CAPE FOLD ECOREGION FISH COMMUNITY ECOLOGY AND RESPONSES TO STRESSORS

Thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

by

CASEY JAY BROOM
(ORCID: 0000-0001-7189-8610)

February 2022

Supervisors:
Dr Josie South
Dr Anthony Thomas Firth Bernard
Prof. Olaf Lawrence Friedrich Weyl (deceased)

DECLARATION

I declare that the work presented in this thesis is entirely my own and that where assistance from others has been accepted, this is fully acknowledged. This study was funded by the National Research Foundation (NRF)—South African Research Chairs Initiative of the Department of Science and Innovation (DSI) (Grant No. 110507). The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

ABSTRACT

Freshwater fishes are in global decline and fish as a group are the most imperilled of all vertebrates. Freshwater systems are among the most threatened globally, largely owing to their comparatively high species and habitat diversity while occupying a minute fraction of the Earth's surface. In South Africa, invasion by non-native fishes has had a devastating effect on freshwater systems across the country. Numerous other stressors and anthropogenic impacts continue to impact these systems, including habitat degradation, water abstraction and global change effects. In general, South African freshwater systems are under-studied and there is a lack of baseline biological and ecological studies on many freshwater fish species. The Cape Fold Ecoregion (CFE) of South Africa is a particularly vulnerable region, with many range-restricted species and highly fragmented native fish ranges following high invasion rates. Within the CFE, the Olifants-Doring River System (ODRS) is of primary concern owing to the high endemism and imperilled status of its freshwater fish species. The Rondegat River in the ODRS is of notable conservation value, as it hosts populations of important endemic CFE species. This river is unique, being the site of the first alien fish eradication programme of its kind in South Africa. Thus the Rondegat River, and in particular its imperilled cyprinid assemblage, is used here as a case study of the responses and community dynamics of recovering freshwater fish species. The members of this cyprinid assemblage are *Sedercypris calidus*, *Pseudobarbus phlegethon* and *Labeobarbus seeberi*. *Sedercypris calidus* and *L. seeberi* are listed by the International Union for the Conservation of Nature (IUCN) as "Near-Threatened", while *P. phlegethon* is listed as "Endangered".

Understanding the dynamics and responses to understudied vulnerable fish communities in the wake of restoration efforts was the overarching goal of this thesis. The first chapter served as a review of current knowledge of the CFE, the Rondegat River, the myriad stressors that have impacted on or are projected to affect this region, and the freshwater fish species on which those stressors act. My first investigations served as an extension of the existing Rondegat River monitoring programme, making use of remote underwater video (RUV) data to assess relative abundance and habitat associations of the focal species (Chapter 2). A relatively limited spatial range of *P. phlegethon* was established, which was suggested to be a result of relatively highly specialised habitat requirements and sensitivity to disturbance. *Sedercypris calidus* was confirmed as a relatively more abundant and ubiquitous species across the length of the Rondegat River, sharing much of the lower and middle reaches with *L. seeberi*.

I sought to use experimental trials of functional response, as a proxy for feeding performance, across representative temperatures and relevant prey types, in the spatially overlapping *S. calidus* and *L. seeberi* (Chapter 3). *Labeobarbus seeberi* generally outcompeted *S. calidus* across temperature treatments and prey types. The ecomorphology and diet of all three species were used to construct trophic profiles, which suggested that there was a high degree of feeding capacity overlap between *L. seeberi* and *S. calidus*, while *P. phlegethon* diverged from the other two species (Chapter 4). Gut content suggested that all three species overlapped broadly in diet. This indicated that the realised trophic niche of these species is similar, despite some morphological specialisation. I then used RUV data to investigate in-situ feeding behaviours, with the aim to disentangle the nuances of community dynamics and mechanisms of coexistence in the cyprinid assemblage (Chapter 5). I found that, despite the higher feeding performance of *L. seeberi* (Chapter 3) and its overlaps in diet and feeding capacity with *S. calidus* (Chapter 4), *S. calidus* is able to mitigate competitive pressures through foraging mode switching and exploitation of allochthonous food inputs. Evidence for further habitat and prey selectivity in *Pseudobarbus phlegethon* was gathered based on dependence on complex habitats and pool refugia for the majority of its feeding, supporting this species as a headwater specialist; alongside signals of its spatial and habitat use patterns (Chapter 2). While *S. calidus* and *L. seeberi* were found to be less habitat-specific than *P. phlegethon*, caution was noted in the potential for ongoing stressors, such as habitat destruction, loss of river connectivity and global change effects, to impact on the reproductive success of these two species.

Stressors affecting the habitats and sensitive invertebrate taxa upon which all three species depend continue to threaten the Rondegat system, highlighting the need to maintain ecosystem integrity through conservation interventions. There remains significant scope to maintain restoration efforts in the Rondegat River and other river systems of the CFE, through direct conservation actions, enhanced community awareness, indigenous riparian vegetation restoration and involvement of local stakeholders in various conservation-centred activities.

ACKNOWLEDGEMENTS

This study is dedicated to the memory of Professor Olaf Weyl, whose guidance and devotion to South African freshwater ecology made the Rondegat River restoration project and all work flowing therefrom possible. To my supervisor Josie – thank you for your awesome insights, plentiful ideas and encouragement. This would not have been possible without you. To Ant, you stepped up and supported me following the passing of Olaf; for this I thank you.

I would like to thank my parents and sister for all their encouragement, positivity and enthusiasm throughout the study, and particularly my mom and dad for helping with fieldwork and their highly appreciated assistance with moving between provinces, and all kinds of selfless support over the years. Thank you from the bottom of my heart.

I would especially like to thank my fiancée Cat, who moved from Cape Town to Makhanda and back with me. She has been through almost all phases of my journey through to my PhD, encouraging and supporting me all the way, sacrificing aspects of her own career to assist with mine. I can’t thank you enough my love.

Sean Marr and Marian Wong are thanked for valuable insights. Angus van Wyk, Rowshyra Castañeda, Alex van Nynatten and Takudzwa Madzivanzira assisted in the field. Munetsi Zvavahera, Dumisani Khosa and Takudzwa Madzivanzira assisted in the lab and during prey collection in the muddy ponds around Makhanda. Francois Lamont was instrumental in supporting the successful maintenance of the oxygen and temperature regulation of the climate-controlled fish holding lab. Olaf’s Freshwater Research Group at SAIAB were all highly encouraging and helpful throughout, from meeting everyone in 2018 at the invaluable Cederberg training and team bonding exercises; through the long grind of the thesis, lab and field work, and they held strong as a tight-knit team through the passing of Olaf and the challenges and isolation as a result of COVID-19.

I specifically wish to thank the Josies (South and Pegg), for stepping up to the massive challenge of keeping everyone afloat and looked after following the loss of Olaf. Leo Nagelkerke is also thanked for his major support of the functional morphology chapter, such as his hosting of the dedicated ecomorphology workshop and assistance with statistics. Leo also provided cross-continental virtual guidance while I made morphological measurements, using borrowed SAIAB equipment from a coffee table in my cottage in Makhanda, during lockdown. Thanks again to Cat for putting up with a dedicated shelf in the freezer for fish dissection samples. I thank Rika du Plessis of CapeNature for facilitating my research activities in the Cederberg Wilderness Area. Jannie and Cecile Nieuwoudt (of Keurbos farm), and Jannie and Katrin Nieuwoudt (Grootkloof/Jamaka farms) are thanked for land access and lively discussions about the history of the Rondegat River valley.

Permission for land access was granted by local conservation authority CapeNature and private land owners. Sampling was governed by collection permit CN44-28-8357 dated 28 March 2019, Western Cape export permit CN16-28-8540, dated 1 April 2019, as well as an import permit for the Eastern Cape issued by the Department of Economic Development, Environmental Affairs and Tourism (DEDEAT) CRO 124/19CR, dated 6 August 2019. In addition, collection of *Labeobarbus seeberi* required a Threatened or Protected Species permit, issued by the DEDEAT with permit number 029705. The experimental work was approved by the Rhodes University Animal Ethics Committee (reference number DIFS2118) and the SAIAB Animal Ethics Committee (reference number 25/4/1/5_2018-10). I hereby acknowledge the use of equipment provided by the NRF-SAIAB Marine Remote Imagery Platform and the funding channelled through the NRF-SAIAB Institutional Support System. Photographs are my own except where otherwise credited.

TABLE OF CONTENTS

Page

DECLARATION .... **ii**

ABSTRACT .... **iii**

ACKNOWLEDGEMENTS .... **v**

TABLE OF CONTENTS .... **vii**

FIGURES & TABLES .... **ix**

Chapter 1: Introduction and thesis motivation **1**

- 1.1 The fish community and ecosystem context of the Rondegat River in the Cape Fold Ecoregion (CFE), Western Cape, South Africa 2
- *1.1.1 Background of the CFE and status of current literature* 2
- *1.1.2 The Rondegat River as a case study and model system* 7
- *1.1.3 Species profiles* 10
- *1.1.4 Climate change and the thermal physiology of CFE fishes* 14
- *1.1.5 The role of stressors in the context of the Rondegat River* 17
- 1.2 Research approach and thesis outline 27

Chapter 2: Habitat associations and abundance of the Rondegat River cyprinid assemblage **32**

- 2.1 Introduction 33
- 2.2 Methods 35
- 2.3 Results 43
- 2.4 Discussion 50

Chapter 3: Prey type and temperature influence functional responses of threatened endemic Cape Fold Ecoregion fishes **56**

- 3.1 Introduction 57
- 3.2 Methods 61
- 3.3 Results 65
- 3.4 Discussion 71

Chapter 4: Ecomorphological niche separation facilitates co-existence of functionally similar threatened fishes **77**

- 4.1 Introduction 78
- 4.2 Methods 80
- 4.3 Results 84
- 4.4 Discussion 89

Chapter 5: Feeding behaviour of a recovering cyprinid fish assemblage revealed using underwater video **93**

- 5.1 Introduction 94
- 5.2 Methods 97
- 5.3 Results 101
- 5.4 Discussion 108

Chapter 6: General discussion **114**

- 6.1 Analysis and further discussion of each chapter 115
- 6.2 Synthesis of chapters 124

6.3 Implications for conservation and recommendations for monitoring of the Rondegat and other CFE systems 127

REFERENCES...**133**

APPENDICES...**149**

FIGURES & TABLES

Figure 1: The location of the Rondegat River within the Olifants-Doring system, part of the Cape Fold Ecoregion of the Western Cape, South Africa. Notable features limiting fish distributions are indicated, as well as the extent of the alien fish eradication efforts utilising the piscicide Rotenone and the position of the focal river in relation to the Clanwilliam Dam and the Olifants River. Adapted from Weyl et al. (2013). 9

Figure 2: The interplay of the core study areas of the thesis. Habitat, functional morphology, behaviour and functional responses are defined here in separate chapters. I demonstrate here the recognition of the overlaps and synergy inherent across these broad disciplines and lines of inquiry as they relate to the current observed distribution and abundance of the Rondegat River cyprinid assemblage and contribute to its resilience in the face of current and future threats. 28

Figure 3: Map of the study system (Rondegat River, Cederberg, South Africa). Important physical barriers to fish movement and the designation of treatment, control and pristine segments of the river is shown. White areas indicate elevation of 200 – 400m, light grey: 400 – 600m, and dark grey areas 600m+. Figure adapted from Castañeda et al. (2020a). 36

Figure 4: Photographs of the study species and habitats of the Rondegat River. (a) fiery redfin *Pseudobarbus phlegethon* (b) Clanwilliam redfin *Sedercypris calidus* (c) Clanwilliam yellowfish *Labeobarbus seeberi* (d) typical vegetated habitat of the upper reaches (e) the fire-affected banks showing the effects on riparian vegetation (f) a section of river in the middle reach, severely altered by a sand plug following fire-assisted erosion, which was previously inhabited by all three species. 37

Figure 5: Longitudinal profile of the distribution of study sites and temperature loggers. Stars indicate the location of temperature loggers, circles show the sampled sites. Parallel vertical lines indicate the position of the Rooidraai waterfall, the upper limit of non-native fish invasion. Elevation ranged from 120 m to a maximum of 521 m above sea level, and sampling covered 21 km of the Rondegat River. The camera monitoring sites for collection of relative abundance and distribution data in April and October 2018 are depicted. 38

Figure 6: A diagram of the mono-camera remote underwater video (RUV) system used in this study. 40

Figure 7: Biplot of the non-metric multidimensional scaling analysis relating environmental constraints and species relative abundances. Species MaxN (relative abundance) score centroids are shown, while standard ellipses containing 40% of the ordinated data denote the primary habitat types. Overlaps are considered to be non-significant. Site status as “protected” or “unprotected” areas is shown using convex hulls. 45

Figure 8: Functional response curves of the two focal fish species (*Sedercypris calidus,* red and *Labeobarbus seeberi*, in blue) across two temperature treatments on *Culex* sp. larvae (**a**) and chironomid larvae (**b**) as prey items. Temperature treatments are depicted using solid lines for 18°C and dashed lines for 25°C, respectively. The shaded areas indicate 95% confidence intervals. 68

Figure 9: Panels A and B: PCAs of the morphological traits of the three focal species; panel A: individual fish morphology distributed across PCA dimensions, panel B: morphological measurements as vectors. Panel C and D: PCAs of the trophic profiles of the focal species; panel C: individual fish trophic profiles as points overlaid on PCA dimensions 1 and 2, panel D: trophic profiles displayed as vectors. 85

Figure 10: Correlations of predicted diet specialisations of the three species (Labeobarbus seeberi, Pseudobarbus phlegethon and Sedercypris calidus) based on their calculated trophic profiles. Horizontal lines indicate the bootstrapped mean values, boxes indicate upper and lower 95% confidence intervals. At least two of the three species were significantly different (P < 0.05) in predicted correlations with all food types. Zooplankton (t) refers to “townet” feeding on zooplankton; Zooplankton (p) refers to “pump” feeding, wherein fish gulp small volumes of

water selectively, in contrast to indescriminate "townet" feeding requiring active swimming. 86
Figure 11: Relative proportions of algae, detritus, plant, insect, and larvae food groups making up the gut content of the focal species Labeobarbus seeberi, Pseudobarbus phlegethon and Sedercypris calidus. Values are derived from the counts of prey items proportionally to their respective volume in gut content. Horizontal lines indicate the bootstrapped mean values, boxes indicate upper and lower 95% confidence intervals. 88
Figure 12: Estimated (Est.) marginal mean abundance-adjusted feeding counts between species and reach types (pools and streams). Blue bars signify confidence intervals; red arrows indicate significance (i.e. overlapping red arrows in the vertical axis indicate a lack of significance, non-overlapping arrows show a significant difference between species means). Significance is based on Tukey-adjusted p-values at an alpha of 0.05 102
Figure 13: Attack rates (feeding counts per unit time) of all three species on different foraging targets. Error bars denote standard errors, horizontal bars denote the median while black circles indicate the mean. 104
Figure 14: Interaction plot of the model predicted probability of feeding between species (*Sedercypris calidus*, red; *Pseudobarbus phlegethon*, green; and *Labeobarbus seeberi*, blue) and reach types (pools or streams). Vertical bars denote 95% confidence intervals around the linear prediction (points). 106
Figure 15: Estimated (Est.) marginal mean odds ratios of feeding probability between species and reach types. Blue bars signify confidence intervals; red arrows indicate significance (i.e. overlapping red arrows indicate a lack of significance, non-overlapping arrows show a significant difference between species means). Significance is based on Tukey-adjusted p-values at an alpha of 0.05. 107

Table 1: Conservation status (IUCN Redlist; EL = extralimital, NN = non-native) and descriptive summary of native and non-native fish species found in the Olifants-Doring River System. Adapted from Ellender et al. (2017) and Jordaan et al. (2020) unless otherwise cited. 6
Table 2: Summary statistics of the relative abundances (MaxN) of the three species. SE = standard error, DR = detection rate, defined as sites with species present/all sites................43
Table 3: Results of the PERMANOVA following non-metric multidimensional scaling (nMDS) ordination. 46
Table 4: Environmental predictors of species abundance after model selection based on AIC values. Fixed predictors and zero-inflated model intercept terms (if applicable) are included for each final model per species. The candidate models are shown in appendix Tables S2a – S2c. 48
Table 5: Model terms for all factors from generalised linear models with a quasi-Poisson error distribution used to determine differences in *Culex* prey consumption with regards to factors "predator species", "temperature" and "prey density", using a Type III Anova and χ2. Chisq indicates the Chi-squared (χ2) value, df indicates degrees of freedom. 67
Table 6: Model terms for all factors from generalised linear models with a quasi-Poisson error distribution used to determine differences in chironomid prey consumption with regards to factors "predator species", "temperature" and "prey density", using a Type III Anova and χ2. Chisq indicates the Chi-squared (χ2) value, df indicates degrees of freedom. 67
Table 7: First order terms and significance levels from logistic regression of the proportion of prey consumed against initial prey density, with functional response type, functional parameters, associated significance levels from Rogers' random predator equation, bias accelerated and corrected 95% confidence intervals for a and h, with regards to Sedercypris calidus and Labeobarbus seeberi predating upon chironomid and Culex larvae under different

temperature treatments. a = attack parameter; h = handling time; 1/h = maximum feeding rate (per one hour) estimate. 69
Table 8: Ecomorphological measurements used in this study. Adapted from Sibbing and Nagelkerke (2001) and Nagelkerke et al. (2018). Most measurements were scaled by standard length (SL) or standard length squared (SL^2) where applicable. 83
Table 9: Feeding behaviour modifiers (as used in BORIS software) with descriptions of the foraging targets and food types based on a simplified ethogram of the Rondegat River cyprinid assemblage. A foraging target and food type, the site-specific details and presence/absence of species other than the focal species was recorded for every observed behaviour. 99
Table 10: Type II ANOVA of the negative binomial linear mixed effects model describing feeding behaviour counts between species, while accounting for fish abundance and the number of unique individuals exhibiting behaviours in a given site. 102
Table 11: Type II ANOVA of the negative binomial linear mixed effects model describing feeding behaviour counts for *S. calidus*, while accounting for fish abundance and the number of unique individuals exhibiting behaviours in a given site. 103
Table 12: Type II ANOVA of the binomial model describing the probability of feeding between species with the main effects of species ID, reach type, substrate and the species ID x reach type interaction term. 105
Table 13: Recovery objectives and performance criteria adapted from Saddlier et al. (2013). The contributions of this thesis or the current status of the objectives are summarised. 131

CHAPTER 1: Introduction and thesis motivation

Sedercypris calidus captured on underwater video in the Rondegat River, Western Cape, South Africa.

1.1 The fish community and ecosystem context of the Rondegat River in the Cape Fold Ecoregion (CFE), Western Cape, South Africa

1.1.1 Background of the CFE and status of current literature

Freshwater systems are among the most threatened globally, largely owing to their comparatively high species and habitat diversity while occupying <1% of the Earth's surface (Dudgeon *et al.*, 2005; Abell, Allan & Lehner, 2007). Globally, drift-feeding stream fishes are some of the most abundant and ecologically important components of freshwater systems (Rincón, Bastir & Grossman, 2007; Matthews, 2012). Stream fishes play an important role in ecosystem functioning and food webs beyond the boundaries of the aquatic environment (Jackson *et al.*, 2016c; Jackson, Pawar & Woodward, 2021). Freshwater fishes are also in global decline and fish as a group are the most imperilled of all vertebrates (Fausch *et al.*, 2002). Freshwater systems are often overlooked in the formation of protected areas and are a low priority in conservation efforts (Jordaan, Chakona & van der Colff, 2020). Additionally, conservation and restoration efforts are often inadequate or poorly implemented (Lintermans, 2013; Jordaan *et al.*, 2020). Globally and within the South African context, effective conservation and restoration of highly impacted stream fish is a multi-faceted, complex, "wicked" problem (Lintermans, 2013; Ellender & Weyl, 2014; Jordaan *et al.*, 2020).

South African freshwater ecosystems are no exception to the vulnerable state of systems further afield and are imperilled by a variety of threats, primarily as a result of anthropogenic interference (Darwall *et al.*, 2009; Weyl *et al.*, 2020). Among these threats, one of the most pressing are invasive organisms introduced to local freshwater systems, particularly predatory fish, alongside anthropogenic climate change and habitat degradation (Abell *et al.*, 2007; Darwall *et al.*, 2009; Tweddle *et al.*, 2009; Filipe, Lawrence & Bonada, 2013; Ellender *et al.*, 2017; Shelton *et al.*, 2018b; Weyl *et al.*, 2020). In South Africa, the Cape Fold Ecoregion (CFE) is recognised as biodiversity hotspot for freshwater fish, as despite a relative paucity of species, there is a very high degree of endemism (Tweddle *et al.*, 2009; Ellender *et al.*, 2017; Shelton *et al.*, 2018b). Current baseline scientific knowledge on the native fishes of the CFE is fairly narrow and tends to focus on specific inquiries or notable biological traits primarily (Ellender *et al.*, 2017). For example, only a single study exists on the physiology of CFE fishes, investigating survival strategies in *Galaxias* sp. when faced with aerial exposure (Chakona, Swartz & Magellan, 2011). Reizenberg et al. (2019) investigated the thermal tolerances and preferences of CFE fishes, with no other examples of studies on critical thermal limits available.

In general, CFE fish literature is lacking, with 103 peer-reviewed articles focusing primarily on taxonomy and biogeography, and to a lesser degree ecology, conservation and human impacts as of 2017 (Ellender *et al.*, 2017). The strong focus on taxonomy and biogeography of researchers thus far is commendable and necessary for conservation of genetic lineages, and particularly for enabling researchers from other disciplines to appropriately define targets of research and conservation endeavours. Native fishes in the CFE are united by a series of pressures such as high levels of invasion, depleted natural range and suitable habitats through water abstraction and damming (but see Beatty et al. (2017) for consideration of dam removal with impacts on artificial refugia under climate change), while research and conservation efforts are usually sporadic and uncoordinated (Ellender *et al.*, 2017). The majority of native small stream fishes have narrow ranges and are highly fragmented, with many recently discovered, genetically distinct and/or reclassified species (Chakona *et al.*, 2020). As a result, knowledge of the exact distributions and available ecological information on these species is limited. The contemporary distributions and biogeographic history of these highly endemic lineages represent an invaluable library of material as to the natural history and freshwater biodiversity heritage of South Africa. This information is crucial for the understanding of freshwater systems both in their present state and in predicting future responses to change (Chakona *et al.*, 2020).

A system of primary concern within the CFE is the Olifants-Doring River System (ODRS), as the system is home to a relatively high diversity of endemic and in most cases threatened or endangered fishes. In particular, the Cederberg Mountains within the ODRS catchment have been identified as "vulnerability hotspots" within an already vulnerable region (Shelton *et al.*, 2018b). Ten of the 19 freshwater fish species endemic to the CFE are found in this system (Weyl *et al.*, 2014), highlighting its importance in the conservation of endemic biodiversity. The native, non-native and extra-limital species currently found in the ODRS are summarised in Table 1. Rivers and catchment areas in the CFE have been devastated by invasive predators, with endemic species being limited to refugia through natural barriers as the only means of survival in much of these systems (Tweddle *et al.*, 2009). The main invasive fish threat in this system are members of the black bass genus *Micropterus* spp. (van der Walt *et al.*, 2016); however, the diversity of invasive fish species in this system is also comparatively high. In the ODRS, an estimated 81% of the natural cyprinid range has been invaded by black bass (van der Walt *et al.*, 2016). It is understood that the mainstem Olifants and Doring Rivers are completely invaded by black bass, and their distribution in the tributaries is primarily limited by natural barriers, which are in most cases the only factor preventing local extirpation of native fishes (van der Walt *et al.*, 2016; Weyl *et al.*, 2020). The bass invasions completely eradicated all small (<10 cm total length) native species and prevented recruitment of species with an adult length of >10 cm (van der Walt *et al.*, 2016). Where bass and other invasive fishes co-occur, local habitat loss to native species is accelerated and multiple predator effects may act to further increase the threat of invasive fish for all local fish life (Wasserman *et al.*, 2016). Currently, relationships between invasive predatory fish and the endemic fish species in this system are only partially understood (Ellender *et al.*, 2017). Within the ODRS, the primary and most damaging interactions of non-native and extra-limital species interactions is direct predation, though some cause displacement of native fishes, introduce novel parasites or diseases, and habitat degradation (Table 1) (Ellender & Weyl, 2014; Weyl *et al.*, 2020).

Another anthropogenic effect on South Africa's freshwater systems is that of climate change (Dallas, 2008; Comte *et al.*, 2013; Beatty *et al.*, 2017), which is expected to have abiotic influences such as fluctuating rainfall patterns and oxygen saturation of aquatic systems, for example. In turn, the abiotic effects of climate change are expected to lead to cascading biotic effects, thus affecting the physiology and behaviour of native and invasive organisms. Current knowledge and predictions around climate change effects on freshwater systems in the CFE are expanded upon in section 1.1.4. Generally, knowledge on the effects of climate change in these systems is limited by the lack of baseline biology and ecology of the native fish species (Dallas & Rivers-Moore, 2014; Reizenberg *et al.*, 2019). Information and predictions of the downstream effects of climate change on important invasive species is somewhat better known, however. For example, trout (*Salmo trutta* and *Oncorhynchus mykiss*) as cool mountain stream-specialized predatory invader species are expected to decrease in abundance and viable population distribution in shrinking refugia within the CFE (Shelton *et al.*, 2018c; Dallas *et al.*, 2020). Conversely, warm-adapted species including major invasive predators, the black bass (*Micropterus* spp.) group, as well as extra-limital tilapia species, may increase in abundance and invasive range (Khosa *et al.*, 2019; Mofu *et al.*, 2019). In addition to direct temperature changes and trends of reduced runoff in the CFE (Dallas & Rivers-Moore, 2014; Dallas *et al.*, 2020), the predicted increase in frequency of extreme events, including flooding, may have major effects on the trajectory and intensity of invasive species through release of captive alien species into natural systems. On the other extreme, drought may have differential effects in different contexts. For example, while drought may reduce available habitat for native species, it may in some cases act as a facilitator of important physical barriers protecting native species through drying of reaches required for the upstream movement of bass (Ellender *et al.*, 2018). In the generally species-depauperate and oligotrophic systems of the ODRS, climate-related abiotic and resultant biotic shifts may have disproportionately devastating effects as a result of relatively minor fluctuations (Shelton *et al.*, 2018b; Reizenberg *et al.*, 2019). Other anthropogenic effects on freshwater systems in the ODRS include habitat degradation through water abstraction, alien riparian vegetation, pollution and damming activities associated with agriculture (Impson *et al.*, 2007), which may act in tandem with climate-related drought as interacting multiplicative stressor events (Jackson *et al.*, 2016a, 2020; Comte *et al.*, 2021).

Table 1: Conservation status (IUCN Redlist; EL = extralimital, NN = non-native) and descriptive summary of native and non-native fish species found in the Olifants-Doring River System. Adapted from Ellender et al. (2017) and Jordaan et al. (2020) unless otherwise cited.

Species	**Status**	**Notes and major threats (impact if non-native)**
Austroglanis barnardi (Skelton, 1981)	EN	Endemic to ODRS. Alien fish, habitat degradation
Austroglanis gilli (Barnard, 1943)	NT	Endemic. Alien fish, habitat degradation
Cheilobarbus serra (Peters, 1864)	NT	Endemic. Alien fish, habitat degradation, utilization
Clarias gariepinus (Burchell, 1822)	EL	Widespread and resilient predator with strong dispersal ability (Skelton, 2001)
Cyprinus carpio Linnaeus, 1758	NN	Widespread in lentic water bodies, degrades habitat through feeding behaviour (Skelton, 2001)
Enteromius anoplus (Weber, 1897)	LC	Widespread, not found in mountain streams of ODRS (Skelton, 2001)
Galaxias zebratus species complex Castelnau, 1861	DD	Endemic to CFE. Ongoing taxonomic revision may classify ODRS populations as genetically distinct. Alien fish, habitat degradation, genetic integrity/population fragmentation (Chakona *et al.*, 2020)
Labeo seeberi Gilchrist & Thompson, 1911	EN	Endemic, found only in the Doring system after extirpation from the Olifants. Alien fish, habitat destruction, migratory barriers (Jordaan *et al.*, 2017a)
Labeobarbus seeberi (Gilchrist and Thompson, 1913)	NT	Endemic, recognised as flagship conservation species. Alien fish, habitat degradation, utilization, physical barriers
Lepomis macrochirus Rafinesque, 1819	NN	Predatory, displaces native species through overpopulation in slow-flowing vegetated systems (Skelton, 2001)
Micropterus dolomieu (Lacepède, 1802)	NN	Predatory, adapted to flowing rocky habitats favoured by native fish (Skelton, 2001)
Micropterus punctulatus (Rafinesque, 1819)	NN	Predatory, less successful than other black bass (Skelton, 2001)
Micropterus salmoides (Lacepède, 1802)	NN	Predatory, prefers lentic systems (Skelton, 2001)
Oncorhynchus mykiss (Walbaum, 1792)	NN	Predatory, cool flowing stream specialist (Skelton, 2001)
Pseudobarbus phlegethon (Barnard, 1938)	EN	Endemic. Alien fish, habitat degradation. Five highly fragmented subpopulations remain, restricted to protected areas (van der Walt, Impson & Jordaan, 2017a)
Pseudobarbus sp. nov. "Doring"	CR	Endemic, currently undergoing taxonomic revision. Alien fish and highly limited distribution
Salmo trutta Linnaeus, 1758	NN	Predatory, cool flowing stream specialist
Sandelia capensis (Cuvier, 1829)	EL	Widespread in the CFE, translocated to the ODRS, threatens sensitive species (Skelton, 2001)
Sedercypris calidus (Barnard, 1938)	NT	Endemic. Alien fish and habitat degradation
Sedercypris erubescens (Skelton, 1974)	CR	Endemic. Alien fish (including extra-limital *S. capensis*), habitat degradation, pollution
Tilapia sparrmanii Smith, 1840	EL	Minor extralimital impact, usually introduced alongside black bass (Skelton, 2001; Ellender & Weyl, 2014)

1.1.2 The Rondegat River as a case study and model system

The Rondegat River (32°24’S; 19°05’E; Fig. 1) is a 25 km long 2nd order perennial tributary of the Olifants River, opening into the Clanwilliam Dam (Lowe *et al.*, 2008; van der Walt, 2014). The catchment area is approximately 111 km^2 and is characterised by winter rainfall falling primarily from June until August (Lowe *et al.*, 2008). Temperatures in the CFE are hottest in February and coldest in June (de Moor & Day, 2013). The river originates as a pristine headwater stream in the Cederberg Mountains, progressing through fynbos and other indigenous terrestrial vegetation and passing areas of alien tree plantations, citrus fruit orchards and associated abstraction points and weirs in the lower reaches (Lowe *et al.*, 2008). The river is characteristic of a typical CFE tributary as a perennial clear, oligotrophic, acidic mountain stream (Swartz, 2000; de Moor & Day, 2013). The Rondegat River is of notable conservation concern, as it hosts populations of important endemic CFE species including *Sedercypris calidus, Pseudobarbus phlegethon*, *Labeobarbus seeberi,* and *Austroglanis gilli* (Woodford *et al.*, 2005; Garrow & Marr, 2012; Weyl *et al.*, 2013). The native fish in this system were in danger of extirpation by non-native fishes present in the system, which led to the extirpation of minnows below a natural barrier (Rooidraai waterfall) and high predation pressure on Clanwilliam Yellowfish recruitment (Woodford *et al.*, 2005; Marr, Impson & Tweddle, 2012). Weyl et al. (2013) estimated fish density in the uninvaded reaches as 97 fish per 100 m^2 compared to 7 per 100 m^2 in the invaded reaches. The Rondegat River served as the site for the first alien fish eradication project in the CFE, performed through a collaboration between the local conservation body CapeNature, the American Fisheries Society and the South African Institute for Aquatic Biodiversity (SAIAB) (Marr *et al.*, 2012; Weyl *et al.*, 2013, 2014, 2016). This project treated a 4 km stretch of the Rondegat River with the piscicide rotenone to remove alien smallmouth bass (*Micropterus dolomieu* Lacepède, 1802) and thus provide additional habitat for the existing native fish populations (Marr *et al.*, 2012; Weyl *et al.*, 2013, 2016; Slabbert, Jordaan & Weyl, 2014).

The Rondegat River restoration project is intended to be the first of four CFE river treatments and was chosen owing to its ideal characteristics (including uninvaded headwater reaches with favourable habitat for native fishes, physical barriers to re-invasion upstream and a lack of angling importance) and invasion situation following an environmental impact assessment (Marr *et al.*, 2012; Weyl *et al.*, 2016). A series of studies and restoration actions were carried out concurrently with the alien fish eradication efforts, such as alien plant clearing and assessments of the effects of the rotenone application on the invertebrate community

(Woodford *et al.*, 2013; Bellingan *et al.*, 2015; Weyl *et al.*, 2016; Fill, Kritzinger-Klopper & van Wilgen, 2018). Alien plant clearing and fynbos restoration efforts resulted in rapid recovery of native shrubs; however, secondary invasion by alien and native grasses prevented a full recovery to a pre-invasion state (Fill *et al.*, 2018). The rapid reinvasion by grasses is thought to be a result of raised nitrogen levels in the riverbank soils, and is likely to require a longer period to recover completely (Fill *et al.*, 2018). This altered nutrient state may have cascading effects on river resource availability and trophic interactions that are difficult to anticipate. The application of rotenone had an immediate impact on invertebrate density, especially reducing the sensitive Ephemeroptera density, with a catastrophic drift event being triggered in response to the rotenone treatment (Woodford *et al.*, 2013). Recolonisation and recovery of the aquatic invertebrate assemblage was anticipated through drift from unaffected upstream populations, and losses in diversity were much less marked than in comparable studies (Woodford *et al.*, 2013). A rapid bioassessment of the impacts of rotenone on the invertebrate community of the river revealed these taxa were both resilient to the treatment (community returned to pre-treatment health) and resistant to lower concentrations of rotenone in the application of the second treatment, despite short term population extirpation (Bellingan *et al.*, 2015). Only one other successful eradication of invasive fishes from a river has been achieved in South Africa, that of the mechanical removal of spotted bass (*Micropterus punctulatus* Rafinesque, 1819) from the Thee river (van der Walt *et al.*, 2019). The Thee shares many characteristics with the Rondegat; it is a similar size, has pristine upper reaches with water abstraction and habitat alteration in the lower reaches, and has a similar fish assemblage (van der Walt, 2014; van der Walt *et al.*, 2019). The specific characteristics of the Thee river and the naïve nature of the spotted bass in this system were ideal for the realisation of success in this specific case, and thus chemical eradication of invasive species is likely to be the preferred method for future restoration actions in the CFE (Weyl *et al.*, 2016; van der Walt *et al.*, 2019).

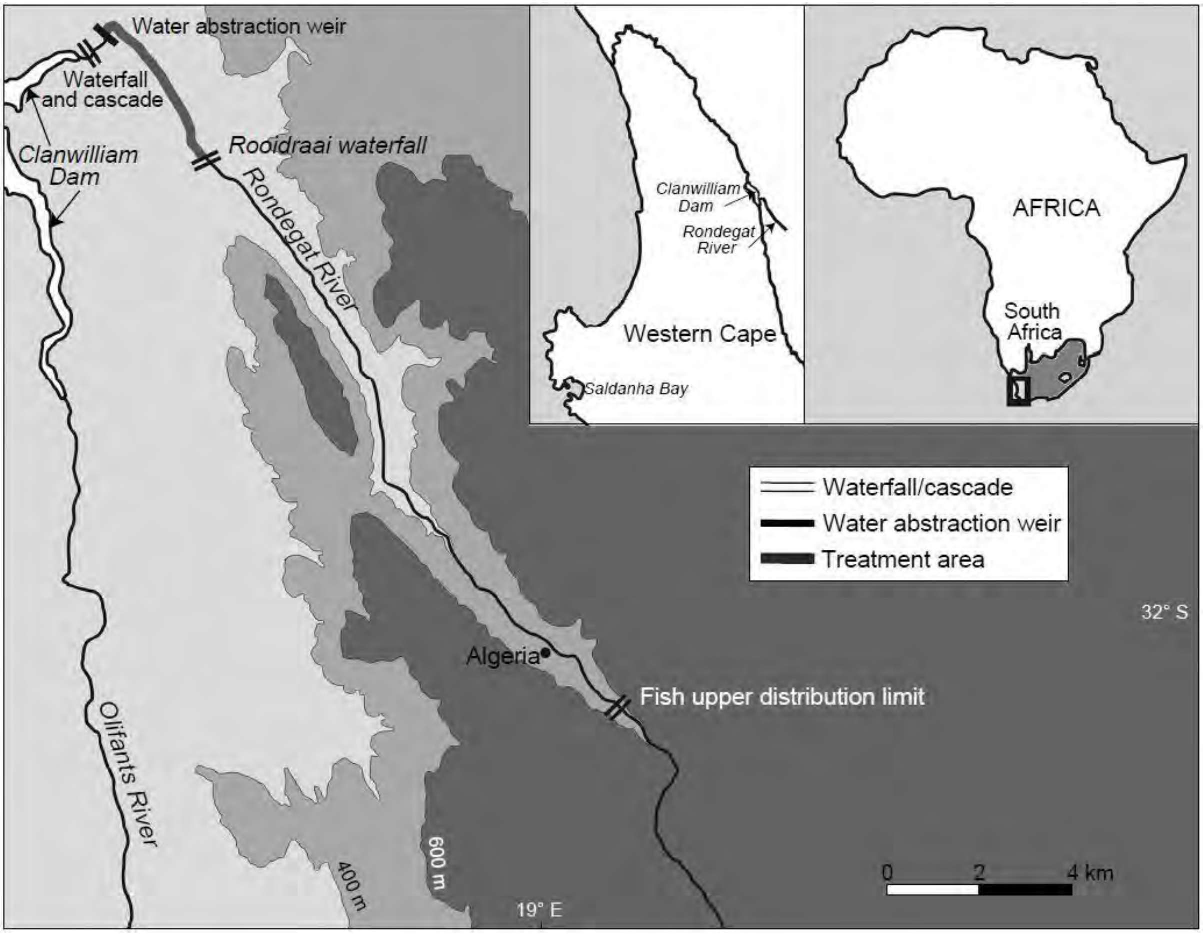

Figure 1: The location of the Rondegat River within the Olifants-Doring system, part of the Cape Fold Ecoregion of the Western Cape, South Africa. Notable features limiting fish distributions are indicated, as well as the extent of the alien fish eradication efforts utilising the piscicide Rotenone and the position of the focal river in relation to the Clanwilliam Dam and the Olifants River. Adapted from Weyl et al. (2013).

A comprehensive assessment of the composition and distribution of native fishes in the Rondegat River prior to the invasive fish removal programme in 2012 was first performed in 2005, followed by another intensive survey in 2013 (Woodford *et al.*, 2005; Weyl *et al.*, 2013, 2016). At this point in the river's history, native fish populations were in direct danger of local extirpation by smallmouth bass. The success of the rotenone treatment has led to the river having significant value for research, representing a natural experiment in terms of an undisturbed headwater section, with the lower reaches now devoid of invasive fish (Weyl *et al.*, 2014). Ongoing monitoring is anticipated to reveal further insights into the recruitment of native fishes and the expansion of natural fish assemblages in a novel environment (Weyl *et al.*, 2016). Currently, the river is host to five recognised teleost species, including three cyprinids, a galaxiid (recently re-discovered following a lengthy period of no sightings), and one austroglanid catfish species.

1.1.3 Species profiles

Sedercypris calidus

Sedercypris calidus (Barnard, 1938), formerly *Barbus* and *Pseudobarbus calidus* (Skelton, Swartz & Vreven, 2018), the Clanwilliam redfin minnow, is currently evaluated as near-threatened using IUCN Red List criteria (van der Walt, Jordaan & Impson, 2017c). This species is only distantly related to the fiery redfin (*Pseudobarbus phlegethon*) with which it co-occurs closely, having a serrated last unbranched dorsal ray and two pairs of barbels as opposed to the soft dorsal ray and one pair of barbels of *P. phlegethon* (Garrow & Marr, 2012; Skelton *et al.*, 2018). In the Rondegat River, *S. calidus* is the more abundant of the two redfins present and primarily feeds on suspended aquatic invertebrates and detritus through drift feeding, in addition to foraging on submerged natural structures (rock, aquatic and immersed vegetation), occasionally taking items from the surface with terminal mouths (Garrow and Marr 2012, pp. 49). *Sedercypris calidus* has notably large eyes, which are supplemented by two pairs of barbels and chemosensory cells on the head, suggesting a variety of food sources and foraging locations within the habitat are used. Nthimo (1997) notes baetid mayfly nymphs and chironomid larvae as the most important prey items for this species based on gut content analysis, with mayfly nymphs constituting >90% of prey items year-round. In addition, a significant intake of terrestrial insects (Formicidae) was found, suggesting terrestrial subsidisation of the diet of this species by feeding from the water surface (Nthimo, 1997). A predominantly carnivorous diet is further supported by a short gut length and smooth pharyngeal teeth (Nthimo, 1997). The summer reproductive season of this species is indicated by the presence of ripe adults (reaching sexual maturity at ~45 mm SL, reflecting that of sister species *S. erubescens* (Nthimo, 1997; Marriott, 1998) from November through to January, with gonad recrudescence initiating in August towards the end of the rainy season (Nthimo, 1997). *Sedercypris calidus* deposits eggs among rock crevices in large spawning aggregations, as opposed to the egg-scattering of the true redfin minnows (*Pseudobarbus* spp.) (Skelton, 2001; van der Walt *et al.*, 2017c). Males develop nuptial tubercles on the head and have enhanced spawning colouration, similarly to other cyprinids.

Pseudobarbus phlegethon

Pseudobarbus phlegethon (Barnard, 1938), the fiery redfin, is endemic to the Olifants River and tributaries (Skelton, 1996). With fewer than ten populations remaining, the *P. phlegethon* is listed as Endangered on the IUCN Red List (van der Walt *et al.*, 2017a). *Pseudobarbus phlegethon* co-occur with Clanwilliam redfin, Clanwilliam yellowfish and the Clanwilliam rock catfish (*Austroglanis gilli*) in the Rondegat River, where they feed on detritus attached to the primarily rocky substrate and aquatic invertebrates (Skelton, 1996; Whitehead, Weyl & Bills, 2007; Garrow & Marr, 2012). Habitat preferences for this species are thought to be slower flowing pool environments with complex structure (Gore, King & Hamman, 1991). A preference for substrate foraging is suggested by this species' sub-terminal mouth orientation (Whitehead *et al.*, 2007). While detritus and algae have been found in gut content analyses of limited numbers of *P. phlegethon*, the gut physiology is better suited to a carnivorous diet of aquatic invertebrate prey (Whitehead *et al.*, 2007). The primary invertebrate food items include Baetid mayflies, Chironomidae, Trichopteran pupae and Coleopteran larvae based on gut contents collected from the Noordhoeks river population (Whitehead *et al.*, 2007). *Pseudobarbus phlegethon* tends to be smaller (up to 90 mm, with most individuals tending towards 45 – 55 mm (Whitehead *et al.*, 2007) and less abundant than *S. calidus* where they co-occur (Skelton, 1996). The two redfin species tend to form transient shoals without apparent aggression behaviours despite overlap in resource and territory use. *Pseudobarbus phlegethon* appears to take advantage of a long summer spawning season with multiple spawning events, with sexual maturity reached at sizes larger than 33 mm (Whitehead *et al.*, 2007). Males defend small territories wherein spawning takes place (Skelton, 1996) and may corral small groups of females into their territories (pers. obs.). Males have larger paired fins and develop bright red fin colouration when in breeding condition, and like *S. calidus* develop nuptial tubercles on the head area (Skelton, 1996).

