0	0	0	0	0	1
Tra-Tra	Tributary	Cobus se Gat	-32.29528, 19.51694	1	1	0	0	0	0	0

River name	River class	Site name	Coordinates	Survey						
				2001 Feb	2001 Oct	2011 Feb	2011 Nov	2013 Oct	2021 Oct	2022 Mar
Biedouw	Tributary	Biedouw top site	-32.17458, 19.16174	0	0	1	0	0	0	0
Biedouw	Tributary	Biedouw 3	-32.16951, 19.16099	0	0	0	0	0	0	1
Biedouw	Tributary	Biedouw 2	-32.16863, 19.16225	0	0	0	0	0	0	1
Biedouw	Tributary	Biedouw 1	-32.16839, 19.16323	0	0	0	0	0	0	1
Biedouw	Tributary	Biedouw 4	-32.15467, 19.18542	0	0	0	0	0	0	1
Biedouw	Tributary	Biedouw Barry road crossing	-32.15330, 19.18645	0	0	1	0	0	0	0
Biedouw	Tributary	Biedouw P02	-32.04722, 19.40889	0	1	0	0	0	0	0
Kransgat	Tributary	Kransgat 2	-31.83280, 19.16394	0	0	0	0	0	0	1
Kransgat	Tributary	Kransgat 1	-31.83303, 19.16831	0	0	0	0	0	0	1
Oorlogskloof	Tributary	S01 (Brakwater)	-31.46454, 19.08092	1	1	1	0	0	0	0
Koebee	Tributary	Rietkuil	-31.58417, 19.07250	1	1	0	1	1	0	0
Gif	Tributary	Gifberg	-31.82897, 18.80500	0	0	1	0	0	0	0
Gif	Tributary	Gif 3	-31.83733, 18.80206	0	0	0	0	0	0	1
Gif	Tributary	Gif 2	-31.83813, 18.80184	0	0	0	0	0	0	1
Gif	Tributary	Gif 1	-31.84859, 18.79915	0	0	0	0	0	0	1

Appendix A 4. Sampling gear used during each ODF survey. Large fyke net: three-winged fyke net (opening: 2 m wide x 1.2 m high; 7 m trap; wings: 22 m long x 2.5 m deep; mesh size: 18 mm); other fyke net: includes any number of smaller fyke nets such as single-winged D-ring nets and several sizes of two-winged square-ring nets; gill nets: a set of four gill nets (mesh sizes 54 mm, 70 mm, 90 mm, and 145 mm). Data origins: February and October 2001, February and November 2011, and October 2013 data were provided by Bruce Paxton; all 2021 and 2022 data were collected by me and a sampling team except for the 2022 Oorlogskloof data, which were collected by Oorlogskloof Nature Reserve staff and volunteers. Table continues for three total pages.

Survey	River name	River class	Site name	Coordinates	Sampling method									
					Large fyke net	Other fyke net	Gill nets	Seine net	Hand net	Angling / fly / lure	Speargun	Electrofisher	Dive transect / snorkel	Visual observation
February 2001	Doring	Mainstem	De Mond	-32.54207, 19.53686			X		X					
	Doring	Mainstem	Aspoort	-32.50270, 19.53516			X	X						
	Doring	Mainstem	Brakfontein (above weir)	-32.34444, 19.48333				X	X					
	Doring	Mainstem	Bos Confluence	-32.12230, 19.48665			X							
	Doring	Mainstem	Uitspankraal	-32.04094, 19.41983				X						
	Doring	Mainstem	Doringbos	-31.97278, 19.22389			X	X		X				
	Doring	Mainstem	Oudrif	-31.85679, 18.91245			X	X		X		X		
	Doring	Mainstem	Melkboom	-31.86016, 18.68714				X				X		
	Olifants	Mainstem	Keerom	-32.86056, 19.08528						X		X	X	
	Olifants	Mainstem	Clanwilliam Dam inlet	-32.33333, 18.95000						X				X
	Olifants	Mainstem	Cascade Pools	-31.97060, 18.74336			X			X	X		X	
	Tra-Tra	Tributary	Cobus se Gat	-32.29528, 19.51694				X						
	Oorlogskloof	Tributary	S01 (Brakwater)	-31.46454, 19.08092			X	X						
	Koebee	Tributary	Rietkuil	-31.58417, 19.07250			X	X						
October 2001	Doring	Mainstem	De Mond	-32.54207, 19.53686			X	X		X			X	
	Doring	Mainstem	Aspoort	-32.50270, 19.53516			X	X		X			X	
	Doring	Mainstem	Bos Confluence	-32.12230, 19.48665			X		X					
	Doring	Mainstem	Uitspankraal	-32.04094, 19.41983			X							
	Doring	Mainstem	Doringbos	-31.97278, 19.22389			X					X		
	Doring	Mainstem	Oudrif	-31.85679, 18.91245			X						X	
	Doring	Mainstem	Melkboom	-31.86016, 18.68714				X	X					
	Olifants	Mainstem	Keerom	-32.86056, 19.08528			X						X	
	Olifants	Mainstem	Clanwilliam Dam inlet	-32.33333, 18.95000									X	

Survey	River name	River class	Site name	Coordinates	Sampling method									
					Large fyke net	Other fyke net	Gill nets	Seine net	Hand net	Angling / fly / lure	Speargun	Electrofisher	Dive transect / snorkel	Visual observation
	Olifants	Mainstem	Cascade Pools	-31.97060, 18.74336			X			X				
	Olifants	Mainstem	Kransgat	-31.83619, 18.62090				X						
	Matjies	Tributary	Matjies Cave	-32.52000, 19.40000									X	
	Tra-Tra	Tributary	Cobus se Gat	-32.29528, 19.51694						X				
	Biedouw	Tributary	Biedouw P02	-32.04722, 19.40889								X		
	Oorlogskloof	Tributary	S01 (Brakwater)	-31.46454, 19.08092			X	X						
	Koebee	Tributary	Rietkuil	-31.58417, 19.07250			X	X				X		
February 2011	Doring	Mainstem	Uitspankraal	-32.04094, 19.41983	X									
	Doring	Mainstem	Bruinkrans	-31.86301, 18.83633	X									
	Tankwa	Tributary	Oudebaaskraal	-32.39681, 19.89952		X								
	Biedouw	Tributary	Biedouw top site	-32.17458, 19.16174		X		X						
	Biedouw	Tributary	Biedouw Barry road crossing	-32.15330, 19.18645								X		
	Oorlogskloof	Tributary	S01 (Brakwater)	-31.46454, 19.08092		X								
	Gif	Tributary	Gifberg	-31.82897, 18.80500		X						X		
November 2011	Doring	Mainstem	De Mond	-32.54207, 19.53686	X	X						X		
	Doring	Mainstem	Elandsvlei	-32.26796, 19.49509	X	X						X		
	Doring	Mainstem	Uitspankraal	-32.04094, 19.41983	X	X						X		
	Doring	Mainstem	Langkuil	-32.01915, 19.30753	X	X						X		
	Doring	Mainstem	Oudrif	-31.85679, 18.91245	X									
	Doring	Mainstem	Melkboom	-31.86016, 18.68714	X							X		
	Olifants	Mainstem	Cascade Pools	-31.97060, 18.74336	X			X						
	Koebee	Tributary	Rietkuil	-31.58417, 19.07250	X									
October / November 2013	Doring	Mainstem	De Mond	-32.54207, 19.53686	X									
	Doring	Mainstem	Elandsvlei	-32.26796, 19.49509	X									
	Doring	Mainstem	Bos Confluence	-32.12230, 19.48665	X									
	Doring	Mainstem	Uitspankraal	-32.04094, 19.41983	X									
	Doring	Mainstem	Langkuil	-32.01915, 19.30753	X									
	Doring	Mainstem	Oudrif	-31.85679, 18.91245	X									
	Doring	Mainstem	Bruinkrans	-31.86301, 18.83633	X									
	Tankwa	Tributary	Blaauheuwel	-32.49603, 20.23697	X									
	Koebee	Tributary	Rietkuil	-31.58417, 19.07250	X									