Labeobarbus seeberi

The Clanwilliam yellowfish (*Labeobarbus seeberi*, formerly *L. capensis*; Smith, 1841) is classified as Vulnerable using IUCN classification criteria and is the largest freshwater fish of the CFE, attaining sizes of up to 1000 mm and 11 kg (Impson, Bills & Wolhuter, 2008; Garrow & Marr, 2012). In the Rondegat River, *L. seeberi* co-occur with redfin minnows, with greater numbers occurring only in the lower reaches, where they survived as older, larger individuals in deeper over-summering pools with a lack of recruitment success owing to the presence of predatory invasive fishes such as *Micropterus dolomieu* (Paxton, 2008; Weyl *et al.*, 2013; van der Walt *et al.*, 2016). *Labeobarbus seeberi* is likely to be dependent on these pools for both over-summering refugia as well as important congregation areas for drift and substrate feeding downstream of riffles (Impson *et al.*, 2008; Paxton, 2008). This species appears to have a more omnivorous diet than that of the other species of the Rondegat assemblage, with a fairly even representation of plant material and invertebrate prey in the limited gut content analyses available (Paxton, 2008). Primary invertebrate groups in gut content analyses by Woodford (2005) were found to be Chironomidae and Corixidae, with high levels of detritus ingested.

Labeobarbus seeberi is a multiple spawning, migratory and riffle-dependent species, making it particularly sensitive to habitat alteration. In addition, it is likely that temperature is a major component of spawning cues for this species, and releases of warmer water from upstream dams may adversely affect the timing of spawning, potentially affecting survival of the offspring (King, Cambray & Impson, 1998; Dallas, 2008). This species is faced with the additional conservation pressures of impacted migratory pathways and disrupted spawning locations owing to both climate-driven drought conditions and anthropogenic influences of water abstraction and damming in crucial riffle habitats (Impson *et al.*, 2008; Paxton, 2008). Damming, water abstraction and other anthropogenic events such as unseasonal fires in the Rondegat River are ongoing, all of which can affect water temperature (King *et al.*, 1998; Dallas, 2008), among other effects.

Austroglanis gilli (Barnard, 1943), the Clanwilliam rock catfish, is a relatively small (<14 cm standard length; hereafter SL) nocturnal species that occurs predominantly in headwaters with complex rocky habitats. It is listed as Near Threatened (van der Walt *et al.*, 2017b). *Austroglanis gilli* is slow-growing, reaching sexual maturity at around three years of age and at a length of approximately 10cm, with some individuals collected from the Rondegat River aged at over twelve years (Mthombeni, 2009). This *K*-selected life history makes *A. gilli* susceptible to habitat alteration, particularly sedimentation, which can result in the reduction of complex habitats that provide both protection from predators in environments where they co-occur with alien piscivores and suitable food sources (Chutter, 1969; Mthombeni, 2009; Garrow & Marr, 2012). The diet of *A. gilli* consists primarily of Ephemeroptera (predominantly baetid mayfly larvae), Diptera (Simulidae, Chironomidae) and Trichoptera (Mthombeni, 2009).

The hydro-geological and climatic history of the CFE river systems has given rise to a hotbed of fine-scale speciation and divergence events (Swartz, Flemming & Mouton, 2004; Swartz, Skelton & Bloomer, 2007, 2009; Chakona, Swartz & Gouws, 2013). *Sedercypris calidus* is likely to have differentiated from its sister species *S. erubescens* in the Doring catchment (Swartz *et al.*, 2004, 2009). Niche preferences, morphological and behavioural differences likely enabled greater dispersal ability in *S. calidus* over *P. phlegethon*, which itself underwent a divergence between the Olifants and Doring catchments (Swartz *et al.*, 2004, 2009; Skelton *et al.*, 2018).

1.1.4 Climate change and the thermal physiology of CFE fishes

As poikilothermic organisms, fish are strongly affected by the thermal environment. In particular, their metabolisms increase as temperatures increase, resulting in a hump-shaped relationship between feeding rate and temperature (Uiterwaal & DeLong, 2020). In rivers, the thermal regime has strong effects on growth rates and distributions of aquatic organisms (Caissie, 2006). This aspect is particularly pronounced in highly fragmented populations that are limited in dispersal and when limited thermal refugia are available. The thermal regime can be affected by natural fluctuations as well as human interference through direct thermal pollution and removal of vegetation or indirectly through anthropogenic climate change (Caissie, 2006). Mean daily temperature increases downstream and the rate of increase in temperature can be particularly significant in smaller streams, owing to their low thermal capacity. In addition to these spatial temperature gradients, headwater streams tend to have lower diel variation than larger streams (Caissie, 2006). Temperature and other environmental variability can affect fish populations through reproductive effects, such as altered triggers for gonad development and spawning behaviours; or recruitment effects, such as decreased egg and larval growth and survival through abnormal habitat conditions (Humphries & Lake, 2000; Paxton, 2008). As fish tend to be adapted to the average long-term conditions of their historical distributions, shifts in conditions beyond the usual scope of their adaptive history may have adverse effects on their ability to time reproductive cycles with prevailing conditions most conducive to successful recruitment and other effects on reproductive cues (Humphries & Lake, 2000; Poff & Zimmerman, 2010).

Mediterranean-climate freshwater biota are likely to be disproportionately affected by climate change, and shifts in distribution, community composition and life-history, particularly in the seasonality of physiological processes, will occur in response (Filipe *et al.*, 2013). To date, anticipated effects of climate change and the general thermal element of variable habitats within the CFE have received very little scientific attention, despite recognition internationally of the threats of climate change to freshwater ecosystems (Abell *et al.*, 2007; Filipe *et al.*, 2013; Comte *et al.*, 2013; Beatty *et al.*, 2017). An aspect of increasing importance in freshwater invasions is that of thermal preferences and tolerances of both the invasive and native organisms. Ellender et al. (2016) present a case focused on salmonid invasion potential based on thermal physiological limits in this group, which is one of the more impactful invasive fish groups within South Africa, particularly S. *trutta* and *O. mykiss*. These species, based on their thermal ranges, are suggested to have a large invasion debt within South Africa, despite having a thermally distinct native origin compared to the introduced range. However, climate change predictions for the Mediterranean-climate CFE region suggest a possible reduction in impact from stenothermic salmonid invasions as rising temperatures decrease suitable habitat both spatially and temporally (Shelton *et al.*, 2018c). It is therefore probable that warm-adapted alien fishes may become a greater threat under future invasion scenarios in the context of rising temperatures, despite the cold-adapted trout species currently being the most abundant invasive fish predators in many CFE systems (Shelton *et al.*, 2018c; Khosa *et al.*, 2019). Black bass have been highlighted as having a high invasion debt as a result of rising temperatures in some areas in tandem with increased dam construction in response to drought (Khosa *et al.*, 2019). The thermal threshold models utilised by Ellender et al. (2016) were also proposed to demonstrate utility in untangling distinct ecological niche utilisation by the two focal species, further improving predictive ability for conservation management efforts. Altitudinal-thermal preferences and extinction likelihood could be differentiated between *S. trutta* and *O. mykiss*, with further utility in climate change scenario applications and invasion potential mapping (Ellender *et al.*, 2016). These thermal thresholds, alongside other ecological limits, can be used to create more accurate invasion models and predict areas of high conservation priority (Ellender *et al.*, 2016; Khosa *et al.*, 2019).

Recent work predicts that many of the CFE fishes will be put under severe strain given "business as usual" climate change scenarios (Dallas, 2013; Dallas & Rivers-Moore, 2014; Shelton *et al.*, 2018b). *Galaxias zebratus* and fish of the genus *Pseudobarbus* (at the time of publication, *S. calidus* was positioned in the *Pseudobarbus* genus as a placeholder pending re-classification (Skelton *et al.*, 2018)) were found to be the most thermally sensitive (Reizenberg *et al.*, 2019). Reizenberg *et al.* (2019) suggest that most CFE species are differentiated in thermal tolerance at the species level, and have not diverged in this trait across river systems (i.e. at the population level). However, the thermal physiological limits of larger cyprinids such as *Labeobarbus seeberi* have not been examined. One key element arising from climate change predictions for southern hemisphere climate change is that river discharge will be strongly affected (Dallas, 2008, 2013; Dallas & Rivers-Moore, 2014; Beatty *et al.*, 2017). Annual rainfall in the south-western Cape is likely to decrease substantially (up to 20% by the end of the 21st century), combining with increased average temperatures and higher demand for water resources from human sources, thus straining an already water-starved region and adding pressure to vulnerable native fishes with highly limited distributions (Fauchereau *et al.*, 2003; Dallas, 2008, 2013; de Moor & Day, 2013; Dallas & Rivers-Moore, 2014; Beatty *et al.*, 2017; Ellender *et al.*, 2017). In the Western Cape, crucial winter rainfall is predicted to decrease by up to 25% (Dallas, 2008; Dallas & Rivers-Moore, 2014) and a shorter rainy season is anticipated (Fauchereau *et al.*, 2003; du Plessis & Schloms, 2017).

Mediterranean-climate areas such as the CFE are especially vulnerable to water diversion owing to their semi-arid and strongly seasonal flow fluctuations (Gasith & Resh, 1999; Filipe *et al.*, 2013; de Moor & Day, 2013). Fish assemblage responses are usually more strongly affected directly by flow variability as opposed to resource or biotic constraints (Gasith & Resh, 1999). Water abstraction and other flow-regulating anthropogenic activities are common in the lower reaches of CFE rivers, and the Rondegat River is one such example (Beatty *et al.*, 2017). de Moor and Day (2013) identify the increasing demand for abstraction as the most pressing threat to aquatic biota in the CFE. Extended drought conditions in recent years are a particular concern (de Moor & Day, 2013), and trends in rainfall going forward will be crucial for management. Rainfall forecasts tend to be less reliable than that of temperature forecasts, though some trends for the CFE region (and the greater Cederberg in particular) have become apparent (Lötter, 2015). General reductions in rainfall over the rainy season (chiefly June, August and October), alongside a shortening of the rainy season, is predicted between 2040 and 2070, tempered by increases in intense short-term rainfall events (Dallas, 2008; Lötter, 2015; du Plessis & Schloms, 2017). Climatic trends indicate that high intensity extreme rainfall events and thus flash flooding are increasing in frequency (Kruger & Nxumalo, 2017). Temperatures have been rising since the beginning of observations (1961) and the hottest 10 year period was consistently reported as the last decade (2000 – 2010) in multiple studies (Lötter, 2015). Maximum and minimum temperatures are likely to increase by up to 2.5°C between 2040 and 2070, and extreme temperature events are predicted to increase in frequency between December and March (Lötter, 2015). Comparing water temperatures of the Driehoeks River in the Doring system between 2005 and 2015 indicates a trend of increasing extreme temperature events (Reizenberg *et al.*, 2019), suggesting the potential for similar scenarios in the Rondegat.

1.1.5 The role of stressors in the context of the Rondegat River

Stressors are defined as novel or extreme environmental changes. Climate changes, such as increased temperatures and frequency of extreme events (e.g. droughts and flooding), as well as biological invasions and habitat destruction, can act synergistically wherein their cumulative effects are non-additive, i.e. “greater than the sum of their parts” (Jackson *et al.*, 2016a, 2020). Freshwaters have been highlighted as particularly sensitive systems in the context of multiple interacting stressors (Jackson *et al.*, 2016a). Multiple stressors have likely played a role in the majority of freshwater species declines, with approximately 81% of studies investigating impacts on freshwater species being found to have nonadditive interaction effects (Jackson *et al.*, 2016a). While management-aided removal of single stressors can result in positive results, defining and eliminating interacting stressors in tandem is likely to result in greater ecological recovery (Jackson *et al.*, 2016a). Below, I highlight some key processes and their related stressors in the context of freshwater streams.

Fausch et al. (2002) describe Schlosser's (1995) dynamic landscape model of stream fish life history: fish move from spawning/incubation habitat, into a mosaic of feeding habitats with favourable growth conditions, and then move into refugia from harsh environmental conditions (such as temperature, flow and turbidity). Each of these habitat classes are moved between over the lifespan of a fish, either to feed, take refuge, or spawn, often as the result of habitat changes and stressors. Streams are highly susceptible to various stressors and are strongly interlinked with their adjacent riparian ecosystems (Fausch & Bramblett, 1991; Fausch, Baxter & Murakami, 2010). Stressors are often tested individually but act synergistically, and can act both directly on the focal fish species and indirectly through changes to prey abundance (Fausch *et al.*, 2010; Jackson *et al.*, 2016a). Furthermore, the characteristics of individual species shape their responses to stressors in often markedly different ways (Fausch *et al.*, 2010). Altered food subsidies can affect fish at the behavioural level (i.e. individual differences) as well as at the population level (i.e. abundance and life history changes). Further, the propensity for diet shifts under stressor-induced conditions as a species trait must be considered (Fausch *et al.*, 2010); for example, in the context of altered terrestrial food subsidies. Species-specific behaviour may alter the strength of stressor effects on resilience; for example, if a fish is readily able to shift prey choice in response to changes in availability (shift from benthic to drift feeding or vice versa depending on stressor involved).

Resource partitioning can be understood as a mediator of species coexistence within ecosystems (Pool *et al.*, 2017). The trophic niche overlap between species can provide an estimate of resource segregation within an environment and can aid in the understanding the structure of a given assemblage. The high dietary plasticity of fishes makes them useful model organisms for exploring how seasonal changes may influence other constituents of food webs in aquatic ecosystems (Pool *et al.*, 2017). Following from the phenomenon of non-additive effects resulting from multiple stressors, these effects can impact on food webs, for example through trophic cascades (Jackson *et al.*, 2017, 2020). Different stressors can have varying strengths of effects in both terrestrial and freshwater systems, with top consumers being most often adversely affected (Jackson *et al.*, 2020). Stable isotope analysis has proven to be a robust method to investigate trophic interactions by using ratios of stable isotopes in animal tissues as a proxy of trophic niche width (Bearhop *et al.*, 2004). Stable isotope analysis has been used to test predictions of impacts from multiple stressors, with land use and climate change effects interacting to alter food webs in South African streams (Jackson *et al.*, 2020; Comte *et al.*, 2021). Jackson et al. (2020) suggest that the predicted decreases in rainfall in South Africa are likely to result in higher vulnerability of freshwater vertebrates to land use patterns such as agriculture. In a literature review of trophic shifts in fishes from seasonally flooding environments and stable isotope analysis of a Cambodian lake, McMeans *et al.* (2019) found a diverse range of responses in foraging behaviour, being highly dependent on the local ecosystem context. Nutrient cycling can be highest in headwaters, particularly in terms of nitrogen productivity, highlighting the importance of headwaters in supporting fisheries downstream (Colvin *et al.*, 2019). Investigating a headwater stream system with a focus on three sympatric fish species, Christian and Adams (2014) found higher partitioning of resources occurred in drying pools between the three species, as well as a lengthening of the gastrointestinal tract in response to isolated pool environments. This showed the potential for rapid phenotypic changes in response to shifting prey availability (Christian & Adams, 2014). While the Mediterranean climate of the CFE may not experience the extremes of tropical seasonal flooding events, winter rainfall spates and resultant flooding are likely to be significant drivers of resource availability within the oligotrophic Rondegat River. Consequently, increasing temperatures, decreasing rainfall and increasing agricultural land use are likely to interact as multiple stressors and impact the food webs of freshwater systems (Jackson *et al.*, 2020; Comte *et al.*, 2021), including the Rondegat River.

Evidence of major sand deposition on the banks of the Rondegat River, following a fire event and heavy rainfall.

In the context of a drought-prone and seasonally variable river system, stream flow is of paramount importance as a “master variable” in terms of abiotic drivers of abundance and distribution (Bunn & Arthington, 2002; Poff & Zimmerman, 2010). The natural stream flow regime can be viewed as one of the abiotic filters that acts in tandem with other filters (such as functional traits and their effects on performance) that dictate the distribution of stream fishes (Poff, 1997; Weiher *et al.*, 2011). The strength of these filters is highly variable across the physical and temporal dimensions. For example, Fausch and Bramblett (1991) describe a system of canyon tributaries in Colorado that are characterised by isolated pools in low-flow scenarios, punctuated by flash floods in the summer. The extreme disturbance patterns of this system have not prevented the successful dispersal of fish, and fish presence or absence is not dependent on the permanence of a given reach, with fish absent in some permanent pools and abundant in pools which were demonstrably ephemeral (Fausch & Bramblett, 1991). Across systems, fish consistently react negatively to alterations of flow magnitude (Poff & Zimmerman, 2010), for example as a result of damming or unseasonal flooding. The life history traits of some species can affect their ability to cope with altered flow, particularly drought-like conditions as a result of human activity (Matthews & Marsh-Matthews, 2003). Damming and other human activities can have strong effects on flow as well as affecting water quality, with associated effects on fish population health, in addition to reducing connectivity of habitats for migratory fishes (Doeg & Koehn, 1994; Beatty *et al.*, 2017). Small-scale damming and the associated effects on flow downstream, as well as weir failures or removals releasing sediment downstream, are a common situation in the middle and lower reaches of the Rondegat River (Woodford, 2005).

While droughts are natural events, with variations in frequency and intensity, human activity in and around river systems can cause disturbances with similar effects (Magoulick & Kobza, 2003). Land use such as agricultural activity has a particularly strong effect as a stressor in freshwater environments through water abstraction and flow disturbance (Schlosser, 1995; Jackson *et al.*, 2020). Unpredictable flow can strongly affect recruitment success in flow-dependent spawning species (Colvin *et al.*, 2019). Conversely, damming and irrigation on rivers can in some cases act as anthropogenic refugia, in the form of deeper pool-like refuge habitats that otherwise might not have been available under drought conditions (Chester & Robson, 2013). In the Rondegat River, multiple small weirs and dams in the lower river reaches are often host to high densities of native fishes, which tend to favour pool environments over flowing runs when available (Woodford, 2005). Despite these artificial refugia, the active abstraction of water that these weirs facilitate is likely to exacerbate already-worsening natural drought effects. The physicochemical stressors and reductions in viable habitat produced by drought can lead to higher incidences of competition in available refugia as fish densities increase (Magoulick & Kobza, 2003; Castañeda *et al.*, 2020a). It is during these “crunch” periods in low-flow conditions within refugia wherein the greatest competition for diminished resources should occur. Usage of refugia is likely to affect population and fish community dynamics, with the size of available refugia and the mobility of the affected fish community being important in ensuring the survival of populations (Magoulick & Kobza, 2003). Fish density in stream refugia through dry periods and during recovery from alien fish invasion was found to be particularly important for the recolonisation of habitat (Castañeda *et al.*, 2020a).

Fish resilience within river reaches is likely enhanced by deep and complex habitats that can be used as refugia during times of both increased (e.g. flash floods) and decreased (e.g. drought) flow (Fausch & Bramblett, 1991). Large, deep and complex pool environments appear to be particularly important in the context of *L. seeberi* during the dry season in the CFE (Paxton, 2008; Weyl *et al.*, 2013). The Rondegat river is currently recovering from a major drought, with areas below a 2m weir designed to prevent re-invasion from the Olifants River (Lowe *et al.*, 2008; Weyl *et al.*, 2013) consisting of isolated pools with confirmed alien fish presence (as of April 2018, the alien fishes in this reach included *Tilapia sparmanii, Lepomis macrochirus* and *Clarias gariepinus*). Summer drought is exacerbated by strong and persistent south-easterly winds that desiccate the landscape and vegetation. These south-easterly winds also increase the probability and intensity of fires in the region (de Moor & Day, 2013). Mediterranean climate areas tend to burn at natural frequencies to which the vegetation is adapted during hot dry summer months. Southey (2009) found that the Cederberg region has a propensity to burn at intervals of less than five years under the influence of anthropogenic accidental ignitions. Natural ignition events vary from 5 to 20 years between fires (Southey, 2009), suggesting that the local vegetation and river fauna are likely adapted to relatively frequent fire events. Vegetation at the time of the February 2018 fire was approximately 50 years from the most recent burn (pers. comm. JH Nieuwoudt), possibly making the fire more intense and damaging to the environment than the less intense but higher frequency of fires usually encountered in the region. Different areas of the river landscape were affected in this fire event than those of the 2009 event, which resulted in progression of a sand slug downstream (DJ Woodford pers. comm.). The fire events of early 2018 and increased rainfall over the winter months is likely to result in high turbidity and altered physical characteristics through the release of a sand slug downstream of burnt areas, as was the case in previous years as a result of fire events followed by winter rainfall.

Burnt and recovering vegetation on the banks of the Rondegat River.

The direct effects of fire on smaller 1st + 2nd order streams are often more pronounced than in larger rivers (Lyon & O'Connor, 2008). Fire can result in multiple concurrent stressors in river environments, such as increased temperatures from reduced riparian vegetation, siltation of pools, altered nutrient inputs from burnt material, as well as altered flow regimes as a result of shifts in vegetation water uptake demands (Bozek & Young, 1994; Burton, 2005; Lyon & O'Connor, 2008). Alongside these, physicochemical changes such as increased pH and decreased O_2 have the potential for to lead to fish kills, especially in smaller streams. Less direct effects of fires may include habitat alteration through reduction of woody debris, followed by increases in woody debris and rocky environments as a result of sediment inputs in later flood events (Burton, 2005; Ebner *et al.*, 2009, 2014). Sediment loads often peak in post-fire rainfall flash flooding as a result of multiple fire-related soil erosion-enhancing effects (Bozek & Young, 1994; Burton, 2005; Lyon & O'Connor, 2008; Ebner *et al.*, 2014). Sediment slugs can in many cases cause near-complete local extirpation of fish populations and loss of habitat complexity as a result of siltation (Chutter, 1969). Habitat and substrate complexity in particular are important components in the reduction of predation success in fish (Swisher, Soluk & Wahl, 1998). Increased siltation and sand deposition will affect all fish species in the Rondegat assemblage; further to the removal of suitable habitat for *A. gilli*, all three cyprinid species are dependent on rocky, complex habitats (redfins) or riffles (yellowfish) for successful spawning. The *P. phlegethon* population of the nearby Noordhoeks River has been impacted by such siltation of suitable habitat brought about by anthropogenic actions (Whitehead *et al.*, 2007). In addition to sediment slugs, a major danger to fish from fire events is increased barriers to re-colonisation of affected areas and their subsequent re-population success during recovery. Thus, the need for barriers to prevent alien fish from extirpating native fish may inadvertently affect successful recovery after fire events (Burton, 2005). Sediment slug formation and siltation of habitats can be a delayed process (Bozek & Young, 1994; Lyon & O'Connor, 2008); which was the situation in the Rondegat in February 2019, a year after a major fire event. Delayed mortality following fire events as observed by Bozek and Young (1994) is a notable conservation concern, as direct fish kills multiple years after an initial event can be devastating and unpredictable. Populations of *G. zebratus* are very small and limited in distribution in the Rondegat River (most recently found in one near-stagnant pool <5km from the river source, with no other reported sightings in the last decade; pers. obs.), and fire events in the upper reaches of the Rondegat may lead to local extirpation. Isolated populations are particularly important for conservation in *G. zebratus*, as very fine-scale differentiation and speciation is commonly observed in this putative species complex (Wishart *et al.*, 2006). The limited distribution and low number of known populations of *P. phlegethon* also warrant attention in the context of fire events affecting the Rondegat River.

Australian freshwater systems and aquatic fauna encounter similar challenges to those of South African systems, and are useful as a knowledge base and template for conservation and restoration actions locally (Lintermans, 2013). Australian native freshwater species are often threatened by a similar suite of threats including alien fish invasions, drought, fire and other anthropogenic impacts, and some survive in fragmented refugia (Koehn & Lintermans, 2012; Saddlier, Koehn & Hammer, 2013; Ward *et al.*, 2020), reflecting the plight of many South African species. Saddlier et al. (2013) provide an account of the conservation and restoration approaches towards confronting the decline of imperilled small native fishes in south-eastern Australia. Important milestones for the restoration of these highly vulnerable species are applicable to South African native species, especially in the context of moving from monitoring programmes into sustainable conservation action. Ten recovery plan objectives are outlined by Saddlier et al. (2013), and below I highlight and briefly describe the progress on these objectives adapted for the Rondegat River fish community.

Objective 1: Determine distribution and abundance

This objective is now well established for the Rondegat River fish community, with monitoring data provided in multiple publications and reports (Weyl *et al.*, 2013, 2014, 2016; Castañeda *et al.*, 2020a) and is covered in Chapter 2 of this thesis. The Rondegat River community is essentially captured within the river itself, without the potential for cross-system migration as a result of the river draining into the highly invaded Clanwilliam Dam and having physical barriers to movement near to the confluence. The *S. calidus* and *L. seeberi* populations are understood to be at equilibrium in terms of recovery and recolonisation of previously invaded areas, while *P. phlegethon* is still in a state of recovery (Castañeda *et al.*, 2020a). The *A. gilli* and *G. zebratus* populations are less well-understood, but are likely to respond to similar conservation interventions, such as prevention of further invasions and habitat degradation through maintaining pristine and protected habitats and prevention of siltation where possible.

Objective 2: Determine the genetic and taxonomic status of populations

The genetic identity of the assemblage is relatively well-defined, though the potential for taxonomic divergence does exist, particularly in the *G. zebratus* population. *Pseudobarbus phlegethon* in the Rondegat is recognised as belonging to the primary western CFE lineage, and is not part of the diverging "Doring" lineage (Swartz *et al.*, 2007, 2009). Similarly for *S. calidus*, the Rondegat population is distinct from *S. erubescens* as a sister species and *L. seeberi* is likewise understood to be a single taxonomic unit (Skelton *et al.*, 2018). However, finer-scale genetic variation between populations across tributaries of the ODRS is possible and is a potential avenue of further research.

Objective 4: Identify and manage potentially threatening processes affecting conservation

The Rondegat River alien species eradication programme represents the most significant direct freshwater conservation intervention in the CFE (Weyl *et al.*, 2014), and was essentially the first step in accomplishing this objective. As mentioned previously, the eradication programme was accompanied by clearing of alien vegetation and construction of more significant barriers to potential alien fish incursions. Other important conservation concerns include more frequent fires, potential pollution and habitat degradation in the lower reaches, as well as global change leading to impacts such as droughts. These are all longer term and less urgent than the (now managed) alien fish invasion, and will require ongoing monitoring, maintaining and potentially expanding the existing protected area covering the upper reaches of the river.

Objective 5: Protect key populations across the range

Beyond the Rondegat River, there are multiple populations of *S. calidus*, *L. seeberi* and *P. phlegethon* which are fully or partially managed within protected areas (Impson, van der Walt & Jordaan, 2017; van der Walt *et al.*, 2017a, c). However, *P. phlegethon* in particular has very few extant and fragmented populations, and ongoing attention and review of possible interventions to conserve these populations should be encouraged. There still remains scope for improvement and expansion of these efforts; for example, the formally protected area in the Rondegat River covers only a fraction of the range of *L. seeberi* across the longitudinal cross-section, while the other four fish species are relatively well represented in the protected section. Further engagement between the local conservation authority and private land owners along the banks of the more vulnerable lower reaches of the river, where *L. seeberi* is more abundant, could present an improvement in the local conservation of this species.

Objective 6: Determine population trends at key sites

As previously mentioned, population trends of the Rondegat River fish assemblage have been relatively well elucidated through continued monitoring efforts and quantified through modelling of occupancy dynamics (Weyl *et al.*, 2013, 2014, 2016; Castañeda *et al.*, 2020a). Increased monitoring of populations of these species in other systems is encouraged using similar monitoring programmes. To this end, a standardised underwater video sampling protocol, such as that adopted for snorkelling surveys (Ellender *et al.*, 2012), is a valid direction for research.

Objective 7: Investigate key aspects of biology and ecology

This objective is a primary focus of this thesis. While some aspects of the biology and ecology of the Rondegat River species are known, efforts are varied in depth and breadth and have significant gaps in knowledge (Ellender *et al.*, 2017). A deeper understanding of the functional morphology, habitat, population, competitive and trophic dynamics, as well as behavioural aspects, are required to develop a more holistic overview of the conservation prognosis for this community. In particular, consideration of the contributions of these aspects to the vulnerabilities to global change and anthropogenic impacts on the Rondegat River community, and a better understanding of its resilience, is needed.

Objective 8: Establish a captive breeding population

Captive breeding of vulnerable CFE species, including those of the Rondegat community, has been a consideration in proposed management plans and scientific reporting in recent years: *L. seeberi* was temporarily captive bred and reintroduced within the Olifants system (Cambray, King & Bruwer, 1997; Swartz, 2000), while *P. phlegethon* (Skelton, 1996), *A. gilli*, *A. barnardi* and *S. erubescens* (Bills & Impson, 2013) have been suggested or tested as candidates for this approach. This approach has had success elsewhere, such as in the case of small fishes threatened by drought conditions in Australia (Lintermans *et al.*, 2015). However, there is an argument for captive breeding populations to be considered a “last resort” owing to the risks of loss of genetic diversity (Swartz, 2000). The establishment of captive breeding populations of highly imperilled species should be encouraged in both research and implementation as a “backstop” in case of future catastrophic population losses where other interventions are unlikely to succeed (Jordaan *et al.*, 2017b). This is particularly true for the most vulnerable CFE species such as *S. erubescens*, *P. burgi* (Boulenger, 1911), genetically distinct populations (e.g. the Cederberg Krom River population of *A. gilli* and *P. phlegethon* “Doring” lineage) and others. To this end, a captive population of *S. erubescens* has been successfully established in the Tuinskloof Dam from an original translocation in 2005 (Jordaan *et al.*, 2017b). However, further investigation is required to better understand the extent to which the small initial population may contribute to a genetic bottleneck in the captive population (Jordaan *et al.*, 2017b). Lessons from the captive breeding of *S. erubescens* may prove useful in the event that captive breeding of *S. calidus* is considered.

Objective 9: Establish new populations

As expanded upon in the previous objective, this objective has had limited success in the South African context. Again, introductions of captive-bred fish should be retained as a last resort option in the conservation toolbox for CFE freshwater species owing to potential impacts on genetic diversity. Similarly, translocations of species to habitats where they previously existed must be done with extreme caution, as well-intentioned translocations have previously led to unforeseen impacts on other species (Impson & Tharme, 1998; Impson *et al.*, 2007, 2008). Similarly to captive-bred fish restocking, translocation of wild fish between systems has its own potentially negative implications for genetic diversity and local adaptation (Impson & Tharme, 1998).

Objective 10: Increase community and stakeholder awareness and involvement

The Rondegat River and its history as the site of an alien fish eradication project has generated fairly significant community and stakeholder engagement, initially as a controversial discussion point with negative public perceptions prior to effective open engagement (Marr *et al.*, 2012; Weyl *et al.*, 2014). More recently, the designation of *L. seeberi* as a flagship conservation species in the CFE, awareness efforts by local conservation authorities and interest groups, and strong local land owner engagement have all contributed to the general perception of relatively high local awareness and involvement.

1.2 Research approach and thesis outline

Understanding the dynamics and responses of understudied vulnerable fish communities in the wake of restoration efforts is the overarching goal of this thesis. To do this, the CFE in general and the Rondegat River in particular, are used as a microcosm and natural experiment with which to explore the application of multiple intertwined theoretical frameworks in the context of imperilled headwater stream fish communities. This thesis investigates the interplay of biological theory as applied to a fragile and recovering system marked by high endemism and multiple interacting stressors (illustrated in Fig. 2). I investigate community assemblage theory through habitat associations and spatial niche partitioning of co-occurring fishes, resilience to global change, habitat loss and future invasion scenarios, inter- and intraspecific competition, trophic niche partitioning, ecomorphological feeding capacity and feeding behaviour to develop a holistic understanding of a keystone fish community in the context of South Africa's freshwater conservation and restoration efforts.

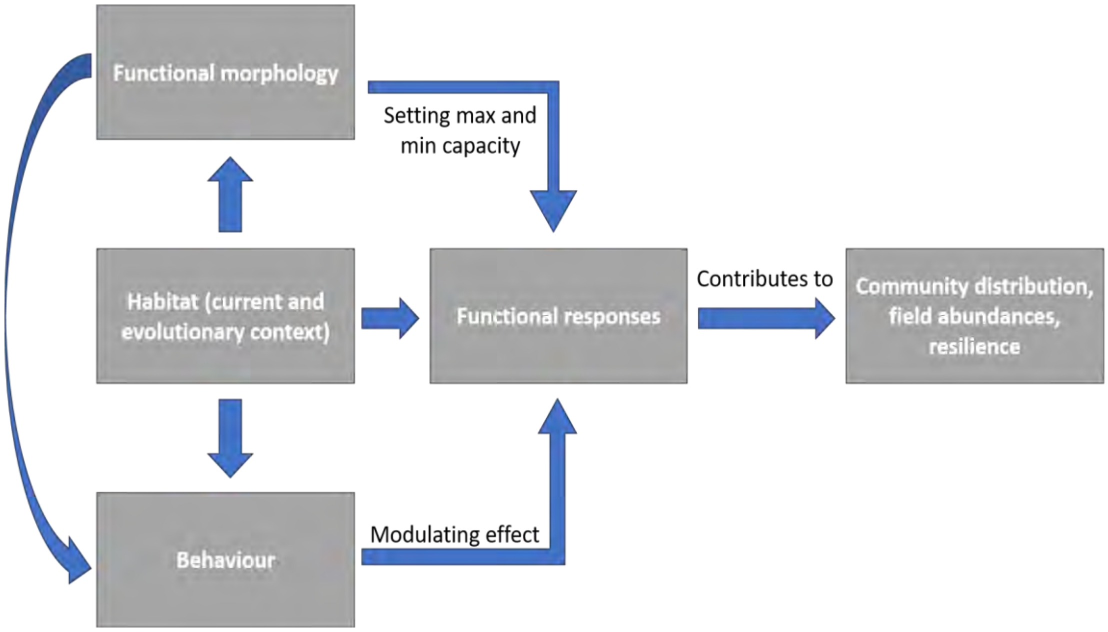

Figure 2: The interplay of the core study areas of the thesis. Habitat, functional morphology, behaviour and functional responses are defined here in separate chapters. I demonstrate here the recognition of the overlaps and synergy inherent across these broad disciplines and lines of inquiry as they relate to the current observed distribution and abundance of the Rondegat River cyprinid assemblage and contribute to its resilience in the face of current and future threats.

In the pursuit of these core areas of inquiry, the thesis has been divided into six chapters. Four of these (Chapters 2 – 5) are data chapters that have been or are intended to be submitted for publication and are thus formally divided into Introduction, Methods, Results and Discussion sections based on the specific investigations. The chapters are individually described below.

Chapter 1 provides the contextual background and underpinnings of the CFE and introduces the Rondegat River as a case study, focusing on the endemic cyprinid assemblage as the primary focal unit throughout the thesis. Current knowledge on the CFE itself, the history of the Rondegat River, particularly in its role as a natural experiment for non-native fish eradication and restoration efforts, native species profiles, and an overview of the contemporary and predicted threats to the system and its inhabitants are provided.

Chapter 2 focuses on the habitat associations, abundance and spatial segregation of the Rondegat River cyprinid assemblage. I used underwater video data collected as part of my contributions to the continued monitoring of the river following the non-native fish eradication programme initiated in 2012. This work is essentially an extension of the long-term monitoring programme and previous examinations of various aspects of the recovering fish community primarily relating to abundance and signals of recovery. Primary examples include Weyl et al. (2013, 2014), and more recently Castañeda et al. (2020a). This approach was inspired by pioneering South African studies into underwater video as a powerful method of inquiry for investigating similar hypotheses, for example Ellender et al. (2012) and Hannweg et al. (2020). Knowledge of imperilled and recovering fish habitat associations under ongoing stressors such as increased turbidity and sedimentation following habitat degradation, fire and drought, are particularly important for assessing the resilience and predicting responses of these species. The importance of protected areas and maintenance of pristine environments for the sustainable recovery of these species is a major consideration in this chapter.

Chapter 3 used a laboratory trial experimental approach to compare the relative feeding performance of *S. calidus* and *L. seeberi* on two different prey types and at two field representative temperatures. Feeding performance was characterised using functional responses (FRs), the relationship between rate of prey consumption and prey density. Functional responses are a useful comparative tool for establishing the relative differences between populations or species of interest. Functional responses are context-dependent and thus powerful for fine-scale comparison for prediction of impacts under relevant field-representative conditions. For these reasons, I intend to use FRs to investigate the competitive differences between two Rondegat River species in terms of their resource uptake rates, and provide insights into the mechanisms of the patterns of spatial co-existence of these species. In addition, testing these responses on differing prey allows for greater understanding of trophic niche separation driven by differential feeding performance. Finally, testing FRs under differing temperature treatments allows for prediction of future changes in performance and competitive dynamics under scenarios of global change.

This chapter has been published: Broom C.J., South J. & Weyl O.L.F. (2021). Prey type and temperature influence functional responses of threatened endemic Cape Floristic Ecoregion fishes. *Environmental Biology of Fishes* **104**(7), 797–810.

Chapter 4 investigates the ecomorphology of all three endemic cyprinids of the Rondegat River assemblage. This approach allows for the integration of functional morphological feeding capacities based on a food-fish model (FFM; Sibbing and Nagelkerke, 2001) with gut content analyses to test predictions of differences between species in feeding capacity as well as actual dietary choice. In essence, the internal and external morphology (focusing on measurements of structures involved in detection, capture and ingestion of food) of the focal fish is used to develop trophic profiles (the capacity to exploit a certain food type) for varying prey types available in the environment. This is particularly useful to better understand how functionally similar headwater stream fishes are partitioning resources and thus reducing interspecific competition through morphological differentiation. Knowledge of the feeding capacity and historical diet of the recovering fish assemblage will allow for better prediction of future vulnerability to global change-induced shifts in prey availability. In particular, this approach provides insights into the contribution of functional morphology to the overall patterns of abundance and distribution, complementing the findings on feeding performance and habitat associations established in the previous chapters.

Chapter 5 explores the feeding behaviour of the Rondegat cyprinid assemblage based on observations extracted from underwater video. Underwater video is a useful method for recording in situ behaviour in clear freshwater streams, and has advantages in terms of reduction or elimination of the observer effect and is particularly valuable as a low-impact method for field observations of vulnerable species. This chapter focuses on differences between fish species in terms of differences in feeding patterns, the use of microhabitat in acquisition of food items, and the types of food items being preferentially targeted by the focal species. Optimal foraging theory and mechanisms of assemblage structure based on differences in species behaviour, particularly in the context of drift feeding stream-dwelling cyprinids, are investigated and can be contrasted with expectations based on ecomorphology, habitat and feeding performance findings of the previous chapters. The findings of this chapter are intended to bridge the theoretical divide between the conclusions of the previous chapters and explore departures from expectations following from theory and prior knowledge. Essentially, the actual in-situ behaviour of the focal assemblage is tested here and the findings thereof will be contrasted with the inferences based on other lines of evidence.

In Chapter 6, I summarise the findings of the previous four data chapters and relate the conclusions of these chapters to the background knowledge outlined in Chapter 1. The findings of the previous chapters are used here as a synergy of multiple lines of evidence to answer baseline theoretical questions on biology and ecology of a recovering endemic fish assemblage. Moreover, I use these findings to predict the responses of these species to various contemporary and projected stressors and threats. Finally, this synthesis considers the case for the sustainable conservation of the Rondegat River system in light of its recovery from non-native fish invasion, as well as the applicability of these findings to the greater CFE region and other imperilled native fish communities.

CHAPTER 2: Habitat associations and abundance of the Rondegat River cyprinid assemblage

The author carrying remote underwater video equipment at the Rooidraai waterfall, in the Rondegat River. Photo: Josie South

2.1 Introduction

Freshwater systems are globally threatened as a result of the increasing and multiple stressors acting upon them (Tickner *et al.*, 2020). In particular, climate change, habitat degradation and invasive species have deleterious effects on aquatic biodiversity (Tickner *et al.*, 2020). Impetus is thus on conserving species which are susceptible to anthropogenic disturbance as a result of endemicity and range restriction as they are constrained in capacity for adaptation. In some cases, restoration interventions can be applied. These include removal of predatory alien invasive species (Slabbert *et al.*, 2014), translocation of populations threatened by habitat change (Hammer *et al.*, 2013; Sayer *et al.*, 2020), or habitat restoration (Lyon *et al.*, 2019). Threat amelioration allows vulnerable fish species to recover; however, to ensure the success of an intervention, robust prior knowledge regarding population dynamics and habitat associations is essential (Van Liefferinge *et al.*, 2019).

The Rondegat River, a tributary of the Olifants River, is of significant conservation value as it hosts five vulnerable endemic Cape Fold Ecoregion (CFE) species (Woodford *et al.*, 2005; Weyl *et al.*, 2013). A major conservation intervention was undertaken through 2012 and 2013, when the piscicide Rotenone was used to remove all aquatic fauna below the Rooidraai waterfall, with the aim to remove alien predatory fish species (Slabbert *et al.*, 2014). The imperilled fish populations have shown considerable recovery within the Rondegat. Two of which, the Clanwilliam yellowfish *Labeobarbus seeberi* (Gilchrist & Thompson, 1913) and the Clanwilliam redfin *Sedercypris calidus* (Barnard, 1938) have reached equilibrium; whereas the Fiery redfin *Pseudobarbus phlegethon* (Barnard, 1938) is yet to (Castañeda *et al.*, 2020a). Despite the successful removal of the alien species threat, the Rondegat River is susceptible to other pervasive disturbances which need to be assessed in order to ensure the persistence of the newly recovered community. Within the Rondegat River, habitat change due to agricultural activities and climate related change such as increased fire events and drought are of particular concern. The long-term viability and persistence is further impacted by the fish species' limited distributions and the vulnerable status of the still recovering Fiery redfin population (Castañeda *et al.*, 2020a). Now that biotic predation threats have been eliminated it is essential to begin to establish the abiotic factors which may ensure the continued recovery of the three cyprinid species. The pre-existing data (i.e. prior to intervention and during recovery phase) is not suitable to draw conclusions as the fish assemblage was not in a natural state, and the studies were conducted using methodology with low accuracy or detection probability, such as snorkel surveys or seine netting (Vaughan & Ormerod, 2010). Long term monitoring with suitable adaptive management after restoration interventions is often neglected which negates the substantial investments in the affected systems (Lintermans, 2013).

I aimed to determine the habitat associations and co-occurrence patterns of the recovered Rondegat River fish community. This follows the recommendations by Castañeda et al. (2020a) and compiles evidence to advise and further monitoring and restoration in the catchment. The fish assemblage at the time of monitoring may be considered as the nearest proxy for a baseline natural state of the river and ought to be assessed prior to further decision making (Castañeda *et al.*, 2020a). In doing so, I also demonstrate the benefits of a novel, low cost, rapid and non-destructive underwater camera methodology to gather high-resolution habitat associations across a longitudinal gradient, both in and out of a protected area. In similar South African headwater stream environments, underwater video has a higher detection probability of threatened fishes than both eDNA and snorkel surveys (Castañeda *et al.*, 2020b a). Thus, it has the potential to be a viable and informative tool in a financially strained conservation landscape. Thus I used three multivariate methods to showcase how the camera data can be used, highlight limitations, and address the environmental factors which drive: 1) community composition, 2) species specific relative abundance and 3) presence/absence of the imperilled cyprinid assemblage. The information is used to recommend further adaptive management of the catchment.

2.2 Methods

Study species and knowledge prior to recovery

Sedercypris calidus – formerly *Barbus* and *Pseudobarbus calidus* (Skelton *et al.*, 2018) – the Clanwilliam redfin minnow, is evaluated as Near Threatened using IUCN Red List criteria (van der Walt *et al.*, 2017c). This species is only distantly related to the fiery redfin (*Pseudobarbus phlegethon*) with which it co-occurs. In the Rondegat River, *S. calidus* is the more abundant of the two redfins present and has previously been associated with deeper pool habitats via snorkel surveys (Woodford et al., 2005).