Survey	River name	River class	Site name	Coordinates	Sampling method									
					Large fyke net	Other fyke net	Gill nets	Seine net	Hand net	Angling / fly / lure	Speargun	Electrofisher	Dive transect / snorkel	Visual observation
October 2021	Groot	Mainstem	Mount Ceder	-32.65789, 19.39831	X					X			X	
	Doring	Mainstem	De Mond	-32.54207, 19.53686	X								X	
	Doring	Mainstem	Brakfontein (below weir)	-32.33360, 19.51657	X									
	Doring	Mainstem	Bos Confluence	-32.12230, 19.48665	X					X				
	Doring	Mainstem	Uitspankraal	-32.04094, 19.41983	X									
	Doring	Mainstem	Langkuil	-32.01915, 19.30753	X					X				
	Doring	Mainstem	Melkbosrug	-31.85897, 18.98331	X					X				
	Doring	Mainstem	Oudrif	-31.85679, 18.91245	X					X				
	Doring	Mainstem	Bruinkrans	-31.86301, 18.83633	X					X				
	Doring	Mainstem	Melkboom	-31.86016, 18.68714	X									
March / April 2022	Matjies	Tributary	Matjies 3	-32.51323, 19.39985		X								
	Matjies	Tributary	Matjies 2	-32.51172, 19.40004		X								
	Matjies	Tributary	Matjies 1	-32.51047, 19.40068		X								
	Tra-Tra	Tributary	Tra-Tra 1	-32.26933, 19.40262		X								
	Biedouw	Tributary	Biedouw 3	-32.16951, 19.16099		X								
	Biedouw	Tributary	Biedouw 2	-32.16863, 19.16225		X								
	Biedouw	Tributary	Biedouw 1	-32.16839, 19.16323		X								
	Biedouw	Tributary	Biedouw 4	-32.15467, 19.18542		X								
	Kransgat	Tributary	Kransgat 2	-31.83280, 19.16394		X								
	Kransgat	Tributary	Kransgat 1	-31.83303, 19.16831		X								
	Oorlogskloof	Tributary	A01	-31.43898, 19.11052		X		X						
	Oorlogskloof	Tributary	S07	-31.49689, 19.11237				X						
	Oorlogskloof	Tributary	S09	-31.49861, 19.11392				X						
	Oorlogskloof	Tributary	S10	-31.49931, 19.11409				X						
	Oorlogskloof	Tributary	S11	-31.50005, 19.11514				X						
	Gif	Tributary	Gif 3	-31.83733, 18.80206		X								
	Gif	Tributary	Gif 2	-31.83813, 18.80184		X								
	Gif	Tributary	Gif 1	-31.84859, 18.79915		X								X

Appendix A 5. Tributary site locations, listed in order from upstream to downstream (in relation to their locations along the Doring River).

River name	Site name	Latitude	Longitude	Elevation (MASL)
Matjies	Matjies 1	-32.510470°	19.400680°	607
Matjies	Matjies 2	-32.511720°	19.400040°	607
Matjies	Matjies 3	-32.513230°	19.399850°	608
Tra-Tra	Tra-Tra 1	-32.269330°	19.402620°	355
Biedouw	Biedouw 1	-32.168390°	19.163230°	376
Biedouw	Biedouw 2	-32.168630°	19.162250°	382
Biedouw	Biedouw 3	-32.169510°	19.160990°	385
Biedouw	Biedouw 4	-32.154670°	19.185420°	341
Kransgat	Kransgat 1	-31.833030°	19.168310°	287
Kransgat	Kransgat 2	-31.832800°	19.163940°	301
Oorlogksloof	A01	-31.438972°	19.110528°	653
Oorlogksloof	S07	-31.496892°	19.112365°	523
Oorlogksloof	S09	-31.498605°	19.113917°	521
Oorlogksloof	S10	-31.499277°	19.114089°	515
Oorlogksloof	S11	-31.500050°	19.115140°	492
Gif	Gif 1	-31.848590°	18.799150°	90
Gif	Gif 2	-31.838130°	18.801840°	122
Gif	Gif 3	-31.837330°	18.802060°	126

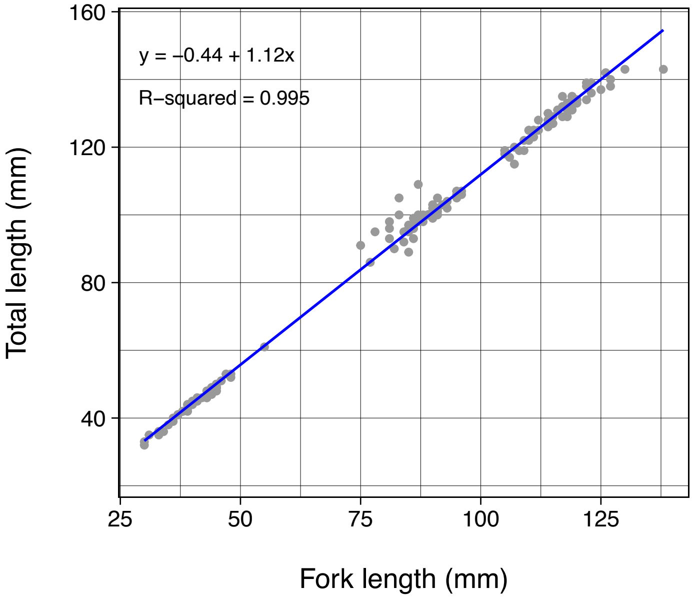
Appendix A 6. Detailed recommendations for the future monitoring of the Olifants and Doring mainstems.

Recommendation category	Specific recommendation
Timing and broad spatial scope	The Olifants and Doring River branches should be monitored concurrently, as actions in one catchment may affect the fish community in the other. A comprehensive Olifants-Doring Fish (ODF) survey should be conducted every five years. Surveys should take place in October or November, depending on flow conditions, when mainstems are not flowing. This concentrates fish in isolated pools, allowing for more effective sampling of the mainstem fish community. Additionally, any potential sandfish recruits from the August-September spawning season are most likely to be detected during this time.
Sites	Doring mainstem (8 sites): De Mond, Brakfontein (above weir), Brakfontein (below weir), Bos confluence, Langkuil, Oudrif, Melkboom. One additional site must be identified between Langkuil and Oudrif to ensure adequate spatial coverage. Olifants mainstem (8 sites): Visgat, Keerom, Noordhoeks confluence, Clanwilliam Dam inlet (above dam), Clanwilliam Dam bridge (below dam), Cascade Pools, Kransgat, Klein confluence.
Methods	• The large three-wing fyke net should be set in the thalweg of the pool to target pelagic species and life stages. It should be set overnight beginning two hours before sunset and retrieved within two hours after sunrise. • Three small single-wing fyke nets should be set along the banks of the pool to target species and life stages that may favour shallow habitats. The placement of these nets should represent the range of available habitats in the pool (e.g. dense vegetation, open water, varied substrates, etc.) • Standardised snorkelling survey: each pool should be snorkelled by a number of samplers appropriate to the channel width. The survey should take place as close to midday as possible to facilitate visual observation. • Environmental DNA samples should be collected according to standardised protocols co-developed with aquatic eDNA specialists. These samples must be collected before any nets are set or personnel enter the water set to avoid contamination.
Metrics	All fish captured by fyke must be identified to species and measured (mm TL). Where more than 30 individuals of a single species are captured, a representative subsample of 30 should be measured.
Mark-recapture	All captured sandfish should be scanned with a hand-held PIT tag scanner and, where a tag is present, the tag ID recorded. If no tag is present, the individual should be anaesthetised and PIT-tagged. Records of tagged sandfish over time will inform assessments of: i) population abundance using an open population mark-recapture model; ii) population trends over time across different size classes (e.g., juveniles, subadults, and adults); iii) longevity; and iv) the relative contribution of head-started individuals to the mainstem population.
Gut dissections of non-native fish	A potential size bias of fyke sampling may arise when predatory fish consume smaller fish in the net. To examine the extent of this bias and, a subsample of non-native fishes (particularly large *Micropterus* spp. and *Clarias gariepinus*) should be either dissected in the field or collected and frozen for later gut content analysis. Gut contents should be examined and, where fish are present, identified to species and measured (if possible).
Additional data	The following habitat characteristics should be recorded: pool length and width; proportions of substrate types, cover types, and vegetation (assessed during snorkelling survey); and turbidity. The following water quality variables should also be recorded (once in the evening and once in the morning): pH, temperature, dissolved oxygen, total dissolved solids, and conductivity.

Appendix A 7. Detailed recommendations for the future monitoring of key Doring River tributaries. The goal of the lower reach surveys (Monitoring Goal 1) is to identify larval or juvenile sandfish, which would confirm that tributary as a spawning ground. The goal of the upper reach surveys (Monitoring Goal 2) is to identify reaches that serve as sandfish refuges and, where sites are located below a bass barrier, to monitor the fish community composition and identify potential downstream dispersal of sandfish from above-barrier reaches.

Monitoring goal	Recommendation category	Specific recommendation
(1) Identify spawning grounds of migratory mainstem population	Timing	Every five years in late September or early October.
	Target tributaries and spatial scope	The Matjies, Tankwa, Tra-Tra, Bos-Wolf, Biedouw, Kransgat, Koebee, and Gif Rivers are the eight major tributaries of the Doring, and therefore likely candidates as spawning tributaries. The Brandewyn (also a major tributary) is not considered suitable due to the large (5 m) waterfall present close to its confluence with the Doring. Surveys should focus on the 10 km reach upstream of each tributary's confluence with the Doring mainstem (or with the Groot River, in the case of the Matjies).
	Sampling methods	Accessible sections of each tributary should be visually assessed from the banks for the presence of larvae and juveniles, where water clarity permits. Where young fish are observed, the area should be sampled by seine, and the fish should be identified to species. In areas where visibility is poor, 10-15 pools suitable for seining (i.e., with an even bottom and no deeper than 1 m) should be sampled using a seine net.
(2) Monitor upper tributary reaches that may play a role as sandfish refuges	Timing	Every five years in spring (September/October) and late summer (March). This timing is intended to identify potential seasonal differences in sandfish reproduction, as well as seasonal variation in fish community composition.
	Target tributaries and spatial scope	In many of the following cases, exploratory surveys will first need to be carried out to identify suitable additional pools to sample. These are specified below. • Matjies: Matjies 1, 2, and 3 should be sampled, as should two additional pools located within the Matjiesrivier Nature Reserve (identified in consultation with CapeNature staff). • Upper Biedouw: In addition to Biedouw 1, 2, 3, and 4, a fifth suitable pool that holds water year-round should be identified and sampled. • Kransgat: There are only two pools that retain water in the Kransgat in the summer. These pools should be sampled during both the spring and summer survey. In the spring survey, three additional pools should be identified in consultation with local landowners. • Oorlogskloof: The regular seine survey of the 36 established survey pools within the Oorlogskloof Nature Reserve (ONR) should resume and be carried out every 1-3 years, as funding allows. Five pools located above the bass barrier (to be selected in consultation with ONR staff) should be sampled using fyke nets according to the proposed sampling schedule. • Upper Koebee: While the Koebee is entirely invaded by centrarchids, it is important to monitor this reach as it may regularly receive downstream dispersers from the ONR. The survey pools should include S36, Rietkuil and three additional pools identified in consultation with ONR staff and local landowners. • Gif: Only three pools are known to retain water through the summer. These pools should be sampled during both surveys. In the spring survey, two additional pools should be identified in consultation with local landowners.
	Metrics	All fish captured by fyke must be identified to species and measured (mm TL). Where more than 30 individuals of a single species are captured, a representative subsample of 30 should be measured.
	Gut dissections	To identify potential in-net predation of smaller fish in areas located below a bass barrier, a subsample of non-native fishes (especially large *Micropterus* spp. and *Clarias gariepinus*) should be either dissected in the field or collected and frozen for later gut content analysis. The contents should be examined and, where fish are present, identified to species and measured (if possible).

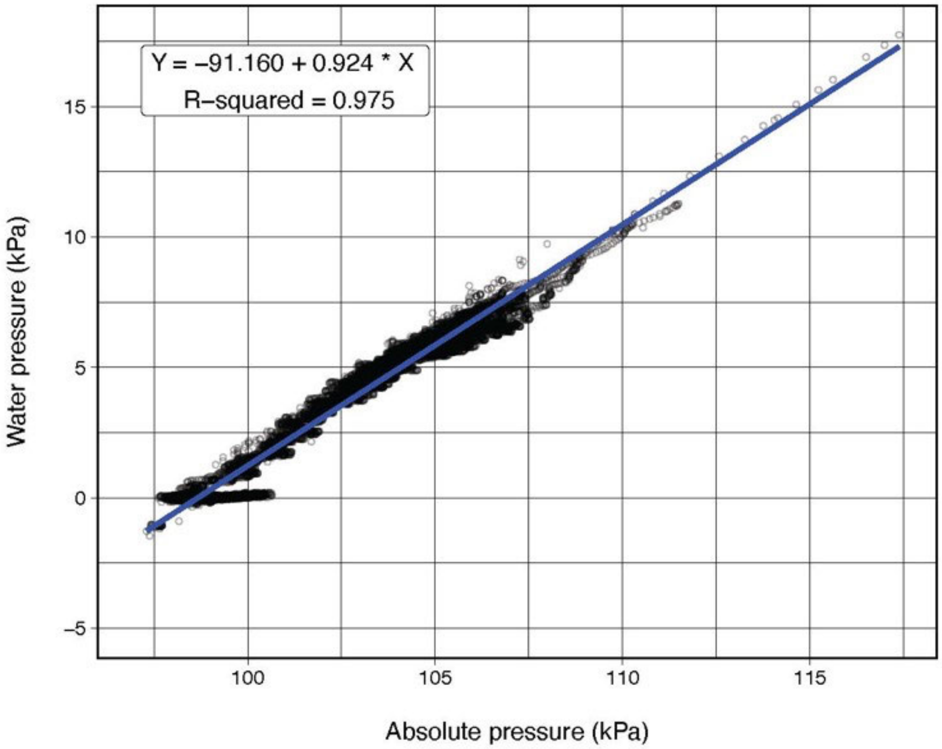
Appendix A 8. Linear regression of fork length versus total length, built using measurements from 158 rescued individuals.



Appendix A 9. Dates and gear used at sanctuary reservoirs during monitoring or release events.