Pseudobarbus phlegethon, the fiery redfin, is endemic to the Olifants River and tributaries (Skelton, 2001 pp 126; Skelton, 1996). With fewer than ten populations remaining, *P. phlegethon* is listed as Endangered on the IUCN Red List (van der Walt *et al.*, 2017a). Habitat preferences for this species are thought to be slower flowing pool environments with complex structure. This is based on legacy snorkel survey data and observations of populations in similar Olifants River tributaries, the Thee and Noordhoeks rivers (Gore *et al.*, 1991).

The Clanwilliam yellowfish (*Labeobarbus seeberi*, formerly *L. capensis*) is the largest fish in the system, and is classified as Near Threatened using IUCN classification criteria (Impson *et al.*, 2017). Prior to alien fish removal *L. seeberi* populations survived in the lower reaches as older, larger individuals in deep over-summering pools with little recruitment success (Woodford, 2005; Weyl *et al.*, 2013; van der Walt *et al.*, 2016). *Labeobarbus seeberi* is likely to be dependent on these pools for both over-summering refugia as well as important congregation areas for drift and substrate feeding downstream of riffles (Woodford, 2005; Impson *et al.*, 2008). *Labeobarbus seeberi* is a multiple spawning, migratory and riffle-dependent species, making it particularly sensitive to habitat alteration.

Study area and sampling methods

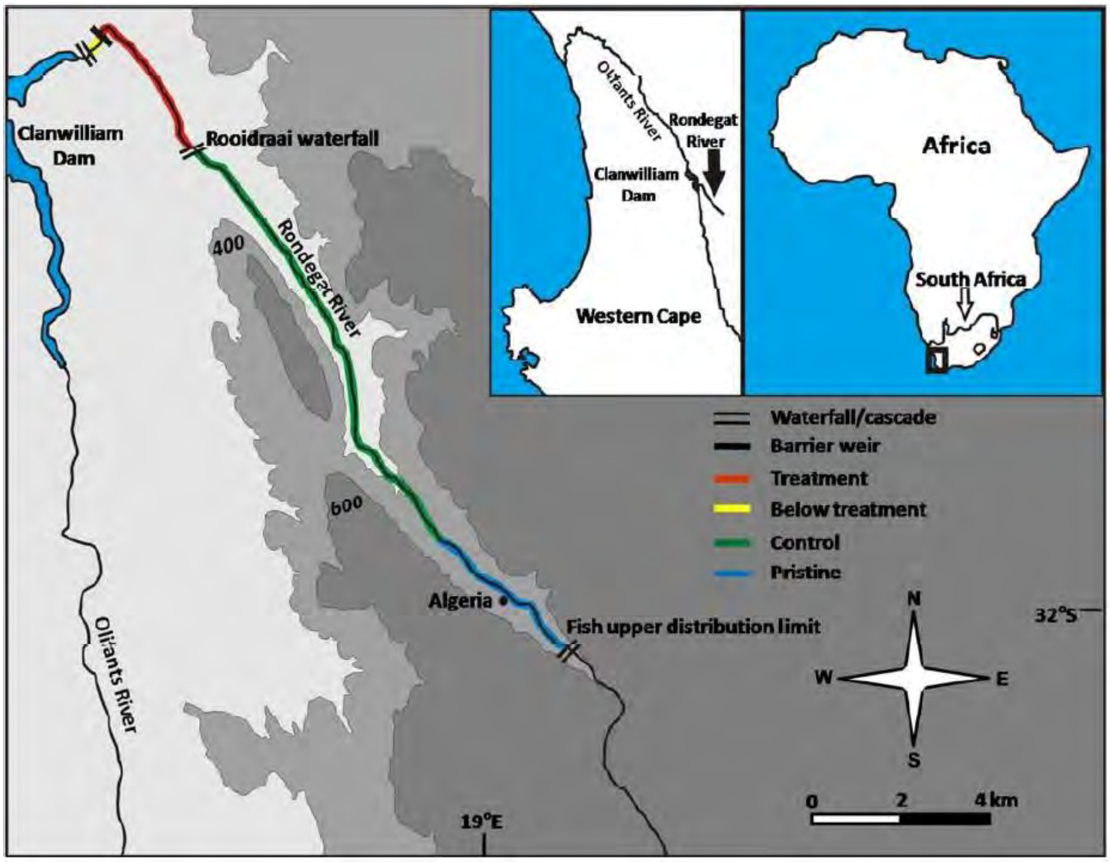

Figure 3: Map of the study system (Rondegat River, Cederberg, South Africa). Important physical barriers to fish movement and the designation of treatment, control and pristine segments of the river is shown. White areas indicate elevation of 200 – 400m, light grey: 400 – 600m, and dark grey areas 600m+. Figure adapted from Castañeda et al. (2020a).

The Rondegat River (32°24'S; 19°05'E) is a 25km long 2nd order perennial tributary of the Olifants River, with the point of confluence at the Clanwilliam Dam (Lowe *et al.*, 2008; van der Walt, 2014) (Fig. 3). The river originates as a pristine headwater stream in the Cederberg Mountains, with the uppermost 4km of the river being classified as a protected area by the local conservation authority CapeNature. The river progresses through undisturbed fynbos vegetation and passes through citrus fruit orchards encroached by alien vegetation in the mid- and lower reaches (Lowe et al., 2008; Woodford, 2005). Typical habitat in the Rondegat River ranges from predominantly cobbled and boulder-dominated upper reaches, to sandy and silted substrates in the abstracted farmland lower reaches. The river is divided in part by the natural Rooidraai waterfall, as well as anthropogenic barriers including an invasive fish exclusion weir near to the confluence at the Clanwilliam Dam and various weirs associated with farmland abstraction sites. The catchment area covers approximately 111 km^2 and is characterised by winter rainfall falling primarily from June until August (Lowe *et al.*, 2008). Temperatures in the CFE are hottest in February and coldest in June (de Moor & Day, 2013). Winter rainfall restricts sampling efforts to months of lesser flow for all sampling methods as a result of high water velocity, turbidity and flooding risks (October – April). In early February 2018, a large unseasonal fire and increased rainfall over the winter months is likely to have resulted in high turbidity and altered physical characteristics through the release of a sand plug downstream of burnt areas (Fig. 4).

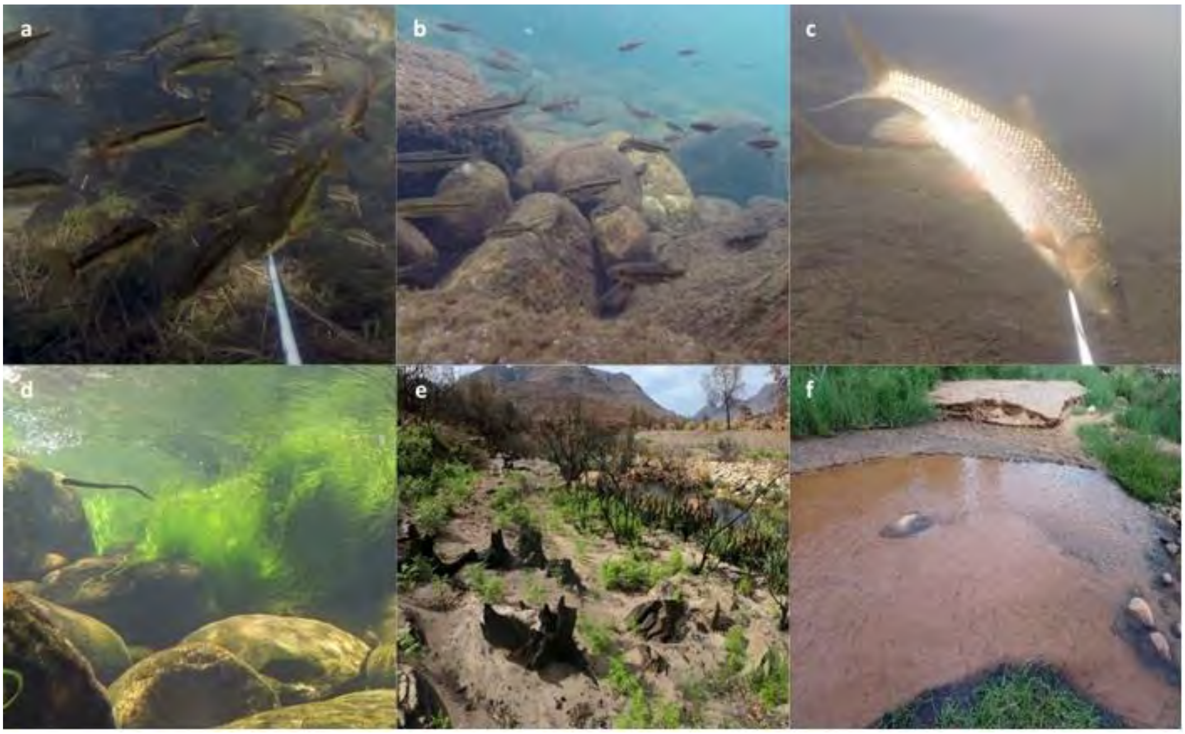

Figure 4: Photographs of the study species and habitats of the Rondegat River. (a) fiery redfin *Pseudobarbus phlegethon* (b) Clanwilliam redfin *Sedercypris calidus* (c) Clanwilliam yellowfish *Labeobarbus seeberi* (d) typical vegetated habitat of the upper reaches (e) the fire-affected banks showing the effects on riparian vegetation (f) a section of river in the middle reach, severely altered by a sand plug following fire-assisted erosion, which was previously inhabited by all three species.

Underwater video was chosen as an ideal sampling methodology for imperilled clear water stream species, given fish mortality risks associated with other sampling methods (Ellender *et al.*, 2012; Castañeda, Weyl & Mandrak, 2020c). This method has been used in several studies in the CFE (Ellender *et al.*, 2012; Weyl *et al.*, 2013, 2016; Castañeda *et al.*, 2020a), and efforts are underway currently to develop a standardised protocol for the use of this method, including the ideal deployment period. Relative abundance (MaxN; or the maximum number of fish counted in a single frame of video) data was extracted from underwater video camera footage that was recorded at 51 sites spanning 21km of the Rondegat River in three sampling instances, the first: 7th - 11th

April, second: 21st - 24th October 2018, as well as 30th September – 4th October 2019 (Fig. 5). GoPro® Hero3+® cameras were deployed approximately central to river flow and facing upstream on Perspex platforms (Fig. 6). Cameras were set to record at a resolution of 1920 x 1080p at 30 frames per second, and using "wide" field-of-view (127°). Recording time per deployment was 15 minutes, with the initial two minutes excluded from analysis to avoid the effects of disturbance or increased turbidity associated with the deployment. The 15 minute deployment period was based on current literature standards, which may span periods of ten minutes and up to an hour of footage in some cases. In the interest of maximising both the accuracy of MaxN estimations and rapid deployment across multiple sites, as well as pilot testing, the 15 minute period was decided upon as the best compromise between approaches. Pilot testing during initial site selection showed that the two minute exclusion has been found to allow fish to return to normal movement and return to the area within the subsequent 15 minute filming period (Hannweg et al., 2020; CJB pers. obs.). The exclusion period in this study was primarily used to limit the effect of minor turbidity increases from river entry and camera placement. GPS coordinates of each site and photographs of camera placement were taken to minimise differences between sampling instances.

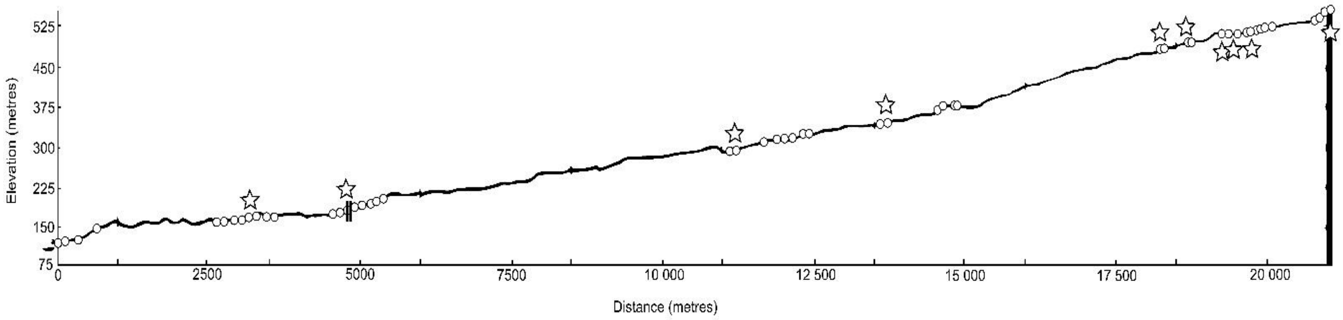

Figure 5: Longitudinal profile of the distribution of study sites and temperature loggers. Stars indicate the location of temperature loggers, circles show the sampled sites. Parallel vertical lines indicate the position of the Rooidraai waterfall, the upper limit of non-native fish invasion. Elevation ranged from 120 m to a maximum of 521 m above sea level, and sampling covered 21 km of the Rondegat River. The camera monitoring sites for collection of relative abundance and distribution data in April and October 2018 are depicted.

Sites were chosen based on suitability for camera deployment and with deployments alternating between pool and riffle/run habitats. Suitability for camera deployment was determined based on the availability of unobstructed viewing angles and clear visibility of the majority of water volume within a selected site, to avoid biases introduced by obscured areas in the video footage and to maximise comparability between sites. Some sites were selected based on prior knowledge of fish distributions, particularly large pools known to be used as refugia for *L. seeberi*. Beyond these opportunistic deployments (n = 3 of 51), the majority of deployments were randomised and agnostic of any prior knowledge of fish distributions. Distance between deployments was determined by the physical characteristics of the locality; cameras were deployed either side of natural barriers or with 10 to 200m gaps between sites where physical barriers were absent. The dominant substratum microhabitat available at each site was classified according to a modified Wentworth scale: sand 1-5 mm; cobble 64-250 mm; boulders >250 mm and bedrock. Other habitats identified were silt (< 1mm particle size) and vegetation (submerged macrophytes). At each site, physicochemical parameters were recorded using an Aquameter® AM-200 multiprobe device (Aquaread Ltd, Kent, United Kingdom). Physicochemical parameters for all sites and sampling events are available in the appendices (Table S1). The length of each site (used as a proxy for site size) was measured by means of a tape measure to the nearest cm. Measurements started at the transition zone between reach changes, moving to the next transition (e.g. where a pool became a riffle, or minor physical barriers spanning the width of the river cross-section). Permission for underwater camera deployment and general research activities was granted by CapeNature under permit number 0056-AAA008-00067.

Video footage was analysed by one observer in EventMeasure software (v5.01, SeaGIS, www.seagis.com.au). MaxN counts are defined as the maximum count of individuals per species in a single frame of video footage. This technique avoids the possibility of counting the same individual more than once and gives conservative relative abundance estimates (Ellender *et al.*, 2012; Campbell *et al.*, 2015).

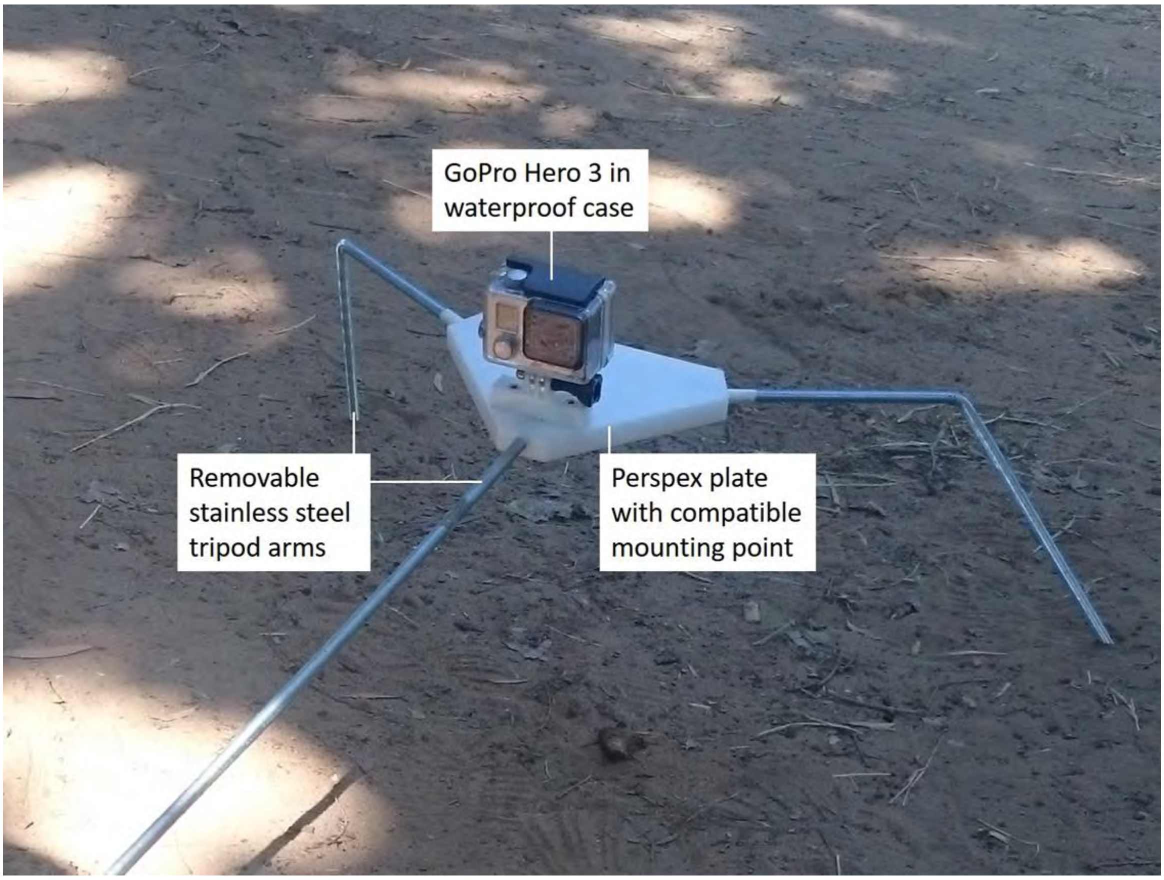

Figure 6: A diagram of the mono-camera remote underwater video (RUV) system used in this study.

Data analysis

All statistical analyses were performed within the R software environment version 3.5.1 (R Core Team, 2018).

Community composition

Focal species relative abundances as the community matrix, and habitat characteristics were visualised using non-metric multidimensional scaling (nMDS) ordination as implemented by the R package 'vegan' version 2.5-5 (Oksanen *et al.*, 2019). The nMDS included reach type, substrate type, distance from uppermost site (metres), electrical conductivity (EC, μS), turbidity (ntu), site length (metres) and protected status (factor defined as 1 = site falls within protected area, 0 = outside of protected area). Correlations between continuous variables were checked and the variables EC and distance from uppermost site were retained, as TDS and salinity were closely correlated with EC ($r > 0.8$) and elevation with distance ($r > 0.8$) (Fig. S1). Including protected status as a variable allows assessment of the focal fish community in accordance with the environmental variables thus taking into account both biotic and abiotic factors driving abundance throughout the river.

PERMANOVA (one-way test using Bray–Curtis non-metric similarity and 1000 permutations), a semi-parametric, permutation-based analogue of traditional ANOVA/MANOVA was used to test for significant effects of habitat characteristics on fish community. The full PERMANOVA model tested the MaxN community data for all three species as response with the variables "substrate type" × "reach type" × "protected status" + turbidity + "site length" + "distance from uppermost site". Community abundance data was square-root transformed and Wisconsin double standardization was applied as implemented via *vegan::metaMDS*, where ordination stress <0.15 was considered appropriate for a two-dimensional biplot (Cousins, Kennard & Ebner, 2017; Oksanen *et al.*, 2019).

Habitat drivers of relative abundance

To test species specific interactions with abiotic variables, regardless of whole community dynamics, generalized linear mixed models (GLMMs) were constructed for each species with relative abundance (MaxN) per site as the response. Error distributions and zero-inflation terms are outlined and candidate models are summarised in the appendices (Tables S2a – S2c). Repeated sampling at sites was accounted for by means of a random effect intercept term of site nested in month ($n = 153$ and $n = 3$, respectively), with environmental variables as fixed effects in GLMMs.

Correlations between continuous variables were checked and the variables salinity, total dissolved solids and elevation were removed based on autocorrelation among variables (appendix Fig. S1). The environmental predictors thus included in the final GLMMs were: reach type, substrate type, turbidity, EC, site length, and distance from the uppermost site, as well as interactions between terms. Continuous predictors were centred and scaled prior to analysis. Exhaustive model selection was applied for each species with the final model chosen based on lowest AIC values (shown in appendix Tables S2a – S2c). Generalised linear mixed models were constructed using R package “glmmTMB” version 0.2.3 (Brooks *et al.*, 2017). Checking of model assumptions was performed using “DHARMa” version 0.2.4 (Hartig, 2019). Log-likelihood stepwise model selection was applied using package “buildmer” version 1.1 (Voeten, 2019) and “MuMIn” version 1.43.17 (Bartoń, 2020). Plotting of mixed model terms was facilitated by package “ggeffects” version 0.11 (Lüdecke, 2018).

Species presence/absence

Classification trees were used to investigate the effects of environmental variables on the presence or absence of each of the study species to identify specific unfavourable drivers and prioritise future intervention efforts (appendix Figs. S2a – S3c). The response variable was presence/absence of the focal species across all sites in both April and October 2018, with the same environmental variables as used in the nMDS analysis used as predictors. Trees were generated by splitting the parent node (all data) based on misclassification costs using the Gini measure of node impurity. To avoid overfitting, the tree pruning was based on misclassification error using 10-fold cross validation and the 1−SE rule (Breiman *et al.*, 1984). The simplest tree within one standard error of the cross validated tree with the lowest estimated error was used to choose the optimal tree (De’ath & Fabricius, 2000). Classification trees were generated and selected using R package “Rpart” version 4.1-15 (Therneau & Atkinson, 2019).

2.3 Results

Relative abundance and distribution of species in the Rondegat River

Relative abundances (MaxN counts) of all three species varied between April and October 2018 (Table 2). *Sedercypris calidus* was the most widespread of the three species, being found in 53% and 71% of monitored sites in April and October, respectively. *Pseudobarbus phlegethon* and *L. seeberi* were less commonly detected in video samples in April, with both species being detected at very low rates of 27% and 29%, respectively. Similarly to the detection rates for *S. calidus*, these species were detected in more sites in October 2018 than in April; *P. phlegethon* was detected in 47% of sites while *L. seeberi* detection increased to 53% in October.

Table 2: Summary statistics of the relative abundances (MaxN) of the three species. SE = standard error, DR = detection rate, defined as sites with species present/all sites.

Species	Month	Mean MaxN	SE	DR
L. seeberi	April 2018	3.92	2.12	0.29
	Oct 2018	1.73	0.34	0.53
	Oct 2019	1.41	0.32	0.37
S. calidus	April 2018	8.63	1.88	0.53
	Oct 2018	6.59	1.29	0.71
	Oct 2019	3.65	1.05	0.47
P. phlegethon	April 2018	2.27	1.18	0.27
	Oct 2018	0.96	0.2	0.47
	Oct 2019	1.51	0.49	0.31
All species	April 2018	4.94	1.04	0.61
	Oct 2018	3.09	0.49	0.86
	Oct 2019	2.19	0.62	0.39

Mean MaxN values for the three species reflected the detection rate trends. *Sedercypris calidus* had the highest relative abundance (mean ± standard error: 8.63 ± 1.86 fish^{-1} and 6.59 ± 1.29 fish^{-1} for April and October, respectively) while *P. phlegethon* was consistently the least abundant (2.27 ± 1.17 and 0.96 ± 0.20). All three species decreased in relative abundance between April and October but increased in site detection rates (Table 2), i.e. fewer fish were found spread across a larger area within the study site in October, suggesting possible dispersion within the river during winter flooding and increased habitat connectivity.

Several sites shifted in primary substrate composition between April and October as a result of deposition of sediments and hydraulic action over the winter rainfall period. Primarily, sand deposition was high; 16 sites (31%) classified as “sand” in April increased to 25 sites (49%) in October. However, the number of silted sites decreased from five sites in April to only one in October, indicative of hydraulic scouring. The trend of dispersed distributions of the focal species continued in October 2019, as none of the focal species exhibited large aggregations at single sites. Detection rates and mean MaxN for all three species decreased from the 2018 results (Table 2).

Environmental constraints associated with species relative abundances

Community composition; Non-metric multidimensional scaling analysis (nMDS)

The three study species separated across dimensions of the nMDS ordination space, indicating differing habitat associations (ordination stress = 0.023; Fig. 7). In the PERMANOVA, the interaction between substrate (habitat) of a site and reach type significantly affected community composition (Table 3). Other significant factors driving changes in community composition were protected status of sites, distance from the uppermost site, site length and turbidity (Table 3).

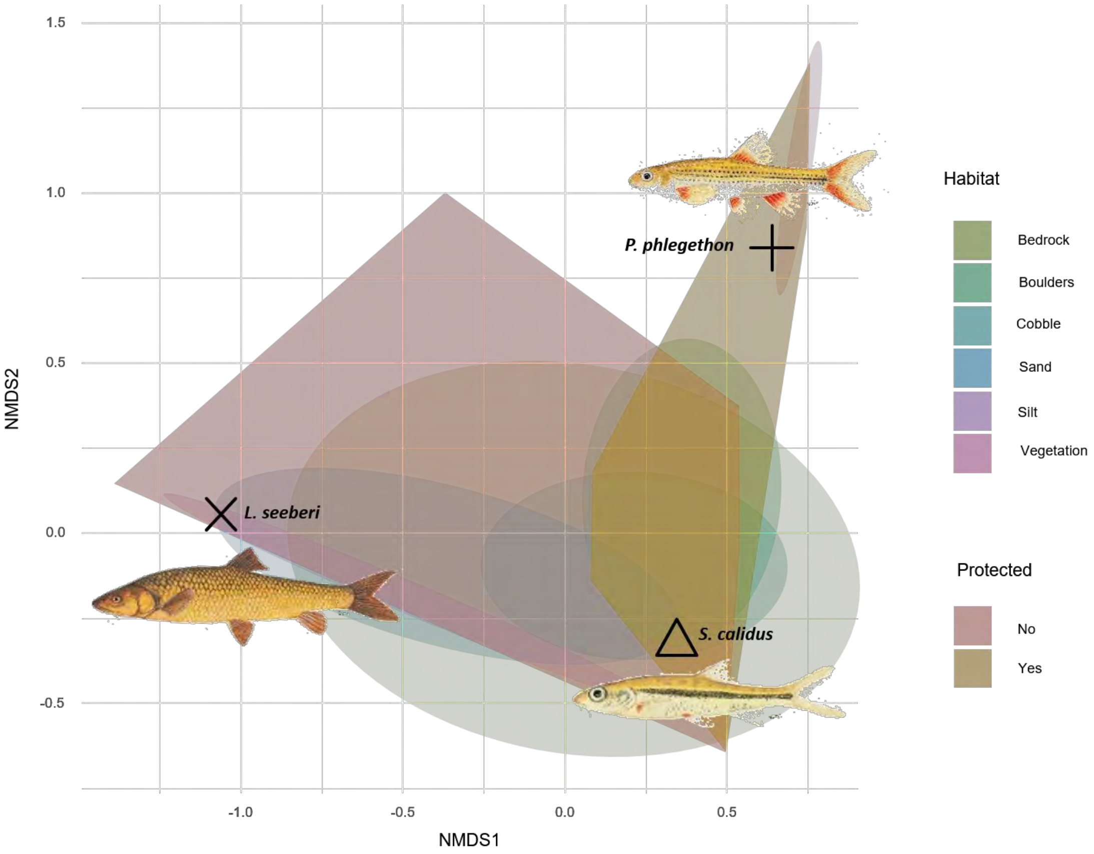

Figure 7: Biplot of the non-metric multidimensional scaling analysis relating environmental constraints and species relative abundances. Species MaxN (relative abundance) score centroids are shown, while standard ellipses containing 40% of the ordinated data denote the primary habitat types. Overlaps are considered to be non-significant. Site status as "protected" or "unprotected" areas is shown using convex hulls.

Table 3: Results of the PERMANOVA following non-metric multidimensional scaling (nMDS) ordination.

Term	Degrees of freedom	Sum of squares	R^2	*F*	*p*
Substrate	**5**	**4.08**	**0.14**	**4.43**	**0.001**
Reach type	**1**	**1.92**	**0.07**	**10.40**	**0.001**
Protected	**1**	**1.97**	**0.07**	**10.70**	**0.001**
Turbidity	**1**	**0.51**	**0.02**	**2.75**	**0.032**
Site length	**1**	**0.74**	**0.03**	**4.01**	**0.002**
Distance	**1**	**0.89**	**0.03**	**4.85**	**0.002**
Substrate x reach type	**1**	**1.91**	**0.07**	**2.10**	**0.005**
Substrate x protected	1	0.41	0.01	1.11	0.354
Reach type x protected	1	0.02	0.00	0.12	0.974
Substrate x reach type x protected	1	0.10	0.00	0.57	0.701
Residual	90	16.60	0.57		
Total	109	29.17	1.00		

The nMDS ordination indicates that higher relative abundance of *L. seeberi* was associated with larger distance from the uppermost site (distance), sand and silt substrates and increased turbidity. *Sedercypris calidus* had higher abundances in a variety of substrates, primarily bedrock, silt and cobbles. Furthermore, increasing relative abundance in *S. calidus* was associated with increasing site lengths (i.e. a proxy for larger site areas) and was associated with protected areas, but to a lesser extent than *P. phlegethon* which was strongly associated with the protected area. In addition, *P. phlegethon* was strongly associated with vegetated sites and shorter site lengths, closer distance to the uppermost site and lower turbidity.

Habitat drivers of relative abundance

Habitat characteristics were modelled to determine their significance as drivers of relative abundances, using GLMMs, for the three species. *Sedercypris calidus* was best modelled by a negative binomial mixed effects model with a zero-inflation component; model selection for *P. phlegethon* and *L. seeberi* indicated that negative binomial mixed effects models without zero inflation terms were most suitable (Table S2a – S2c). All species abundances were significantly predicted by distance from the uppermost site, reflecting a longitudinal shift in the fish community distribution between species (Table 4). All species were significantly more abundant in pools and less abundant in run or riffle (i.e. stream) habitats (Table 4). *Sedercypris calidus* relative abundance was predicted by site length in addition to reach type (being associated with pools rather than stream sections) and distance from uppermost site, with higher predicted abundances in longer sites. In addition, this species was more abundant in turbid conditions. *Pseudobarbus phlegethon* abundance was significantly predicted by substrate (microhabitat) type. In particular, this species had a strong association with vegetated and boulder-dominated sites and was very unlikely to be abundant in silt-dominated environments. Distance from the uppermost site and pool environments were significant predictors of *L. seeberi* relative abundance, with the predicted abundance of this species increasing with greater distance and in pools as opposed to stream sites (Table 4). The final model for *L. seeberi* included a distance × site length interaction, suggesting a combined role of spatial and physical predictors in its abundance patterns (Table 4). There was a disordinal interaction where abundance was positively related to site size in the upper reaches but negatively related to site size in the lower reaches. However, in the lower reaches, the opposite was true and *L. seeberi* abundance increased from large sites to smaller sites.

Table 4: Environmental predictors of species abundance after model selection based on AIC values. Fixed predictors and zero-inflated model intercept terms (if applicable) are included for each final model per species. The candidate models are shown in appendix Tables S2a – S2c.

Predictors	**Estimate**	**SE**	***Z*-value**	***p***
Sedercypris calidus				
(Intercept)	2.21	1.57	12.76	**<0.001**
Distance	-0.65	0.08	-4.36	**<0.001**
Stream	-1.24	0.07	-5.32	**<0.001**
Site length	0.29	0.15	2.61	**0.009**
Turbidity	0.45	0.23	3.01	**0.003**
Zero-inflated model				
Intercept	0.31	0.13	-2.73	**0.006**
Pseudobarbus phlegethon				
(Intercept)	-1.64	0.67	-2.42	**0.015**
Distance	-1.86	0.33	-5.62	**<0.001**
Boulders	1.17	0.59	1.98	**0.048**
Cobble	0.83	0.62	1.33	0.184
Sand	1.35	0.75	1.80	0.073
Silt	-18.46	10143.50	-0.00	1.000
Vegetation	2.17	0.69	3.13	**0.002**
Stream	-1.30	0.30	-4.27	**<0.001**
Labeobarbus seeberi				
(Intercept)	0.95	1.02	2.39	**0.017**
Distance	0.83	0.53	3.64	**<0.001**
Stream	-1.54	0.08	-3.97	**<0.001**
Site length	-0.02	0.22	-0.08	0.94
Distance × site length	-0.67	0.13	-2.72	**0.006**

Habitat effects on species presence/absence

The final classification tree for *S. calidus* indicated the distance from the uppermost sampling location (close to the river source) as the only significant variable explaining species presence or absence. Overall, *S. calidus* was likely to be present over most of the length of the river, with the exception of the extreme lower reaches. This species was present in 62% of total sampling events (detection rate across both sampling periods). *Sedercypris calidus* was predicted to be absent in 16% of sites when distance from the uppermost site was greater than 18km (i.e. predicted to be absent in the lower ~3km of sampled river length). For sites within 18km of the uppermost site (84% of cases), this species is likely to be present (67% likelihood).

The classification tree for *P. phlegethon* reduced to distance from the uppermost site as the single explanatory variable. This species was present in only 35% of all sites. For distances greater than approximately 4.5km from the uppermost site, the species was likely to be absent in 69% of cases with a probability of 86%. For distances less than 4.5km, the species was 81% likely to be present in the remaining 31% of cases.

Labeobarbus seeberi presence/absence was best characterised by distance from uppermost site. Across all cases, *L. seeberi* was more likely to be absent (60% probability) than present. 25% of the total sample is completely absent in the upper 2.2km of river. In sites further than 2.2km from the source (75% of the remaining sites), this species was present in the majority (54%) of cases. For the remaining 75% of cases, at distances greater than 18km from the uppermost site, *L. seeberi* was 87% likely to be absent in 16% of cases, while for distance less than 18km, the species was 64% likely to be present.

2.4 Discussion

Long term monitoring of aquatic systems after intervention is rarely achieved due to the cost and effort to sustain it (Lintermans, 2013). This undermines both the initial economic outlay and the ecological objectives of maintaining the ongoing recovery of the system. The Rondegat River has clear conservation value and a considerable sunk cost in restoration interventions to remove alien fishes. Yet freshwaters are threatened by multiple stressors, indeed within the unprotected areas of the Rondegat there are signs of pervasive threats, discussed here, which may continue to hamper conservation efforts. The present study used underwater videos to demonstrate the utility of such a non-invasive and low cost method to understand how a recovering and vulnerable cyprinid assemblage interacts with the abiotic variables in the Rondegat River, South Africa. I found that several factors affect the distribution and abundance across the longitudinal gradient and determine possible future threats to the recently recovered populations. Consequently, the data gathered is the most ecologically accurate in the system to date, as much of the biology and baseline ecological knowledge of South African native fishes remains anecdotal (Ellender *et al.*, 2017). Thus this study can contribute evidence for critical areas for the management of keystone species in a conservation hotspot (Skelton, 2000).

The utility of underwater video monitoring in aquatic environments has been pioneered in marine community assessments and is increasingly being adopted in freshwater settings (Ebner *et al.*, 2014). As a result, many protocols and statistical approaches to monitoring have been established and overcome (Harvey & Mladenov, 2001; Cappo *et al.*, 2003; Coghlan *et al.*, 2017). I was able to use the data to determine detection rates between seasons and maximise the utility of the data by characterising habitats from the collected footage. Underwater video has previously been shown to have the highest detection probability in South African headwater streams, despite being hampered by turbidity (Castañeda *et al.*, 2020c). Camera deployment can be prohibitive in small, shallow streams and riffle environments, but these conditions are also a hindrance when snorkel sampling. Yet the method is non-destructive and more reliable than snorkel or netting approaches as the fish are less disturbed; all of which are key elements when working with threatened species to generate ecologically relevant data (Ellender *et al.*, 2012). In this study, fish were not noticeably impacted by the presence of the camera, with all three species continuing natural behaviours (pers. obs.) and any disturbance effects ameliorated by the two minute acclimation period (Nanninga *et al.*, 2017a; Hannweg *et al.*, 2020). Thus, I propose that underwater video makes an ideal candidate for long-term low cost monitoring initiatives which can be archived for posterity and used in future research (e.g. behavioural analysis; see Chapter 5). The data provides a unique insight into behavioural and ecological aspects of aquatic communities under natural conditions (Ebner *et al.*, 2009, 2014), increasing the scientific value of monitoring programmes beyond those currently possible with other monitoring techniques.

The suitability of river habitat is severely affected by major fire events through the formation of sand plugs as a result of vegetation loss and bank erosion. It is unclear whether shifts in all species detection rates and relative abundances (i.e., the general increase in detection rates but decrease in relative abundance) were a natural annual phenomenon or were driven/exacerbated by a major fire event in February 2018. Further, there was an increase in sandy substrates in October following the winter rainfall. Greater deposition of sand following fire and vegetation clearing have occurred previously in this system (J Nieuwoudt pers. comm.). These events reduce habitat availability particularly in the lower reaches (i.e. become more shallow) (Woodford, 2005) and inflows of ash and sediment were observed during the April sampling efforts. Despite this, no conspicuous stress or mortality was seen in the footage from this period for any species. Furthermore, no reports of fish kills were made by either local land owners or conservation authorities for the period. Species which have evolved within the stochastic CFE fynbos ecosystem (i.e. fire adapted) are resilient to unpredictable conditions (Ellender & Weyl, 2015) and it can be assumed that this particular event was not the main driver of seasonal change in fish abundance. Rather, the shift may be a result of natural seasonal movements and varying habitat availability following increased sediment deposition. Continued monitoring and assessment of largescale burns on aquatic resources should be a research priority as changing climates exacerbate burn frequency globally (Carvalho *et al.*, 2011; Ward *et al.*, 2020; Gallagher *et al.*, 2021). The video approach utilised in this study also provides an unprecedented opportunity to continue monitoring with the fire event in mind and may present opportunities in the future to compare rates of habitat change in the system. Turbidity affected community composition overall (per PERMANOVA) and turbid conditions seem to increase *S. calidus* abundance (per the GLMMs). This may be linked to high abundance in a small number of larger, downstream pool sites, which tend to be more turbid than small stream sites in the upper reaches (evidenced by the collinearity of turbidity and distance from uppermost site; see appendix Fig. S1). When considering aggregative species which accumulate in specific individual sites, abundance metrics alone should be complemented with other multivariate models (i.e. NMDS, canonical correlation analysis, etc.) to avoid erroneous conclusions.

Sedercypris calidus is clearly a dominant player in the system, being both the most widely distributed and most abundant of the three cyprinids present. This pattern has been observed in prior studies using both underwater video and traditional fyke netting and snorkel surveys (Weyl *et al.*, 2013; Castañeda *et al.*, 2020c). This species' physiology appears to be well-suited to its generalist resource use profile, utilising all habitat types in significant numbers across the environmental factors measured in this study. The finding of increased abundance under turbid conditions was surprising, but may be linked to this species being highly abundant in a small number of larger, downstream pool sites, which tend to be more turbid than small stream sites in the upper reaches. This potential source of bias, i.e. very large numbers of individuals found in individual sites with specific conditions, should be taken into account when drawing conclusions from overall models based around abundance. A direct preference for turbid conditions in this species is also possible. The purported greater dispersal ability of *S. calidus* over that of *P. phlegethon* suggested by Swartz et al. (2004) may apply to the patterns observed within the Rondegat River. Specifically, the changes in distribution between seasons (Table 1) show a higher frequency of this species being found across more sites within the study system over time, while this effect is less pronounced in *P. phlegethon*, which remains abundant truly only in the upper protected areas of the river. In contrast to the ubiquity of *S. calidus*, *P. phlegethon* is found in much lower abundance and is more restricted within the course of the river regardless of seasonal changes in distribution.

Taking into account the results from all analyses of habitat associations in this study, *P. phlegethon* appears to be the most habitat-dependent and sensitive to disturbed habitats of the cyprinid assemblage. I suggest that this sensitivity can be inferred from the changes in this species distribution and abundance between April and October, and the concurrent fire disturbance over that time frame. The sand flows and siltation (i.e. 16 sites classified as "sand" in April increased to 25 sites in October) that followed the fire events and heavy rains were a form of habitat disturbance. As this species appears to be the most dependent on vegetated and boulder-dominated habitats, this habitat shift would be classified as disturbance. The sub-terminal mouth of *P. phlegethon* indicates a preference for substrate foraging on detritus, which may be disrupted in turbid environments with high sedimentation due to smothering of plant matter (Skelton, 1996; Whitehead *et al.*, 2007). Therefore, these associations should be further tested by restorative action in the mid and lower reaches to limit silting and sand deposition in order to create more suitable habitat for the still recovering populations of *P. phlegethon* (Castañeda *et al.*, 2020a). In addition, the finding that this species was abundant in protected areas further indicates a sensitivity to disturbance beyond that of the other two species studied here. The apparent habitat specificity of this species is a possible contributory factor to the finding that this species has not fully recovered, based on models of population dynamics and occupancy patterns (Castañeda *et al.*, 2020a). The sensitivity of *P. phlegethon* to turbid habitats is supported by work on the biology of the closely related *P. afer*, which was found to be highly dependent on vision for food acquisition (Cambray, 1994).

Pseudobarbus phlegethon is thought to have shared a common ancestor with the *P. afer* species complex originating in the Gouritz River, diverging following the last glacial maximum (Swartz *et al.*, 2007, 2009; Chakona & Skelton, 2017). It is probable that strong similarities in physiology and habitat preferences exist between these closely related lineages. Given the relative lack of mtDNA differentiation within *P. phlegethon* and the isolated populations leading to a lack of contemporary gene flow (Swartz *et al.*, 2007), it could be suggested that *P. phlegethon* is less able to adapt to conditions outside of its narrow distribution range compared to the more widespread and interconnected *S. calidus* and *L. seeberi*. This is further corroborated by *P. phlegethon's* strong association with protected areas.

Labeobarbus seeberi is a flagship conservation species in South Africa, with considerable economic relevance as a sport fish (Skelton, 2000). There was clear evidence in all lines of analyses showing that *L. seeberi* only proliferates in the lower reaches of the stream. Exclusion from the uppermost reaches is caused by a natural barrier close to the Algeria campsite, approximately 3km downstream of the uppermost observed site. However, below the barrier, occurrence and abundance of *L. seeberi* changed considerably between the sampling events. Large aggregations primarily in the larger pools of the lower reaches characterise the late summer April sample, shifting to more widely distributed smaller groups in October. The spawning behaviour and migratory nature of this species could be an explanatory factor in this late-year pattern. Large over-summering aggregations and movement to riffle habitats for spawning in November to January have been anecdotally observed (Cambray *et al.*, 1997; Impson *et al.*, 2008), and the current study provides evidence of this seasonal activity. The interaction of distance from uppermost site and site length suggests that in the upper reaches *L. seeberi* has lower abundance in general and that small sites are particularly unsuitable. This may be primarily driven by very shallow habitats and partial physical barriers. Where *L. seeberi* is found in the upper reaches, they tend to be abundant only in larger sites with more suitable dimensions for larger-bodied, and generally adult, fish (pers. obs.). In the lower reaches, *L. seeberi* juveniles tend to gather in large shoals in small river sections. Conversely, larger sites in the lower reaches have fewer large adults (pers. obs.). It is plausible that the current distribution of *L. seeberi* reflects a situation wherein distinct mainstem and stream populations have emerged which consist of resident and migratory components. Similar scenarios in brook trout have been suggested (Huntsman & Petty, 2014; Huntsman *et al.*, 2016) and further investigation in this system is warranted. Source-sink population dynamics between mainstem and tributary systems have been observed in many migratory fishes, particularly salmonids (Schlosser, 1995; Huntsman & Petty, 2014; Huntsman *et al.*, 2016). Habitat preferences suggested by the current study lend support to a scenario of *L. seeberi* being a mainstem-adapted species, and may not be perfectly suited to headwater environments. *Labeobarbus seeberi* is faced with the pressures of impacted migratory pathways and disrupted spawning locations owing to both climate-driven drought conditions and anthropogenic influences of water abstraction and damming in crucial riffle habitats (Impson *et al.*, 2008). Further investigation regarding water level and environmental flows in this system is warranted, considering that experimental floods promoted spawning, and the construction of the Clanwilliam Dam and the associated flood reduction was implicated in the rapid population decline of *L. seeberi* (Cambray *et al.*, 1997; Skelton, 2000; Impson *et al.*, 2008). This raises concerns regarding sand plugs and siltation causing substrate change in the lower reaches and the ecological implications of habitat loss associated with flow disruptions.

The upper ~4 km of the river runs within a formally protected area that did not burn in the 2018 fire event. This stretch of the river is free from agricultural runoff and has limited anthropogenic influence on the river conditions. This aspect influences the abundance and distribution of all species, but is particularly notable in the case of *P. phlegethon*. This species was strongly associated with submerged vegetation, which was only found commonly in the upper reaches. Submerged vegetation and woody debris were found to be positively correlated with this species' abundance in a previous study (Woodford et al., 2005). Furthermore, *P. phlegethon* was associated with shorter site lengths, indicating a preference for smaller runs between riffles over the large, deep pools. It is unclear whether the protected status of the upper reach or the natural characteristics of this reach (high submerged vegetation, riparian integrity, boulders) are facilitating the population of *P. phlegethon*. Nonetheless, this species has a stronghold in this section of the river and has been identified as the most vulnerable of the fish assemblage (van der Walt *et al.*, 2017a). More concerning, the population has not yet reached equilibrium, despite other native species recovering to this extent over multiple years since conservation intervention (Castañeda *et al.*, 2020a). Thus I recommend cohesive management and small scale interventions throughout the Rondegat River which extend beyond the current boundaries of the CapeNature protected area to focus on priority habitat restoration (i.e. submerged and riparian vegetation, as well as reduced turbidity). This combined with regular seasonal monitoring will be able to detect any net gain in *P. phlegethon* abundance outside of the protected area.

Overall, the results show clear habitat preferences in *P. phlegethon* and *L. seeberi* whereas *S. calidus* is a generalist throughout the stream. All three species are able to co-occur but the extent is mediated by abiotic factors. Maintaining habitat integrity for *P. phlegethon* by reducing runoff and sedimentation to promote submerged vegetation in the lower reaches may be a constructive advance and drive population equilibrium. Future extreme drought and fire events are likely to continue to reduce flow and promote sand slugs which threaten *L. seeberi* persistence and reproduction. Thus management measures in the lower reaches should concentrate on identifying crucial stretches of the stream which include as many key habitats as possible and to limit agricultural activities such as irrigation pumping to maintain water depth and natural flow regimes. By prioritising the species constrained to either extreme of the stream gradient, the management interventions will benefit *S. calidus* coincidentally. In order to gain a holistic picture, more research should be carried out to determine the biotic interactions which interact with the abiotic factors documented in this study to drive species abundance and distribution trends. Consistent underwater video surveys are feasible to inform whether these actions cause a positive change and can be carried out by both environmental managers and citizen scientists, especially land owners, as important stakeholders in any conservation project. Furthermore, enhancing video surveys using stereo remote underwater video systems, adapted from their applications in marine and lentic systems, could provide valuable in-situ data on ontogenetic habitat preferences and population structure.

In conclusion, spatial niche segregation is likely to exist between species of the cyprinid assemblage of the Rondegat River. This was supported by differences in presence/absence data and relative abundances across species among reaches of the river, as well as between dominant substrate types. These differences require further investigation. Primarily, differences in resource use need to be investigated, which may provide insights into competition or trophic niche differentiation in this system of functionally similar fishes. Differences in feeding performance, for example, would reveal relative differences in the strength of feeding performance when the focal species and resources overlap. Furthermore, differences in functional morphology and behaviour need to be quantified to demonstrate the mechanisms of trophic niche differentiation if it is found. Investigation into the ecomorphology of all three species may indicate how trophic niche separation and possible divergent adaptations of this assemblage are determined. Finally, characterising the strength of trophic interactions and food web structure along the gradient of the stream would determine how species abundance and the interplay of terrestrial and aquatic resources determine spatial segregation. Further research striving for a more comprehensive understanding of habitat preferences is required in order to mitigate the effects of habitat degradation and other anthropogenic effects on this vulnerable community.

CHAPTER 3: Prey type and temperature influence functional responses of threatened endemic Cape Fold Ecoregion fishes

The holding aquaria and functional response trial arenas used in this chapter, based at the South African Institute for Aquatic Biodiversity, Makhanda.

3.1 Introduction

Freshwater systems represent some of the most threatened systems on Earth, characterised by comparatively high species and habitat diversity despite comprising a miniscule fraction of the globes surface (Vörösmarty *et al.*, 2010; Albert *et al.*, 2020). Due to the bounded nature of freshwater ecosystems, they are essentially biogeographic islands which makes them among the most vulnerable to biodiversity loss of all systems (Tickner *et al.*, 2020). Mediterranean climate regions, and particularly their freshwater systems are renowned for their high levels of endemism (Ellender *et al.*, 2017). Unfortunately, highly endemic species have a far higher extinction risk from multiple pressures such as invasive species, climate change and habitat degradation (Tickner *et al.*, 2020). In particular, shifts in temperature as a result of climate change have an important role to play in long-term dynamics of resource use and resulting species interactions (Vasseur *et al.*, 2014; Gilbert *et al.*, 2014). The metabolism of poikilotherms is highly dependent on their surrounding thermal conditions and other physical parameters such as body size. Furthermore, shifts in the thermal environment can alter the phenology of fish and their prey resources (Dell, Pawar & Savage, 2014). This multitude of stressors as a result of changing temperatures, in addition to the aforementioned anthropogenic impacts, bring sharp focus toward the need for accurate predictive capacity in the construction of effective conservation frameworks. Special attention needs to be paid towards the prediction of changes in resource use in imperilled species under different scenarios of thermal stress.

In the Cape Fold Ecoregion (CFE), many fish assemblages are made up of functionally similar sympatric species constrained to narrow distribution ranges (Ellender *et al.*, 2017). The competitive relationships and niche partitioning strategies of fish assemblages in the CFE are largely unknown (but see Kadye et al., 2016; Shelton et al., 2018a), with some evidence for differentiated habitat use and spatial segregation (Woodford, 2005; Kadye, Chakona & Jordaan, 2016; Broom, South & Weyl, in review). Shelton et al. (2018a) found evidence for diet partitioning among three coexisting CFE species, with some overlap in trophic niches. Conversely, there was little evidence to suggest niche partitioning or habitat filtering in two redfin species (Kadye *et al.*, 2016). It was instead inferred that high intraspecific variability in phenotype and ontogeny was driving broad ecological niche exploitation with regards to both habitat and trophic position (Kadye *et al.*, 2016). The river systems of the CFE are near-universally oligotrophic, leading to low primary productivity, and freshwater fauna is highly adapted to these conditions (de Moor & Day, 2013). This set of constraints suggests plausibly higher levels of competition for scarce resources amongst similarly-adapted freshwater fish species.

Predicting the strength and direction of biotic interactions is multi-faceted, and made even more cumbersome when dealing with threatened species. Thus, methods which can produce effective results with little perturbation and animal use are preferred. Functional responses (FRs) are defined as the relationship between density of prey and consumption rate of the predator (Solomon, 1949; Holling, 1959, 1965; Dick *et al.*, 2014, 2017; Wasserman *et al.*, 2016). These responses are illustrated by logistic curves showing the number of prey consumed against pre-determined prey densities as a function of the attack parameter, handling time and experimental period (Dick *et al.*, 2014, 2017). Environmental context dependencies such as temperature, which alter the efficacy of a consumer, can be integrated into the FR experimental design (Dickey *et al.*, 2020). Much of the modern literature making use of functional responses has focused on invaded systems and comparisons of functional responses between invaders and native trophic analogues; but see South et al. (2018) for application regarding native species thermal comparisons. The main merit of this approach is due to the relative comparison aspect, in that while laboratory derived feeding experiments are not fully realistic, the magnitude differences in the parameters [a, h, $1/h$; *sensu* FR terminology] can be used to make inferences regarding context dependent interaction strength.

Dickey et al. (2020) provide multiple examples of the uses for this method. In particular, they note that the abundances of interacting species, species exclusions and coexistence are driven by interspecific competition, which are in turn driven by patterns of resource use. Environmental context dependencies such as temperature which alter the efficacy of a consumer can be integrated into the FR. Shifts in phenology as a result of warming cues, affecting the timing of increased prey abundance (Dell *et al.*, 2014), can also be integrated into predictions of predator impacts. Mofu et al. (2019) illustrate the utility of this method for comparing the competitive ability of an invasive fish with a functionally analogous native fish, finding that the invader was able to outcompete native fish for food resources in the presence of a competitor. Jackson and Britton (2013) found variation in the trophic overlaps between invasive *Pseudorasbora parva* and sympatric cyprinids using stable isotope analysis. FR analysis could be used in scenarios where trophic overlap is suspected or observed, to determine the relative performance between invasive species and a native species. Alternatively, FRs can be investigated between native species with the goal to determine the strength of competition in the extant populations, allowing prediction of the impacts of a given potential invader. FRs are a useful comparative tool for establishing the relative differences between populations or species of interest, and are furthermore context-dependent and thus powerful for fine-scale comparison for prediction of impacts under relevant field-representative conditions.

An important modifier to the FR in ectotherms is the temperature under which prey consumption takes place as well as the thermal history and level of acclimation to conditions of the participants in these interactions (Englund *et al.*, 2011; Dell *et al.*, 2014; Uszko *et al.*, 2017; Daugaard, Petchey & Pennekamp, 2019; Uiterwaal & DeLong, 2020). The mechanism of the temperature dependence of FRs is described primarily by the metabolic theory of ecology (MTE), wherein metabolic processes of organisms generally increase with temperature and are maximised at an optimal temperature to which organisms are adapted (Englund *et al.*, 2011; Uiterwaal & DeLong, 2020). This universal temperature dependence in ectotherms is driven by a shared metabolic biochemistry across aerobic organisms (Englund *et al.*, 2011). FRs in general have a unimodal mild temperature optimum between 15 and 25°C (Uiterwaal & DeLong, 2020). In addition, the components of a FR, such as attack rate and handling time, have differing thermal optima and are most significant at opposite ends of the continuum of resource availability. From this, it is clear that changing prey population sizes will have corresponding shifting thermal optima (Uiterwaal & DeLong, 2020).

Determining the FRs of native species under different contexts can be used to infer changes in interaction strengths and population dynamics under predicted environmental change (Dickey *et al.*, 2020). This is especially relevant when dealing with functionally analogous species in resource limited habitats. Due to the factorial nature of FR experiments there is the possibility to test both the MTE and trophic niche partitioning of native species of interest. Considering the multiple stressors acting on freshwaters, experimental data provides powerful fine-scale, factorial, comparisons of potential impacts under relevant field conditions, which can then be verified with food web and abundance survey data (Orr *et al.*, 2020).

In this study, the feeding performance of two native species is investigated at two representative test temperatures to better understand the dynamics of the Rondegat River system in the CFE. In particular, this study will aid in understanding competition for food resources in an oligotrophic system, given the putative similar feeding niche of the two focal species: *Sedercypris calidus*, the Clanwilliam redfin minnow, and *Labeobarbus seeberi*, the Clanwilliam yellowfish. These two species have a high degree of spatial and habitat use overlap within the Rondegat River (Weyl *et al.*, 2013; Broom *et al.*, in review). This situation suggests that a mechanism of niche segregation or equilibrium of species interactions exists in this scenario. The Rondegat River conservation intervention represents one of very few long-term recovery projects that is both successful and monitored throughout. This uniquely positions the river as a useful “living experiment” from which to generate knowledge applicable to the wider CFE and freshwater systems generally. An improved understanding of threats can aid in focusing conservation efforts, increasing the feasibility and success of those efforts and reducing their cost impacts on South Africa’s scarce resources. Uniting these approaches (longitudinal habitat and abiotic measurements, functional morphology, functional responses and behaviour) will contribute to an understanding of species abundance distribution across rivers in the CFE. Therefore, I intend to use FR methods to test how resource use differs dependent on resource type as well as the potential for thermally modulated resource use changes at both intra- and inter-specific levels. This allows the prediction and increased understanding of threats to vulnerable species’ persistence under environmental change.

I hypothesised that these species would differ in their feeding performance between different prey types and prey occupying different niches and distinct predation avoidance strategies (i.e. benthic chironomids with a burrowing escape strategy and water surface-dwelling mosquito larvae using movement for escape). Benthic and pelagic prey (and the substrate of the environment) present distinct challenges to successful capture by predators, and this aspect of prey behaviour can significantly affect the ability of predators to effectively exploit prey resources and therefore performance in FR trials (Gebauer *et al.*, 2019; South *et al.*, 2019). Furthermore, I expected that there would be species-specific context dependency of the FR owing to spatial differentiation between the species, i.e. warmer lower reaches where *L. seeberi* is most abundant *versus* cooler upper reaches where *L. seeberi* is excluded.

3.2 Methods

Study area

The Rondegat River (32°24’S; 19°05’E) is a 25km long tributary of the Olifants River, opening into the Clanwilliam Dam in the Western Cape, South Africa (Lowe *et al.*, 2008). The river originates as a pristine headwater stream in the Cederberg Mountains, progressing through indigenous terrestrial vegetation, with the lower reaches dominated by agriculture and associated abstraction points and weirs in the lower reaches (Lowe *et al.*, 2008). The native fish in this system were threatened by predatory non-native fishes, which led to the extirpation of native fish below a natural barrier (Woodford, 2005; Marr *et al.*, 2012). The Rondegat River served as the site for the first alien fish eradication project in the CFE, treating a 4km stretch of the river with the piscicide rotenone to remove alien smallmouth bass (*Micropterus dolomieu*) (Marr *et al.*, 2012; Weyl *et al.*, 2013, 2014). The goal of the intervention was to recover invaded habitat for the existing native fish populations (Marr *et al.*, 2012; Weyl *et al.*, 2013). Currently the system is in a state of recovery following the successful alien eradication project. The Rondegat River conservation intervention represents one of very few long-term recovery projects that is both successful and monitored throughout. This uniquely positions the river as a “living experiment” from which to generate knowledge applicable to the wider CFE and freshwater systems generally.

Sampling and holding conditions

Sedercypris calidus (n = 75) and *L. seeberi* (n = 50) were collected from the Rondegat River in October 2019 using fyke nets. Fish were size matched to minimize confounding of results, with *S. calidus* having a mean (± standard error) fork length of 75.4 ± 1.5 mm and *L. seeberi* 80.6 ± 2.3 mm. Juvenile *L. seeberi* and adult *S. calidus* were chosen to maximise size similarity between species in experimental conditions; juvenile *L. seeberi* are commonly found in mixed shoals with adult *S. calidus* in the Rondegat system. It should be noted that it is likely that ontogenetic factors may partly confound the interpretation of results because of the use of adult *S. calidus* with juvenile *L. seeberi*. However, the higher abundance of juvenile *L. seeberi* compared to adult *S. calidus* and the widespread coexistence of juvenile *L. seeberi* across the system with adult *S. calidus*, noted from field observations, suggests that this comparison is ecologically more relevant to direct interactions between the species. The sheer size difference between adult *L. seeberi* and adult *S. calidus* and likely shifts in diet in the adult *L. seeberi* exclude them from direct interactions with *S. calidus*. In addition, the relative rarity of *L. seeberi* adults make them less impactful as a competitor species to the relatively abundant *S. calidus* in the Rondegat River. Overlaps in distribution and habitat associations between these co-occurring species were found to be common in this system through underwater camera monitoring (Chapter 2; Broom *et al.*, in review). All sampling was carried out in accordance with the local conservation authority (CapeNature) guidelines, including restrictions on total number of fish per species and taking no more than seven fish per collection locality to reduce impacts on any single river section. Sampling was governed by collection permit CN44-28-8357 dated 28 March 2019, Western Cape export permit CN16-28-8540, dated 1 April 2019, as well as an import permit for the Eastern Cape issued by the Department of Economic Development, Environmental Affairs and Tourism (DEDEAT) CRO 124/19CR, dated 6 August 2019. In addition, collection of *L. seeberi* required a Threatened or Protected Species permit, issued by the DEDEAT with permit number 029705. Permission for land access was granted by CapeNature and private land owners where applicable. The experimental work was approved by the Rhodes University Animal Ethics Committee (reference number DIFS2118) and the SAIAB Animal Ethics Committee (reference number 25/4/1/5_2018-10).

Fish were transported in 150 L plastic containers with lockable lids, provided with continuous aeration using battery-powered air pumps and temperatures were kept low using ice packs to reduce stress levels. Temperature, oxygen and other physicochemical parameters were monitored using an Aquameter® AM-200 multi-probe device (Aquaread Ltd, Kent, United Kingdom). Partial water changes were carried out at 2-3 h intervals using collected river water to maintain optimal conditions.

Fish were introduced slowly to laboratory holding aquaria (dimensions: 900 mm x 380 mm x 330 mm) over a 12 h period to allow for acclimation to conditions in captivity and fed ad libitum on flake food and frozen chironomids. The laboratory was kept at a constant temperature of 18 °C based on long-term data collected by Reizenberg et al. (2019) and temperature loggers deployed in the Rondegat River (see Fig. S3 of the appendices for raw temperature data between April 2018 and October 2020). The study species are likely adapted or acclimated to a mean temperature of approximately 18 °C based on long-term temperature data, and are likely able to withstand high daily fluctuations in temperature, with most CFE species having upper thermal tolerances of 32-33 °C (Reizenberg *et al.*, 2019). The higher test temperature of 25 °C in this study is based on the known thermal tolerance of *S. calidus* and peak summer temperatures in the Rondegat River (Reizenberg et al., 2019; see appendix Fig. S3). Acclimation to the higher test temperature was achieved by increasing temperatures by 1 °C per day for seven days. Once the higher holding and test temperature was attained, all fish were further acclimated for another seven days. Holding aquaria contained collected river, rain and filtered reverse osmosis water to provide optimal conditions of pH (~7.0) and electrical conductivity (<200 µS). Holding aquaria were filtered with 400 L/h pumps and provided constant aeration using air pumps and a dedicated compressor air line for redundancy. Aquaria were maintained with 50% water changes twice weekly using rainwater and RO, with nitrate, nitrite and free ammonia levels monitored using Sera™ chemical test kits. Aquaria were furnished with collected river cobble stone and paving bricks to provide shelter. Laboratory lighting was on a 12 : 12 h light : dark schedule to simulate diurnal lighting conditions.

Experimental arenas, prey details and functional response trials

Seven replicate functional response arenas (dimensions: 600 mm x 400 mm x 200 mm) were partially filled with 24 L of water sourced from holding tanks. Aeration was provided to each arena by means of an air pump. A tight-fitting plastic mesh was used to prevent escape by fish while still allowing laboratory lighting to enter the arena. Each arena was provided with a 5 mm deep river sand substrate to simulate natural feeding conditions and limited cover for prey items. Food was withheld from fish for 24 h prior to trials to standardise hunger levels. Individual fish were transferred from holding aquaria into experimental arenas for one hour before introduction of prey and initialisation of the trial to allow acclimation to the environment. After one hour had passed, prey items were added at the density required for the trial. Fish were given two hours to consume prey before concluding the experiment by capturing the fish and transferring them to holding aquaria. Prey were then counted to establish the amount consumed within the trial period. Counts of prey were done by thorough examination of the experimental arena, sifting through the sand layer completely with a 1 mm mesh size hand net to allow passage of sand but capturing any concealed prey items. Fish were re-used in experiments, with the frequency of re-use reduced by rotation of fish between holding aquaria with fish recently used in trials being held in one aquarium until all fish in the unused pool had been used in trials. A single fish would experience a maximum of two trials in one round of experiments at one temperature treatment. Re-use of animals was unavoidable because of constraints in sample size by permit limitations, ethics approval, and conservation considerations.

Experimental prey were mosquito larvae (*Culex* sp., mean length ± standard error = 7.69 ± 0.19 mm) and chironomid spp. larvae (12.42 ± 0.28 mm). Prey were not combined in FR trials to consider differences in prey exploitation by the focal fish species. Prey were chosen based on their dietary relevance in natural conditions (chironomids and other aquatic larvae are the primary food group of both species: Nthimo 1997; Woodford 2005), distinct location in the water column (benthic vs pelagic), differing escape responses, and availability for capture and culturing. Chironomid larvae were harvested using kick sampling from ponds on the outskirts of Makhanda, Eastern Cape, and rinsed in RO water to remove contaminants. Mosquito larvae were cultured at the South African Institute for Aquatic Biodiversity. Egg rafts deposited in rainwater-filled containers were collected and placed individually into jars, with the resulting larvae identified to genus level and transferred to shallow trays. Larvae were reared to the 4th instar to standardise size before use as prey in experiments. Prey were allowed to acclimate to laboratory conditions in rainwater for at least 24 h prior to use in functional response trials. Experimental trials involved seven replicates of 2, 4, 8, 16, 32, 64, 96 and 128 prey density bins. Experimental controls were full sets of the replicate trials at low density (two prey items) and high density (32 prey items) for both *Culex* and chironomid prey. Experimental trials and controls were run at acclimation temperature (18°C) and high temperature (25°C) treatments.

Data analysis

All statistical analyses were carried out in R software version 4.0.0 (R Core Team 2020). A full generalised linear model (GLM) was used to assess prey consumption (i.e. *Culex* sp. and chironomid larvae) using the fixed factors “predator species”, “temperature”, “prey type” and “prey density”. All fixed factors were found to interact significantly ($p < 0.05$). Two separate GLMs were then used to assess consumption per prey type to investigate the change in interaction strength under different temperature conditions on single prey resources, rather than between prey types, as prey switching capacity was not part of these experiments. Quasipoisson error distributions were used for both GLMs as data were over-dispersed. Differences between factor levels were assessed using the R package emmeans (Lenth *et al.*, 2018) using linear contrasts post-hoc. Differences in variance of individual prey density consumption were evaluated using F-tests.

Primary functional response curve fitting and hypothesis testing was done using the R package *frair* (v. 0.5.1) (Pritchard *et al.*, 2017). Functional responses were modelled using maximum likelihood estimation (Bolker, 2010) and Rogers’ (1972) Random Predator Equation (Equation 1), as prey were not replaced as they were consumed. If the proportion of prey consumed decreased with an increase in prey density, the logistic regression produced a significantly negative result (i.e. the FR would be classified as Type II); a significantly positive result would indicate the response should be classified as Type III (Juliano, 2001). As all FRs were found to be of Type II, only the equation for this response is given. Rogers' (1972) random predator equation for a Type II FR was derived as:

$$Ne = N_0(1 - exp\,(a(N_e h - T))) \qquad (1)$$

where Ne is the number of prey eaten, N_0 is the initial density of prey, a is the attack parameter, h is the handling time and T is the individual trial duration (i.e. two hours).

The data were non-parametrically bootstrapped (n = 2000) to construct 95% confidence intervals around the mean functional response curve for each treatment. This approach enables population-level inferences to be made based on the separation or overlapping of confidence intervals. Differences in FR component parameters were compared for intraspecific treatments for each predator species to compare mechanistic differences in FR influenced by temperature using *frair::frair—compare* z-tests of optimised coefficients for Type II FR models (see Juliano 2001; Pritchard et al. 2017).

3.3 Results

Prey survival was 100% under control treatment conditions, indicating that all prey losses recorded in experimental conditions were likely the result of predation by a fish.

Factor interactions and post-hoc analyses

Culex larvae

There were significant two way interaction effects of both 'temperature × prey density' and 'predator species × prey density' on consumption of *Culex* larvae (Table 5). Temperature did not have a significant main effect on *Culex* larvae consumption (Table 5). At 18°C more prey were consumed by *L. seeberi* than *S. calidus* at prey density = 96 ($Z = -2.56$, $p = 0.01$, Fig. 8), while this effect was not present at 25°C. At prey density = 8, feeding performance was higher at 18°C than 25°C across both fish predator species ($Z = 2.25$, $p < 0.05$); this was also true for prey density = 32 ($Z = 2.28$, $p < 0.05$).

Chironomid larvae

There was a significant interaction effect of ‘predator species × prey density’ on consumption of chironomid larvae (Table 6). Similar to *Culex* larvae, *L. seeber*i consistently consumed more prey than *S. calidus* at both temperature treatments at prey high densities (d = 96: $Z = -6.91$, $p < 0.001$; d = 128: $Z = -5.04$, $p < 0.001$; Fig. 8). Temperature had a significant main effect on prey consumption (Table 6) where both species reduced consumption of chironomid larvae at 25 °C compared to 18 °C ($F = 7.69$, $p < 0.001$; Fig. 8).

Overall, *S. calidus* consumed less of both prey types compared to *L. seeberi* at $d = 96$ ($Z = 6.90$, $p < 0.001$) and $d = 128$ ($Z = 8.03$, $p < 0.001$) regardless of temperature (Fig. 8).

Table 5: Model terms for all factors from generalised linear models with a quasi-Poisson error distribution used to determine differences in *Culex* prey consumption with regards to factors “predator species”, “temperature” and “prey density”, using a Type III Anova and χ2. Chisq indicates the Chi-squared (χ2) value, df indicates degrees of freedom.

Model term	**Chisq**	**df**	***p*-value**
Predator species	**175.2**	**1**	**<0.001**
Temperature	15.9	1	0.077
Prey density	**5067.1**	**7**	**<0.001**
Predator species × Temperature	14.3	1	0.093
Predator species × Prey density	**108.2**	**7**	**<0.01**
Temperature × Prey density	**73.4**	**7**	**0.043**
Predator species × Temperature × Prey density	42.6	7	0.297

Table 6: Model terms for all factors from generalised linear models with a quasi-Poisson error distribution used to determine differences in chironomid prey consumption with regards to factors “predator species”, “temperature” and “prey density”, using a Type III Anova and χ2. Chisq indicates the Chi-squared (χ2) value, df indicates degrees of freedom.

Model term	**Chisq**	**df**	***p*-value**
Predator species	**147.2**	**1**	**<0.001**
Temperature	**22.4**	**1**	**<0.01**
Prey density	**5923.8**	**7**	**<0.001**
Predator species × Temperature	0.3	1	0.738
Predator species × Prey density	**79.0**	**7**	**<0.001**
Temperature × Prey density	16.3	7	0.591
Predator species × Temperature × Prey density	7.0	7	0.934

FR results

Across all prey types and temperature treatments, significant Type II functional responses for both *S. calidus* and *L. seeberi* were observed (Table 7; Fig. 8).

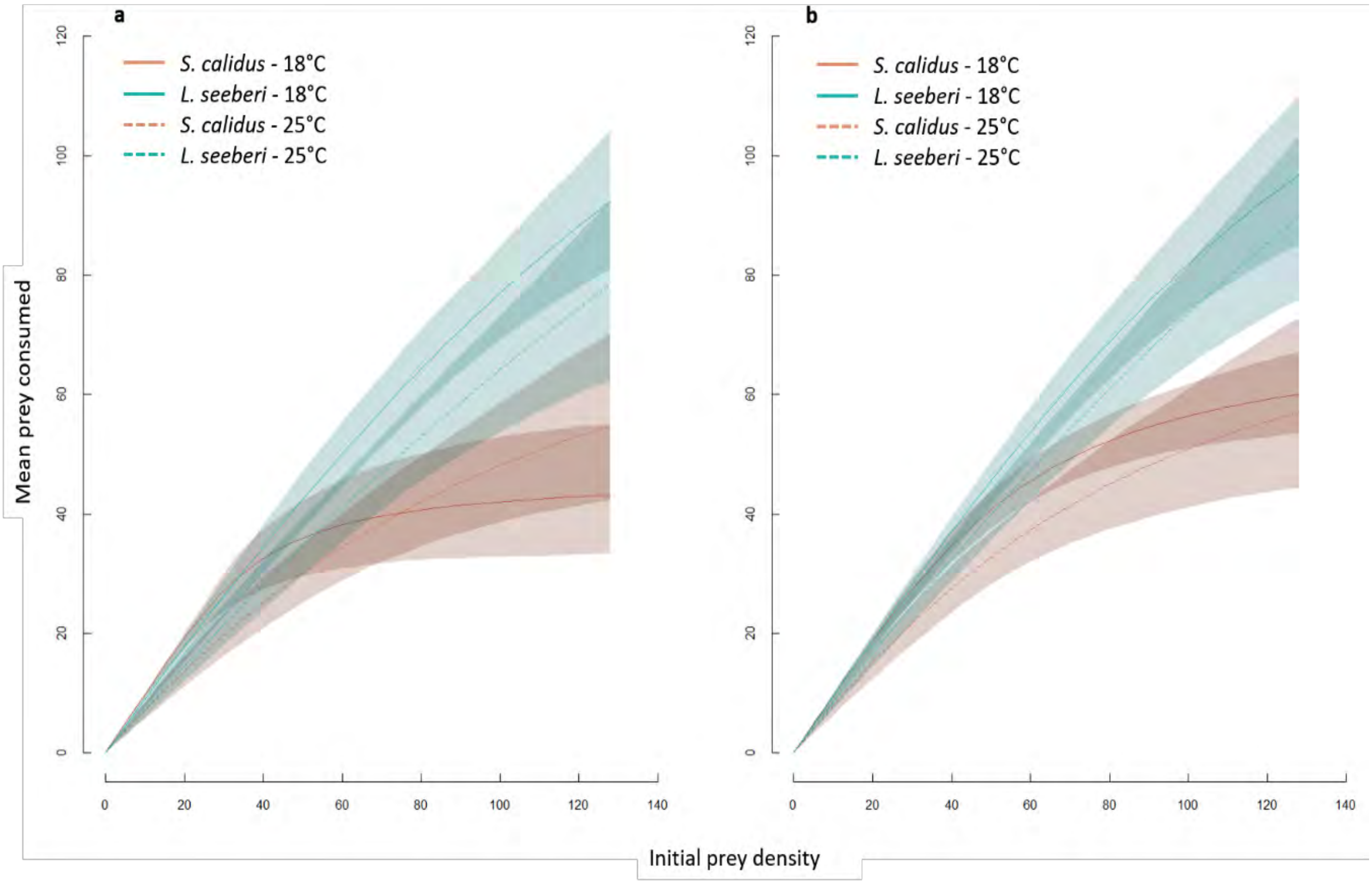

Figure 8: Functional response curves of the two focal fish species (*Sedercypris calidus,* red and *Labeobarbus seeberi*, in blue) across two temperature treatments on *Culex* sp. larvae (**a**) and chironomid larvae (**b**) as prey items. Temperature treatments are depicted using solid lines for 18°C and dashed lines for 25°C, respectively. The shaded areas indicate 95% confidence intervals.

Sedercypris calidus attack parameters, when feeding on *Culex* larvae, were significantly higher at 18°C than 25°C (Z = 8.03, p<0.001; Table 7), although handling time significantly decreased at 25 °C compared to 18 °C (Z = 9.53, p<0.001; Table 3). In contrast, increased temperature significantly increased the attack parameter (Z = 6.83, p<0.001; Table 7) and significantly reduced the handling parameter (Z = 2.38, p=0.017; Table 7), for *S. calidus* preying on chironomid larvae at 25 °C compared to 18°C.

For *L. seeberi* feeding on chironomid larvae, attack parameters were significantly higher at 18 °C than 25 °C (Z = 6.14, p<0.001; Table 7) and handling times were significantly longer at the lower temperature (Z = 2.46, p=0.014; Table 7). For *L. seeberi* feeding on *Culex* prey, the attack parameter was significantly higher at 18°C than 25°C (Z = 5.89, p<0.001; Table 3). However, handling time for *L. seeberi* feeding on *Culex* was not significantly different between 18°C and 25°C treatments (Z = 1.18, p=0.235; Table 7).

Table 7: First order terms and significance levels from logistic regression of the proportion of prey consumed against initial prey density, with functional response type, functional parameters, associated significance levels from Rogers' random predator equation, bias accelerated and corrected 95% confidence intervals for a and h, with regards to *Sedercypris calidus* and *Labeobarbus seeberi* predating upon chironomid and *Culex* larvae under different temperature treatments. a = attack parameter; h = handling time; 1/h = maximum feeding rate (per one hour) estimate.

Predator species	**Prey**	**Temp. (°C)**	**First order term (*p*-value)**	**FR Type**	***a* (*p*-value)**	***a* 95% BCa CI**	***h* (*p*-value)**	***h* 95% BCa CI**	**Maximum Feeding Estimate (1/*h*)**
S. calidus	Chironomids	18	-0.025 (<0.001)	II	1.95, <0.001	1.27-2.82	0.03 (<0.001)	0.02-0.03	33.33
L. seeberi	Chironomids	18	-0.012 (<0.001)	II	1.67, <0.001	0.89-2.68	0.01 (<0.001)	0.00-0.02	100
S. calidus	Chironomids	25	-0.013 (<0.001)	II	0.85, <0.001	0.52-1.35	0.02 (<0.001)	0.00-0.04	50
L. seeberi	Chironomids	25	-0.007 (<0.001)	II	0.96, <0.001	0.64-1.50	0.01 (<0.001)	0.00-0.02	100
S. calidus	*Culex*	18	-0.031 (<0.001)	II	2.79, <0.001	1.48-7.49	0.04 (<0.001)	0.03-0.06	25
L. seeberi	*Culex*	18	-0.007 <0.001	II	1.21, <0.001	0.67-1.92	0.01 (<0.001)	0.00-0.02	100
S. calidus	*Culex*	25	-0.01 (<0.001)	II	0.68, <0.001	0.43-1.04	0.02 (<0.001)	0.00-0.04	50
L. seeberi	*Culex*	25	-0.005 (<0.001)	II	0.69, <0.001	0.46-1.05	0.01 (<0.001)	0.00-0.02	100

Confidence intervals around the FR overlap, signifying a lack of significant difference on a phenomenalistic basis, between temperature conditions for each predator species (Fig. 8a,b). *Sedercypris calidus* showed either higher or similar attack parameters (the initial curve steepness) compared to *L. seeberi*, however, the consistently lower handling time of *L. seeberi* resulted in higher maximal feeding in *L. seeberi* regardless of prey type or temperature conditions (Table 3; Fig. 8a,b). There was no significant difference in FR curves between 18 °C and 25 °C for either predator species, however, across the board there were trends in which the FR magnitude was greater at 18 °C compared to 25 °C for all treatments, besides *S. calidus* preying more on *Culex* sp. larvae at 25 °C (Fig. 8a,b). When preying on *Culex* sp. larvae, the FRs of *L. seeberi* and *S. calidus* were similar under the lower prey densities at all temperatures, however, at higher densities they diverge wherein the FR for *S. calidus* at 18 °C reaches a plateau around prey density 60 (Fig. 8a). When preying upon chironomid larvae the FR curves diverge again around prey density 80 and maintain the trend that *L. seeberi* is able to consume more prey at higher prey densities than *S. calidus*, regardless of temperature treatment (Fig. 8b).

Sedercypris calidus, despite generally higher attack parameters, and exhibiting the highest attack parameter in the case of *Culex* prey at 18°C, did not exceed the maximum feeding rate per hour estimate of *L. seeberi*. This was found even when comparing its best performance (50 maximum feeding rate per hour estimate) against *L. seeberi*'s lowest estimate (100; Table 3, Fig. 8). The highest feeding rate estimates were consistently found in *L. seeberi*, primarily as a result of lower handling times than *S. calidus* in all scenarios. However, the FR curves for *L. seeberi* in both sets of prey and temperature treatments did not reach an asymptote and therefore the model handling parameter estimates, and consequently the maximum feeding estimates, are likely unreliable due to underestimation.

3.4 Discussion

I compared the relative feeding performance of two native fish species occurring in sympatry in a recovering river system, affected previously by invasive predatory fish, and currently by ongoing abiotic pressures from climate change and habitat degradation. These stressors are likely causing shifts in both resource availability and resource phenology (de Moor & Day, 2013; Bellingan *et al.*, 2019). In particular, the findings of this study assist in understanding competition for shared food resources in an oligotrophic system. This knowledge will assist conservation efforts in this system and the wider CFE through greater insight into the magnitude of species interactions, variation between sympatric species and the impact of anthropogenic stressors on these interactions. This study compared the ability of two local species (*L. seeberi* and *S. calidus*) to successfully consume two typical but distinct prey types (benthic and pelagic invertebrates) across representative baseline (18°C) and elevated summer (25°) temperature profiles.

I found that both species, *L. seeberi* and *S. calidus*, exhibit Type II FRs under all test conditions, with the caveat of spatial and environmental limitations imposed by the laboratory environment. In general, *L. seeberi* outcompeted *S. calidus* in maximum feeding rate per hour estimates regardless of temperature or prey type. In summary, at the tested temperatures, the FRs of the two species did not differ significantly intraspecifically for either prey type. However, consumption of chironomid prey did diverge between species, with *L. seeberi* being the better performer on this benthic prey. Ecological impacts on floating or pelagic prey (i.e. *Culex* in this study) are likely to equally impact the two fish species investigated here. Shifts in flow regime as a result of drying through climate change or land use changes could impact pelagic or drifting prey (Schlosser, 1995; Boddy, Booker & McIntosh, 2020). While FR experiments are constrained by laboratory simplicity, they have been proven to show high predictive impact where differences are regarded as relative rather than absolute. As such, the method is ideal for rapid assessment of vulnerable species which can be verified through further field studies. Consequently, these data represent the first quantitative estimates of the feeding efficiency of these species, which may provide predictive insight into their overlapping distributions and resilience to disturbance of natural competitive hierarchies and prey availability shifts.

Sedercypris calidus appeared to rapidly reach an asymptote in feeding performance, ultimately being limited by longer handling times, which resulted in it being outperformed by *L. seeberi* when higher densities of prey were available. The higher performance of *L. seeberi* when feeding on chironomid larvae may be explained by adaptation to feeding in sandy substrates. The species is often observed sifting through the substrate for food items in the wild, and in experimental trials chironomid larvae were observed to partially or fully burrow into the arena substrate. It is possible that *L. seeberi* is better adapted, morphologically and behaviourally, to this mode of feeding and the handling of prey items found in such substrates (i.e. sand, silt, gravel or detritus-dominated substrates) (Impson *et al.*, 2008). In comparison, the likely boulder/cobble-adapted *S. calidus* is thought to be more effective at foraging on prey items on the surfaces and between the crevices of these environments, which was not tested here (Impson et al. 2008; pers. obs.). Another plausible explanation regarding the better handling of high prey densities by *L. seeberi* is the migratory nature of the species. This species makes migratory journeys to spawning sites in riffle habitats in summer, which are likely to have a lower abundance of typical food items. In spring and summer, there is a marked increase in the abundance of aquatic insects (Bellingan *et al.*, 2019). *Labeobarbus seeberi* are likely well-adapted to take advantage of high densities of aquatic insect prey in this pre-spawning season, to ensure sufficient nutrient and energy intake for both the migratory journey and the maximisation of energy investment into reproduction. Evaluation of this possibility would likely need to be confirmed through further field observations and/or stable isotope analysis. Juvenile *L. seeberi* growth requirements likely necessitate a similar suite of adaptation that can be carried forward into adulthood for taking advantage of high prey densities prior to spawning migrations, increasing the advantage of this species in this study in terms of FRs. Ontogenetic shifts from primarily aquatic invertebrates in juveniles towards a higher degree of omnivory in adult *L. seeberi* are likely partly responsible for this difference in performance (Impson *et al.*, 2008). However, higher juvenile *L. seeberi* numbers associated with adult *S. calidus* aggregations are often observed, and the multiple spawning nature of *L. seeberi* throughout summer (Cambray *et al.*, 1997; King *et al.*, 1998) ensures that juveniles are present where both species co-occur across most of the river (see Chapter 2).

The higher attack parameter at 18 °C compared to 25 °C may be explained by the temperate origins of both species, with physiologies adapted to moderate temperatures and decreased efficiency at extremes of temperature. Both species are also known to be primarily insectivorous, and adapted to very similar prey profiles. There is also a general trend of feeding performance being highest at moderate or intermediate temperatures (Englund *et al.*, 2011; Dell *et al.*, 2014; Uiterwaal & DeLong, 2020). The attack parameter, handling time and thus the maximum feeding rate per hour estimate have been found to be thermally dependent (Englund *et al.*, 2011). Attack parameters appear to be strongly moderated by temperature, with higher temperatures showing consistently lower attack parameters in both species. This was unexpected in light of most literature indicating generally higher attack parameters with increasing temperatures (Rall *et al.*, 2010; Englund *et al.*, 2011; Gilbert *et al.*, 2014; Pepi *et al.*, 2018). This may be related to the metabolic trade-offs involved in making foraging decisions under stressed conditions (Vasseur *et al.*, 2014; Gilbert *et al.*, 2014). As temperature increases, as does oxygen consumption and metabolism of poikilotherms. Concordantly, the energetic budget of the individual is then increased which thus leaves a deficit (Gilbert *et al.*, 2014). Foraging behaviour consumes energy and may not be energetically worthwhile to engage in when prey densities are low, thus driving the low attack parameter and low handling time exhibited here. Consequently, when these species are under thermal stress in environments where prey resources are not in high abundance they may be disadvantaged in terms of energetic requirements (Killen, 2011; Gilbert *et al.*, 2014).

Contrary to expectations derived from the metabolic theory of ecology (Huey & Stevenson, 1979; Englund *et al.*, 2011), there was no significant increase in maximal feeding rates with temperature. These findings indicate some resilience to thermal change in these species which may potentially mitigate some aspects of future thermal stressors as a result of climate warming. The handling time of prey items was consistent across temperatures and prey types in *L. seeberi*, but fluctuated in *S. calidus*, which may be indicative of thermal sensitivity. In *S. calidus*, handling time improved at higher temperatures, while attack parameter decreased; this may be indicative of an energetic trade-off in the search phase (Pörtner *et al.*, 2006; Neubauer & Andersen, 2019). For example, fish could exhibit lower activity levels at higher temperatures, when energetic payoffs are greater at higher prey densities that require less energetic expenditure than when searching for low density prey). It must be noted that a fairly high degree of inter-individual variability in feeding performance was observed, which echoes the suggestion by Kadye et al. (2016) that the persistence of CFE fishes under harsh conditions is partially attributable to inter-individual variation. Raw feeding results are presented in the appendices (Figs. S4 and S5) to illustrate this variability.