Reservoir	Dates	Event	Gear used
Clay	Mar 2021	Monitoring	(4) Two 1-wing fyke nets, two 2-wing fyke nets
	Mar 2022	Monitoring	(4) Four 1-wing fyke nets
	Sep 2022	Release to wild	(5) One 3-wing fyke net, two 2-wing fyke nets
	Oct 2022	Release to wild	(9) One 3-wing fyke net, three 2-wng fyke nets, five 1-wing fyke nets
Enjo	Jun 2021	Release to wild	(2) Two 2-wing fyke nets
	Aug 2021	Release to wild	Not recorded
	Sep 2021	Release to wild	Not recorded
	Oct 2021	Survival study	Not recorded
	Nov 2021 (1)	Survival study	Not recorded
	Nov 2021 (2)	Survival study	(2) Two 1-wing fyke nets
	Mar 2022	Monitoring	(3) Three 1-wing fyke nets
	Sep 2022	Release to wild	(6) Four 1-wing fyke nets, two 2-wing fyke nets
Hartsbesluit	Jul 2023	Monitoring	(3) Three 1
	Aug 2023	Release to wild	(7) Four 1-wing fyke nets, three 2-wing fyke nets
Mertenhof	Mar 2021	Monitoring	(6) Three 1-wing fyke nets, three 2-wing fyke nets
	Sep 2021	Release to wild	Not recorded
	Mar 2022	Monitoring	(4) Two 1-wing fyke nets, two 2-wing fyke nets
	Apr 2022	Monitoring	(5) Three 1-wing fyke nets, two 2-wing fyke nets
	Aug 2022	Release to wild	(8) One 3-wing fyke net, four 1-wing fyke nets, three 2-wing fyke nets
	Sep 2022	Release to wild	(1) One 3-wing fyke net
	Oct 2022 (1)	Release to wild	(1) One 3-wing fyke net
	Oct 2022 (2)	Release to wild	(3) One 3-wing fyke net, two 2-wing fyke nets
	Aug 2023	Release to wild	(2) Two 2-wing fyke nets
Syfer	Mar 2022	Monitoring	(4) Four 1-wing fyke nets

Appendix A 10. Linear regression generated from known absolute pressure readings and barometrically compensated water pressure readings.



Appendix A 11. Size ranges of measured subsamples from the second, third, and fourth rescue seasons. Sandfish rescued in 2020 were not measured.

Rescue season	Rescue date range	Total length (mm)			
		n	Minimum	Maximum	Mean
2021/22	25 Nov 2021 – 1 Mar 2022	215	32	143	79.05 ± 2.55
2022	7 Nov 2022 – 3 Dec 2022	296	23	66	35.47 ± 0.50
2023	28-29 Nov 2023	30	41	60	51.33 ± 0.85

Appendix A 12. Size ranges of fish rescued during the three rescue windows in the 2021/22 rescue season.

Window	Dates	N	Min	Max	Mean TL (mm)
1	25 – 27 Nov 2021	109	32	61	44.29 ± 0.50
2	10 – 11 Feb 2022	96	86	143	113.03 ± 1.67
3	1 March 2022	10	119	142	131.60 ± 2.32

Appendix A 13. Size ranges and growth rates of fish rescued and relocated to Enjo Reservoir in 2020 that were resampled during subsequent sampling events. Event codes: M (monitoring); W (release to wild).

				Total length (mm)					
Window	Dates	Event	n	Min	Max	Mean ± SE	Days since last sample	Growth (mm)	Growth rate (mm/day)
1	26 – 27 Jun 2021	W	101	150	214	185.50 ± 1.34	-	-	-
2	30 Aug – 3 Sep 2021	W	291	146	211	183.85 ± 0.74	65	- 1.66	- 0.03
3	12 Sep 2021	W	183	145	210	184.08 ± 0.91	13	+ 0.23	0.02
4	6 Nov 2021	M	81	159	218	199.75 ± 1.38	55	+ 15.67	0.28
5	2 Mar 2022	M	121	210	280	258.95 ± 0.92	116	+ 59.20	0.51

Appendix A 14. Size ranges and growth rates of fish rescued and relocated to Mertenhof Reservoir in the 2020, 2021/22, and 2022 rescue seasons that were resampled during subsequent sampling events. Event codes: M (monitoring); R (rescue); W (release to wild).

					Total length (mm)					
Rescue cohort	Window	Dates	Event	n	Min	Max	Mean ± SE	Days since last sample	Growth (mm)	Growth rate (mm/day)
2020	1	21 Mar 2021	M	105	90	160	127.05 ± 1.75	-	-	-
	2	1 – 3 Sep 2021	W	684	111	200	155.74 ± 0.53	164	+ 28.61	0.17
	4	1 Mar 2022	M	18	182	216	197.94 ± 2.64	181	+ 42.20	0.23
	5	21 Apr 2022	M	23	182	218	204.17 ± 2.16	51	+ 6.23	0.12
	6	31 Aug – 3 Sep 2022	W	166	185	232	208.19 ± 0.73	132	+ 4.02	0.03
	7	18 – 20 Oct 2022	W	142	186	245	211.47 ± 0.73	48	+ 3.28	0.07
	9	30 – 31 Aug 2023	W	269	230	285	253.32 ± 0.63	316	+ 41.85	0.13
2021/22	3	25 Nov 2021	R	55	34	54	43.76 ± 0.66	-	-	-
	4	1 Mar 2022	M	59	83	103	93.17 ± 0.55	96	+ 49.41	0.51
	5	21 Apr 2022	M	27	82	122	97.59 ± 1.65	51	+ 4.42	0.09
	6	31 Aug – 3 Sep 2022	W	30	83	138	105.57 ± 1.42	132	+ 7.98	0.06
	7	18 – 20 Oct 2022	W	30	102	125	109.27 ± 1.05	48	+ 3.70	0.08
	9	30 – 31 Aug 2023	W	360	170	227	189.43 ± 0.43	316	+ 80.16	0.25
2022	8	8 – 9 Nov 2022	R	61	23	43	30.61 ± 0.53	-	-	-
	9	30 – 31 Aug 2023	W	69	106	153	118.93 ± 0.86	295	+ 88.32	0.30

Appendix A 15. Size ranges and growth rates of fish rescued and relocated to Clay Reservoir in 2020 that were resampled during subsequent sampling events. Event codes: M (monitoring); W (release to wild).

				Total length (mm)					
Window	Dates	Event	n	Min	Max	Mean ± SE	Days since last sample	Growth (mm)	Growth rate (mm/day)
1	22 Mar 2021	M	57	110	180	152.81 ± 2.19	-	-	-
2	10 Mar 2022	M	4	255	289	269.50 ± 7.27	353	+ 116.69	0.33
3	2 Sep 2022	W	12	255	320	288.75 ± 4.87	176	+ 19.25	0.11
4	19 Oct 2022	W	21	272	325	299.48 ± 3.11	47	+ 10.73	0.23

Appendix A 16. Size ranges and growth rates of fish rescued and relocated to Hartsbesluit Reservoir during the 2022 rescue season that were resampled during subsequent sampling events. Because the sampling during windows 1 and 2 took place during separate stocking events, the change in total length from Window 1 to Window 2 cannot be said to represent growth rate in Hartsbesluit Reservoir, but rather reflects growth rate in the wild between the two dates. Event codes: M (monitoring); R (rescue); W (release to wild).

				Total length (mm)			Days since			
Window	Dates	Event	n	Min	Max	Mean ± SE	Reference window	Number of days	Growth (mm)	Growth rate (mm/day)
1	7 – 9 Nov 2022	R	204	23	47	34.28 ± 0.43		-	-	-
2	3 Dec 2022	R	31	39	66	52.90 ± 1.40		-	-	-
3	12 Jul 2023	M	31	120	151	135.65 ± 1.55	1	247	+ 101.37	0.41
4	16 – 18 Aug 2023	W	439	98	165	138.71 ± 0.42	3	35	+ 3.06	0.09

Appendix A 17. Minimum, maximum, and mean (± SE) total length (mm) and counts of fish released in 2021, 2022, and 2023. Some fish were released without tags, and some were released prior to being measured (or both) due to various reasons discussed in section 4.2.2.4. These are listed under the appropriate categories.

	Tagged					Not tagged					Total
	Measured				Not measured	Measured				Not measured	
		Total length (mm)					Total length (mm)				
	n	Min	Max	Mean ± SE	n	n	Min	Max	Mean ± SE	n	n
2021	992	142	214	175.70 ± 0.47	2	265	111	205	142.86 ± 0.65	17	1276
2022	354	131	325	217.27 ± 1.57	0	1	145	145	*NA*	2	357
2022	1189	98	296	185.30 ± 1.38	0	1	118	118	*NA*	3	1193

Appendix A 18. Summary of freshwater fish translocations carried out in South Africa and Lesotho. The table continues on the next pages.

Species	Date	Moved from	Moved to	# fish moved	Responsible party	Purpose	Outcome	Source
Treur River barb *Enteromius treurensis* (Critically endangered)	~1982	Upper Blyde River, Limpopo catchment, Mpumalanga Province, South Africa	Treur River (a tributary of the Blyde River) above New Chum waterfalls, Limpopo catchment, Mpumalanga Province, South Africa	50-70	Dr Johann Engelbrecht, former Transvaal Directorate of Nature Conservation	*E. treurensis* was presumed locally extinct in the Treur River in the 1970s/80s due to the introduction of non-native fishes into the Blyde and Treur Rivers. It was reintroduced in specified reaches to reestablish a population in the Treur River.	Unsuccessful. Extensive surveys in 1984 and 1994 failed to find any *E. treurensis* in the Treur River.	(Kleynhans 1987; Engelbrecht & Roux 1998; Diedericks 2019)
Treur River barb *Enteromius treurensis* (Critically endangered)	March 1995	Blyde River below Christmas Pools Falls, Limpopo catchment, Mpumalanga Province, South Africa	Clearstream Pool in the Treur River above Clearstream Falls, Limpopo catchment, Mpumalanga Province, South Africa	504	Mondi Forests and the former Transvaal Directorate of Nature Conservation	As above.	Successful. Surveys in 1998 and 1999 confirmed successful recolonisation of the Treur River, with their distribution reaching 7 km downstream and 1 km upstream from the reintroduction site. A 2019 survey showed *E. treurensis* had successfully recolonized 12.4 km of the main Treur River and sampled tributaries.	(Kleynhans 1987; Engelbrecht & Roux 1998; Diedericks 2019)
Clanwilliam sandfish *Labeo seeberi* (Endangered)	2013	Lower reach of the Biedouw River, Doring catchment, Western Cape Province, South Africa	Upper Biedouw Gorge, above a waterfall that excludes non-native fishes, Doring catchment, Western Cape Province, South Africa	338	Endangered Wildlife Trust Cape Critical Rivers	Very low juvenile recruitment was observed during a 2013 survey of the Doring River mainstem. When, during this survey, young sandfish were discovered in the lower reaches of the Biedouw River, they were translocated to the upper gorge in order to increase chances of survival (as they would have succumbed to desiccation and predation by non-native fishes).	Small numbers of subadult and adult sandfish were sampled in the Biedouw gorge (above and below the alien fish barrier) in 2020 and 2022. It is unclear if they are a direct result of the 2013 translocation effort.	(Endangered Wildlife Trust 2015)
Clanwilliam sandfish *Labeo seeberi* (Endangered)	2019	Lower reach of the Biedouw River, Doring catchment, Western Cape Province, South Africa	Upper Biedouw Gorge, above a waterfall that excludes non-native fishes, Doring catchment, Western Cape Province, South Africa	610	Freshwater Research Centre	To increase chances of survival after observation of minimal survival in the lower reaches due to predation by non-native fishes and river desiccation.	Small numbers of subadult and adult sandfish were sampled in the Biedouw gorge (above and below the alien fish barrier) in 2020 and 2022. It is unclear if they are a direct result of the 2019 translocation effort.	(J. Shelton, unpublished data)

Species	Date	Moved from	Moved to	# fish moved	Responsible party	Purpose	Outcome	Source
Bushveld smallscale yellowfish *Labeobarbus polylepis* (Least concern)	1993-1996	Komati River, Incomati System, Mpumalanga Province, South Africa	Elands River (upstream of Ngodwana at Hemlock) and manmade Ngodwana Lake, Incomati System, Mpumalanga Province, South Africa	30-50	Mpumalanga Parks and Tourism Agency	Attempt to increase local population which had been reduced by the 1989 SAPPI chemical spill into the Elands River by supplementing with individuals from the Komati River.	Successful. The local population in the Elands River is stable and increasing in abundance.	(O'Brien et al. 2014)
Clanwilliam yellowfish *Labeobarbus seeberi* (Near threatened)	Mid-1980s	Clanwilliam hatchery, Olifants-Doring River system, Western Cape Province, South Africa	Above Twee River 'fourth' waterfall, into a zone where, prior to fish introductions, only the native Twee River redfin *Sedercypris erubescens* (critically endangered) and Cape Galaxias *Galaxias zebratus* (data deficient) occurred. Olifants-Doring River system, Western Cape Province, South Africa	Unknown	Chief Directorate Nature Conservation of the Cape Province (predecessor to CapeNature)	To improve conservation status of *L. seeberi*	*L. seeberi* have become established and numerous in their introduced range, which provides a source of *L. seeberi* for the Doring system. Native *S. erubescens* and *G. zebratus* have been negatively impacted by introduced *L. seeberi* and other introduced fish species, with populations of both native species declining to concerning levels since the 1980s.	(Impson 2001; Impson et al. 2007; Swartz 2008; Marr et al. 2009)
Clanwilliam yellowfish *Labeobarbus seeberi* (Near threatened) Clanwilliam sawfin *Cheilobarbus serra* (Near threatened)	March 2010	Beaverlac Dam and the Ratels River, a tributary of the Olifants River, Olifants-Doring River system, Western Cape Province, South Africa	3 dams in upper Ratels catchment: Berghhof, Pampoenfontein and Beaverlac dams, Olifants-Doring River system, Western Cape Province, South Africa	12 *L. seeberi* 151 *P. serra*	CapeNature & Western Cape Yellowfish Working Group	To create additional populations of *L. seeberi* and C. *serra* as a safeguard against extinction and to promote angling for native species.	Unknown, as no follow up surveys have been undertaken	(Impson 2010)

Species	Date	Moved from	Moved to	# fish moved	Responsible party	Purpose	Outcome	Source
Clanwilliam yellowfish *Labeobarbus seeberi* (Near threatened) Clanwilliam sawfin *Cheilobarbus serra* (Near threatened) Clanwilliam redfin *Sedercypris calidus* (Near threatened)	1997	Biedouw River	Boontjies River (a tributary of the Brandewyn River) above Meidegat Waterfall in Bushmans Kloof Private Nature Reserve, Olifants-Doring River system, Western Cape Province, South Africa	1 *L. seeberi* 17 *P. serra* 19 *P. calidus*	CapeNature and Bushmans Kloof Private Nature Reserve	To promote the ecotourism value of indigenous fishes and establish a further refuge population of threatened endemic fishes.	Has not been formally assessed, but Dean Impson (personal communication) has confirmed that *L. seeberi* and C. *serra* have become established in the introduced range.	(Impson & Tharme 1998; Swartz 2008; D. Impson, personal communication)
Maloti minnow *Pseudobarbus quathlambae* (Endangered)	Early 1970s	Below waterfall in Tsoelikane River in Sehlabathebe National Park, Lesotho	Above waterfall in Tsoelikane River in Sehlabathebe National Park, Lesotho	Unknown	South African National Parks (SANParks)	Unknown	Unknown	(P. Skelton, personal communication)
Maloti minnow *Pseudobarbus quathlambae* cf. Mohale (Endangered)	2002	Six localities within the Senqunyane, Bokong, and Jordane Rivers within the Mohale Dam catchment in the Lesotho Highlands, Lesotho	Sixteen localities within the Makhaleng, Jordane, Maletsunyane, and Quthing Rivers in the Lesotho Highlands, Lesotho	1,711	South African Institute for Aquatic Biodiversity (SAIAB)	To preserve the unique Mohale ESU lineage of *P. quathlambae* by pre-emptively establishing viable populations outside of areas predicted to be impacted by the Lesotho Highlands Water Project (ahead of the LHWP's implementation in the area)	Successful. According to a 2017 survey, *P. quathlambae* cf. Mohale is likely extirpated from its native range, mostly likely due to predation by smallmouth yellowfish, whose introduction was facilitated by the LHWP. *P. quathlambae* cf. Mohale populations persist in three of the four translocation localities (Makhaleng, Jordane, and Maletsunyane Rivers), while the fourth translocation (into Quthing River) has likely failed.	(Rall 2005; Rall & Sephaka 2008; McCafferty et al. 2018)