The differences in maximum feeding rate estimates may be related to the use of juvenile *L. seeberi,* in comparison with adult *S. calidus,* in this study. As juveniles, higher feeding rates would be required to maintain fast growth rates during this developmental phase, as a priority for energy use. Similarities in performance may reflect plasticity in feeding behaviour and may play a role in the propensity for these species to co-occur frequently across their range (and across life stages) in the Rondegat River. As body temperature in ectotherms varies with environmental temperature, so too does their body velocity (Dell *et al.*, 2014), affecting both the predators and prey in this study. While a general trend of increased metabolic rates, increased consumption rates and higher activity levels can be predicted under increased temperatures (Dell *et al.*, 2014), the specific context of the players in ecological interactions must be well understood in order for accurate predictions to be made. For example, the success of introduced rainbow trout *Oncorhynchus mykiss* as an invasive species in South Africa has been limited, and is predicted to be further diminished by increasing temperatures under climate change (Shelton *et al.*, 2018c). This introduced species' impacts and density were increased under cooler spring and autumn conditions, and were limited in the warmer summer months (Shelton *et al.*, 2018c). This is in contrast to a plausible expectation (per the metabolic theory of ecology) of invasive species impacts being exacerbated through greater metabolic capacity and activity levels under higher temperature conditions.

Accurate prediction of outcomes based on thermal conditions is dependent on the foraging strategy and the thermy of the focal species in these interactions (Dell *et al.*, 2014). Generally, one would expect both escapes and failed attacks to be more likely under lower temperatures, leading to lower attack parameters and thus lower maximal feeding rates (Dell *et al.*, 2014). The results of the present study do not seem to support this trend, particularly in the case of *S. calidus*, which was consistently capable of higher attack parameters than *L. seeberi* at the lower test temperature, across both prey types. It is possible that *S. calidus*, being more uniformly distributed across the longitudinal span of the Rondegat River, is more adapted metabolically (i.e. having a thermal optimum for activity) to the mean temperature profile of the river (Reizenberg *et al.*, 2019) upon which the holding and lower test trials were based. Given this possible thermal advantage over *L. seeberi* at low prey densities the difference in attack parameters can be partially explained. While performance between the two species is similar at low prey densities, *L. seeberi* appears to be able to exploit higher prey densities through lower handling times, despite similar or lower attack parameters compared to *S. calidus*. This may infer possible niche partitioning as well as differential environmental filtering processes acting on the spatial and trophic segregation of the two focal species, especially given *L. seeberi* migration patterns. However, caution should be applied in extrapolation of the results found here in juveniles to adult *L. seeberi*. Establishing the level of trophic overlap or lack thereof could be achieved using stable isotope analysis of the food web available in the Rondegat River. Recent work on the trophic profiles of co-occurring piranha species indicated that, in contrast to predicted trophic overlap, species were near-completely trophically segregated (Alves *et al.*, 2017). Further work on the trophic relationships of the Rondegat cyprinid assemblage will be needed to determine the mechanisms of species co-existence.

The pelagic mosquito larvae prey used in this study are functionally analogous to drifting or surface prey items. Flow regime changes such as increased drying through climate change, or ongoing anthropogenic disturbance through land use change in CFE streams (such as the Rondegat), could impact the behaviour and abundance of this prey type, influencing the availability of an important prey resource in stream fishes (Bunn & Arthington, 2002; Boddy *et al.*, 2020; Meijer, Warburton & McIntosh, 2021). Predicted warming under current models of climate change is likely to affect CFE river communities in variable, but generally negative, ways (Shelton *et al.*, 2018b). The primary impacts are expected to be increased water temperatures and decreased total runoff (Dallas, 2013; Dallas & Rivers-Moore, 2014; Shelton *et al.*, 2018b). *Labeobarbus seeberi* is likely to be particularly vulnerable, as it is sensitive to temperature cues to facilitate migratory spawning; this is further exacerbated by the fragmented nature of the CFE (Shelton *et al.*, 2018b). *Labeobarbus seeberi* may be further vulnerable to increased temperatures through increased metabolic requirements, and in turn needing to expend energy resources through increased foraging time in order to remedy this energy deficit. The consistently lower prey handling times found in *L. seeberi* in this study are one such mechanism by which *L. seeberi* may mitigate this adaptive challenge. Under higher temperature scenarios, fish are more likely to shift from Type II to Type III FRs, wherein trade-offs between energy expenditure and foraging effort require efficient use of high density prey resources (Uszko *et al.*, 2017). In the case of *L. seeberi*, this effect may be more pronounced as it has relatively a large maximum body size and consequently higher energetic and respiratory requirements. Changes in performance in fish predators through climate change may be coupled with increased escape ability and altered phenology of aquatic invertebrate populations (Dell *et al.*, 2014), and the combined effect of these changes may reflect in drastically altered competitive dynamics in the context of food resources for this species in relation to other actors in the ecosystem.

In this study, a high degree of overlap in confidence interval estimates around the two species' FR curves was observed. This appears to be driven by high intraspecific variability in fish feeding performance. It is possible that this intraspecific variability in resource exploitation could echo the findings of Kadye et al. (2016) in the context of other CFE minnow species, which also coexist without clear evidence of niche partitioning. This was suggested to be primarily driven by intraspecific morphological differences in closely related redfin minnow species. Manna et al. (2020) found that morphological differences in loricariid fishes suggested trophic segregation, but diet analyses revealed high levels of trophic overlap. Spatial segregation through differences in habitat use was suggested instead to be the mechanism enabling coexistence (Manna *et al.*, 2020). Morphological and habitat differences, primarily in the context of resource use, should thus be ascertained in the case of *S. calidus* and *L. seeberi* to explore the role of morphology in this crucial aspect of their functional ecology.

Comparisons of functional responses and traits of threatened and range restricted endemic fish species under contrasting thermal conditions can be used to predict changes to species interactions and persistence under global change scenarios. The comparative functional morphology of the cyprinid assemblage in particular will provide extensive insight into the species interactions and differences in aspects of their functional response; as morphology will influence attack parameters and prey handling times of various resources (Luger *et al.*, 2020). Understanding the nuanced effects of temperature on imperilled fishes will allow researchers to predict changes in trophic interactions under future climate change scenarios.

CHAPTER 4: Ecomorphological niche separation facilitates co-existence of functionally similar threatened fishes

A large *Labeobarbus seeberi* specimen is measured in the field. Photo: Alex van Nynatten

4.1 Introduction

Anthropogenic and climate change-induced disturbances affecting resource availability may result in shifts in abundances and distribution as populations respond to these changes. Freshwaters in particular suffer from increased levels of biodiversity loss as a consequence of degradation (Tickner *et al.*, 2020). Increasing environmental stressors such as sedimentation, climate warming and other anthropogenic impacts (Shelton *et al.*, 2018b; Reizenberg *et al.*, 2019) will further act to alter competitive dynamics, feeding performance and ultimately the entire community (Comte *et al.*, 2013). Thus understanding and predicting the forces which shape current species assemblages, persistence and co-existence within these stressful environments is necessary to develop conservation strategies.

The coexistence of functionally similar organisms in resource-limited environments is at least partially explained by the partitioning of resources between species. Limiting similarity occurs through trait divergence to ameliorate competitive exclusion within a patch (Hutchinson, 1961; Leibold & McPeek, 2006). Habitat partitioning occurs when species have similar morphology, use the same set of resources but exploit a different habitat niche; this is affected by dispersal ability which, if low, may drive trait divergence and radiation such as in the African Great Lakes (Villéger *et al.*, 2017). Habitat filtering in contrast suggests that the environmental characteristics favour specific trait expressions within a community which allows species coexistence with reduced competitive exclusion (Chave, 2004; McGill *et al.*, 2006; Kraft *et al.*, 2015). Beyond these interactions between consumers for the same resources, prey species may apply their own pressures in consumer-resource interactions. For example, the characteristics of prey may create an adaptive pressure on a consumer that favours larger body size in the consumer (Cohen *et al.*, 1993; Cohen, Jonsson & Carpenter, 2003). In turn, other competing consumers may evolve other functional traits (in the long term) or exhibit resource-switching behaviour (in the short term) to reduce competition (i.e. interspecific character displacement) (Grant, 1972; Kraft *et al.*, 2015). Strategies to reduce intraspecific competition can include within-species morphological variation, variable resource use and / or microhabitat segregation within that species (Svanbäck & Bolnick, 2007; Bolnick *et al.*, 2007; Kraft *et al.*, 2015).

Functional traits are increasingly used as a powerful tool to facilitate generalisation of community assemblage rules without bias of geographic differences in taxonomy and phylogeny (Luiz *et al.*, 2019). However, trophic interactions are rarely incorporated into the many trait-based multiple stressor frameworks (but see Keppeler et al. (2020)). In order to make sense of the inherent complexity of natural systems, one approach is by means of a “periodic table of niches”, used to simplify and group organisms using functional traits (Pianka, 1974; Winemiller *et al.*, 2015). Ecomorphology is defined as the relationship between the functional morphology of an organism and its ecological performance (Winemiller, Kelso-Winemiller & Brenkert, 1995). Morphology forms the “hardware” of the organism, modulated by the “software” (i.e. behaviour) leading to an “output’’, a performance metric (for example, in the form of a functional response) (Sibbing *et al.*, 1998). The goal of ecomorphology is to provide a mechanistic approach to quantify and predict interspecific trophic relationships in a community of organisms. Thus, ecomorphology is a crucial pillar in the formulation of a periodical table of niches as envisioned by Winemiller et al. (2015).

The Rondegat River in the Cape Fold Ecoregion (CFE) of South Africa was the venue for a flagship restoration project for native fish threatened by invasive fish (Weyl *et al.*, 2013, 2014; Castañeda *et al.*, 2020a). Following the successful eradication of invasive centrarchid species, the recovering, yet still vulnerable, endemic cyprinid species remaining in the Rondegat River include the Clanwilliam yellowfish Labeobarbus seeberi (Gilchrist & Thompson, 1913) and two redfin minnow species, the Fiery redfin *Pseudobarbus phlegethon* (Barnard, 1938) and the Clanwilliam redfin *Sedercypris calidus* (Barnard, 1938). Shared phylogeny has been posited to result in similar niche usage and therein high competition to limited resources (Schoener, 1974; Skelton *et al.*, 2018; Keppeler & Winemiller, 2020). Nonetheless, this may not hold true in all systems; see Sibbing et al. (1998) and Sibbing and Nagelkerke (2001), where the closely related cyprinid assemblage of Lake Tana exhibits a high degree of morphological divergence. Kadye et al. (2016) suggested that the common scenario of co-occurrence of closely related species of redfins in CFE streams is driven by niche-based processes. Cape Fold Ecoregion systems are low in primary productivity (Dallas & Day, 2007; de Moor & Day, 2013), which suggests that co-occurring species in such environments are likely to compete for highly limited food resources. However, relatively small differences in morphology could allow divergence in feeding specialisations to reduce the strength of competition (Schoener, 1974; Wikramanayake, 1990; Winemiller *et al.*, 1995; Keppeler & Winemiller, 2020). Spatial partitioning does exist to some degree in the Rondegat River community. All three cyprinid species co-occur with each other, however *P. phlegethon* is most abundant mainly in the upper reaches and L. seeberi primarily in the lower reaches, while *S. calidus* broadly co-occurs with both other species across the length of the river (Weyl *et al.*, 2013). Furthermore, habitat associations largely mirror the spatial patterns observed (Chapter 2).

In a scenario of spatial overlap between these three species, including the still-recovering *P. phlegethon* (according to patterns in recolonisation based on occupancy modelling: Castañeda *et al.*, 2020a), determining the mechanisms of co-existence in the Rondegat cyprinid assemblage is important for informing potential conservation interventions. Furthermore, determining the mechanisms of co-existence will aid in understanding and predicting the responses of these vulnerable species to anthropogenic stressors and further habitat degradation. Using ecomorphology as a tool and a legacy gut contents dataset as proof of concept, I aim to test the limiting similarity hypothesis. Under this framework, it would be expected that the co-existence of the three species distributed throughout the river is driven by trophic niche partitioning (i.e. diet as a proximate mechanism) as a result of ecomorphological separation (i.e. ultimate mechanism of co-existence). In particular, I expect that species with a high degree of spatial overlap will also show a higher degree of interspecific morphological differentiation and in turn more divergent trophic niches. Thus I predict that *S. calidus*, as the most ubiquitous species, will show a higher degree of separation in trait space and diet from the other species. Furthermore, in terms of the degrees of specialisation in diet among the three species, I expect that *P. phlegethon* will have the most specialised diet, followed by *L. seeberi* as a more generalist species but preferring prey taken from the benthic environment primarily; finally, *S. calidus* is expected to have the most variable (i.e. generalist) diet, being able to exploit the widest feeding niche of the three species.

4.2 Methods

All three species were collected from the Rondegat River (32°24'S; 19°05'E) in October 2019 using fyke nets and following local conservation authority CapeNature guidelines as described in Broom et al. (2021) and Chapter 3.

Ethical considerations

Sampling was carried out in accordance with relevant South African provincial collection, live import and export permits. Permission for land access was granted by CapeNature and private land owners where applicable. The work was approved by the Rhodes University Animal Ethics Committee (reference number DIFS2118) and the SAIAB Animal Ethics Committee (reference number 25/4/1/5_2018-10).

Morphological measurements

Ten individuals of each species were euthanised using an overdose of 400 mg/L clove oil and immediately frozen. The selected cohorts were: adult *P. phlegethon* n = 10 (min – max standard length SL: 42 – 51 mm); adult *S. calidus* n = 10 (SL = 58 – 90 mm); juvenile *L. seeberi* n = 10 (SL = 63 – 116 mm). I applied the Food-Fish Model (FFM) to relate feeding-associated morphological traits of the focal fish species with the properties of aquatic food types (such as size, shape and escape characteristics) (Sibbing & Nagelkerke, 2001). This method quantitatively links a fish predators' feeding structures to the characteristics of different prey types, and can be used to objectively compare and rank feeding specialisations between species. The feeding-associated morphological traits measured followed Sibbing and Nagelkerke (2001) and Nagelkerke et al. (2018; Table 8). This set of traits was chosen because they relate to capacity to prey on relevant aquatic prey types and were appropriate for the three cyprinid species investigated. Measurements were performed using a standard dissection kit, a balance (CQT 202) for weight measurements, a set of digital callipers for length measurements, and a dissection microscope (Leica).

All statistical analyses were performed in the R software version 4.0.0 (R Core Team, 2020). The FFM (Sibbing & Nagelkerke, 2001) was used to relate a set of functional traits of individual fishes (Table 8) to their ability to exploit different prey types. Functional trait measures were scaled by dividing them by the standard length (SL). Surface areas were divided by SL, while ratios between traits and angles were not scaled (Nagelkerke *et al.*, 2018; Luger *et al.*, 2020). The FFM used here followed Luger et al. (2020). Theoretically optimal trait values for consuming each of the relevant aquatic food resources were systematically compared with the actual trait values in each of the fish, resulting in a trophic profile for each individual expressing its relative capacity to feed on each of the food types (details in Luger et al., 2020 and Nagelkerke et al. 2018). The morphology of all three species was compared using principal components analysis (PCA) on functional feeding traits. This approach is also used for trophic profiles to provide insight into differences in feeding specialisation between species. Means of trophic profiles were bootstrapped (n=1000) and 95% confidence intervals calculated, where lack of overlap in bootstrapped mean indicated significant difference.

Dietary data collection

Dietary data used in this study were collected in September – November 2003 and April 2004 (Woodford, 2005), and were partially summarised by Lowe et al. (2008). This represents the only existing dataset of the focal species' diet given the vulnerable status of the fishes and the strict conditions of fish use set out in the collection permits. These gut content data are used here as a proof of concept "snapshot" of diets. This gives insight into the realised niche of the focal species at that point in time, in contrast to the capacities for feeding niche exploitation, which is inferred from the species' morphology. Fish were collected using electrofishing, seine and fyke nets across the length of the river and processed within two hours of capture to avoid digestion of gut contents. Cyprinids lack a true stomach, thus the contents of the entire gut were analysed by dissection using scissors and forceps and contents placed into a Petri dish. Food item groups were determined and then sorted using a binocular dissecting microscope.

To make the identified gut contents from Woodford (2005) more easily comparable to the predictions generated by the FFM, food items were categorised into broad functional food groups (see appendix Table S3). These food groups were "Algae", "Plant matter", and "Detritus" in addition to the insect and larvae functional groups as part of the broader "Invertebrates" group, which were directly measured as volume percentages of the gut content in Woodford (2005). Invertebrate prey counts were converted to volume percentages by dividing the counts of invertebrate food items by the total gut content volume of invertebrates, and assigning these values to "Larvae" (dipteran and trichopteran larvae), or to "Insects" (encompassing both terrestrial and aquatic invertebrates). In effect, higher counts of individual invertebrates could be compared to higher volumes of detritus, algae or plant matter on a similar scale.

An analysis of similarities (ANOSIM) was used to determine whether species had significant differences in diet based on proportional food group data. A pairwise multilevel comparison permutational analysis of variance (PERMANOVA) was used to determine diet differences between species pairs post-hoc, using the R package "pairwiseAdonis" (Arbizu, 2020). P-values (0.05 significance level) were adjusted for multiple testing using a Bonferroni correction. Permutational analyses used 999 permutations to establish confidence intervals. Differences in proportional gut content of the broad food groups were illustrated using bootstrapped mean values and 95% confidence intervals.

Table 8: Ecomorphological measurements used in this study. Adapted from Sibbing and Nagelkerke (2001) and Nagelkerke et al. (2018). Most measurements were scaled by standard length (SL) or standard length squared (SL^2) where applicable.

Morphological trait	Abbreviation / description	Unit	Scaled by
Anterior barbel length	ABaL	mm	SL
Body depth	BD	mm	SL
Body width	BW	mm	SL
Caudal peduncle depth	CPD	mm	SL
Eye diameter	ED	mm	SL
Gape size /area	OGAr	mm^2	SL^2
Gill arch resistance	GiRL/GiRD: ratio between gill raker length and gill raker distance	ratio	-
Gill raker length	GiRL: Average length of 10 rakers, lateral on arch 2	mm	SL
Gut length	GuL: measured from pharyngeal jaw to vent	mm	SL
Head length	HL	mm	SL
Hyoid length	HyL: Length hyoid bar (ceratohyal and hypohyal bones)	mm	SL
Lower jaw closing force efficiency	Ljin/Ljout: the ratio between input and output closing lever of the lower jaw	ratio	-
Lower jaw length	LJL: From anterior tip to its posterior joint	mm	SL
Operculum area	OpAr: Postorbital length × Operculum depth	mm^2	SL^2
Operculum depth	OpD: From skull to point where sub-operculum and inter-operculum meet	mm	SL
Oral gape axis	OGAx: Ranging from terminal to sub- or supra-terminal; measured as 90° – OGAx	degrees	-
Palatal organ area	PalOAr: Trapezium between the first and fourth gill-arches, left and right	mm^2	SL^2
Postlingual organ width	PLOW: Width of the oral floor between the left and right second gill-arch	mm	SL
Postorbital length	POrL	mm	SL
Protrusion length	ProtL: extension of the upper jaw when opening the mouth	mm	SL
Relative gape area	OGAr/Bar: ratio between oral gape area and body area (body width × body depth)	ratio	-
Standard length	SL	mm	-
Velocity suction capacity	HyL/LJSL: ratio between hyoid and lower jaw-suspensorium length	ratio	-
Volume capacity operculum	POrL/OpD: ratio between postorbital length and operculum depth	ratio	-

4.3 Results

Ecomorphology

The morphology of the three species differed strongly according to the PCA, with 66.8% of the variance in morphological traits explained on the first and second axes (Fig. 9a). *Sedercypris calidus* and *L. seeberi* were similar in terms of principal component axis 1, but diverged from one another across axis 2. *Pseudobarbus phlegethon* separated strongly from the other two species along both axes and was primarily characterised by a deeper caudal peduncle and shorter head length (Fig. 9b). Eye diameter and gape size were larger in *S. calidus*, while *L. seeberi* had a longer head length and larger opercular volume capacity than the other two species (Fig. 9b).

The trophic profiles PCA (Fig. 9c), with 84.1% of variation explained in the first two axes, indicated a stronger similarity in trophic profile between *L. seeberi* juveniles and *S. calidus*, with *P. phlegethon* separating from these two species. In particular, the results suggest that *P. phlegethon* is more specialised towards feeding on larvae, plant matter, seeds, crustaceans, molluscs and detritus. *Sedercypris calidus* and *L. seeberi* appear to be more suited to townet feeding (i.e. "overswimming" of prey items, when the fish engulfs prey items through keeping the mouth open to filter large volumes of water) on zoo- and phytoplankton and fish (pursuit feeding mode) (Fig. 9d). All three species had significantly different trophic profiles for all prey types (Fig. 10).

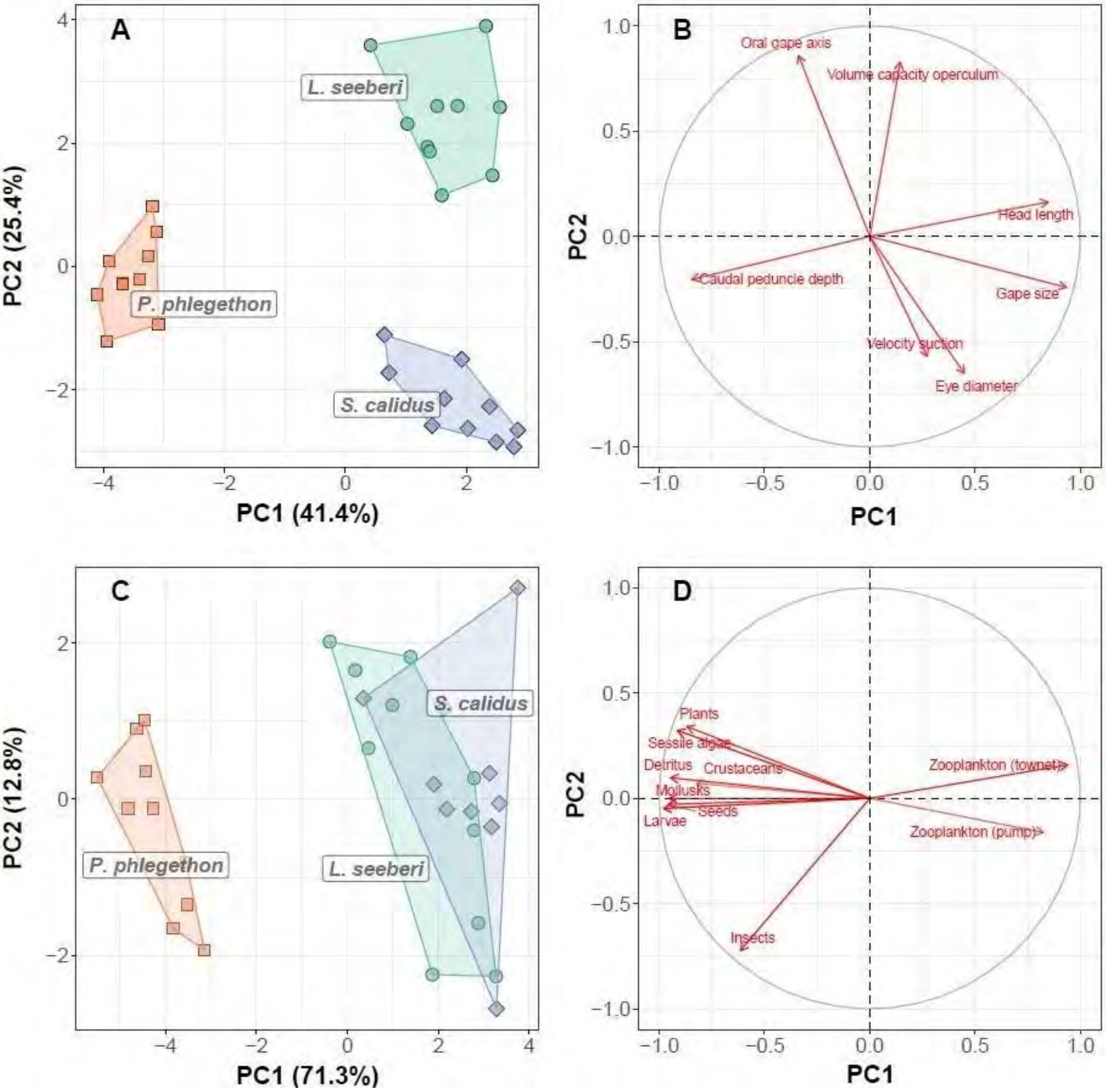

Figure 9: Panels A and B: PCAs of the morphological traits of the three focal species; panel A: individual fish morphology distributed across PCA dimensions, panel B: morphological measurements as vectors. Panel C and D: PCAs of the trophic profiles of the focal species; panel C: individual fish trophic profiles as points overlaid on PCA dimensions 1 and 2, panel D: trophic profiles displayed as vectors.

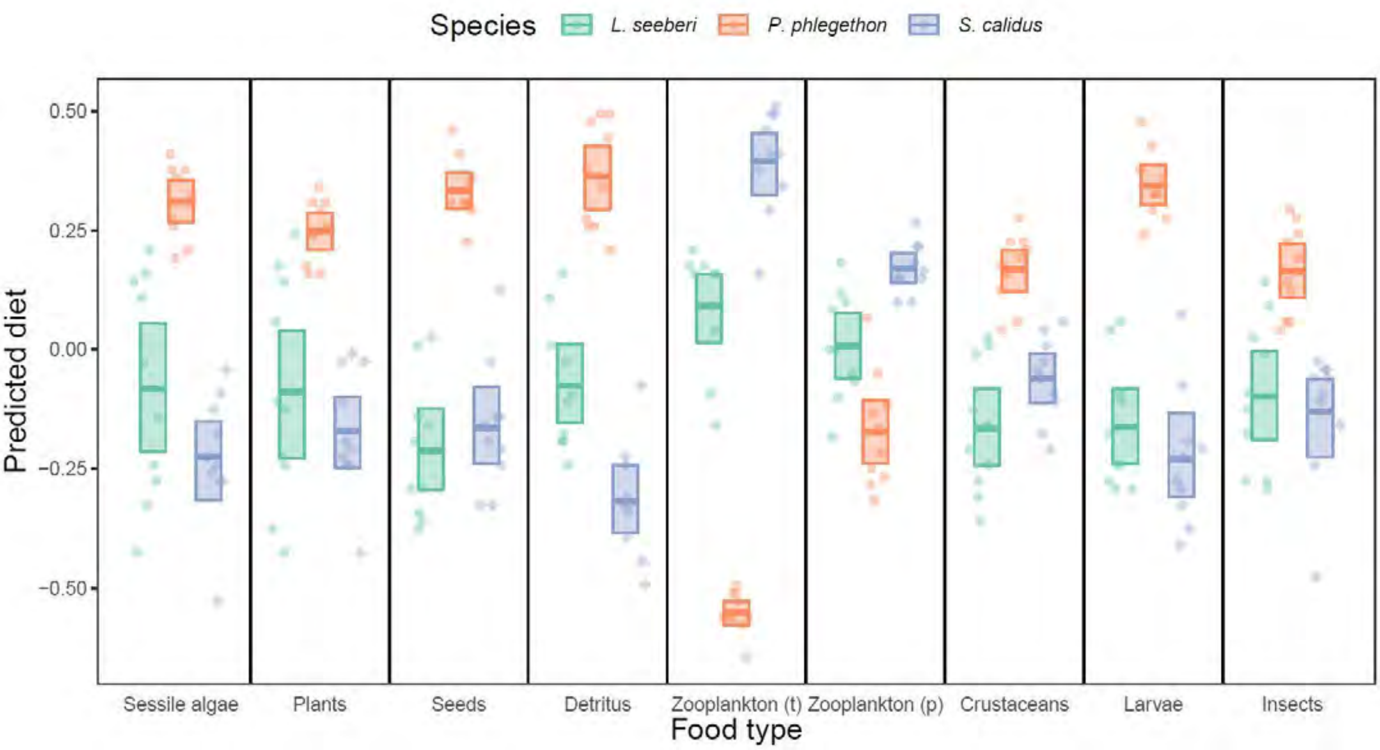

Figure 10: Correlations of predicted diet specialisations of the three species (*Labeobarbus seeberi*, *Pseudobarbus phlegethon* and *Sedercypris calidus*) based on their calculated trophic profiles. Horizontal lines indicate the bootstrapped mean values, boxes indicate upper and lower 95% confidence intervals. At least two of the three species were significantly different ($P < 0.05$) in predicted correlations with all food types. Zooplankton (t) refers to "townet" feeding on zooplankton; Zooplankton (p) refers to "pump" feeding, wherein fish gulp small volumes of water selectively, in contrast to indescriminate "townet" feeding requiring active swimming.

Diet

The ANOSIM revealed that the three species were significantly dissimilar in their gut content based on broad food groups, although with some notable similarity inferred through the low R value (R = 0.18, p = 0.001). Pairwise comparison of the gut contents indicated significant differences in diet between *L. seeberi* and *P. phlegethon* ($F_{(1,\,73)} = 6.03$, $R^2 = 0.09$, $p_{adj} = 0.012$), and *P. phlegethon* and *S. calidus* ($F_{(1,\,73)} = 10.86$, $R^2 = 0.20$, $p_{adj} = 0.003$), but not between *L. seeberi* and *S. calidus* ($F_{(1,\,73)} = 3.44$, $R^2 = 0.08$, $p_{adj} = 0.075$). However, all three species show overlap in diet, based on the broad food groups of detritus, algae, plant matter, insects and larvae (Fig. 11). The diet of *P. phlegethon* shows less intraspecific variation than the other two species and appears to be most specialised on larvae, while insects, algae and plant matter are consumed less. *Labeobarbus seeberi* and *S. calidus* both show more intraspecific variation in diet and thus can be considered as more generalist, although *S. calidus* on average had more insects and low detritus content in its diet. *Labeobarbus seeberi* did not show any specialisation among the three species (Fig. 11).

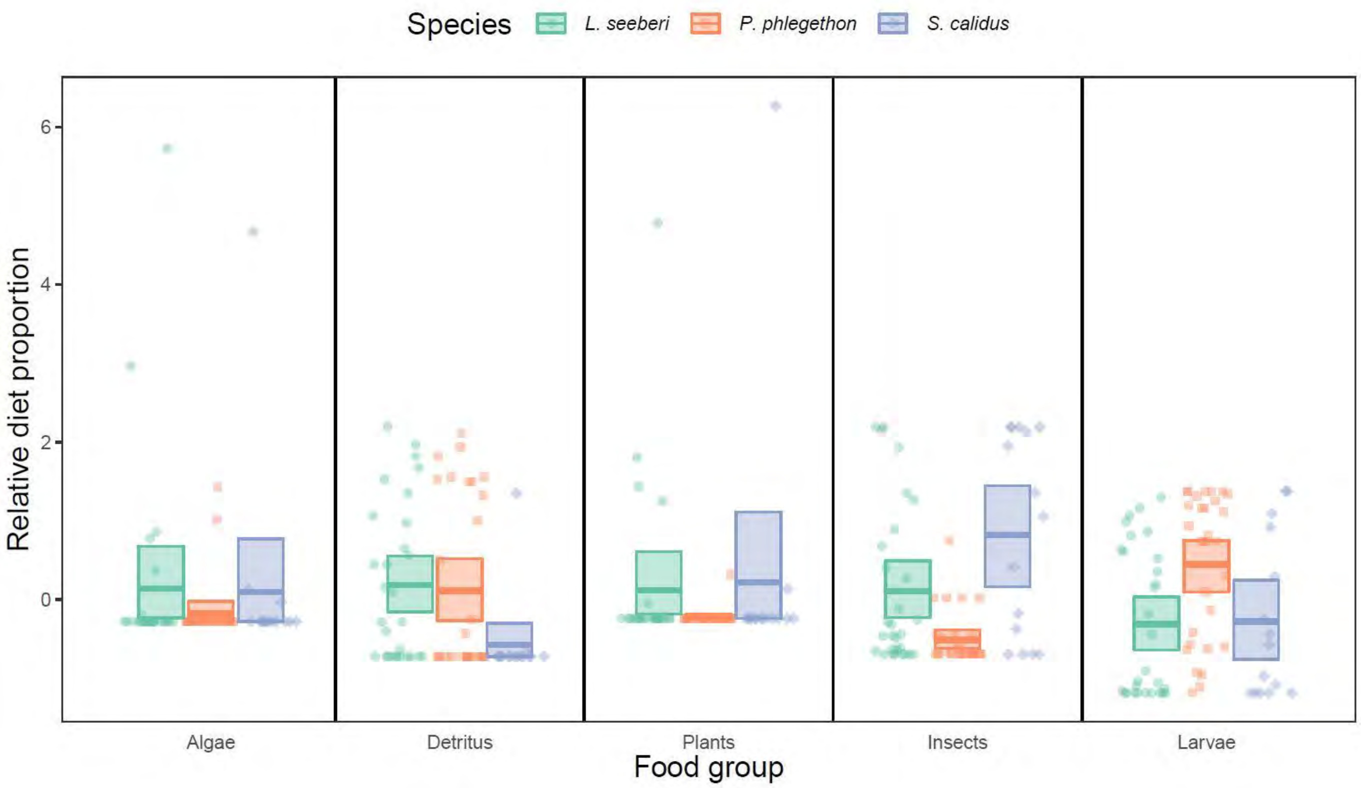

Figure 11: Relative proportions of algae, detritus, plant, insect, and larvae food groups making up the gut content of the focal species *Labeobarbus seeberi*, *Pseudobarbus phlegethon* and *Sedercypris calidus*. Values are derived from the counts of prey items proportionally to their respective volume in gut content. Horizontal lines indicate the bootstrapped mean values, boxes indicate upper and lower 95% confidence intervals.

4.4 Discussion

Determining mechanistic process which govern ecological community organisation is crucial to predict changes in ecosystem functioning following perturbations. My hypothesis was that species with high levels of spatial overlap will have stronger trophic niche differentiation to mitigate competition through limiting similarity. The results, however, indicate that the two species with higher spatial overlap have higher similarity in feeding niche (predicted and actual). Whereas *P. phlegethon*, currently only abundant in the upper reaches, has a more specialised feeding niche. The FFM was thus able to accurately predict the diet specialisations of each species within the Rondegat River system and these findings can be used to discuss the implications for the conservation of the assemblage.

Overlap in niche was expected to increase from morphology to trophic profile to actual diet data, as each level increases in complexity and accounts for behavioural and environmental plasticity. All species separated out in morpho-trait space; however, the species with the higher spatial overlap, *L. seeberi* and *S. calidus*, mostly overlap in trophic profiles and actual diets whereas *P. phlegethon* diverges consistently. In another CFE example, three coexisting species (*Pseudobarbus burchelli*, *Sandelia capensis* and *Galaxias zebratus*) were compared in their degree of trophic segregation and diet partitioning as mechanisms driving community structure (Shelton *et al.*, 2018a). Shelton et al. (2018a) found similar results to those found here, using a stable isotope diet approach alongside gut contents: all three species occupied different trophic niches, but with some degree of overlap. Notably, Shelton et al. (2018a) suggest that resource partitioning and biotic interactions are more important than environmental filters in CFE streams. Coexistence patterns in other CFE cyprinids are partially driven by intraspecific phenotypic differences, particularly in species with larger ecological niches (Kadye *et al.*, 2016). A study of frugivorous fishes in the Amazon floodplains used gut content data and stable isotope analysis to determine the extent of diet overlap, finding evidence for temporal shifts in feeding niche in response to flood pulses and other disturbances (Correa & Winemiller, 2014). Another study used phylogenetic relatedness, morphology, gut content and stable isotope analysis to argue that a combination of all of the above approaches achieved the highest resolution of assemblage trophic structure and feeding niches (Keppeler & Winemiller, 2020). Here, evidence of multiple interacting processes affecting species coexistence in the Rondegat River cyprinid assemblage has been found. Primarily, some degree of micro-habitat segregation and spatial sorting are likely occurring, especially between the two species with highly overlapping diets, *S. calidus* and *L. seeberi*. Ontogenetic shifts are likely to occur in maturing

L. seeberi juveniles towards a more omnivorous diet (Impson *et al.*, 2008, 2017); however, the multiple-spawning nature of this species throughout summer (Cambray *et al.*, 1997; King *et al.*, 1998) ensures that juvenile *L. seeberi* cohorts are continuously in contact with adult *S. calidus* across much of the system. Secondly, evidence for limiting similarity is found through the specialized morphology of all three species, given the patterns of coexistence across the length of the river. Finally, the trophic niches based on ecomorphology and gut content-based field data suggest some degree of habitat filtering acting on this fish community.

I propose that competition between *L. seeberi* and *S. calidus* is likely reduced via microhabitat separation as inferred from the proportional differences in detritus and insect consumption between the two. Field observations and the limited available literature on *S. calidus* diet indicate a generalist feeding profile with a strong capacity for drift feeding, primarily taking prey from the water column and striking at surface prey (i.e. drift feeding) and detritus sifting (Woodford, 2005; Broom *et al.*, 2021). Drift feeding capacity and drifting prey capture performance are strongly affected by morphology (Rincón *et al.*, 2007) and the FFM results mirror this drift-feeding specialisation (defined here as “townet” feeding). Body shape in fish with drift feeding specialisations reflects a trade-off between strong swimming ability to hold position in stream currents, while maintaining high agility for successful prey capture (Grossman *et al.*, 2002; Rincón *et al.*, 2007). In comparison, *L. seeberi* has the least specialised diet, consistent with its morphological traits which indicate preference for benthic prey and detritus (though ontogenetic shifts between juvenile and adult *L. seeberi* may complicate this relationship, depending on local abundances and site-specific characteristics). This is supported by experimental data showing that *L. seeberi* outperforms *S. calidus* in functional response on both benthic and open-water invertebrate prey when available in high densities, indicating a competitive advantage for *L. seeberi* under this scenario (Broom et al., 2021; Chapter 3). Conversely, *S. calidus* was notably better adapted to feeding on drift and surface prey when these are available at low densities (Broom *et al.*, 2021; Chapter 3).

Pseudobarbus phlegethon appears to have adapted towards a specialised feeding profile in headwater stream, complex benthic environments. Indeed, the uppermost reaches of the river have the highest *P. phlegethon* abundance (Weyl *et al.*, 2013). In contrast to the two other species, *P. phlegethon* appears to be more specialised towards feeding on more sessile prey and particularly suited to small, primarily benthic prey items. Limited literature on the diet of this species suggests that it feeds almost exclusively on aquatic invertebrates (primarily dipteran larvae), as well as some ingestion of detritus and filamentous algae, with seasonal variation being observed (Skelton, 2001; Woodford, 2005; Whitehead *et al.*, 2007). This is in line with predictions based on the ecomorphology of this species and gut content data. Further, the relatively wide caudal peduncle depth of *P. phlegethon* may also point to a headwater specialisation and manoeuvring in the associated more complex boulder-cobble habitat (Oliveira *et al.*, 2010). Thinner caudal peduncles are usually found in fish with stronger endurance swimming ability through reduced drag (Sibbing & Nagelkerke, 2001), and the relatively thick caudal peduncle may assist this species with short-range speed bursts to capture small agile prey items. In particular, *P. phlegethon* appears to be best suited to feeding in the interstitial spaces between the submerged structures of the benthic environment. These microhabitats are most commonly available in the pristine upper reaches of the system, where this species is most abundant. This signal of relative specialisation in feeding morphology and behaviour should be confirmed through in-situ observations of feeding behaviour. This specialisation sets *P. phlegethon* apart from *L. seeberi* and *S. calidus*, both of which show evidence of more variable use of the environment, switching between water column feeding and feeding on benthic surfaces.

There are some caveats to the findings arising from this study. It could be argued that diets of fish from invaded reaches may have been impacted by the alien fish invasion. However, owing to the invaded area of the river being only the lowermost four kilometres between the river mouth and the Rooidraai waterfall, and only *L. seeberi* adults being found in this area at the time, the actual impact of the invasion on diet in the majority of the river is likely to be negligible. Further research is required; I suggest the use of stable isotope analysis or other longer-term diet indicators to further the understanding of diet in this system and compare it to the uncovered feeding capacities derived from ecomorphology here. Additional direct comparisons of this system to others within the CFE and in other comparable Mediterranean-climate ecosystems are further encouraged.

The Rondegat River and the CFE are under numerous anthropogenic pressures which continue to threaten the persistence of these populations. *Pseudobarbus phlegethon* in particular has very few extant populations, is highly restricted range and vulnerable to non-native predatory fishes (Skelton, 1996; van der Walt *et al.*, 2017a). This population has not yet reached equilibrium since the piscicide intervention and is considered as still in recovery (Castañeda *et al.*, 2020a). The present results indicate a specialised diet with high dependence on sensitive invertebrate taxa (Trichoptera), highlighting the importance of stream health and habitat integrity. It could therefore be considered *P. phlegethon* persists in the protected upper reaches due to a higher abundance of ecologically sensitive prey compared to the more disturbed lower reaches. Another conservation concern is the increased turbidity in the Rondegat River as a result of widespread summer fire events in 2009 and 2018, as well as localised dam construction and removal in the mid and lower reaches of the river (Woodford *et al.*, 2005; Weyl *et al.*, 2014). Key river health bioindicator taxa such as Hydropsychidae, Philopotamidae and Polycentropodidae (grouped under larvae in the gut content analysis) are strongly associated with the stones-in-current biotope and would have been smothered by the sedimentation that moved down the river between the 2009 fire and the 2012 rehabilitation. Surface drift feeding on terrestrial insects in *S. calidus* points to an enhanced ability to exploit cross-ecosystem energy subsidies, making this species potentially more resilient than the other cyprinids to long-term environmental change such as climate warming or reduced flows that might negatively affect availability of instream resources (Uno & Power, 2015; McIntosh *et al.*, 2018).

My predictions based on the ecomorphology of the three species are in partial agreement with the conclusions drawn from the diet data but there are discrepancies and caveats that need to be explored. The prey biota are under selection pressure in response to fish predators (and other stressors) and exhibit their own phenotypic variation, which consequently affects the increase in niche width of the fish predators as their spatial sorting manifests over the course of the river. This demonstrates the importance of taking into account both consumer/predator and resource dynamics simultaneously to explain possible spatial and co-existence patterns. Nonetheless, the FFM was able to predict the actual diet remarkably well and indicates dependencies on certain resource types. Thus this method could be posited as a rapid and low intensity assessment tool for threatened fish species ecology.

CHAPTER 5: Feeding behaviour of a recovering cyprinid fish assemblage revealed using underwater video

A mixed group of feeding *Sedercypris calidus*, *Pseudobarbus phlegethon* and *Labeobarbus seeberi* captured on underwater video in the Rondegat River, Western Cape, South Africa.

5.1 Introduction

Freshwater biodiversity is in decline globally, and is characterised both by high rates of endemism and a multitude of interacting anthropogenic threats (Dudgeon *et al.*, 2006; Abell *et al.*, 2007; Tickner *et al.*, 2020; Harper *et al.*, 2021). Freshwater systems are often disregarded in conservation planning, despite the provision of vital services to communities (Dudgeon *et al.*, 2006; Albert *et al.*, 2020; Tickner *et al.*, 2020). When conservation interventions are implemented, a holistic and multi-faceted approach is required to adequately address the issue; from choice and scale of intervention through to the restoration of the affected ecosystem post-intervention (Lintermans, 2013; Harper *et al.*, 2021). Restoration efforts in particular tend to lack effective long-term monitoring and in-depth investigation of the fundamental mechanisms and species interactions that underpin community function (Lintermans, 2013; Penaluna *et al.*, 2017; Harper *et al.*, 2021). Trophic interactions and predation in particular are primary drivers of community structure and function (Sih, Englund & Wooster, 1998; Schmitz, 2007). Trophic interactions are often the first to be affected by environmental (biotic and abiotic) stressors, and trophic cascades following from the disruption or loss of these interactions are a major catalyst for biodiversity loss and reduced resilience (Stachowicz, 2001; Jackson *et al.*, 2016b, 2017).

Trophic interactions in natural ecosystems reflect a significant portion of the total interactions responsible for community dynamics. These interactions often include knock-on effects across species and trophic levels that should be considered in order to gain a holistic understanding of a given system (Jackson *et al.*, 2016b, 2017). Methods to understand trophic interactions in aquatic ecosystems are diverse and have various advantages or drawbacks to their use. Two examples are gut content analyses and stable isotope analysis, both of which inform on trophic position and resource acquisition patterns. Gut content analysis was applied in Chapter 4, alongside a morphometric predictive approach and low sample numbers, with the aim to improve upon traditional approaches and reduce the negative impacts of sampling. However, these methods can be destructive and stressful, which is undesirable in sensitive ecosystems and when working with imperilled species. Beyond trophic interactions, competition for resources is often an important factor in speciation and thus exerts strong effects on community composition over time (Robinson, 2000; Pritchard & Schluter, 2001; Ward, Webster & Hart, 2006; Bolnick *et al.*, 2007). Competitive interactions between species are expected when there is a high degree of morphological and ecological similarities within the assemblage (Usio & Nakano, 1998). In the case of the Rondegat assemblage, the findings of previous chapters show significant overlap in the spatial distribution, habitat associations, feeding capacities and diet, particularly between *Sedercypris calidus* and *Labeobarbus seeberi*.

In-situ observational studies are valuable as a complement to well-designed experimental tests of ecological theory and as validation of conclusions derived from laboratory or desktop-based studies. Remote underwater video (RUV) allows a relatively unbiased in-situ method of analysing real-time fish behaviours and inter-species interactions with minimal observer effect (Ebner *et al.*, 2009). Since the inception of the field of animal behaviour research, primarily using direct observation by researchers, efforts have been potentially confounded by the observer effect, i.e. that the presence of the researcher alters the observed behaviours of the focal animals. Most studies of this effect have concluded that some form of observer effect is indeed being found in studies of animal behaviour both in the field and in laboratory settings (Nanninga *et al.*, 2017b; Branconi, Wong & Buston, 2019). Additionally, RUV is low-impact and well-suited to imperilled and threatened fish species that may be sensitive to capture and handling (Ebner *et al.*, 2009, 2014; Ellender *et al.*, 2012). Remote underwater video is also valuable for aquatic biodiversity, population dynamics and habitat use studies (Ellender *et al.*, 2012; Ebner & Morgan, 2013; Ebner *et al.*, 2014; Broom *et al.*, in review). In comparing direct (e.g. scuba diving or snorkelling) to indirect (e.g. remote underwater video) observation approaches in the marine environment, Branconi et al. (2019) found that rapid, repetitive behaviours are better scored by a video camera system, while behaviours exhibited close to the coral habitat were better scored by a diver. When video camera systems are used, the observer effect is not entirely removed as a consideration, and a species-specific acclimation time is recommended after deployment to obtain unbiased behavioural data (Nanninga *et al.*, 2017b).

The Rondegat River cyprinid assemblage reflects a commonly observed situation in stream fish communities where ecologically similar fish species co-occur with overlapping spatial distribution and resource use (see Chapters 2, 3 and 4), in contrast to expectations based on community assemblage theory (Grossman, Moyle & Whitaker, 1982; Grossman, Dowd & Crawford, 1990; Fausch & Bramblett, 1991). Indeed, the findings of Chapter 4 suggest that strong competition driven by very similar diets and feeding capacities, and differing feeding performance, particularly between *S. calidus* and *L. seeberi*, would be expected. It is possible that trophic niche differentiation is occurring through shifts in microhabitat use and targeting of different prey types between species (*sensu* Nakano, Fausch & Kitano, 1999), as no obvious competition-reduction strategies have otherwise been observed for this assemblage. Thus, my objective was to investigate the fine-scale differences in in-situ feeding behaviour that facilitate coexistence by reducing competition.

The RUV approach facilitates a more natural quantification of microhabitat/ foraging target choice and the types of food selected by each species. The study hypotheses are as follows, driven by the findings of the feeding capacities and actual diet derived from gut content in Chapter 4:

Hypothesis 1: It is expected that foraging targets will differ significantly between species, and within species, feeding targets are significantly differentiated. In particular, *S. calidus* is expected to feed primarily in the water column as a drift feeding-adapted species; *L. seeberi* is expected to feed most often on the substrate, with some water column feeding as a generalist species; and *P. phlegethon* is expected to feed primarily on submerged structures as a specialist in complex, vegetated and cobble-dominated microhabitats.

Hypothesis 2: At least one species is expected to significantly differ in the frequency that prey/food types are consumed. Insect and invertebrate larvae are predicted to make up a large portion of the diet of *S. calidus*; *L. seeberi* is expected to feed on a mixed diet of invertebrates and detritus; and *P. phlegethon* is predicted to feed on aquatic invertebrate larvae primarily.

Taken together, the above hypotheses and predictions of actual fish behaviour are intended to serve as an in-situ test of the conclusions and concepts developed in the previous chapters.

5.2 Methods

Underwater video was chosen as an ideal sampling methodology for imperilled clear water stream species, given fish mortality risks associated with other sampling methods (Ellender *et al.*, 2012; Castañeda *et al.*, 2020c). This method has been used in several studies in the CFE, primarily for monitoring recovering fish populations (Ellender *et al.*, 2012; Weyl *et al.*, 2013, 2016; Castañeda *et al.*, 2020a).

For this study, a subset of videos used in Chapter 2 were utilised. Hence, the details of site selection and camera deployments have been outlined previously and I reiterate the core methods here. Underwater video cameras were deployed during routine monitoring surveys dated 25th – 27th February, 24th – 26th April and 30th September – 4th October 2019. GoPro® Hero3+® cameras were deployed approximately central to river flow and facing upstream on Perspex platforms. Cameras were set to record at a resolution of 1920 x 1080p at 30 frames per second, and using "wide" field-of-view (127°). Recording time per deployment was 15 minutes, with the initial two minutes excluded from analysis to avoid the possible disturbance of fish behaviour associated with the deployment (video analysis time = 13 minutes). A two to three-minute exclusion period has been recommended to allow fish to return to normal movement and return to the area within the subsequent 15 minute filming period (Hannweg *et al.*, 2020). A two minute period has been found to be adequate for the return of brook charr to foraging behaviours following disturbance (Grant & Noakes, 1987; McLaughlin, 2001). The median time from camera placement to first recorded fish behaviour was 1 minute (interquartile range 0 – 2 minutes) across all videos with recorded behaviours ($n = 52$). This suggests that the two minute exclusion time is appropriate for recording genuine behaviours that should be minimally affected by the camera placement process. The location of each site was recorded with GPS and photographs of camera placement were taken to minimise differences between sampling instances.

Fish behaviour data were extracted from video footage using Behavioral Observation Research Interactive Software (BORIS) (Friard & Gamba, 2016). Behaviours for individual fish were recorded in 30 second segments, with 30 second breaks between recording segments, resulting in a total of 7 min 30 s of potential behaviours extracted from each video (to reduce the total time required while aiming to aggregate a representative sample of behaviours). As analysis of the recorded behaviours took into account the relative abundance of fish in videos (explained further in the Data Analysis section below), and short spates of repeated behaviours were scored as a single behaviour for each fish, skewing of the data by differences in fish abundance and outliers in repeated behaviours by some individuals was minimised. An ethogram was constructed for feeding behaviours of the three cyprinid species. This included a set of descriptions for observed feeding actions on a foraging target and the associated food type that was targeted by a focal fish (Table 9). The number and identity of other species present in relation to the focal species per observation were also recorded. At each site, and sampling event, physicochemical parameters were recorded using an Aquameter® AM-200 multiprobe device (Aquaread Ltd, Kent, United Kingdom) as utilised in Chapter 2. See Table S1 of the appendices for all physicochemical parameters recorded. Permission for underwater camera deployment and general research activities was granted by CapeNature under permit number 0056-AAA008-00067.

Table 9: Feeding behaviour modifiers (as used in BORIS software) with descriptions of the foraging targets and food types based on a simplified ethogram of the Rondegat River cyprinid assemblage. A foraging target and food type, the site-specific details and presence/absence of species other than the focal species was recorded for every observed behaviour.

Feeding behaviour modifier	Category	Description
Foraging target	Water surface	Fish strikes food item at surface.
	Water column	Fish moves towards food item and consumes in water column.
	Structure	Fish picks at food particles on submerged vegetation/rock/wood.
	Substrate	Fish picks at or sifts for food particles on primary substrate of the focal site.
Food type	Detritus	Indeterminate flocculent food items >1 mm in diameter.
	Insects	Adult aquatic or terrestrial insects.
	Larvae	Aquatic invertebrate larvae (e.g. chironomids).
	Small particle/planktons	Indeterminate food items <1 mm in diameter (phyto- or zooplankton).
	Algae	Green algae growing on submerged structures or the substrate.
	Fish (ambush)	Fish consumed by means of a sit-and-wait strategy.
	Fish (pursuit)	Fish consumed by means of chase and capture.

Data analysis

A linear mixed effects negative binomial model was used to evaluate the effects of different environmental parameters on differences between species in feeding behaviour. Interactions were not included in the final model owing to convergence errors. The model included feeding behaviour count as a response, with fixed predictors: species ID, substrate type, reach type, foraging target and food type. An offset term was used in the model to account for the number of fish presenting behaviours and differences in abundance between sites. This was taken as the log of unique fish involved in behaviours divided by that species' abundance in a given site (i.e. the prevalence of behaviours is standardised by the number of fish per species per video). A random effect term of site nested in sampling date was also included to account for repeated sampling. A similar modelling approach was used to investigate the effects of the environmental predictor variables on feeding behaviour within individual species. Model selection was applied for each species with the final model chosen based on the lowest Aikake information criterion (AIC) values (ΔAIC = 2; i.e models with a difference in AIC <2 were considered to be equivalent) (Burnham & Anderson, 2004). In addition, a non-parametric Kruskal-Wallis test was used to compare attack rates (the count of feeding behaviours per unit of observation time, i.e. 7.5 minutes) between different foraging targets per species. This approach was used as data were not normally distributed and could be used to explore pairwise differences in the absence of interactions in the mixed model.

A binomial mixed effect model was then used to predict the probability of fish feeding based on environmental factors. This model was used to further explore aspects of fish behaviour that could not be evaluated because of convergence issues when including interactions in the negative binomial mixed effects model. A proportional response variable was created using a 1/0 binary response of the feeding behaviour being present or absent, divided by the number of unique fish involved in feeding behaviours, creating a proportion between 0 and 1. Variables included in the model were species ID, reach type, substrate type and an interaction term between reach type and species ID. Foraging target was not included in these analyses based on the findings of the previous linear mixed effects negative binomial model. A random effect of site nested in sampling date was used to account for repeated measures, and the model was weighted by fish abundance (MaxN) as an upper bound for the proportional response.

5.3 Results

All species

Species differed significantly in the counts of feeding behaviours (Table 10; Fig. 12). While a lack of model convergence was encountered when including a reach x species interaction term in the linear mixed effects model, reach type had a significant effect on feeding counts, with feeding behaviours being significantly higher in pool habitats when observations were grouped across all species (est. marginal mean 0.81 ± 0.2, t(193) = 3.994, $p < 0.001$; Fig. 12). Tukey's HSD post-hoc testing on the estimated marginal means indicated that the feeding counts of *P. phlegethon* (mean ± SE: 2.79 ± 2.45) were lower than *S. calidus* (3.20 ± 2.82); model estimated marginal mean (est.) ± SE = -0.71 ± 0.18, t(193) = -3.86, $p < 0.001$). Pairwise behaviour counts for *S. calidus* and *L. seeberi* (est. mean ± SE = -0.32 ± 0.23, t(193) = -1.37, p = 0.359), as well as *L. seeberi* and *P. phlegethon* (est. mean ± SE = -0.40 ± 0.27, t(193) = 1.46, p = 0.315), were not significantly differentiated.

Table 10: Type II ANOVA of the negative binomial linear mixed effects model describing feeding behaviour counts between species, while accounting for fish abundance and the number of unique individuals exhibiting behaviours in a given site.

Term	Chi-square	df	p
Species	**15.49**	**2**	**<0.001**
Substrate	2.15	5	0.828
Reach type	**15.95**	**1**	**<0.001**
Foraging target	7.27	3	0.064
Food type	6.17	3	0.103

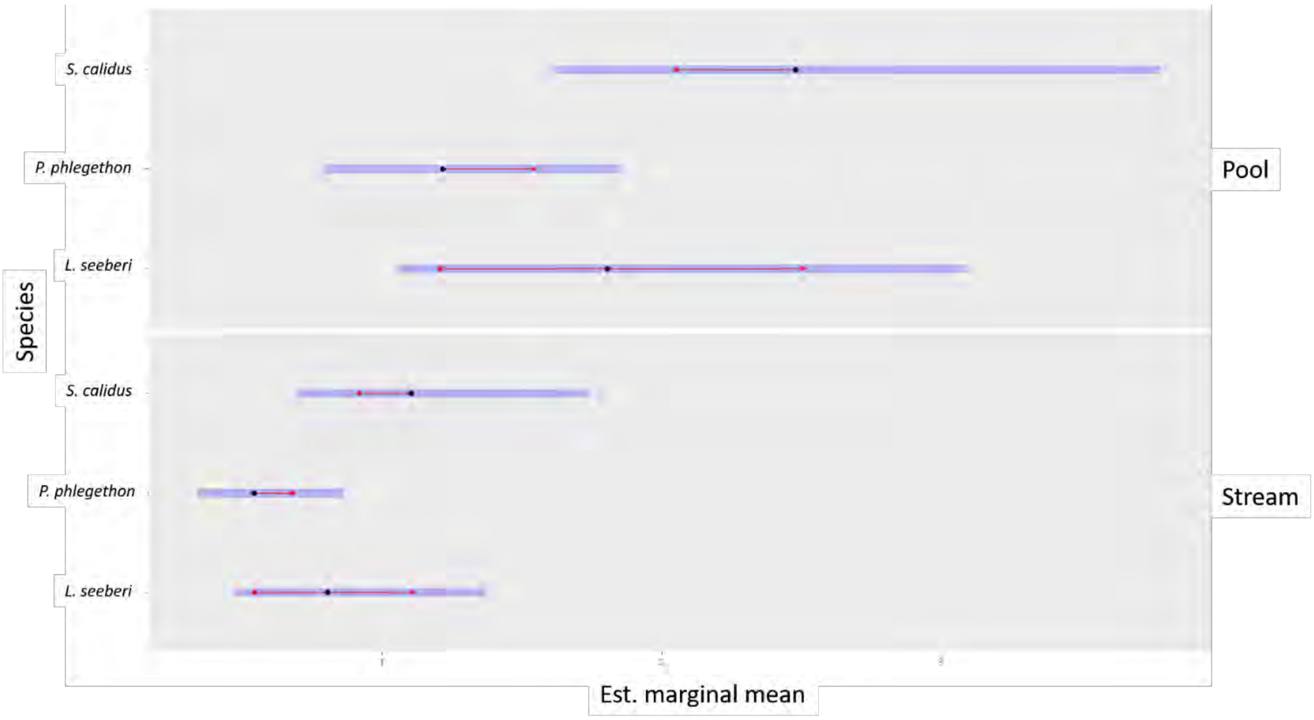

Figure 12: Estimated (Est.) marginal mean abundance-adjusted feeding counts between species and reach types (pools and streams). Blue bars signify confidence intervals; red arrows indicate significance (i.e. overlapping red arrows in the vertical axis indicate a lack of significance, non-overlapping arrows show a significant difference between species means). Significance is based on Tukey-adjusted p-values at an alpha of 0.05.

Sedercypris calidus:

For *S. calidus*, reach type was a significant predictor of feeding behaviour counts (Table 11), with pool reaches yielding higher feeding counts than stream reaches (model est. marginal mean ± SE = 1.12 ± 0.27, t(71) = 4.09, $p < 0.001$). Furthermore, this species had much higher feeding counts in boulder substrate habitats (mean ± SE:3.45 ± 2.77) than in bedrock-dominated habitats (est. mean ± SE = -2.49 ± 0.76, t(71) = -3.29, p = 0.019). Notably, *S. calidus* was the only species recorded feeding from the surface (Fig. 13). In addition, this species accounted for the majority of feeding events in the water column (Fig. 13). Investigating attack rates between foraging targets for all species, there were significant differences between foraging targets only for *S. calidus* (Fig. 13; Kruskal-Wallis chi-squared = 8.25, df = 3, p = 0.041). Surface feeding attack rates (0.20 ± 0.15) were significantly lower than water column attack rates (0.52 ± 0.41, model est. mean: -0.32 ± 0.10, Z = -3.28, $p < 0.01$; Fig. 13) in this species.

Table 11: Type II ANOVA of the negative binomial linear mixed effects model describing feeding behaviour counts for *S. calidus*, while accounting for fish abundance and the number of unique individuals exhibiting behaviours in a given site.

Term	Chi-square	df	p
Reach type	**16.75**	**1**	**<0.001**
Substrate	**14.42**	**5**	**0.013**
Foraging target	1.30	3	0.729
Food type	0.35	3	0.950

Pseudobarbus phlegethon:

For *P. phlegethon*, the most parsimonious model included only reach type and the offset and random effect terms. Pool reaches (mean ± SE = 2.88 ± 2.62) had higher feeding behaviour counts than stream environments (2.59 ± 2.06; model est. mean: 0.90 ± 0.33, t(95) = 2.72, $p < 0.01$; Fig. 12). *Pseudobarbus phlegethon* fed most often on submerged structures (Fig. 13). This species was not observed feeding on the surface, and no significant differences were found between the remaining foraging targets (Fig. 13; Kruskal-Wallis chi-squared = 4.30, df = 2, p = 0.117).

Labeobarbus seeberi:

For *L. seeberi*, no terms had a significant effect on feeding counts. As all observed feeding events were on detritus, the food type term could not be included in the GLMM. Similarly to

P. phlegethon, this species was not observed feeding on the surface, and no significant differences were found between the remaining foraging targets (Fig. 13; Kruskal-Wallis chi-squared = 4.06, df = 2, p = 0.132).

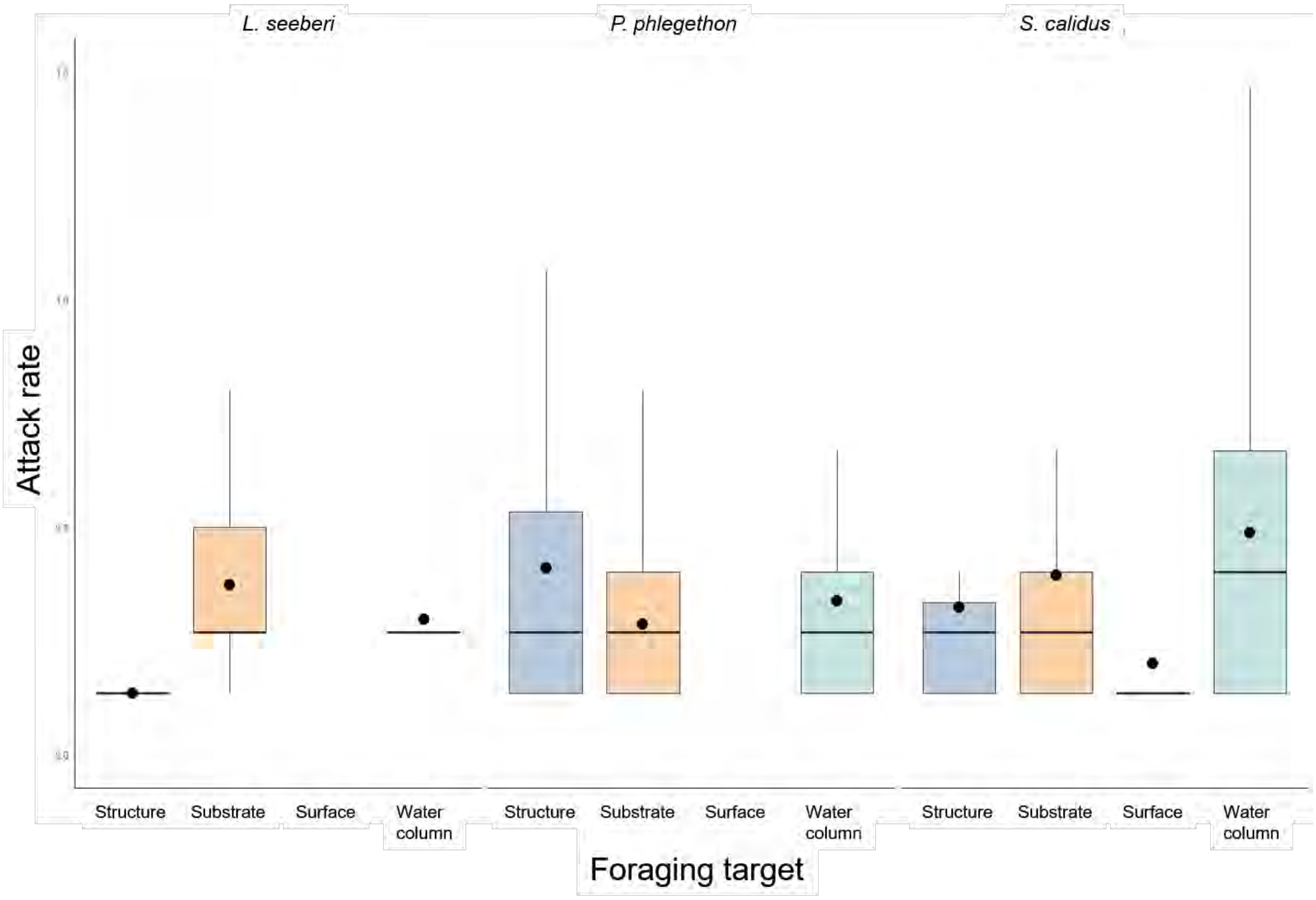

Figure 13: Attack rates (feeding counts per unit time) of all three species on different foraging targets. Error bars denote standard errors, horizontal bars denote the median while black circles indicate the mean.

The binomial model results indicated that the probability of feeding behaviours occurring was significantly predicted by species ID and the interaction between species and reach type (Table 12). Exploring the species × reach interaction, significant differences in the probability of feeding were found between species in pool environments (Fig. 14). Specifically, *S. calidus* was significantly less likely to feed compared to *P. phlegethon* (Fig. 15; odds ratio = 0.57 ± 0.12, t(119) = -2.71, p = 0.021) and *L. seeberi* (odds ratio = 0.43 ± 0.13, t(119) = -2.72, p = 0.021) in pool environments. In stream environments, there were no significant differences in probability of feeding (Fig. 15). Within species, *S. calidus* was found to have a significant difference in feeding probability, with a significantly higher probability of feeding in stream environments over pools (marginal mean = -0.906 ± 0.36, t(119) = -2.514, p = 0.013). No significant differences in the probability of feeding in pools *versus* streams were found in *L. seeberi* or *P. phlegethon* (p > 0.05, Fig. 15).

Table 12: Type II ANOVA of the binomial model describing the probability of feeding between species with the main effects of species ID, reach type, substrate and the species ID x reach type interaction term.

Term	Chi-square	df	p
Species	**9.76**	**2**	**<0.01**
Reach type	2.08	1	0.150
Substrate	8.69	5	0.122
Species × Reach	**6.77**	**2**	**0.034**

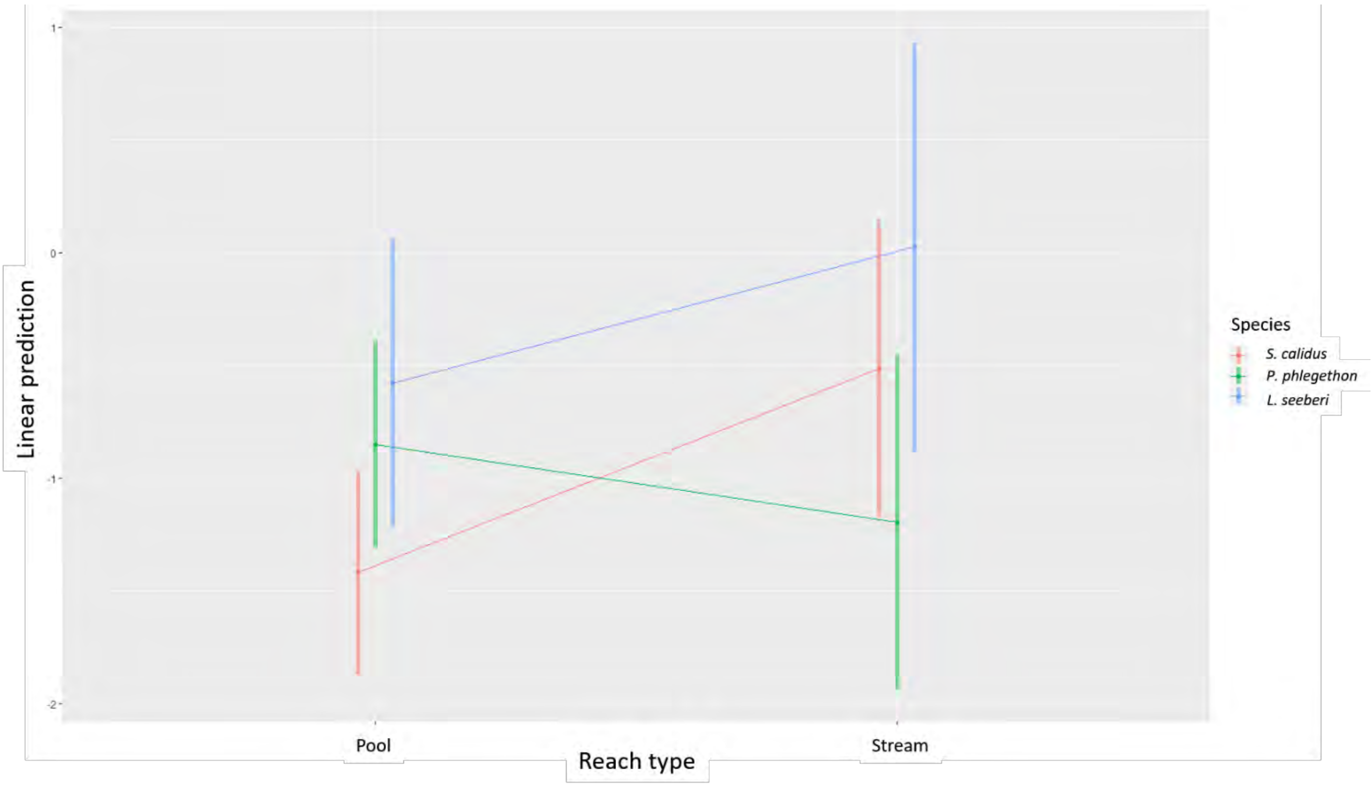

Figure 14: Interaction plot of the model predicted probability of feeding between species (*Sedercypris calidus*, red; *Pseudobarbus phlegethon*, green; and *Labeobarbus seeberi*, blue) and reach types (pools or streams). Vertical bars denote 95% confidence intervals around the linear prediction (points).

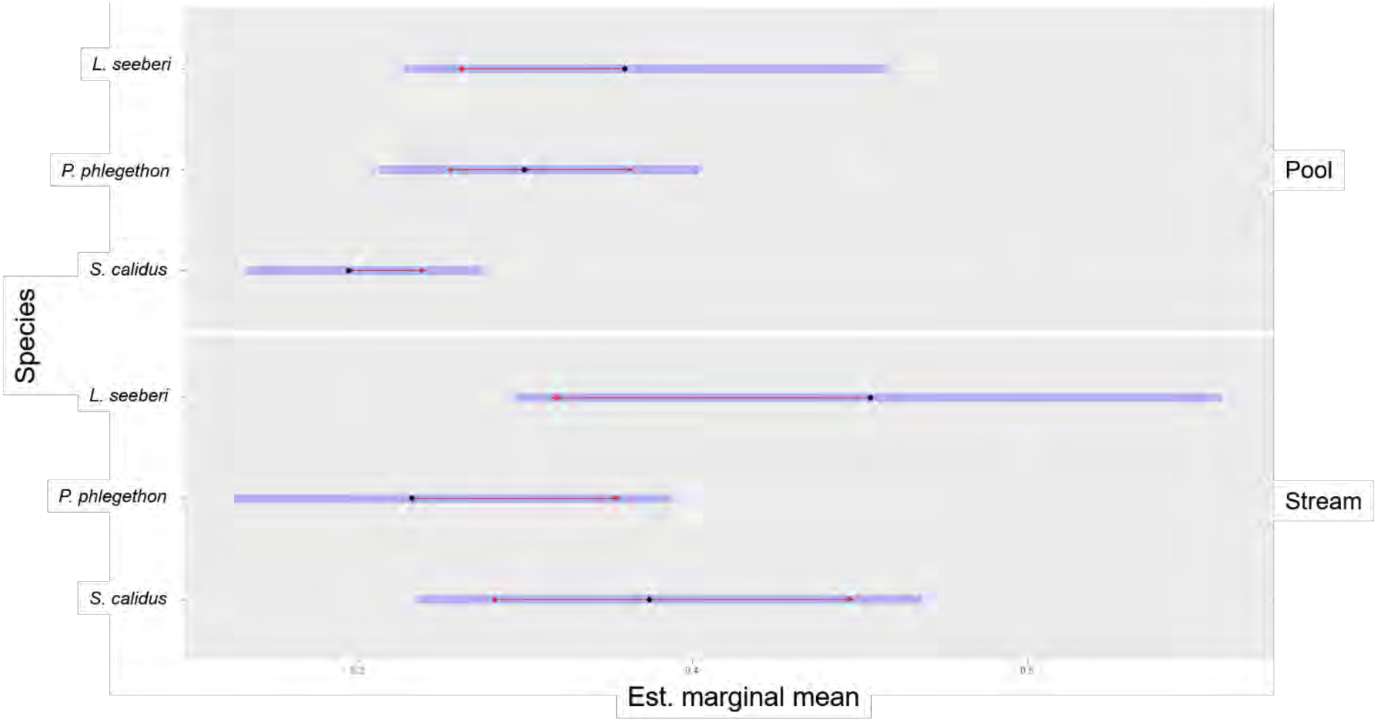

Figure 15: Estimated (Est.) marginal mean odds ratios of feeding probability between species and reach types. Blue bars signify confidence intervals; red arrows indicate significance (i.e. overlapping red arrows indicate a lack of significance, non-overlapping arrows show a significant difference between species means). Significance is based on Tukey-adjusted p-values at an alpha of 0.05.

5.4 Discussion

In-situ feeding behaviour between members of the cyprinid assemblage of the Rondegat River was successfully assessed using RUV footage. It was expected that these species would be selecting foods and targeting different microhabitats to reduce interspecific competition. Some evidence was found in support of both hypotheses; however, the resolution of data able to be gathered from the RUV footage lead to some single events of specific feeding behaviours, complicating statistical analyses. There were also some difficulties encountered in trying to differentiate small resource items, such as planktons or invertebrates at further distances. Pool environments in particular seem to be important for feeding for all three species. Nonetheless, there was evidence of niche specialisation via microhabitat separation which would not have been possible to deduce from traditional methods.

The first hypothesis predicted that there would be differences in the counts of feeding events among foraging targets and between species. Given the results of the all-species model, I found that there were indeed differences in feeding counts between species, but foraging targets did not significantly differ. Instead, reach type (i.e. pool versus stream environments) was influential in the observed feeding behaviour counts. Overall, feeding was more commonly observed in pool environments, even when accounting for higher fish abundance and the number of unique individuals. The differences in feeding counts through microhabitat selection observed here are similar to results found for other stream fishes, particularly in the case of salmonids switching between holding station in the current foraging opportunistically on drifting prey (i.e. stream reaches here), in contrast to individuals roaming in a search area on benthic prey in pools (Fausch, Nakano & Kitano, 1997; Nakano et al., 1999a).

For the second hypothesis, I predicted that at least one species would be expected to differ in the counts of feeding events by each species across food types. Overall, there were no discernible differences in feeding frequency on different food types. This was likely driven by the observed diets being dominated by feeding attempts on detritus, which was the most commonly observed food source among all three species, and the only food that *L. seeberi* was observed to feed on. A detritus-centred diet is to be expected in *L. seeberi* based on its morphological profile and generalist diet following from Chapter 4. Whitehead et al. (2007) suggest that *P. phlegethon*, with a short gut length indicating a carnivorous specialisation, ingests fairly large amounts of detritus through foraging, which is congruent with my observations here. Only a few observed attempts to feed on clearly defined algae, insects and invertebrate larvae were observed across all species, despite many of these items making up the known diets of the study species. It should be noted that some feeding observations recorded as being on detritus may have been on another food source, but this was not clearly discernible, and “detritus” was used as the fall-back option when absolute clarity on the food item was not available. This may reflect a potential limitation of underwater video in this application, but which may be remedied by longer deployment times or other methodological refinements. Notably, the occasional surface feeding on drifting insects in *S. calidus* do support the second hypothesis. Surface feeding on allochthonous invertebrates is an important aspect of stream fish trophic ecology (Garman, 1991), and these inputs can be a major determinant of energy flow in stream ecology, potentially shifting fish abundance patterns (Kawaguchi, Taniguchi & Nakano, 2003) and playing a key role in affecting the entire stream community through control of trophic cascades (Nakano, Miyasaka & Kuhara, 1999b).

In contrast to the higher attack rates in pool environments, the probability of feeding derived from the binomial model increased significantly for *S. calidus* in stream environments. This difference in observed feeding counts in relation to feeding probability between reach types was also observed in *L. seeberi*, but the difference was not significant. It could be suggested that these species are able to switch foraging mode effectively under different environments in response to prey availability; i.e. *S. calidus* shifts within sites to faster flowing stream sections to avoid competition with *L. seeberi* under certain conditions. This apparent discrepancy between feeding counts and probability of feeding between pool and stream environments could come about through a scenario wherein each individual fish had a lower probability of feeding in pools, but individuals that did feed, fed repetitively. In streams, *S. calidus* individuals were more likely to feed at least once, but fewer feeding instances were counted per individual. Hence, this switching may explain a trend of more likelihood of feeding in stream environments (with fewer actual feeding events per individual), compared to deliberate repeated feeding in fewer pool environments, when conditions allow. Terrestrial insects obtained from drift feeding could serve as the more nutritionally / energetically preferable but less abundant food source in streams, while more abundant, lower quality food sources are available in pool environments. Investigations of the functional morphology of these two species showed a high degree of overlap in feeding capacity and diet for drift feeding (Chapter 4), lending further support for a higher probability of feeding in higher flow environments as a result of higher allochthonous inputs. Optimal foraging theory predicts that fish will select habitats that maximise energy intake, therefore they should be expected to select high flow habitats given the assumption of higher drift prey availability (Bunn & Arthington, 2002; Grossman *et al.*, 2002; Rincón *et al.*, 2007). When drift-adapted fishes are not selecting high flow environments, other processes such as interspecific competition, predation or other niche-based segregation in response to patch dynamics and resource abundance may be more influential on habitat selection in the fish community (Grossman et al., 1982, 2002). In the Rondegat River, all three of the cyprinid species are primarily associated with low flow and/ or pool environments (Woodford, 2005; see Chapter 2). A potential reason for this phenomenon is that of the higher energetic requirements in high flow conditions, reducing the energy budget available to perform behaviours because of maintaining position in the current (Grossman et al., 2002). Consequently, it could be suggested that the cyprinid species of the Rondegat River are behaviourally selecting foods and targeting different foraging microhabitats (i.e. trophic segregation) to reduce interspecific competition. In particular, *S. calidus* and *L. seeberi* could be expected to differentiate their feeding microhabitats and prey choice to reduce competition where they co-occur, based on their morphology and diet in Chapter 4. One caveat that should be noted is that the use of underwater video in faster-flowing stream environments may be less reliable than in more static pool reaches, potentially requiring longer deployment times to capture less frequent and dynamic behaviours in stream reaches.

Given the high counts of water column feeding and being the only species observed to feed on surface insects, *S. calidus* is supported as a drift feeding-specialised species, as suggested by the feeding capacities uncovered in Chapter 4. Investigating the expectations of behavioural differences in feeding patterns between species based on past and current work, niche differentiation is to be expected between *S. calidus* and *L. seeberi*, with *S. calidus* being more able to exploit prey at low densities in the water column and the surface (Chapter 3; Broom *et al.*, 2021). *Labeobarbus seeberi* is in turn more able to exploit prey at high densities, particularly in pool environments. Additionally, *S. calidus* appears to be most able to opportunistically exploit sporadic higher food inputs into the system, particularly in the form of invertebrate drift events and terrestrial (i.e. surface) invertebrate availability based on Chapter 4. Exploitation of higher flow stream environments to take advantage of these events may allow *S. calidus* to compete more effectively with the generally higher-performing *L. seeberi.* Similar shifts in opportunistic use of higher allochthonous prey inputs and shifts in drift density have been found in sympatric charr species (Fausch et al., 1997; Nakano et al., 1999a, b).

This dynamic has been observed in other stream fishes, with one group positioning for drift feeding, and the other using an active search of the benthos for prey (Nakano et al., 1999a). The ability of *S. calidus* to exploit allochthonous terrestrial invertebrate prey subsidies likely allows it to further differentiate from *L. seeberi* and reduce competition, as found in other stream species by Kawaguchi et al. (2003) when investigating the effects of experimentally controlled surface prey inputs. The higher degree of specialisation in *S. calidus*, in contrast to the highly generalist nature of *L. seeberi* based on morphology, diet and now behaviour, points to a mechanism of trophic niche differentiation in this assemblage. *Pseudobarbus phlegethon* also showed some signals of specialisation to complex environments as suggested by habitat associations, morphology and diet (Chapter 2 and Chapter 4), through the high proportion of feeding on submerged structures. In contrast to the other two species, *P. phlegethon* fed more often on submerged structures, with no surface feeding events recorded. Furthermore, investigation of morphology and gut content differentiated this species from the other two species in both feeding capacity and diet, being specialised in feeding on invertebrate larvae prey (Chapter 4). Taking these aspects into consideration, in addition to the submerged structure foraging targets based on in-situ behaviour here, suggests that *P. phlegethon* is exploiting complex habitat used by sensitive aquatic larvae, in contrast to the drifting, surface and substrate prey exploited by the other species. This dependence on complex structure and refugia has recently been observed in the closely related *P. afer*, which showed clear behavioural preference for both natural and artificial refugia (Magellan, Weyl & Booth, 2021).

The low counts of food items beyond detritus make direct inference in prey choice difficult in the current study. As such, the results and conclusions of Chapter 4 based on gut content are still the best approximation of actual diet, while the current study can provide insight into microhabitat foraging specialisation between the species. The relative difficulty of identifying prey in a dynamic natural system using underwater video (and thus identifying trends in abundance of different prey types) presents a challenge if using this approach exclusively. As prey abundance is variable and sporadic increases in prey abundance are valuable in oligotrophic systems, effective prey switching in response to varying prey abundance is an important aspect of behaviour (Murdoch, 1969; Warburton, Retif & Hume, 1998; McCard et al., 2021). In the Rondegat system, *S. calidus* is likely exhibiting prey switching behaviours through opportunistic surface feeding when stochastic events cause a higher abundance of prey to become available, facilitated through their functional morphology (based on feeding capacity and actual diet; Chapter 4).

Recent efforts to characterise the Rondegat fish community recovery through occupancy modelling showed that density-dependent effects of fish aggregations may have increased local competition, thus incentivising dispersion and colonisation (Castañeda et al., 2020a). However, the exact mechanisms and interactions driving community structure in this recovering fish community are unknown, particularly where multiple species aggregate in higher densities. A common theme arising from the results here is support for the preference of all three species for low flow, pool environments over higher velocity stream reaches in general (see Chapter 2 in terms of relative abundance in stream *versus* pool environments). Notably, the results of Chapter 5 suggest that frequency of feeding was higher in pools, while the probability of feeding was higher in stream environments for *S. calidus*. This suggests that a behavioural switch may occur when foraging in these two environments, and which is likely to be of increased importance in oligotrophic environments (Correa & Winemiller, 2014). In particular, the low productivity and prey availability of these environments likely necessitates exploitation of stochastic and rare higher prey abundance events, as fish switch between generalist feeding modes in normal conditions and specialise on temporarily abundant food sources (Stephens & Krebs, 1986; Perry & Pianka, 1997; Warburton et al., 1998; Correa & Winemiller, 2014). It is likely that *S. calidus* exploits the inlet portions of pool environments, targeting prey entering the pool reach from upstream higher flow reaches, thus balancing energy intake with reduced energy usage for holding position in currents (Grossman et al., 2002). Inlet portions of pool environments have been proposed as ideal for maximisation of food intake, and are often defended by dominant individuals in competitive interactions (Nakano et al., 1999a).

The results of this study reveal some of the strengths and limitations of underwater video as a method for observing fish behaviour. The method is low-cost, and rapidly deployable; and behaviours can be effectively recorded using free software (e.g. BORIS). However, the method does require clear water and high visibility, and is only able to record diurnal behaviours. Some of the limitations identified include a degree of difficulty in identifying prey items without some ambiguity, owing to high speed, small size and insufficient video resolution, or some combination of these factors. Some of these problems could be resolved using multiple cameras watching the same area, and longer deployment times to increase the probability of recording sporadic, low-frequency behaviours, such as that employed by Hannweg et al. (2020) under similar conditions. For these reason, underwater video is perhaps best used as a complementary line of evidence to studies using more traditional approaches, and the nature of the data necessitates strong ecological knowledge of the system in question for effective interpretation.

In conclusion, I have found evidence for some mechanisms determining community structure through niche differentiation between the species of the Rondegat River cyprinid assemblage, as a confirmatory approach employed alongside other lines of evidence (in particular, based on the findings of Chapters 2 and 4). The trophic interactions observed here will need further assessment to establish their persistence given the sporadic nature of some aspects of feeding dynamics in this variable system. In particular, the highly imperilled *P. phlegethon* is particularly vulnerable to disturbance through its specialised feeding adaptations and behaviours. In contrast, the broad niche of *L. seeberi* and ability to exploit sporadic high prey abundance events and terrestrial subsidies of *S. calidus* make these species potentially more resilient under a scenario of ongoing disturbance and global change affecting the western CFE.

CHAPTER 6: General discussion

One of the camera deployment sites in the Rondegat River. Photo: Josie South

Through the preceding chapters, a wide array of evidence has been gathered and synthesised towards understanding the species interactions and resilience of the Rondegat River fish community. This is a recovering fish community following the first invasive fish eradication programme using chemical methods in a natural river system in South Africa. Knowledge of the population trends, baseline community dynamics and trophic interactions with the surrounding environment are thus crucial for a sound understanding of the mechanisms underpinning current community structure and predicting responses to potential disturbances going forward in the Anthropocene epoch.

This general discussion is made up of three parts: an analysis and further discussion of the preceding chapters to summarise and consolidate the findings; a synthesis of the findings into a holistic understanding of the Rondegat system through the linkages and synergies of the chapters; and finally a discussion of the implications for conservation and recommendations for future conservation interventions. Throughout this general discussion, I intend to demonstrate the extent to which these research chapters contributed to an understanding of key biological and ecological aspects of the Rondegat assemblage. Thus, this chapter will elaborate on how the recommendations of Saddlier et al. (2013) and the overall thesis aim of understanding the dynamics and responses of understudied vulnerable fish communities in the wake of restoration efforts, have been met.