Species	Date	Moved from	Moved to	# fish moved	Responsible party	Purpose	Outcome	Source
Twee River redfin *Sedercypris erubescens* (Critically endangered)	2005	Twee River catchment, Olifants-Doring River System, Western Cape Province, South Africa	Tuinskloof Dam, Twee River Catchment, Olifants-Doring River System, Western Cape Province, South Africa	48	CapeNature	*S. erubescens* populations have declined dramatically since at least the 1980s following the introduction of non-native fish species and due to the impacts of water over-abstraction and agrichemical pollution. Translocations were carried out to establish refuge populations in artificial off-channel impoundments to guard against probable extinction.	Successful establishment in Tuinskloof Dam. Survey in 2015 sampled 2,838 *S. erubescens* of a variety of size classes, indicating spawning is taking place.	(Impson et al. 2007; Jordaan et al. 2017)
Twee River redfin *Sedercypris erubescens* (Critically endangered)	February 2016	Tuinskloof Dam, Twee River catchment, Olifants-Doring-River system, Western Cape Province, South Africa	Two off-stream dams on Suurvlak farm in upper Suurvlei catchment, Olifants-Doring-River system, Western Cape Province, South Africa	270 into Suurvlak dam 1 200 into Suurvlak dam 2	CapeNature	As above.	Unknown as no follow up surveys have been undertaken	(D. Impson & J. van der Walt, personal communication)
Berg-Breede River whitefish *Cheilobarbus capensis* (Endangered)	Unknown	Unknown	Several off-stream impoundments in the Berg River catchment (including Voëlvlei Dam), Western Cape Province, South Africa	Unknown	CapeNature	*C. capensis* (endemic to the Berg and Breede River systems) has suffered major declines since the 1930s and likely became extinct in the Berg River system in the 1990s due primarily to predation by non-native fishes and exacerbated by water over-abstraction and pollution. Translocations have been carried out to establish sanctuary stocks for re-stocking river areas when appropriate.	Unknown	(Impson 2008, 2011)
Berg-Breede whitefish *Cheilobarbus capensis* (Endangered)	October 2007, November 2007, February 2008	In-stream Brandvlei Dam, Breede River, Western Cape Province, South Africa	In-stream Berg River Dam, upper Berg River, Western Cape Province, South Africa	95-115	CapeNature	Attempted reintroduction of *C. capensis* into its previous range in the Berg River system, where it was declared extinct in the 1990s.	Partially successful. Thirteen *C. capensis* were caught on three occasions (2008, 2009, 2011), showing that introduced fish had survived since 2007-2008 introduction. No juveniles had been sampled as of 2011, indicating there has been no successful spawning and recruitment since introduction.	(Impson 2011)

Species	Date	Moved from	Moved to	# fish moved	Responsible party	Purpose	Outcome	Source
Verlorenvlei redfin *Pseudobarbus verloreni* (Endangered) Cape galaxias *Galaxias zebratus* (Data deficient)	November 2016	Lower Krom Antonies River, a tributary of the Verlorenvlei River, Western Cape Province, South Africa	A large instream dam in the upper Bergvallei River, a tributary of the Verlorenvlei River, Western Cape Province, South Africa	120 *P. verloreni* 30 *G. zebratus*	CapeNature	Remaining wild populations of *P. verloreni* are threatened by non-native fish species and habitat loss. The translocation took place to establish a refuge population and to rescue individuals that were at risk of desiccation in intermittent pools.	November 2022 fyke survey of dam yielded 2,200+ *P. verloreni* (largest 132mm) but no *G. zebratus*.	(Jordaan 2016; J. van der Walt, personal communication)
Lowveld largemouth *Serranochromis meridianus* (Endangered)	1972	Sabie River, Kruger National Park, Mpumalanga Province, South Africa	Mtsawu Dam, Sabie River catchment, Kruger National Park, Mpumalanga Province, South Africa	13	SANParks	Create refuge population.	Successful in the years following initial stocking. Thousands of individuals were sampled from the Mtsawu dam in 1974, and several hundred were taken from there and stocked into Mestel, Nyamundwa and Mhlanganzwane Dams in the Sabie catchment. The current status of the translocated populations is unknown.	(Pienaar 1978)
Orange-fringed largemouth *Chetia brevis* (Endangered)	1970s	Dams along the edge of the Lomati River, Incomati River catchment, exact localities unknown (Lomati River crosses South Africa and Eswatini)	Stolsnek, Newu and Mpondo Dams, Crocodile River catchment, Kruger National Park, Mpumalanga, Province, South Africa	100+	SANParks	Native habitat threatened by water abstraction, pollution, and siltation. Translocations were carried out to create refuge populations.	Somewhat successful. Initial translocations successful, but Mpondo and Newu Dams have since dried up. Stolsnek Dam still holds water. The population in Stolsnek Dam was reportedly thriving in the 1980s. Local fishermen reported sightings in 2021 but surveys have not confirmed the species' presence.	(Pienaar 1978; de Moor & Bruton 1988; Bills 2021)
Kneria sp. nov. 'South Africa', (Endangered)	1983	Alexander Spruit River, a tributary of the Crocodile River, Mpumalanga Province, South Africa	A tributary of the Elands River, itself a tributary of the Crocodile River, Mpumalanga Province, South Africa	200	Unknown Transvaal Chief Directorate of Nature Conservation?	Attempt to establish a refuge population in the Elands River system following the destruction of habitat associated with the construction of man-made Kwena Lake in their native range.	Successful. A 2007 survey indicated the relocated population still exists in at least two tributaries of the Elands River.	(O'Brien et al. 2014)

Species	Date	Moved from	Moved to	# fish moved	Responsible party	Purpose	Outcome	Source
Incomati suckermouth *Chiloglanis bifurcus* (Critically endangered)	1992	Elands River, Incomati System, Mpumalanga Province, South Africa	Ngodwana River, a tributary of the Elands River, Incomati System, Mpumalanga Province, South Africa	100	The former JLB Smith Institute of Ichthyology (now the South African Institute for Aquatic Biodiversity)	Attempt to establish a refuge for the species following the 1989 SAPPI chemical spill into the Elands River and the associated decline in their abundance and distribution.	Somewhat successful. While numbers have not recovered to pre-1989 levels, the populations persist at low but stable numbers.	(Kleynhans et al. 1992; O'Brien et al. 2014)
Spotted killifish *Nothobranchius orthonotus* (Least concern) Rainbow killifish *Nothobranchius rachovii* (Least concern)	1975 – 1985	Pool in headwaters of Mtomene River, N'wambiyanapan and Pumbe Picket pan, Kruger National Park, South Africa	Pool in the headwaters of the Nhlangulene spruit (Incomati catchment) and several pans in the KNP: Xirohembepan, two Nwambiyapans, Machayipan, Mathlakusapan, N'wambiyanapan, Klawerpan, Pumbe Picket pan, South Africa	~ 80 *N. orthonotus* ~ 280 *N. rachovii*	SANParks	As annual species with extremely narrow habitat requirements, *Nothobranchius* sp. are extremely vulnerable to disturbance and habitat alteration (and therefore very rare in the KNP). Over the course of 10 years, several translocations of *N. orthonotus* and *N. rachovii* took place to increase their distribution and chances of survival.	A survey in 2001 indicated that all translocations had been unsuccessful, with no individuals found at any of the translocation sites.	(Pienaar 1978; de Moor & Bruton 1988; Watters 2003)
Rainbow killifish *Nothobranchius rachovii* (Least concern)	February and March 2001	Captive stock from specimens collected in 1984 from Pumbe Picket pan in Kruger National Park, South Africa	Pumbe Picket pan, Kruger National Park, South Africa	34 in February 13 in March	Brian R. Watters (University of Regina, Canada) and SANParks	No *Nothobranchius* species had been found in the Pumbe Picket pan (only known habitat of *N. rachovii* in the KNP) since 1985. It was decided to reintroduce the captively bred specimens to reestablish a population in the KNP.	During the second introduction in March 2001, no specimens from the February introduction were found. The long-term outcome is unknown, but likely unsuccessful.	(Watters 2003; R. Petersen, personal communication)

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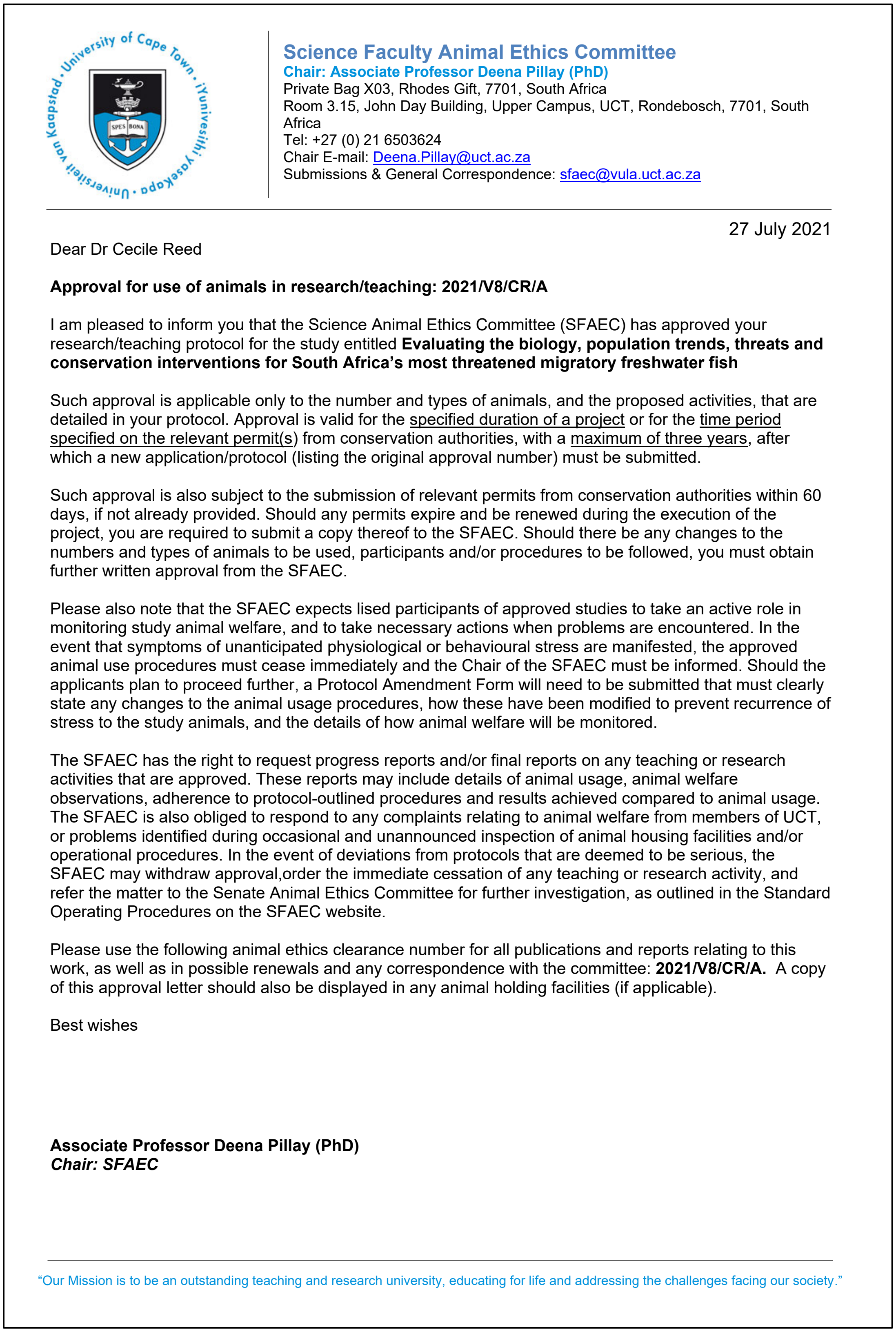
Appendix B 1. Permits.

All work was carried out with permits from CapeNature (CN54-28-14847, CN54-28-14848, CN54-28-22956, CN54-28-19451, CN54-28-19450, CN54-28-19448, CN54-28-19449, CN44-87-17976) and ethical clearance from the University of Cape Town (2021/V8/CR/A). The Department of Water and Sanitation issued a General Authorization for the rotenone treatments.

Appendix B 2. University of Cape Town Science Faculty Animal Ethics Committee approval letter (2021/V8/CR/A).



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27 July 2021

Dear Dr Cecile Reed

Approval for use of animals in research/teaching: 2021/V8/CR/A

I am pleased to inform you that the Science Animal Ethics Committee (SFAEC) has approved your research/teaching protocol for the study entitled **Evaluating the biology, population trends, threats and conservation interventions for South Africa's most threatened migratory freshwater fish**

Such approval is applicable only to the number and types of animals, and the proposed activities, that are detailed in your protocol. Approval is valid for the specified duration of a project or for the time period specified on the relevant permit(s) from conservation authorities, with a maximum of three years, after which a new application/protocol (listing the original approval number) must be submitted.

Such approval is also subject to the submission of relevant permits from conservation authorities within 60 days, if not already provided. Should any permits expire and be renewed during the execution of the project, you are required to submit a copy thereof to the SFAEC. Should there be any changes to the numbers and types of animals to be used, participants and/or procedures to be followed, you must obtain further written approval from the SFAEC.

Please also note that the SFAEC expects lised participants of approved studies to take an active role in monitoring study animal welfare, and to take necessary actions when problems are encountered. In the event that symptoms of unanticipated physiological or behavioural stress are manifested, the approved animal use procedures must cease immediately and the Chair of the SFAEC must be informed. Should the applicants plan to proceed further, a Protocol Amendment Form will need to be submitted that must clearly state any changes to the animal usage procedures, how these have been modified to prevent recurrence of stress to the study animals, and the details of how animal welfare will be monitored.

The SFAEC has the right to request progress reports and/or final reports on any teaching or research activities that are approved. These reports may include details of animal usage, animal welfare observations, adherence to protocol-outlined procedures and results achieved compared to animal usage. The SFAEC is also obliged to respond to any complaints relating to animal welfare from members of UCT, or problems identified during occasional and unannounced inspection of animal housing facilities and/or operational procedures. In the event of deviations from protocols that are deemed to be serious, the SFAEC may withdraw approval,order the immediate cessation of any teaching or research activity, and refer the matter to the Senate Animal Ethics Committee for further investigation, as outlined in the Standard Operating Procedures on the SFAEC website.