6.1 Analysis and further discussion of each chapter

Chapter 1

In Chapter 1, I introduced a broad outline on the existing literature surrounding the Rondegat River and the Cape Fold Ecoregion, to which the findings of this thesis may be applied. I provided an overview of the challenges facing freshwaters globally and in the South African context, the fish species, history of the river as the site of a collaborative non-native fish eradication effort, and a summary of the various stressors and current state of conservation efforts in relation to the Rondegat River. This chapter was used as the scaffolding from which the other chapters are derived in order to answer various questions surrounding the concepts dictating the mechanisms of community structure, interactions and resilience in the face of global change. Finally, I adapted a framework of freshwater ecosystem restoration (Saddlier et al. 2013), from identification of the current situation and threats, through to the establishment of long-term sustainable conservation of the imperilled species identified, to the situation of the

Rondegat River as a microcosm of the greater CFE area. Within this framework, I identified the key aspects of biology and ecology driving community structure (Objective 7 of 10), as the main thrust of this thesis, and as a foundation from which to further accomplish the other recognised conservation objectives in the final stages of the restoration interventions required for the Rondegat River. The following chapters address aspects of the biology and ecology of the recovering cyprinid assemblage, in line with objective 7 (Saddlier et al. 2013), focusing on community dynamics and trophic ecology.

Chapter 2

Chapter 2 investigated the current distribution, status and abundance patterns of the Rondegat cyprinid assemblage, with a particular focus on how these factors relate to habitat associations. This study broadly expanded on the findings of previous authors engaged in the monitoring of the river, immediately prior to and in the years following the non-native fish eradication and related restoration efforts. In particular, the recent findings of Castañeda et al. (2020a) were used as a motivating factor, whereby the relatively well recovered *Sedercyrpis calidus* and *Labeobarbus seeberi* contrasted with the still-recovering *Pseudobarbus phlegethon*. The work was based on underwater video assessments of fish abundance and the associated habitat and physicochemical data were utilised to establish the relative habitat niche breadth and preferences of the three species.

In the results of this study, distance from the uppermost site in the river was the most significant predictor of all three species' abundance, indicating spatial segregation and varying overlap between species. The protected status of sites in the upper reaches, vegetated substrates and size of individual sites were the most important predictors of abundance for the endangered *P. phlegethon*. In addition, this study indicated that underwater video monitoring is an effective and low cost approach that can monitor species abundance distributions and contribute to conservation recommendations. I suggested that reducing runoff and sedimentation in the lower reaches may be useful interventions to maintain key habitats of submerged vegetation. Finally, I concluded that consistent underwater video surveys are a feasible and low cost method to inform whether these conservation actions cause a positive change.

One of the most important findings of this initial investigation of the distribution, abundance and habitat associations of the three species of the Rondegat cyprinid assemblage; was that distance from the uppermost site in the river was the most significant predictor of overall species abundance. This was interpreted to indicate that spatial segregation between species is a possible driver of current distributions following the alien fish eradication and restoration efforts. Furthermore, there is varying overlap between species across the river both spatially and in habitat associations: *Labeobarbus seeberi* and *S. calidus* are more often found together in the lower reaches, while *P. phlegethon* and *S. calidus* are more often closely associated in the upper reaches. Essentially, there is a very high degree of segregation between *P. phlegethon* and *L. seeberi*, while *S. calidus* is ubiquitous across the river and the varying microhabitats therein. A further finding was that the protected status of sites in the upper reaches and vegetated microhabitats were the most important environments for the endangered *P. phlegethon*. It is important to note that the protected areas of the river largely, but not completely incorporate the most appropriate other habitat factors (high levels of submerged vegetation, pools and complex habitat). As *P. phlegethon* abundances can be seen to decrease outside of protected areas but still within otherwise suitable habitat, it could suggest that the protected status and its associated effects (reduction in disturbance and a lack of agricultural activity) are likely to play an important role in the recovery and sustained abundance of this species. When comparing stream and pool reaches, a clear pattern of higher relative abundances in deeper pool environments was found in all three species, highlighting the importance of these refugia for conservation.

Another important finding derived from Chapter 2 is that underwater video monitoring is a highly viable technique for long-term monitoring of fish populations as part of restoration efforts. In addition, the video footage produced from this approach can be used to answer other scientific questions, such as the habitat associations revealed here (but see also Chapter 5 on the behaviour data extracted from underwater video). Underwater video was also useful in the tracking of habitat change as a result of sedimentation brought about by the fire event of 2018, with much of the mid and lower reaches of the river being drastically changed from previously cobbled or boulder-dominated sites into shallow, sand-inundated sites. While this study was successful in establishing abundance patterns and habitat associations, and the overall rehabilitation of the Rondegat River following the alien fish eradication intervention has been mostly achieved, the ongoing pressures of drought and anthropogenic habitat destruction effects (i.e. local damming, water abstraction and fire events) are now at the forefront of threats to the system. I suggested that the reduction of runoff and sedimentation in the lower reaches would likely be the most impactful interventions to maintain key habitats, especially the complex rocky and vegetated habitats favoured by *P. phlegethon*. In effect, this would require the possible expansion of protected areas and greater engagement between local conservation authorities and landowners in the area. In addition, engagement with local government management, with the ability to respond to fire events in a more pro-active manner, is required. However, many municipalities in South Africa (including the Cederberg municipality responsible for functions in the area) are under-resourced and may be insufficiently equipped to adequately respond to uncontrolled fire events. A possible solution to this challenge could be the implementation of volunteer programmes from the local rural populace to expand firefighting or fire prevention efforts.

Chapter 3

Chapter 3 took a laboratory experimental approach, focusing on feeding dynamics through functional responses. In this chapter, I investigated the closely co-occurring *S. calidus* and *L. seeberi*, which often co-occur across much of the river; usually aggregations of juvenile *L. seeberi* and adult *S. calidus* in relatively high abundance. The dynamics of trophic segregation, strength of competition for limited resources in an oligotrophic environment, and interactions of prey type and thermal conditions on feeding performance were explored. The functional responses of these species were compared between two prey types (mosquito and chironomid larvae) at different field-representative temperatures (18°C and 25°C) in laboratory trials, with the aim to infer response and resilience to global change. *Labeobarbus seeberi* consistently outperformed *Sedercypris calidus* across prey types and temperature treatments at high prey densities. This was driven primarily by shorter handling times in *L. seeberi*, despite higher attack parameters in *S. calidus* under certain prey/temperature scenarios. The higher temperature treatment had differential effects on prey consumption dependent on fish species and prey type. However, neither species showed significant intra-specific differences in functional response between temperature conditions for either prey species, indicating community resilience to thermal change in the CFE. An implication of this study was that context dependent experiments can be used in tandem with field data to identify conditions of potential ecological tipping points in imperilled systems.

The results of this chapter represent the first feeding estimates of feeding efficiency in these species, and the findings provide some signals of inter-species and species-prey interactions which are further investigated in Chapters 4 and 5. The finding of overall stronger feeding performance in *L. seeberi* over *S. calidus*, especially on the surface and water-column representative mosquito larvae prey, was a surprising result. It was expected that a clear separation of feeding performance between these prey types would occur as part of adaptive trophic niche segregation between these species. However, a noteworthy consideration to recall from the results of Chapter 3, was the differences in attack parameter, especially the high values for *S. calidus* on mosquito larvae. This was interpreted to be a signal of this species having sensory advantages over *L. seeberi*, which translate into a faster search phase in the acquisition of prey (translating to the higher attack parameter), while the consistently lower handling times of *L. seeberi* allowed a higher overall feeding rate. The sensory advantages of *S. calidus* are intuitively apparent, with large eyes and two pairs of barbels instead of the one pair found in *L. seeberi*. I expand on the relative feeding capacities and in-situ diets of these two species in Chapter 4. While the overall feeding rate advantage of *L. seeberi* is strongly evident, it must be contextualised in the environment of the Rondegat River. The river system is oligotrophic and food items are low in abundance; thus, subtle differences in feeding capacity, especially in the detection/search and locomotion to capture prey will play a highly significant role in the actual acquisition of prey in real-world conditions. This may indicate that some degree of microhabitat segregation, with a drift-feeding focus in *S. calidus* and an active foraging behaviour in *L. seeberi*, may be at play. This assertion is examined in Chapters 4 and 5.

A key aspect of Chapter 3 was that of the temperature treatments used in experiments and the implications of the feeding performance impacts these temperatures may have in real-world conditions. While there were no detected differences in feeding performance between temperatures for *L. seeberi*, the sensitivity of *S. calidus*' attack parameter and maximal feeding rates to temperature may indicate a previously-undetected temperature sensitivity in this species. This thermal sensitivity in feeding rate, combined with the established reproductive thermal sensitivity in *L. seeberi* suggested by Shelton et al. (Shelton *et al.*, 2018b), as well as the relatively low thermal tolerance of the true redfins *P. phlegethon* and *P. afer* (Reizenberg *et al.*, 2019), suggest that the Rondegat assemblage (and possibly other species across the CFE) is likely to experience a myriad of different impacts in response to rising temperatures. In particular, a rise in temperatures may result in a reduction in abundance of *S. calidus* over time, through both a reduction in feeding performance and reductions in abundance of sensitive invertebrate taxa or alterations in their phenology. Simultaneously, the accelerated loss of suitable spawning habitat of *L. seeberi* could result in multiple failed reproductive attempts, causing a reduction in abundance or local population crashes. For *P. phlegethon*, while rising temperatures are unlikely to reach the thermal threshold within the foreseeable future (Reizenberg *et al.*, 2019), this species is limited in the available thermal refugia in the small upper headwaters to which it is now restricted. Thus, longer term acceleration of temperature increases and extreme weather events are a concern for this species.

Chapter 4

Chapter 4 combined a predictive framework based on morphology with historical (2004 - 2005) diet data to establish feeding capacities and in-situ trophic niches of the three species. I used a combination of functional morphological feeding capacities based on a food-fish model with gut content analyses to test predictions of differences between species in feeding capacity as well as dietary choice from field data. I found that all three species were morphologically distinct. However, the constructed trophic profiles suggested that there was a high degree of feeding capacity overlap between *L. seeberi* and *S. calidus*, while *P. phlegethon* diverged from the other two species. Gut content suggested that all three species overlapped broadly in diet, though *P. phlegethon*'s diet was predominantly larvae, *S. calidus* fed primarily on larger free-swimming or terrestrial insects (i.e. diet was subsidised by terrestrial invertebrates taken from the surface), while *L. seeberi*'s diet was relatively unspecialised. The results showed that the realised trophic niche of these species is similar, despite morphological differentiation. Using a composite of mechanistic approaches, this study confirmed some signals of limiting similarity through trait divergence on the fish assemblage despite overlap of actual diet through opportunistic feeding. It was concluded that the threatened and endangered species of this community, primarily *P. phlegethon*, rely on sensitive invertebrate taxa and are vulnerable to anthropogenic disturbances impacting prey abundance, despite a degree of diet plasticity.

A primary finding was that, despite fairly strong morphological divergence, the trophic niches of *L. seeberi* and *S. calidus* showed strong overlap; in contrast *P. phlegethon* displayed a signal of a relatively high degree of specialisation in diet. This finding reinforced the emerging perception of *P. phlegethon* as requiring a more specific set of conditions for continued recovery and for the maintenance of healthy populations, especially pristine, complex habitat that can support their dietary needs. This chapter also established strong evidence for *S. calidus* being a drift-feeding specialist in contrast to the generalist feeding tendencies of *L. seeberi*; however, a high degree of plasticity in behaviour through prey switching based on conditions and presence of other species was suggested, owing to the feeding capacities uncovered here. Essentially, this chapter defined the scope of differences and overlaps in feeding capacity and incorporated insights into actual feeding based on the dietary data to develop a set of predictions for in-situ behaviour between the three species. This was informed further by the findings of Chapters 2 (habitat and spatial patterns) and 3 (feeding performance in the putative direct feeding competitors *S. calidus* and *L. seeberi*). The specialist trophic niche of *P. phlegethon* reinforces this species as needing particular attention in conservation considerations, such as preservation of protected areas and investigation into the viability of extending existing protected areas. It is highly likely given the current and historical distributions of the three species across the length of the river that anthropogenic disturbance, particularly agricultural activity, are strong predictors of declines in abundance of *P. phlegethon*. The recolonisation and recovery of *L. seeberi* and *S. calidus* over time following the removal of alien species (Castañeda *et al.*, 2020a), despite other ongoing disturbances in the affected reaches, suggests a higher degree of resilience in these species, and the findings of this chapter further reinforce this, particularly in the feeding plasticity and generalist nature of these two species. This chapter suggested that microhabitat segregation and prey switching are likely responsible for the coexistence of these two species despite their diet overlaps. These aspects were further investigated in Chapter 5.

Sedimentation is a particularly concerning impact for all three species; however, the dietary profiles and feeding capacities uncovered here suggest mechanisms for how *L. seeberi* and *S. calidus* may be able to mitigate impacts to some degree. In particular, *L. seeberi* could be expected to continue to forage effectively in sandy and silted environments, with the ability to sift detritus and buried food items effectively. Similarly, drifting food items may be reduced for *S. calidus*, but complete loss of this food source is unlikely unless the entirety of the river is inundated with sediment. Both species will however be impacted in reproduction or be required to migrate further for effective recruitment; adult *L. seeberi* would need to find unaffected riffles further upstream, while *S. calidus* would require intact boulder or cobble habitat for crevice spawning. *Pseudobarbus phlegethon* in contrast would be severely impacted in both feeding and reproductive success, with a loss of stones-in-current biotope and the associated loss of sensitive taxa on which this species appears to be specialised. This is particularly important for the *P. phlegethon* population, which is one of only five recognised in the CFE (van der Walt *et al.*, 2017a). Consequently, the importance of vigilance and response to uncontrolled fire events and limiting of anthropogenic activity in and around the river is again recommended in the interests of maintenance of the Rondegat River cyprinid assemblage.

Chapter 5

In the final data chapter I used in-situ remote underwater video (RUV) to directly observe and quantify the feeding-specific behaviours of the Rondegat cyprinid assemblage. This chapter focused on differences between fish species in terms of feeding patterns, the use of microhabitat in acquisition of food items, and the types of food items being preferentially targeted by the focal species. Exploring the findings, it was clear that identification of different food types was less informative than expected. Fish were primarily observed feeding opportunistically on detritus or indistinct food items, particularly in the case of *L. seeberi*. Quantification of feeding in different microhabitats (surface, water column, submerged structure and substrate) was the more illuminating approach, where differences between and within the species were evident. *Sedercypris calidus* showed clear signals of a drift-feeding, opportunistic feeding mode, with this species being the single observed member of the Rondegat cyprinid assemblage to feed from the surface in this study, as well as the highest frequency of water column feeding. This was contrasted by *L. seeberi* having no clear trend in feeding counts between microhabitats, suggesting an unspecialised feeding mode. *Pseudobarbus phlegethon* had higher feeding counts in submerged cover structures, particularly in pool environments, suggesting a strong preference for complex refugia habitat, and indicating a degree of specialisation towards this feeding mode and prey associated with these environments. Given these trends in feeding behaviour, it was suggested that *P. phlegethon* is likely to be the most sensitive to habitat degradation or other environmental change through this specialisation relative to the generalist and opportunistic strategies of *L. seeberi* and *S. calidus*, respectively. In particular, *S. calidus* could be the most able to exploit shifts in allochthonous nutrient inputs through perturbation of the system and the surrounding terrestrial landscape.

One of the main findings of this chapter was that, even when accounting for fish abundance differences, *S. calidus* and *P. phlegethon* had higher feeding counts in pool environments compared to stream environments, when examining each species' feeding counts individually. This could be explained easily for *P. phlegethon* based on insights from previous chapters, with clear habitat associations, feeding capacity and diet that align well with the pool environment. Evidence for a pool environment preference in the Rondegat cyprinid assemblage follows from previous studies (Woodford, 2005) and current work on habitat preferences and abundance patterns of these species (see Chapter 2). In particular, *P. phlegethon*'s preference for deeper environments is corroborated by similar findings in other *Pseudobarbus* redfin lineages (Magellan *et al.*, 2021). The higher abundance of conspecifics and thus social feeding cues in pool environments are likely to contribute to the increased foraging rates in pools through reduced predation risk as observed in other shoaling species (Ryer & Olla, 1991; Brown & Laland, 2002). The increased feeding counts in pool environments were unexpected in *S. calidus*, which appears to target prey in the water column primarily, as it would be expected that drifting prey is more abundant in higher flow environments (Grossman *et al.*, 2002). The higher probability of feeding in stream environments in *S. calidus* gives further evidence for a possible shift in foraging mode between pool and stream environments. In particular, this species could be exploiting more abundant, low-value foods in pool environments at a higher feeding rate, while utilising multiple adjacent stream environments for opportunistic, low-frequency but high-value invertebrate prey. Alternatively, *S. calidus* may preferentially position towards the inlet portion of pools, downstream of riffles, to maximise the capture probability of drifting food items while reducing the energetic cost of maintaining position directly within the stronger currents of those environments. This chapter also provided direct evidence of some behavioural mechanisms of trophic niche divergence at play in the Rondegat cyprinid assemblage. Primarily, the complete lack of surface feeding in *P. phlegethon* and *L. seeberi* suggests that this mode of feeding is an important aspect of the behaviour and morphological feeding capacity of *S. calidus* in competing with the other species. Additionally, *P. phlegethon* showed further evidence for being a headwater specialist, through habitat associations, morphology and diet, and finally in behaviour: this species had a high affinity for feeding on submerged structure, presumably foraging for invertebrate larvae primarily found in this microhabitat, i.e. boulders, cobble, woody debris and aquatic vegetation.

While the synthesis of findings above suggest a degree of niche segregation between the three species, some caveats must be noted to place these findings in context. In particular, trophic niche segregation understood in general theoretical terms implies an equilibrium or near "steady-state" has been achieved between the ecological players in a system. However, given the intrinsic lack of stability in Mediterranean climate river systems, commonly having both predictable and unpredictable disturbances, it can be difficult to accurately determine the stability of observed ecological differences between species. Highly variable resource abundances can force species to opportunistically converge in feeding on a lower variety of resources than would naturally occur. For example, widespread disturbance may reduce the availability of sensitive EPT taxa, leaving only a small subset of the usually available invertebrates in sufficient abundance for exploitation. Conversely, an unseasonal flooding event may widen the pool of available resources and allow higher levels of trophic segregation as species dynamically adjust feeding patterns in response to shifts in resource abundance. The constant of unpredictability in the environment is likely to be an evolutionary driver for Mediterranean species towards opportunistic or generalist feeding modes in comparison to more stable and predictable systems. In particular, Mediterranean river fish species may show a tendency toward high plasticity in order to derive sufficient persistence and resilience under the prevailing evolutionary constraints. Thus, long-lived, longitudinal studies are required to develop a more complete understanding of community dynamics in these systems.

Considering the sedimentation scenario explored earlier, this affinity for feeding in structurally complex, low-flow, deeper pool environments, suggests vulnerability in the adaptive ability of

P. phlegethon. Sedimentation would affect this species through both the loss of preferred complex structure habitat along with the associated sensitive invertebrate larvae food source, as well as creating a shallower, more stream-like environment. This species would then be further disadvantaged in competition with *S. calidus*, which would be able to use its superior drift feeding capacity and more generalist trophic profile to adapt to the shifted resource availability in a widespread sedimentation scenario. Similarly, *P. phlegethon* lacks the ability to effectively sift through sand for food items like *L. seeberi*, and its small mouth size would further limit the ability to opportunistically switch to different prey. Furthermore, in a scenario of increased anthropogenic disturbance and agricultural land use patterns, the sensitive taxa that this species depends upon would be highly susceptible to pollutants and turbidity.

Some challenges were encountered with the use of RUV methods to observe behaviours, including identification of prey items, lack of utility in turbid or very shallow environments, and statistical challenges in the nature of the collected data such as zero-inflation and multi-level factors. However, many of the insights gained were still useful as part of a suite of information gained through other methods and inquiries. Some aspects of the study could be improved, such as a finer-scale investigation of individual fish behaviours rather than grouped counts of behaviours in a given video sample. This would allow for greater flexibility in how the resulting data could be utilised and statistically managed.

6.2 Synthesis of chapters

When considering the findings of these chapters as a whole thesis, multiple conceptual synergies are evident. I have developed multiple lines of evidence that have better established the relative distributions, trophic niches, competitive dynamics and ecological interactions of the sparsely studied Rondegat River cyprinid assemblage. Broadly, the significantly larger *L. seeberi* is best adapted to the lower and middle reaches of the river, with a generalist feeding niche and strong ability to shift between available prey types; furthermore, the species is best able to exploit rare high prey abundances in support of a migratory reproductive life history. However, further research into the diet and functional response specifically in adult *L. seeberi* (in contrast to the juveniles focused on in Chapters 3 and 4) will be required for a full picture of this species' dynamics. *Sedercypris calidus* shares much of the river with both other species as an important player in higher abundance in the aquatic ecosystem across all reaches. It appears that *S. calidus* fulfils a similar niche to juvenile *L. seeberi* through strong overlaps in feeding niche between aggregations of similarly sized *L. seeberi* and *S. calidus*. However, *S.*

calidus is better adapted to drift feeding on prey available in low densities in the water column, and its diet is subsidised by terrestrial insects captured on the surface. The species was also shown to have some advantages in searching for and acquiring prey at low densities, likely through sensory advantages and suitable morphology for prey pursuit, in comparison to the low prey handling times and high-density prey feeding advantages of *L. seeberi* (Chapter 3). The habitat associations and distributions of *S. calidus* and *L. seeberi* showed them to be ideal for comparison in terms of competitive performance through the proxy of feeding performance (Chapter 2); hence the decision to include these two species specifically in functional response trials. In particular, these two species overlapped spatially over much of the river and had very similar habitat preferences, while *P. phlegethon* is more separated from the other species; spatially in the case of *L. seeberi*, and in habitat specialisations when compared to *S. calidus*.

Taking into account evidence from all data chapters, and considering *S. calidus* and *L. seeberi* specifically, a clear pattern of niche differentiation emerged. As *S. calidus* was acknowledged as a drift-feeding adapted ubiquitous species, filling a niche that *L. seeberi*, despite similar feeding capacities, habitat associations and higher overall feeding performance, is unable to fully exploit. Specifically, I highlighted the similarities in habitat preferences and spatial distribution, with the exception of the exclusion of *L. seeberi* above a historical physical barrier weir (below the Algeria conservation site) within the protected area (Chapter 2). I then investigated an aspect of competition between the species and suggested a possible niche differentiation mechanism to explain the apparent stable equilibrium of both species' coexistence, despite the spatial overlap and apparent competitive advantages of *L. seeberi* (Chapter 3). Following this evaluation of relative feeding performance, I investigated the theoretical feeding capacities of both species and combined this with in-situ diet information (Chapter 4). Thus, the fundamental feeding niche of both species was established by functional morphology, and the realised niche was represented by diet data. My findings also reinforced the need for direct confirmation of niche differentiation through the evidence uncovered for limiting similarity; i.e. the morphological differences, especially between *S. calidus* and *L. seeberi*, are a mechanism that may explain how competition is mediated between these species. The observed in-situ behaviours then confirmed differentiated microhabitat usage of *L. seeberi* and *S. calidus*, as suggested by the laboratory-based Chapters 3 and 4 in response to an initial question of community dynamics established by the in-situ abundance patterns established by Chapter 2. Through a combination of investigations of spatial abundance, habitat associations, feeding performance, morphology, diet and behaviour, a much greater understanding of the community dynamics between *L. seeberi* and *S. calidus* as vitally important conservation species in a unique post-intervention scenario was thus obtained. Thus, significant contributions to the ecological knowledge required for the recovery of these species have been made, particularly through objective 7 (baseline biology and ecology) and other objectives as outlined by Saddlier et al. (2013), and in the stated aims of this thesis.

I now shift focus to *P. phlegethon*. I built on a framework established following work on the occupancy dynamics of the Rondegat cyprinid assemblage (Castañeda *et al.*, 2020a) which demonstrated that the *P. phlegethon* population is still recovering, in contrast to the populations of *S. calidus* and *L. seeberi* being in a state of equilibrium in terms of recolonisation and migration between sites. To explore this, I investigated the habitat associations and distributions of the three species and a clear trend was established whereupon *P. phlegethon* was strongly associated with pristine, upper headwater habitats, particularly the vegetated deeper pools between riffles. Furthermore, even when suitable habitat was available, the protected status of the reach and the associated lack of anthropogenic disturbance was noted as a significant predictor of presence and abundance for this species. Supporting this trend was a clear negative association of this species with turbid conditions, commonly associated with disturbance. This set of findings was further reinforced by enhancing the understanding of the diet and ecomorphologically defined feeding capacity of *P. phlegethon* (Chapter 4). *Pseudobarbus phlegethon*'s trophic niche was shown to trend towards relatively high specialisation on small aquatic larval prey in complex, structure-rich habitat, with a small subterminal mouth appropriate for this prey type, and a larger caudal peduncle suggesting manoeuvrability in complex environments. Observations of *P. phlegethon* behaviour further crystallises the relatively specialised niche of this species as distinct from that of *L. seeberi* and *S. calidus*. The markedly higher foraging frequency of this species on submerged structures, as opposed to water column or substrate feeding, links well with the habitat associations, morphology and diet uncovered here. This set of findings form a comprehensive trend of evidence for a higher sensitivity to habitat disturbance and other anthropogenic effects in *P. phlegethon*, notwithstanding specific vulnerabilities of *S. calidus* and *L. seeberi* which I will discuss in the following section.

6.3 Implications for conservation and recommendations for monitoring of the Rondegat and other CFE systems

Taking into account the multiple lines of evidence gathered throughout this body of work, clearer recommendations for the conservation of this recovering endemic fish assemblage can be formulated. Following the 2012 and 2013 rotenone applications, eradication of non-native species from the Rondegat River, and the subsequent recolonisation and rebound of the affected populations, continued monitoring revealed a positive recovery prognosis (Weyl *et al.*, 2013, 2014; Bellingan *et al.*, 2019; Castañeda *et al.*, 2020a). In particular, the populations of *L. seeberi* and *S. calidus* showed strong initial recovery rapidly following the eradication programme, while *P. phlegethon* had a less dramatic recolonisation and population resurgence. This initial finding was confirmed as an ongoing trend, with occupancy dynamics modelling revealing *P. phlegethon*'s slow recovery and failure to fully stabilise by 2016, three years after the second rotenone treatment (Castañeda *et al.*, 2020a). The work here, particularly in Chapter 2, reinforced these findings for the more recent 2018 – 2019 time period; *P. phlegethon* lags behind the other species in abundance and is conspicuously underrepresented in the previously invaded reach. The most likely explanation for this trend is that of *P. phlegethon* being the most sensitive of the three species to habitat degradation and other anthropogenic effects impacting on the river, in addition to the legacy of the initial local extirpation by invasive species. This argument is supported by Chapters 2, 4 and 5, with evidence gathered through abundance patterns, habitat associations, trophic niche and behaviour. In particular, sedimentation, turbidity, water abstraction and land use changes have been identified as the primary drivers of the impacts on this species' struggle to regain a foothold in previously suitable habitat.

Given the slow recovery situation and the nature of ongoing threats to the river, it is clear that while *S. calidus* and *L. seeberi* have been sufficiently protected from the effects of invasive species, all three species are still in danger of further declines in the event that current anthropogenic effects continue to increase in severity. These local and shorter term effects are liable to be compounded by the differential impacts of global climate change on the three species over time, as previously acknowledged. With the knowledge gained here and building upon previous work on the Rondegat River as a case study of alien fish eradication in South Africa, recommendations as to the future of the project from a conservation standpoint can be offered. First, continued monitoring is recommended; this could be less frequent than that of the years immediately following the rotenone application and twice-yearly monitoring employed in this project. This would serve primarily to evaluate the recovery of *P. phlegethon*, and perhaps as an early warning system in case declines in *L. seeberi* or *S. calidus* are detected. The continued and historical monitoring data should be integrated with modern data capturing and accession systems, such as the Freshwater Biodiversity Information System (FBIS) (Dallas *et al.*, 2021). In tandem with continued monitoring by local conservation authorities and researchers, consideration should be given to the implementation of small-scale conservation interventions that can mitigate the new set of challenges facing the aquatic fauna of the Rondegat River, beyond the threat of non-native species invasions.

First, maintenance and any necessitated improvements to alien fish barriers in the lower reaches should be considered, to prevent encroachment of the ever-present alien species below the previously invaded/ treatment zone. Second, the possibility of expanding protected areas to encourage the recruitment and sustainability of the *P. phlegethon* population, and provisioning additional refugia for the other vulnerable fish species of the river, should be investigated. This may require the forfeit of some land from local land owners, at least in a limited radius around the river in the expanded section. Any interventions of this nature would require careful consultation and engagement with all stakeholders, with sensible protocols for implementation and maintenance of the expanded boundary. A further intervention recommendation would be the cooperative (i.e. between local conservation authorities and land owners) construction of fish migration passageways alongside the seasonal damming carried out for various functions along the length of the river in the agricultural land-use areas. This may require minimal changes to how these activities are currently carried out, with a major benefit for colonisation and access to refugia and suitable spawning sites for many of the fish species in the river (benefits to *A. gilli* and *G. zebratus* are unclear at the current level of knowledge, but are likely to be less impactful for these species than for the cyprinid assemblage). Unrestricted movement within the river is likely to be of particular value to the conservation of the fish fauna under the current status of the river, being highly sedimented and considerably lower in ideal habitat in much of the mid and lower reaches following the 2018 fire event. Connectivity is well understood as a crucial aspect of river ecosystem health (Fausch *et al.*, 2002; Falke *et al.*, 2012; Beatty *et al.*, 2017), and given the reduction in suitable habitat in a significant portion of the Rondegat River, the construction of fish passageways should be highlighted as a particularly high-priority intervention. Concurrently with the efforts to increase river connectivity, continued restoration and maintenance of indigenous riparian vegetation and extension of the current alien tree removal efforts is recommended. This focus on indigenous vegetation may assist with reduction in sedimentation, reducing the severity of fire events, and potentially increase allochthonous food biomass flows into the river system (Nakano *et al.*, 1999b; Lowe *et al.*, 2008). Alongside these high-priority options, enhanced conservation awareness campaigns, fire prevention and response cooperation between conservation authorities and local government services would be of value to mitigate a large portion of the ongoing and potential threats to the river ecosystem. Finally, the contentious issue of subsistence fishing by the underprivileged local populace, particularly on the highly protected *L. seeberi*, is a sensitive problem to overcome. Informal fishing by the local populace has been anecdotally reported by members of the public, and evidence of ad-hoc damming and deployment of makeshift seine nets has been found during my own monitoring efforts (specifically during a snorkel survey in February 2020). While removal is prohibited as a strictly no-take species under the NEM:BA – Threatened or Protected Species (TOPS) regulations, enforcement is problematic owing to the disadvantaged nature of the affected populace. Enforcement is further hampered through the stretching of the resources of local authorities and the property rights involved in areas that are not officially protected; i.e. much of the lower river reaches where congregations of adult *L. seeberi* are most vulnerable to opportunistic fishing.

Taking into consideration the other fish species of the Rondegat freshwater community, *A. gilli* and *G. zebratus*, some recommendations can be put forward based on shared requirements with the cyprinid assemblage and previous work on these species. Both species present difficulty for the monitoring technique used here (underwater video); *A. gilli* owing to nocturnal and cryptic behaviour, and *G. zebratus* owing to a highly limited distribution (this species was only found at one site through all underwater camera deployments and snorkel surveys, and has previously been only intermittently detected in surveys). However, during sampling of the river for stable isotope fin clip samples, *A. gilli* were found in quite high abundance in fyke nets deployed overnight in most sites. It is likely that this species is quite stable and well-recovered with strong recruitment into the treatment area from the unaffected reaches; however, it is highly vulnerable to sedimentation, perhaps even more so than the cyprinid species (Bills, 1998). *Galaxias zebratus* in the Rondegat River is fairly elusive, and is found only in the extreme upper reaches in slow-flowing bedrock-dominated pools. Owing to the highly fragmented nature of the *G. zebratus* species complex, and following the trend of widespread cryptic speciation in this species complex (Wishart *et al.*, 2006; Chakona *et al.*, 2013, 2020), it is likely that the *G. zebratus* population of the Rondegat River is genetically distinct. This population may be distinct from the Jan Dissels River and other nearby systems' populations on the opposite slope of the mountain from which both rivers originate (A. Chakona, pers. comm.). For this reason, further research into the genetic identity and a fine-scale appraisal of this population's distribution and abundance in the protected area of the Rondegat is recommended. The current distribution, within pristine and protected habitat and with low probability of significant anthropogenic disturbance, suggests that this population may be relatively safe from extirpation. However, cognisance of the very small distribution range and likelihood of unique genealogy of this population should be encouraged, as well as sustained protection of this population through maintenance of the conservation area.

Regarding the conservation guidelines outlined by Saddlier et al. (2013) and the overall goals of this body of work, key gaps in the knowledge of the ecology and ongoing conservation requirements of the fish community of the Rondegat River have been identified and addressed (Table 13). Insights gained include varying aspects of the habitat associations, abundance and spatial patterns, ecomorphology, feeding ecology and behaviour of all three cyprinid species. Conclusions as to the current status and predicted trends in the recovery of the cyprinid assemblage, as well as their ecological interactions and responses to stressors have been put forward, utilising multiple lines of evidence through a variety of research techniques. Looking to the future, greater focus on the conservation of fish communities of the CFE will be required given the myriad accelerating stressors expected to impact this hotspot of endemism (Dallas, 2013; Dallas & Rivers-Moore, 2014; Shelton *et al.*, 2018b). While the severe Western Cape drought conditions appear to have lifted as of 2020 / 2021, these conditions may well return and intensify in the coming years, and the eastern CFE regions are still affected by very low rainfall (Dallas & Rivers-Moore, 2014; Shelton *et al.*, 2018b; van Wilgen *et al.*, 2020). As such, careful water management based on sound biological information, as presented here, will be required (Magoulick & Kobza, 2003; Crook *et al.*, 2010). In particular, further work on the biology of CFE species will be required to better ascertain their resistance and resilience to drought and other Anthropocene-related stressors (Crook *et al.*, 2010).

Table 13: Recovery objectives and performance criteria adapted from Saddlier et al. (2013). The contributions of this thesis or the current status of the objectives are summarised.

Recovery objective	Performance criterion	Thesis contribution / status
1. Determine distribution and abundance.	Gain additional knowledge of population numbers and sizes and their distribution.	Improved understanding of the current abundance and spatial distributions of the focal species has been gained (**Chapter 2**).
2. Determine genetic and taxonomic status of populations.	An understanding of the current population genetics and fine-scale taxonomy of the focal species.	Knowledge fairly robust and not investigated here; however, fine scale comparisons with other populations are recommended.
3. Determine habitat characteristics and requirements.	Knowledge of the habitat use across the range of all focal species, including at different life-history stages.	A greater understanding of habitat use in the three focal species was gained (**Chapters 2 and 5**). Further work on habitat use in different life-history stages is recommended particularly in the case of *L. seeberi*.
4. Identify and manage potentially threatening processes having an impact on conservation.	Improvement in the understanding of existing and potential threats to the system, followed by an increase in abundance and range in the focal species.	Ongoing and forecasted threats to the recovery of the focal species were identified and discussed in relation to my findings (**Chapters 2 - 5**). Monitoring following future conservation actions will assess population trends in response to threats and interventions.
5. Protect key populations across the range.	Identification of key populations, ideally including genetically distinct units.	Refugia and crucial parts of species' ranges in the Rondegat River were identified (**Chapter 2**).
6. Determine population trends at key sites.	Establishing long-term monitoring programmes to assess population trends and impacts of threats and conservation efforts.	This thesis contributed to the long term programmes already established in the Rondegat River (**Chapter 2**).
7. Investigate key aspects of biology and ecology.	Knowledge generated on the baseline biology and ecology that can inform conservation actions.	All data chapters (**2 - 5**) have contributed to a greater understanding of the ecological interactions affecting community dynamics.
8. Establish a captive breeding population.	Vulnerable species identified, successfully maintained in captivity and breeding programmes established.	Not addressed here; however, the contributed knowledge from this thesis may inform conservation efforts.
9. Establish new populations.	New populations established in appropriate and protected habitat.	Not addressed here; however, the contributed knowledge from this thesis may inform conservation efforts.
10. Increase community and stakeholder awareness and involvement.	Increased awareness and established conservation volunteer programmes in the local populace.	Conservation-relevant data generated and recommendations put forward (**Chapter 6**).

Considering the sparse nature of scientific knowledge on South African freshwater fishes, my findings allow for recommendations to be made towards priorities for future ecological research on the focal species. Of the three species, *P. phlegethon* is likely to be the most in need of conservation actions both within the Rondegat River and in the wider CFE. My findings here suggest that the species is the most dependent on undisturbed habitat; furthermore, it is still recovering in this system according to occupancy dynamics modelling (Castañeda *et al.*, 2020a), and only four other populations are known in the CFE. I suggest that the most urgent research priorities for this species are thus investigations into the potential for successful holding in captivity, requirements for robust breeding programmes, and careful consideration as to where this species could potentially be (re)established.

Labeobarbus seeberi, as a flagship conservation species, has had comparatively higher research efforts directed towards it. However, gaps in knowledge remain, particularly in the potential for differing requirements between juveniles and adults' ideal habitat requirements. In addition, a fine-scale investigation of the impacts of reduced habitat connectivity on *L. seeberi* is required. Another avenue of research that could inform conservation efforts would be a large-scale tracking programme to better understand priority areas for spawning and potential shifts in breeding migrations over time. Currently, migratory spawning of this species is understood to occur in parts of the Olifants River, but movements of adults between tributaries and the main stem are largely unknown.

Finally, *S. calidus* characterises a scenario wherein the species has a very small distribution range; however, where it is still found following range reductions through the introduction of non-native predatory species, it has fairly robust populations. The potential for future work on this species could include a similar approach to *P. phlegethon* in terms of gaining knowledge on consistent methods to ensure survival and breeding in captivity, with the aim to re-establish this species into areas it may have occupied prior to invasion impacts. Any (re-)introductions of native species should, however, be carefully implemented in order to avoid potential for loss of genetic diversity or other unintended consequences in remaining local populations. The acquisition of this knowledge for many imperilled CFE fishes, and the subsequent implementation of safe haven areas and successful breeding programmes, may help to mitigate the current pressures and uncertain future threats to these highly endemic and valuable species.

REFERENCES

Abell R., Allan J.D. & Lehner B. (2007). Unlocking the potential of protected areas for freshwaters. *Biological Conservation* **134**, 48–63. https://doi.org/10.1016/j.biocon.2006.08.017

Albert J.S., Destouni G., Duke-Sylvester S.M., Magurran A.E., Oberdorff T., Reis R.E., *et al.* (2020). Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio*. https://doi.org/10.1007/s13280-020-01318-8

Alves G.H.Z., Figueiredo B.R.S., Manetta G.I., Sacramento P.A., Tófoli R.M. & Benedito E. (2017). Trophic segregation underlies the coexistence of two piranha species after the removal of a geographic barrier. *Hydrobiologia* **797**, 57–68. https://doi.org/10.1007/s10750-017-3159-6

Arbizu P.M. (2020). *pairwiseAdonis: Pairwise Multilevel Comparison using Adonis*.

Bartoń K. (2020). *MuMIn: Multi-Model Inference. R package version 1.43.17. http://R-Forge.R-project.org/projects/mumin/.*

Bearhop S., Adams C.E., Waldron S., Fuller R.A. & Macleod H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis: Stable isotopes as measures of niche width. *Journal of Animal Ecology* **73**, 1007–1012. https://doi.org/10.1111/j.0021-8790.2004.00861.x

Beatty S., Allen M., Lymbery A., Jordaan M.S., Morgan D., Impson D., *et al.* (2017). Rethinking refuges: Implications of climate change for dam busting. *Biological Conservation* **209**, 188–195. https://doi.org/10.1016/j.biocon.2017.02.007

Bellingan T., Woodford D., Gouws J., Villet M. & Weyl O. (2015). Rapid bioassessment of the effects of repeated rotenone treatments on invertebrate assemblages in the Rondegat River, South Africa. *African Journal of Aquatic Science* **40**, 89–94. https://doi.org/10.2989/16085914.2014.984651

Bellingan T.A., Hugo S., Woodford D.J., Gouws J., Villet M.H. & Weyl O.L.F. (2019). Rapid recovery of macroinvertebrates in a South African stream treated with rotenone. *Hydrobiologia* **834**, 1–11. https://doi.org/10.1007/s10750-019-3885-z

Bills I.R. & Impson N.D. (2013). *Conservation biology of endangered freshwater fishes - linking conservation of endangered freshwater fishes with river conservation, focusing on the Cederberg: report to the Water Research Commission*. Water Research Commission, WRC Report No. KV 305/12, Pretoria.

Bills R. (1998). Biology and conservation status of the Clanwilliam rock catfish and spotted rock catfish. 23

Boddy N.C., Booker D.J. & McIntosh A.R. (2020). Heterogeneity in flow disturbance around river confluences influences spatial patterns in native and non-native species co-occurrence. *Biological Invasions* **22**, 3457–3475

Bolker B. (2010). *bbmle: Tools for general maximum likelihood estimation*. R package version 0.9.

Bolnick D.I., Svanback R., Araujo M.S. & Persson L. (2007). Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proceedings of the National Academy of Sciences* **104**, 10075–10079. https://doi.org/10.1073/pnas.0703743104

Bozek M.A. & Young M.K. (1994). Fish mortality resulting from delayed effects of fire in the greater Yellowstone ecosystem. *The Great Basin Naturalist* **54**, 91–95

Branconi R., Wong M.Y.L. & Buston P.M. (2019). Comparison of efficiency of direct observations by scuba diver and indirect observations *via* video camera for measuring reef-fish behaviour. *Journal of Fish Biology* **94**, 489–497. https://doi.org/10.1111/jfb.13921

Breiman L., Friedman J., Olshen R. & Stone C. (1984). *Classification and Regression Trees*. Wadsworth International Group, Belmont, California, USA.

Brooks M.E., Kristensen K., Benthem K.J. van, Magnusson A., Berg C.W., Nielsen A., *et al.* (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* **9**, 378–400

Broom C.J., South J. & Weyl O.L.F. (2021). Prey type and temperature influence functional responses of threatened endemic Cape Floristic Ecoregion fishes. *Environmental Biology of Fishes* **104**, 797–810. https://doi.org/10.1007/s10641-021-01111-w

Broom C.J., South J. & Weyl O.L.F. (in review).

Brown C. & Laland K. (2002). Social enhancement and social inhibition of foraging behaviour in hatchery-reared Atlantic salmon. *Journal of Fish Biology* **61**, 987–998. https://doi.org/10.1111/j.1095-8649.2002.tb01857.x

Bunn S.E. & Arthington A.H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental management* **30**, 492–507

Burnham K.P. & Anderson D.R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological methods & Research* **33**, 261–304. https://0-doi.org.wam.seals.ac.za/10.1177/0049124104268644

Burton T.A. (2005). Fish and stream habitat risks from uncharacteristic wildfire: Observations from 17 years of fire-related disturbances on the Boise National Forest, Idaho. *Forest Ecology and Management* **211**, 140–149. https://doi.org/10.1016/j.foreco.2005.02.063

Caissie D. (2006). The thermal regime of rivers: a review. *Freshwater Biology* **51**, 1389–1406. https://doi.org/10.1111/j.1365-2427.2006.01597.x

Cambray J.A. (1994). Effects of turbidity on the neural structures of two closely related redfin minnows, *Pseudobarbus afer* and *P. asper*, in the Gamtoos River System, South Africa. *South African Journal of Zoology* **29**, 126–131. https://doi.org/10.1080/02541858.1994.11448337

Cambray J.A., King J.M. & Bruwer C. (1997). Spawning behaviour and early development of the Clanwilliam yellowfish (Barbus capensis; Cyprinidae), linked to experimental dam releases in the Olifants River, South Africa. *Regulated Rivers: Research & Management* **13**, 579–602. https://doi.org/10.1002/(SICI)1099-1646(199711/12)13:6<579::AID-RRR486>3.0.CO;2-F

Campbell M.D., Pollack A.G., Gledhill C.T., Switzer T.S. & DeVries D.A. (2015). Comparison of relative abundance indices calculated from two methods of generating video count data. *Fisheries Research* **170**, 125–133. https://doi.org/10.1016/j.fishres.2015.05.011

Cappo M., Harvey E., Malcolm H. & Speare P. (2003). Potential of video techniques to monitor diversity, abundance and size of fish in studies of marine protected areas. In: *In: Beumer, J.P., Grant, A. & Smith, D.C. (eds) Aquatic protected areas: What works best and what do we know? Proceedings of the World Congress on Aquatic Protected Areas*. pp. 455–464.