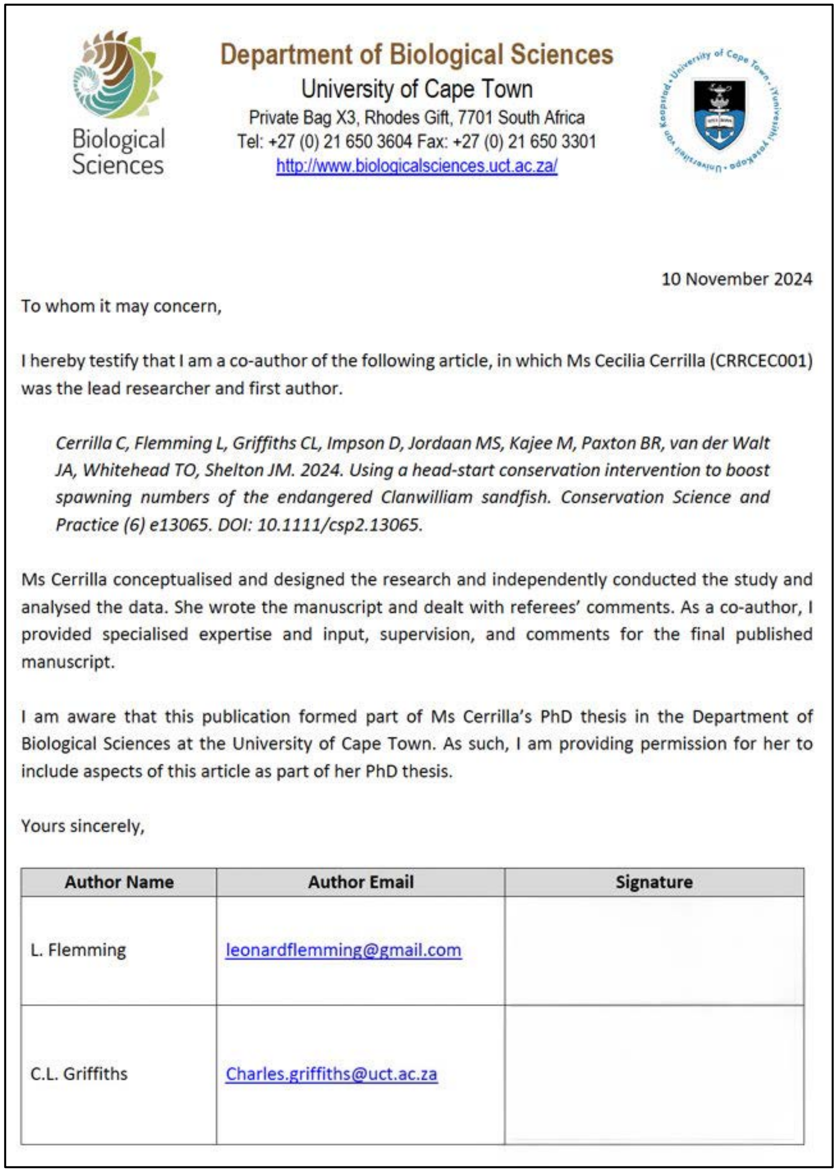
Please use the following animal ethics clearance number for all publications and reports relating to this work, as well as in possible renewals and any correspondence with the committee: **2021/V8/CR/A.** A copy of this approval letter should also be displayed in any animal holding facilities (if applicable).

Best wishes

Associate Professor Deena Pillay (PhD)
Chair: SFAEC

"Our Mission is to be an outstanding teaching and research university, educating for life and addressing the challenges facing our society."

Appendix B 3. Author permission letter for Cerrilla et al. (2024) published in *Conservation Science and Practice* 6:1. DOI: 10.1111/csp2.13065.



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10 November 2024

To whom it may concern,

I hereby testify that I am a co-author of the following article, in which Ms Cecilia Cerrilla (CRRCEC001) was the lead researcher and first author.

> *Cerrilla C, Flemming L, Griffiths CL, Impson D, Jordaan MS, Kajee M, Paxton BR, van der Walt JA, Whitehead TO, Shelton JM. 2024. Using a head-start conservation intervention to boost spawning numbers of the endangered Clanwilliam sandfish. Conservation Science and Practice (6) e13065. DOI: 10.1111/csp2.13065.*

Ms Cerrilla conceptualised and designed the research and independently conducted the study and analysed the data. She wrote the manuscript and dealt with referees' comments. As a co-author, I provided specialised expertise and input, supervision, and comments for the final published manuscript.

I am aware that this publication formed part of Ms Cerrilla's PhD thesis in the Department of Biological Sciences at the University of Cape Town. As such, I am providing permission for her to include aspects of this article as part of her PhD thesis.

Yours sincerely,

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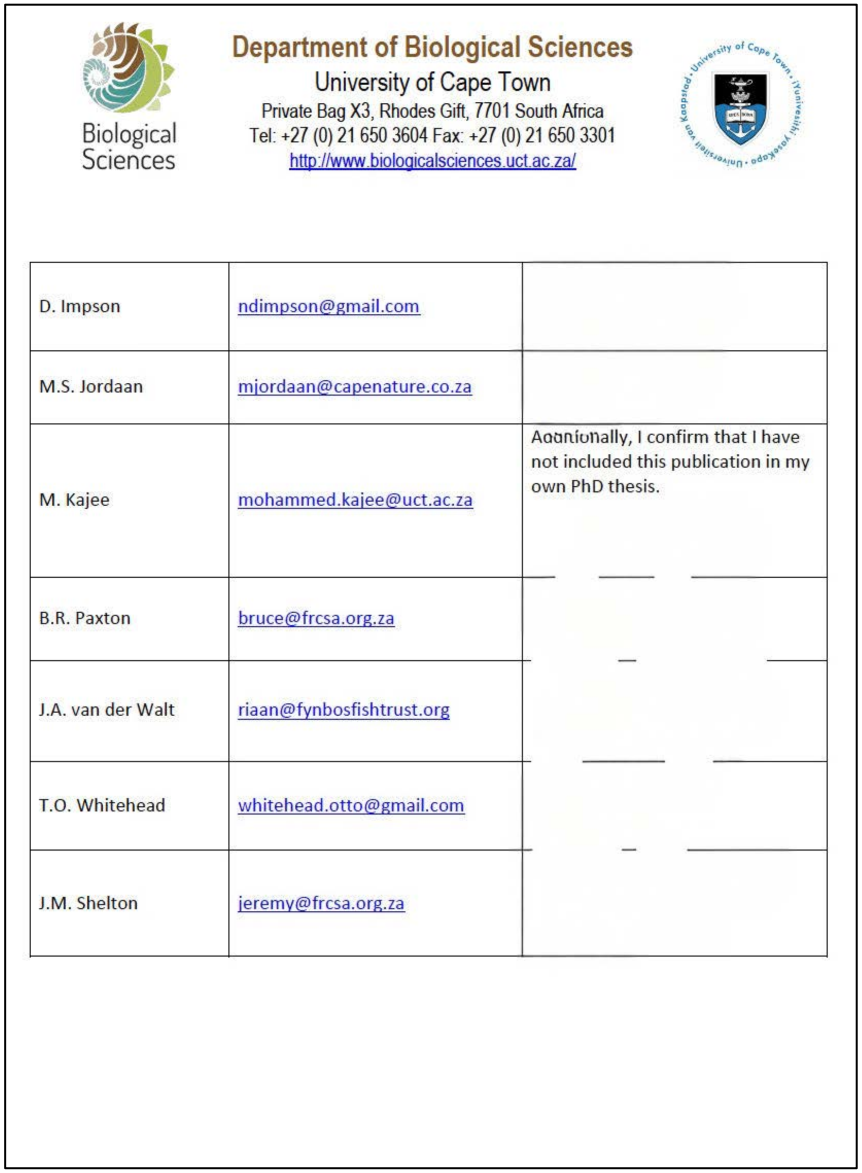

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