Carvalho A.C., Carvalho A., Martins H., Marques C., Rocha A., Borrego C., *et al.* (2011). Fire weather risk assessment under climate change using a dynamical downscaling approach. *Environmental Modelling & Software* **26**, 1123–1133. https://doi.org/10.1016/j.envsoft.2011.03.012

Castañeda R.A., Mandrak N.E., Barrow S. & Weyl O.L.F. (2020a). Occupancy dynamics of rare cyprinids after invasive fish eradication. *Aquatic Conservation: Marine and Freshwater Ecosystems* **30**, 1424–1436. https://doi.org/10.1002/aqc.3364

Castañeda R.A., Van Nynatten A., Crookes S., Ellender B.R., Heath D.D., MacIsaac H.J., *et al.* (2020b). Detecting native freshwater fishes using novel non-invasive methods. *Frontiers in Environmental Science* **8**, 29. https://doi.org/10.3389/fenvs.2020.00029

Castañeda R.A., Weyl O.L.F. & Mandrak N.E. (2020c). Using occupancy models to assess the effectiveness of underwater cameras to detect rare stream fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, aqc.3254. https://doi.org/10.1002/aqc.3254

Chakona A., Gouws G., Kadye W.T., Jordaan M.S. & Swartz E.R. (2020). Reconstruction of the historical distribution ranges of imperilled stream fishes from a global endemic hotspot based on molecular data: Implications for conservation of threatened taxa. *Aquatic Conservation: Marine and Freshwater Ecosystems* **30**, 144–158. https://doi.org/10.1002/aqc.3251

Chakona A. & Skelton P.H. (2017). A review of the Pseudobarbus afer (Peters, 1864) species complex (Teleostei, Cyprinidae) in the eastern Cape Fold Ecoregion of South Africa. *ZooKeys* **657**, 109–140. https://doi.org/10.3897/zookeys.657.11076

Chakona A., Swartz E.R. & Gouws G. (2013). Evolutionary Drivers of Diversification and Distribution of a Southern Temperate Stream Fish Assemblage: Testing the Role of Historical Isolation and Spatial Range Expansion. *PLoS ONE* **8**, e70953. https://doi.org/10.1371/journal.pone.0070953

Chakona A., Swartz E.R. & Magellan K. (2011). Aerial exposure tolerance of a newly discovered galaxiid. *Journal of Fish Biology* **78**, 912–922. https://doi.org/10.1111/j.1095-8649.2011.02913.x

Chave J. (2004). Neutral theory and community ecology. *Ecology letters* **7**, 241–253

Chester E.T. & Robson B.J. (2013). Anthropogenic refuges for freshwater biodiversity: Their ecological characteristics and management. *Biological Conservation* **166**, 64–75. https://doi.org/10.1016/j.biocon.2013.06.016

Christian J.M. & Adams G.L. (2014). Effects of pool isolation on trophic ecology of fishes in a highland stream: effects of pool isolation on trophic ecology. *Journal of Fish Biology* **85**, 752–772. https://doi.org/10.1111/jfb.12453

Chutter F.M. (1969). The effects of silt and sand on the invertebrate fauna of streams and rivers. *Hydrobiologia* **34**, 57–76. https://doi.org/10.1007/BF00040323

Coghlan A.R., McLean D.L., Harvey E.S. & Langlois T.J. (2017). Does fish behaviour bias abundance and length information collected by baited underwater video? *Journal of Experimental Marine Biology and Ecology* **497**, 143–151. https://doi.org/10.1016/j.jembe.2017.09.005

Cohen J.E., Jonsson T. & Carpenter S.R. (2003). Ecological community description using the food web, species abundance, and body size. *Proceedings of the National Academy of Sciences* **100**, 1781–1786. https://doi.org/10.1073pnas.232715699

Cohen J.E., Pimm S.L., Yodzis P. & Saldaña J. (1993). Body sizes of animal predators and animal prey in food webs. *Journal of Animal Ecology* **62**, 67–78

Colvin S.A.R., Sullivan S.M.P., Shirey P.D., Colvin R.W., Winemiller K.O., Hughes R.M., *et al.* (2019). Headwater Streams and Wetlands are Critical for Sustaining Fish, Fisheries, and Ecosystem Services. *Fisheries* **44**, 73–91. https://doi.org/10.1002/fsh.10229

Comte L., Buisson L., Daufresne M. & Grenouillet G. (2013). Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. *Freshwater Biology* **58**, 625–639. https://doi.org/10.1111/fwb.12081

Comte L., Olden J.D., Tedesco P.A., Ruhi A. & Giam X. (2021). Climate and land-use changes interact to drive long-term reorganization of riverine fish communities globally. *Proceedings of the National Academy of Sciences* **118**, e2011639118. https://doi.org/10.1073/pnas.2011639118

Correa S.B. & Winemiller K.O. (2014). Niche partitioning among frugivorous fishes in response to fluctuating resources in the Amazonian floodplain forest. *Ecology* **95**, 210–224. https://doi.org/10.1890/13-0393.1

Cousins S., Kennard M.J. & Ebner B.C. (2017). Depth-related composition and structuring of tropical riverine fish assemblages revealed by baited video. *Marine and Freshwater Research* **68**, 1965. https://doi.org/10.1071/MF16278

Crook D.A., Reich P., Bond N.R., McMaster D., Koehn J.D. & Lake P.S. (2010). Using biological information to support proactive strategies for managing freshwater fish during drought. *Marine and Freshwater Research* **61**, 379. https://doi.org/10.1071/MF09209

Dallas H. (2008). Water temperature and riverine ecosystems: An overview of knowledge and approaches for assessing biotic responses, with special reference to South Africa. *Water SA* **34**, 393–404

Dallas H., Shelton J., Paxton B., Weyl O., Reizenberg J.-L., Bloy L., *et al.* (2020). *Assessing the Effect of Climate Change on Native and Non-Native Freshwater Fishes of the Cape Fold Ecoregion, South Africa*. Water Research Commission, WRC Report No. TT 801/19, Pretoria, South Africa.

Dallas H., Shelton J., Sutton T., Tri Cuptura D., Kajee M. & Job N. (2021). The Freshwater Biodiversity Information System (FBIS) – mobilising data for evaluating long-term change in South African rivers. *African Journal of Aquatic Science*, 1–16. https://doi.org/10.2989/16085914.2021.1982672

Dallas H.F. (2013). Ecological status assessment in Mediterranean rivers: complexities and challenges in developing tools for assessing ecological status and defining reference conditions. *Hydrobiologia* **719**, 483–507. https://doi.org/10.1007/s10750-012-1305-8

Dallas H.F. & Day J.A. (2007). Natural variation in macroinvertebrate assemblages and the development of a biological banding system for interpreting bioassessment data—a preliminary evaluation using data from upland sites in the south-western Cape, South Africa. *Hydrobiologia* **575**, 231–244

Dallas H.F. & Rivers-Moore N. (2014). Ecological consequences of global climate change for freshwater ecosystems in South Africa. *South African Journal of Science* **110**. https://doi.org/10.1590/sajs.2014/20130274

Darwall W.R.T., Smith K.G., Tweddle D. & Skelton P. (2009). *The status and distribution of freshwater biodiversity in southern Africa*. IUCN, Gland.

Daugaard U., Petchey O.L. & Pennekamp F. (2019). Warming can destabilize predator–prey interactions by shifting the functional response from Type III to Type II. *Journal of Animal Ecology* **88**, 1575–1586. https://doi.org/10.1111/1365-2656.13053

De'ath G. & Fabricius K.E. (2000). Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* **81**, 3178–3192. https://doi.org/10.1890/0012-9658(2000)081[3178:CARTAP]2.0.CO;2

Dell A.I., Pawar S. & Savage V.M. (2014). Temperature dependence of trophic interactions are driven by asymmetry of species responses and foraging strategy. *Journal of Animal Ecology* **83**, 70–84. https://doi.org/10.1111/1365-2656.12081

Dick J.T.A., Alexander M.E., Jeschke J.M., Ricciardi A., MacIsaac H.J., Robinson T.B., *et al.* (2014). Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. *Biological Invasions* **16**, 735–753. https://doi.org/10.1007/s10530-013-0550-8

Dick J.T.A., Laverty C., Lennon J.J., Barrios-O'Neill D., Mensink P.J., Robert Britton J., *et al.* (2017). Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. *Journal of Applied Ecology* **54**, 1259–1267. https://doi.org/10.1111/1365-2664.12849

Dickey J.W.E., Cuthbert R.N., South J., Britton J.R., Caffrey J., Chang X., *et al.* (2020). On the RIP: using Relative Impact Potential to assess the ecological impacts of invasive alien species. *NeoBiota* **55**, 27–60. https://doi.org/10.3897/neobiota.55.49547

Doeg T. & Koehn J. (1994). Effects of draining and desilting a small weir on downstream fish and macroinvertebrates. *Regulated Rivers: Research & Management* **9**, 263–277

Dudgeon D., Arthington A.H., Gessner M.O., Kawabata Z.-I., Knowler D.J., Lévêque C., *et al.* (2005). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**, 163–182. https://doi.org/10.1017/s1464793105006950

Dudgeon D., Arthington A.H., Gessner M.O., Kawabata Z.-I., Knowler D.J., Lévêque C., *et al.* (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**, 163. https://doi.org/10.1017/S1464793105006950

Ebner B.C., Clear R., Godschalx S. & Beitzel M. (2009). In-stream behaviour of threatened fishes and their food organisms based on remote video monitoring. *Aquatic Ecology* **43**, 569–576. https://doi.org/10.1007/s10452-008-9192-9

Ebner B.C. & Morgan D.L. (2013). Using remote underwater video to estimate freshwater fish species richness. *Journal of Fish Biology* **82**, 1592–1612. https://doi.org/10.1111/jfb.12096

Ebner B.C., Starrs D., Morgan D.L., Fulton C., Donaldson J.A., Doody J.S., *et al.* (2014). Emergence of field-based underwater video for understanding the ecology of freshwater fishes and crustaceans in Australia. *Journal of the Royal Society of Western Australia* **97**, 287–296

Ellender B.R., Becker A., Weyl O.L.F. & Swartz E.R. (2012). Underwater video analysis as a non-destructive alternative to electrofishing for sampling imperilled headwater stream fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems* **22**, 58–65. https://doi.org/10.1002/aqc.1236

Ellender B.R., Rivers-Moore N.A., Coppinger C.R., Bellingan T.A. & Weyl O.L.F. (2016). Towards using thermal stress thresholds to predict salmonid invasion potential. *Biological Invasions* **18**, 3513–3525. https://doi.org/10.1007/s10530-016-1244-9

Ellender B.R., Wasserman R.J., Chakona A., Skelton P.H. & Weyl O.L.F. (2017). A review of the biology and status of Cape Fold Ecoregion freshwater fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems* **27**, 867–879. https://doi.org/10.1002/aqc.2730

Ellender B.R. & Weyl O.L.F. (2014). A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions* **9**, 117–132. https://doi.org/10.3391/ai.2014.9.2.01

Ellender B.R. & Weyl O.L.F. (2015). Resilience of imperilled headwater stream fish to an unpredictable high-magnitude flood. *Koedoe* **57**, 1–8. https://doi.org/10.4102/koedoe.v57i1.1258

Ellender B.R., Weyl O.L.F., Alexander M.E., Luger A.M., Nagelkerke L.A.J. & Woodford D.J. (2018). Out of the pot and into the fire: Explaining the vulnerability of an endangered small headwater stream fish to black-bass *Micropterus* spp. invasion. *Journal of Fish Biology* **92**, 1035–1050. https://doi.org/10.1111/jfb.13562

Englund G., Öhlund G., Hein C.L. & Diehl S. (2011). Temperature dependence of the functional response. *Ecology Letters* **14**, 914–921. https://doi.org/10.1111/j.1461-0248.2011.01661.x

Falke J.A., Bailey L.L., Fausch K.D. & Bestgen K.R. (2012). Colonization and extinction in dynamic habitats: an occupancy approach for a Great Plains stream fish assemblage. *Ecology* **93**, 858–867. https://doi.org/10.1890/11-1515.1

Fauchereau N., Trzaska S., Rouault M. & Richard Y. (2003). Rainfall Variability and Changes in Southern Africa during the 20th Century in the Global Warming Context. *Natural Hazards* **29**, 139–154

Fausch K.D., Baxter C.V. & Murakami M. (2010). Multiple stressors in north temperate streams: lessons from linked forest-stream ecosystems in northern Japan. *Freshwater Biology* **55**, 120–134. https://doi.org/10.1111/j.1365-2427.2009.02378.x

Fausch K.D. & Bramblett R.G. (1991). Disturbance and Fish Communities in Intermittent Tributaries of a Western Great Plains River. *Copeia* **1991**, 659. https://doi.org/10.2307/1446392

Fausch K.D., Nakano S. & Kitano S. (1997). Experimentally induced foraging mode shift by sympatric charrs in a Japanese mountain stream. *Behavioral Ecology* **8**, 414–420

Fausch K.D., Torgersen C.E., Baxter C.V. & Li H.W. (2002). Landscapes to Riverscapes: Bridging the Gap between Research and Conservation of Stream Fishes. *BioScience* **52**, 483. https://doi.org/10.1641/0006-3568(2002)052[0483:LTRBTG]2.0.CO;2

Filipe A.F., Lawrence J.E. & Bonada N. (2013). Vulnerability of stream biota to climate change in Mediterranean climate regions: a synthesis of ecological responses and conservation challenges. *Hydrobiologia* **719**, 331–351. https://doi.org/10.1007/s10750-012-1244-4

Fill J.M., Kritzinger-Klopper S. & van Wilgen B.W. (2018). Short-term vegetation recovery after alien plant clearing along the Rondegat River, South Africa: Short-term vegetation recovery. *Restoration Ecology* **26**, 434–438. https://doi.org/10.1111/rec.12585

Friard O. & Gamba M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution* **7**, 1325–1330. https://doi.org/10.1111/2041-210x.12584

Gallagher R.V., Allen S., Mackenzie B.D., Yates C.J., Gosper C.R., Keith D.A., *et al.* (2021). High fire frequency and the impact of the 2019–2020 megafires on Australian plant diversity. *Diversity and Distributions* **0**, 1–14. https://doi.org/10.1111/ddi.13265

Garman G.C. (1991). Use of terrestrial arthropod prey by a stream-dwelling cyprinid fish. *Environmental Biology of Fishes* **30**, 325–331. https://doi.org/10.1007/BF02028848

Garrow C. & Marr S. (2012). *Swimming on the Edge of Extinction: The Perilous State of the Indigenous Freshwater Fishes of the Western Cape*. NISC.

Gasith A. & Resh V.H. (1999). Streams in Mediterranean Climate Regions: Abiotic Influences and Biotic Responses to Predictable Seasonal Events. *Annual Review of Ecology and Systematics* **30**, 51–81. https://doi.org/10.1146/annurev.ecolsys.30.1.51

Gebauer R., Veselý L., Vanina T., Buřič M., Kouba A. & Drozd B. (2019). Prediction of ecological impact of two alien gobiids in habitat structures of differing complexity. *Canadian Journal of Fisheries and Aquatic Sciences* **76**, 1954–1961. https://doi.org/10.1139/cjfas-2018-0346

Gilbert B., Tunney T.D., McCann K.S., DeLong J.P., Vasseur D.A., Savage V., *et al.* (2014). A bioenergetic framework for the temperature dependence of trophic interactions. *Ecology Letters* **17**, 902–914. https://doi.org/10.1111/ele.12307

Gore J., King J. & Hamman K. (1991). Application of the instream flow incremental methodology to Southern African rivers: Protecting endemic fish of the Olifants River. *Water SA* **17**, 225–236

Grant J.W. & Noakes D.L. (1987). Escape behaviour and use of cover by young-of-the-year brook trout, Salvelinus fontinalis. *Canadian Journal of Fisheries and Aquatic Sciences* **44**, 1390–1396

Grant P.R. (1972). Convergent and divergent character displacement. *Biological journal of the Linnean Society* **4**, 39–68

Grossman G.D., Dowd J.F. & Crawford M. (1990). Assemblage stability in stream fishes: A review. *Environmental Management* **14**, 661–671. https://doi.org/10.1007/BF02394716

Grossman G.D., Moyle P.B. & Whitaker, J.O. (1982). Stochasticity in Structural and Functional Characteristics of an Indiana Stream Fish Assemblage: A Test of Community Theory. *The American Naturalist* **120**, 423–454. https://doi.org/10.1086/284004

Grossman G.D., Rincon P.A., Farr M.D. & Ratajczak R.E. (2002). A new optimal foraging model predicts habitat use by drift-feeding stream minnows: Optimal foraging in stream fishes. *Ecology of Freshwater Fish* **11**, 2–10. https://doi.org/10.1034/j.1600-0633.2002.110102.x

Hammer M.P., Bice C.M., Hall A., Frears A., Watt A., Whiterod N.S., *et al.* (2013). Freshwater fish conservation in the face of critical water shortages in the southern Murray–Darling Basin, Australia. *Marine and Freshwater Research* **64**, 807–821. https://doi.org/10.1071/MF12258

Hannweg B., Marr S.M., Bloy L.E. & Weyl O.L.F. (2020). Using action cameras to estimate the abundance and habitat use of threatened fish in clear headwater streams. *African Journal of Aquatic Science* **45**, 372–377. https://doi.org/10.2989/16085914.2019.1701404

Harper M., Mejbel H.S., Longert D., Abell R., Beard T.D., Bennett J.R., *et al.* (2021). Twenty-five essential research questions to inform the protection and restoration of freshwater biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems*, aqc.3634. https://doi.org/10.1002/aqc.3634

Hartig F. (2019). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*.

Harvey E. & Mladenov P. (2001). The uses of underwater television and video technology in fisheries science: a review. In: *Video sensing of the size and abundance of target and non-target fauna in Australian fisheries-a national workshop*. p. 90.

Holling C.S. (1959). The components of predation as revealed by a study of small - mammal predation of the European Pine Sawfly. *The Canadian Entomologist*, 293–320

Holling C.S. (1965). The functional response of predators to prey density and its role in mimicry and population regulation. *The Memoirs of the Entomological Society of Canada* **97**, 5–60

Huey R.B. & Stevenson R. (1979). Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist* **19**, 357–366

Humphries P. & Lake P.S. (2000). Fish larvae and the management of regulated rivers. *Regulated Rivers: Research & Management* **16**, 421–432. https://doi.org/10.1002/1099-1646(200009/10)16:5<421::AID-RRR594>3.0.CO;2-4

Huntsman B.M. & Petty J.T. (2014). Density-Dependent Regulation of Brook Trout Population Dynamics along a Core-Periphery Distribution Gradient in a Central Appalachian Watershed. *PLoS ONE* **9**, e91673. https://doi.org/10.1371/journal.pone.0091673

Huntsman B.M., Petty J.T., Sharma S. & Merriam E.R. (2016). More than a corridor: use of a main stem stream as supplemental foraging habitat by a brook trout metapopulation. *Oecologia* **182**, 463–473. https://doi.org/10.1007/s00442-016-3676-4

Hutchinson G.E. (1961). The Paradox of the Plankton. *The American Naturalist* **95**, 137–145

Impson D. (2016). Capacity development. Have our provincial aquatic scientists become critically endangered? *Water Wheel* **15**, 20–23

Impson N. & Tharme R. (1998). Translocation of indigenous freshwater fishes for conservation and flyfishing: the Boontjies River, Western Cape as a case study. *Piscator* **130**, 87–92

Impson N.D., Bills I.R. & Wolhuter L.E. (2008). *Technical report on the state of yellowfishes in South Africa 2007*. Water Research Commission, Pretoria, South Africa.

Impson N.D., Marriott M., Bills I.R. & Skelton P.H. (2007). Conservation biology and management of a critically endangered cyprinid, the Twee River redfin, *Barbus erubescens* (Teleostei: Cyprinidae), of the Cape Floristic Region, South Africa. *African Journal of Aquatic Science* **32**, 27–33. https://doi.org/10.2989/AJAS.2007.32.1.4.141

Impson N.D., van der Walt J.A. & Jordaan M.S. (2017). Labeobarbus seeberi. *The IUCN Red List of Threatened Species 2017: e.T63290A100163027*. http://dx.doi.org/10.2305/IUCN.UK.2017- 3.RLTS.T63290A100163027.en

Jackson M., Wasserman R., Grey J., Ricciardi A., Dick J.T. & Alexander M. (2017). Novel and disrupted trophic links following invasion in freshwater ecosystems. *Advances in ecological research* **57**, 55–97

Jackson M.C. & Britton J.R. (2013). Variation in the trophic overlap of invasive *Pseudorasbora parva* and sympatric cyprinid fishes. *Ecology of Freshwater Fish* **22**, 654–657. https://doi.org/10.1111/eff.12063

Jackson M.C., Fourie H.E., Dalu T., Woodford D.J., Wasserman R.J., Zengeya T.A., *et al.* (2020). Food web properties vary with climate and land use in South African streams. *Functional Ecology* **34**, 1653–1665. https://doi.org/10.1111/1365-2435.13601

Jackson M.C., Loewen C.J.G., Vinebrooke R.D. & Chimimba C.T. (2016a). Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Global Change Biology* **22**, 180–189. https://doi.org/10.1111/gcb.13028

Jackson M.C., Loewen C.J.G., Vinebrooke R.D. & Chimimba C.T. (2016b). Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Global Change Biology* **22**, 180–189. https://doi.org/10.1111/gcb.13028

Jackson M.C., Pawar S. & Woodward G. (2021). The Temporal Dynamics of Multiple Stressor Effects: From Individuals to Ecosystems. *Trends in Ecology & Evolution* **36**, 402–410. https://doi.org/10.1016/j.tree.2021.01.005

Jackson M.C., Woodford D.J., Bellingan T.A., Weyl O.L.F., Potgieter M.J., Rivers-Moore N.A., *et al.* (2016c). Trophic overlap between fish and riparian spiders: potential impacts of an invasive fish on terrestrial consumers. *Ecology and Evolution* **6**, 1745–1752. https://doi.org/10.1002/ece3.1893

Jordaan M.S., Chakona A. & van der Colff D. (2020). Protected Areas and Endemic Freshwater Fishes of the Cape Fold Ecoregion: Missing the Boat for Fish Conservation? *Frontiers in Environmental Science* **8**, 502042. https://doi.org/10.3389/fenvs.2020.502042

Jordaan M.S., Lubbe A., Bragg C., Paxton B.R., Schumann M., Van Der Walt R., *et al.* (2017a). Labeo seeberi (errata version published in 2020). *The IUCN Red List of Threatened Species 2017: e.T11071A174778890.* https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T11071A174778890.en

Jordaan M.S., van der Walt J.A., Brink Z., Erasmus S. & Weyl O.L.F. (2017b). Conservation implications of establishment success of the Critically Endangered Twee River redfin ‘ <i>Pseudobarbus’ erubescens</i> (Skelton,) in an artificial impoundment in South Africa. *Aquatic Conservation: Marine and Freshwater Ecosystems* **27**, 886–889. https://doi.org/10.1002/aqc.2747

Juliano S.A. (2001). Nonlinear curve fitting: predation and functional response curves. *Design and analysis of ecological experiments* **2**, 178–196

Kadye W.T., Chakona A. & Jordaan M.S. (2016). Swimming with the giant: coexistence patterns of a new redfin minnow *Pseudobarbus skeltoni* from a global biodiversity hot spot. *Ecology and Evolution* **6**, 7141–7155. https://doi.org/10.1002/ece3.2328

Kawaguchi Y., Taniguchi Y. & Nakano S. (2003). Terrestrial invertebrate inputs determine the local abundance of stream fishes in a forested stream. *Ecology* **84**, 701–708. https://doi.org/10.1890/0012-9658(2003)084[0701:TIIDTL]2.0.CO;2

Keppeler F.W., Montaña C.G. & Winemiller K.O. (2020). The relationship between trophic level and body size in fishes depends on functional traits. *Ecological Monographs* **90**. https://doi.org/10.1002/ecm.1415

Keppeler F.W. & Winemiller K.O. (2020). Can ancestry and morphology be used as surrogates for species niche relationships? *Ecology and Evolution* **10**, 6562–6578. https://doi.org/10.1002/ece3.6390

Khosa D., Marr S.M., Wasserman R.J., Zengeya T.A. & Weyl O.L.F. (2019). An evaluation of the current extent and potential spread of Black Bass invasions in South Africa. *Biological Invasions* **21**, 1721–1736. https://doi.org/10.1007/s10530-019-01930-0

Killen S. (2011). Energetics of foraging decisions and prey handling. In: *Encyclopedia of fish physiology: From Genome to Environment*. pp. 1588–1595. Academic press, San Diego.

King J., Cambray J.A. & Impson N.D. (1998). Linked effects of dam-released floods and water temperature on spawning of the Clanwilliam yellowfish *Barbus capensis*. *Hydrobiologica* **384**, 245–265

Koehn J.D. & Lintermans M. (2012). A strategy to rehabilitate fishes of the Murray-Darling Basin, south-eastern Australia. *Endangered Species Research* **16**, 165–181

Kraft N.J., Adler P.B., Godoy O., James E.C., Fuller S. & Levine J.M. (2015). Community assembly, coexistence and the environmental filtering metaphor. *Functional ecology* **29**, 592–599

Kruger A.C. & Nxumalo M.P. (2017). Historical rainfall trends in South Africa: 1921–2015. *Water SA* **43**, 285. https://doi.org/10.4314/wsa.v43i2.12

Leibold M.A. & McPeek M.A. (2006). Coexistence of the niche and neutral perspectives in community ecology. *Ecology* **87**, 1399–1410. https://doi.org/10.1890/0012-9658(2006)87[1399:COTNAN]2.0.CO;2

Lenth R., Singmann H., Love J., Buerkner P. & Herve M. (2018). *Emmeans: Estimated marginal means, aka least-squares means*.

Lintermans M. (2013). A review of on-ground recovery actions for threatened freshwater fish in Australia. *Marine and Freshwater Research* **64**, 775–791. https://doi.org/10.1071/MF12306

Lintermans M., Lyon J.P., Hammer M.P., Ellis I. & Ebner B.C. (2015). Underwater, out of sight: lessons from threatened freshwater fish translocations in Australia. In: *Advances in reintroduction biology of Australian and New Zealand fauna.* pp. 237–253. CSIRO Publishing, Victoria, Australia.

Lötter D. (2015). *Potential implications of climate change for Rooibos (A.Linearis) production and distribution in the greater Cederberg Region, South Africa*. University of Cape Town, Cape Town.

Lowe S.R., Woodford D.J., Impson D.N. & Day J.A. (2008). The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. *African Journal of Aquatic Science* **33**, 51–62. https://doi.org/10.2989/AJAS.2007.33.1.6.390

Lüdecke D. (2018). ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. *Journal of Open Source Software* **3**, 772. https://doi.org/10.21105/joss.00772

Luger A.M., South J., Alexander M.E., Ellender B.R., Weyl O.L.F. & Nagelkerke L.A.J. (2020). Ecomorphology of largemouth bass relative to a native trophic analogue explains its high invasive impact. *Biological Invasions* **22**, 2223–2233. https://doi.org/10.1007/s10530-020-02252-2

Luiz O.J., Olden J.D., Kennard M.J., Crook D.A., Douglas M.M., Saunders T.M., *et al.* (2019). Trait-based ecology of fishes: A quantitative assessment of literature trends and knowledge gaps using topic modelling. *Fish and Fisheries* **20**, 1100–1110. https://doi.org/10.1111/FAF.12399

Lyon J.P., Bird T.J., Kearns J., Nicol S., Tonkin Z., Todd C.R., *et al.* (2019). Increased population size of fish in a lowland river following restoration of structural habitat. *Ecological Applications* **29**, e01882. https://doi.org/10.1002/eap.1882

Lyon J.P. & O'Connor J.P. (2008). Smoke on the water: Can riverine fish populations recover following a catastrophic fire-related sediment slug? *Austral Ecology* **33**, 794–806. https://doi.org/10.1111/j.1442-9993.2008.01851.x

Magellan K., Weyl O. & Booth A. (2021). Preference for Artificial Refugia over Natural Refugia in an Endangered Fish. *Diversity* **13**, 635. https://doi.org/10.3390/d13120635

Magoulick D.D. & Kobza R.M. (2003). The role of refugia for fishes during drought: a review and synthesis. *Freshwater Biology* **48**, 1186–1198. https://doi.org/10.1046/j.1365-2427.2003.01089.x

Manna L.R., Miranda J.C., Rezende C.F. & Mazzoni R. (2020). Feeding strategy and morphology as indicators of habitat use and coexistence of two loricariid fishes from a Brazilian coastal stream. *Biota Neotropica* **20**, e20190764. https://doi.org/10.1590/1676-0611-bn-2019-0764

Marr S.M., Impson N.D. & Tweddle D. (2012). An assessment of a proposal to eradicate non-native fish from priority rivers in the Cape Floristic Region, South Africa. *African Journal of Aquatic Science* **37**, 131–142. https://doi.org/10.2989/16085914.2012.666654

Marriott M.S. (1998). *Conservation biology and management of the Twee River redfin, Barbus erubescens (Pisces: Cyprinidae)*. Rhodes University, Grahamstown.

Matthews W.J. (2012). *Patterns in freshwater fish ecology*. Springer Science & Business Media.

Matthews W.J. & Marsh-Matthews E. (2003). Effects of drought on fish across axes of space, time and ecological complexity. *Freshwater Biology* **48**, 1232–1253. https://doi.org/10.1046/j.1365-2427.2003.01087.x

McCard M., South J., Cuthbert R.N., Dickey J.W.E., McCard N. & Dick J.T.A. (2021). Pushing the switch: functional responses and prey switching by invasive lionfish may mediate their ecological impact. *Biological Invasions* **23**, 2019–2032. https://doi.org/10.1007/s10530-021-02487-7

McGill B.J., Enquist B.J., Weiher E. & Westoby M. (2006). Rebuilding community ecology from functional traits. *Trends in ecology & evolution* **21**, 178–185

McIntosh A.R., McHugh P.A., Plank M.J., Jellyman P.G., Warburton H.J. & Greig H.S. (2018). Capacity to support predators scales with habitat size. *Science Advances* **4**, 1–8. https://doi.org/10.1126/sciadv.aap7523

McLaughlin R.L. (2001). Behavioural diversification in brook charr: adaptive responses to local conditions. *Journal of Animal Ecology* **70**, 325–337. https://doi.org/10.1111/j.1365-2656.2001.00491.x

McMeans B.C., Kadoya T., Pool T.K., Holtgrieve G.W., Lek S., Kong H., *et al.* (2019). Consumer trophic positions respond variably to seasonally fluctuating environments. *Ecology* **100**, e02570. https://doi.org/10.1002/ecy.2570

Meijer C.G., Warburton H.J. & McIntosh A.R. (2021). Disentangling the multiple effects of stream drying and riparian canopy cover on the trophic ecology of a highly threatened fish. *Freshwater Biology* **66**, 102–113. https://doi.org/10.1111/fwb.13620

Mofu L., South J., Wasserman R.J., Dalu T., Woodford D.J., Dick J.T.A., *et al.* (2019). Inter-specific differences in invader and native fish functional responses illustrate neutral effects on prey but superior invader competitive ability. *Freshwater Biology* **64**, 1655–1663. https://doi.org/10.1111/fwb.13361

de Moor F.C. & Day J.A. (2013). Aquatic biodiversity in the Mediterranean region of South Africa. *Hydrobiologia* **719**, 237–268. https://doi.org/10.1007/s10750-013-1488-7

Mthombeni V.G. (2009). *The biology of Austroglanis gilli and Austroglanis barnardi (Siluriformes: Austroglanididae) in the Olifants River system, South Africa*. Rhodes University, Grahamstown.

Murdoch W.W. (1969). Switching in General Predators: Experiments on Predator Specificity and Stability of Prey Populations. *Ecological Monographs* **39**, 335–354. https://doi.org/10.2307/1942352

Nagelkerke L.A.J., van Onselen E., van Kessel N. & Leuven R.S.E.W. (2018). Functional feeding traits as predictors of invasive success of alien freshwater fish species using a food-fish model. *PLOS ONE* **13**, e0197636. https://doi.org/10.1371/journal.pone.0197636

Nakano S., Fausch K.D. & Kitano S. (1999a). Flexible niche partitioning via a foraging mode shift: a proposed mechanism for coexistence in stream-dwelling charrs. *Journal of Animal Ecology* **68**, 1079–1092. https://doi.org/10.1046/j.1365-2656.1999.00355.x

Nakano S., Miyasaka H. & Kuhara N. (1999b). Terrestrial–aquatic linkages: riparian arthropod inputs alter trophic cascades in a stream food web. *Ecology* **80**, 2435–2441. https://doi.org/10.1890/0012-9658(1999)080[2435:TALRAI]2.0.CO;2

Nanninga G.B., Côté I.M., Beldade R. & Mills S.C. (2017a). Behavioural acclimation to cameras and observers in coral reef fishes. *Ethology* **123**, 705–711. https://doi.org/10.1111/eth.12642

Nanninga G.B., Côté I.M., Beldade R. & Mills S.C. (2017b). Behavioural acclimation to cameras and observers in coral reef fishes. *Ethology* **123**, 705–711. https://doi.org/10.1111/eth.12642

Neubauer P. & Andersen K.H. (2019). Thermal performance of fish is explained by an interplay between physiology, behaviour and ecology. *Conservation Physiology* **7**. https://doi.org/10.1093/conphys/coz025

Nthimo M. (1997). *The reproductive and feeding biology of Clanwilliam redfin minnow, Barbus calidus (Pisces: Cyprinidae)*. Rhodes University, Grahamstown.

Oksanen J., Blanchet F.G., Friendly M., Kindt R., Legendre P., McGlinn D., *et al.* (2019). *vegan: Community Ecology Package*.

Oliveira E.F., Goulart E., Breda L., Minte-Vera C.V., Paiva L.R. de S. & Vismara M.R. (2010). Ecomorphological patterns of the fish assemblage in a tropical floodplain: effects of trophic, spatial and phylogenetic structures. *Neotropical Ichthyology* **8**, 569–586. https://doi.org/10.1590/S1679-62252010000300002

Orr J.A., Vinebrooke R.D., Jackson M.C., Kroeker K.J., Kordas R.L., Mantyka-Pringle C., *et al.* (2020). Towards a unified study of multiple stressors: divisions and common goals across research disciplines. *Proceedings of the Royal Society B: Biological Sciences* **287**, 20200421. https://doi.org/10.1098/rspb.2020.0421

Paxton B.R. (2008). *The influence of hydraulics, hydrology and temperature on the distribution, habitat use and recruitment of threatened cyprinids in a Western Cape river, South Africa*. University of Cape Town.

Penaluna B.E., Olson D.H., Flitcroft R.L., Weber M.A., Bellmore J.R., Wondzell S.M., *et al.* (2017). Aquatic biodiversity in forests: a weak link in ecosystem services resilience. *Biodiversity and Conservation* **26**, 3125–3155. https://doi.org/10.1007/s10531-016-1148-0

Pepi A., Grof-Tisza P., Holyoak M. & Karban R. (2018). As temperature increases, predator attack rate is more important to survival than a smaller window of prey vulnerability. *Ecology* **99**, 1584–1590. https://doi.org/10.1002/ecy.2356

Perry G. & Pianka E.R. (1997). Animal foraging: past, present and future. *Trends in Ecology & Evolution* **12**, 360–364

Pianka E.R. (1974). *Evolutionary Ecology*, First Edition. Harper and Row, New York.

du Plessis J.A. & Schloms B. (2017). An investigation into the evidence of seasonal rainfall pattern shifts in the Western Cape, South Africa. *Journal of the South African Institution of Civil Engineering* **59**, 47–55. https://doi.org/10.17159/2309-8775/2017/v59n4a5

Poff N.L. (1997). Landscape Filters and Species Traits: Towards Mechanistic Understanding and Prediction in Stream Ecology. *Journal of the North American Benthological Society* **16**, 391–409. https://doi.org/10.2307/1468026

Poff N.L. & Zimmerman J.K.H. (2010). Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows: Review of altered flow regimes. *Freshwater Biology* **55**, 194–205. https://doi.org/10.1111/j.1365-2427.2009.02272.x

Pool T., Holtgrieve G., Elliott V., McCann K., McMeans B., Rooney N., *et al.* (2017). Seasonal increases in fish trophic niche plasticity within a flood-pulse river ecosystem (Tonle Sap Lake, Cambodia). *Ecosphere* **8**, e01881. https://doi.org/10.1002/ecs2.1881

Pörtner H.O., Bennett A.F., Bozinovic F., Clarke A., Lardies M.A., Lucassen M., *et al.* (2006). Trade-offs in thermal adaptation: the need for a molecular to ecological integration. *Physiological and Biochemical Zoology* **79**, 295–313. https://doi.org/10.1086/499986

Pritchard D.W., Paterson R.A., Bovy H.C. & Barrios-O'Neill D. (2017). FRAIR: an R package for fitting and comparing consumer functional responses. *Methods in Ecology and Evolution* **8**, 1528–1534. https://doi.org/10.1111/2041-210X.12784

Pritchard J.R. & Schluter D. (2001). Declining interspecific competition during character displacement: Summoning the ghost of competition past. *Evolutionary Ecology Research* **3**, 209–220

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rall B.C., VUCIC-PESTIC O., Ehnes R.B., Emmerson M. & Brose U. (2010). Temperature, predator–prey interaction strength and population stability. *Global Change Biology* **16**, 2145–2157

Reizenberg J.-L., Bloy L.E., Weyl O.L.F., Shelton J.M. & Dallas H.F. (2019). Variation in thermal tolerances of native freshwater fishes in South Africa's Cape Fold Ecoregion: examining the east-west gradient in species' sensitivity to climate warming. *Journal of Fish Biology*. https://doi.org/10.1111/jfb.13866

Rincón P.A., Bastir M. & Grossman G.D. (2007). Form and performance: body shape and prey-capture success in four drift-feeding minnows. *Oecologia* **152**, 345–355. https://doi.org/10.1007/s00442-006-0651-5

Robinson B. (2000). Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. *Behaviour* **137**, 865–888

Rogers D. (1972). Random search and insect population models. *The Journal of Animal Ecology* **41**, 369–383. https://doi.org/10.2307/3474

Ryer C.H. & Olla B.L. (1991). Information transfer and the facilitation and inhibition of feeding in a schooling fish. *Environmental Biology of Fishes* **30**, 317–323. https://doi.org/10.1007/BF02028847

Saddlier S., Koehn J.D. & Hammer M.P. (2013). Let's not forget the small fishes – conservation of two threatened species of pygmy perch in south-eastern Australia. *Marine and Freshwater Research* **64**, 874. https://doi.org/10.1071/MF12260

Sayer C.D., Emson D., Patmore I.R., Greaves H.M., West W.P., Payne J., *et al.* (2020). Recovery of the crucian carp Carassius carassius (L.): Approach and early results of an English conservation project. *Aquatic Conservation: Marine and Freshwater Ecosystems* **30**, 2240–2253. https://doi.org/10.1002/aqc.3422

Schlosser I.J. (1995). Critical landscape attributes that influence fish population dynamics in headwater streams. *Hydrobiologia* **303**, 71–81

Schmitz O.J. (2007). Predator diversity and trophic interactions. *Ecology* **88**, 2415–2426. https://doi.org/10.1890/06-0937.1

Schoener T.W. (1974). Resource Partitioning in Ecological Communities. *Science* **185**, 27–39. https://doi.org/10.1126/science.185.4145.27

Shelton J.M., Bird M. & Marr S. (2018a). Evidence for diet partitioning among three coexisting native freshwater fishes in South Africa's Cape Fold Ecoregion. *African Journal of Aquatic Science* **43**, 89–100. https://doi.org/10.2989/16085914.2018.1470490

Shelton J.M., Weyl O.L.F., Chakona A., Ellender B.R., Esler K.J., Impson N.D., *et al.* (2018b). Vulnerability of Cape Fold Ecoregion freshwater fishes to climate change and other human impacts. *Aquatic Conservation: Marine and Freshwater Ecosystems* **28**, 68–77. https://doi.org/10.1002/aqc.2849

Shelton J.M., Weyl O.L.F., Esler K.J., Paxton B.R., Impson N.D. & Dallas H.F. (2018c). Temperature mediates the impact of non-native rainbow trout on native freshwater fishes in South Africa's Cape Fold Ecoregion. *Biological Invasions* **20**, 2927–2944. https://doi.org/10.1007/s10530-018-1747-7

Sibbing F.A., Nagelkerke L.A., Stet R.J. & Osse J.W. (1998). Speciation of endemic Lake Tana barbs (Cyprinidae, Ethiopia) driven by trophic resource partitioning; a molecular and ecomorphological approach. *Aquatic Ecology* **32**, 217–227

Sibbing F.A. & Nagelkerke L.A.J. (2001). Resource partitioning by Lake Tana barbs predicted from fish morphometrics and prey characteristics. *Reviews in Fish Biology and Fisheries* **10**, 393–437

Sih A., Englund G. & Wooster D. (1998). Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution* **13**, 350–355. https://doi.org/10.1016/S0169-5347(98)01437-2

Skelton P.H. (2001). *A complete guide to the freshwater fishes of southern Africa.*, 2nd edn. Struik, Cape Town, South Africa.

Skelton P.H. (2000). Flagships and fragments—perspectives on the conservation of freshwater fishes in southern Africa. *Southern African Journal of Aquatic Sciences* **25**, 37–42. https://doi.org/10.2989/160859100780177929

Skelton P.H. (1996). Threatened fishes of the world: *Pseudobarbus phlegethon* (Barnard, 1938) (Cyprinidae). *Environmental Biology of Fishes*, 214

Skelton P.H., Swartz E.R. & Vreven E.J. (2018). The identity of *Barbus capensis* Smith, 1841 and the generic status of southern African tetraploid cyprinids (Teleostei, Cyprinidae). *European Journal of Taxonomy* **410**, 1–29. https://doi.org/10.5852/ejt.2018.410

Slabbert E., Jordaan M. & Weyl O. (2014). Analysis of active rotenone concentration during treatment of the Rondegat River, Cape Floristic Region, South Africa. *African Journal of Aquatic Science* **39**, 467–472. https://doi.org/10.2989/16085914.2014.981144

Solomon M. (1949). The natural control of animal populations. *The Journal of Animal Ecology* **18**, 1–35. https://doi.org/10.2307/1578

South J., McCard M., Khosa D., Mofu L., Madzivanzira T.C., Dick J.T.A., *et al.* (2019). The effect of prey identity and substrate type on the functional response of a globally invasive crayfish. *NeoBiota* **52**, 9–24. https://doi.org/10.3897/neobiota.52.39245

South J., Welsh D., Anton A., Sigwart J.D. & Dick J.T.A. (2018). Increasing temperature decreases the predatory effect of the intertidal shanny *Lipophrys pholis* on an amphipod prey: temperature reduces *lipophrys pholis* functional response. *Journal of Fish Biology* **92**, 150–164. https://doi.org/10.1111/jfb.13500

Southey D. (2009). *Wildfires in the Cape Floristic Region: Exploring vegetation and weather as drivers of fire frequency*. University of Cape Town, Cape Town.

Stachowicz J.J. (2001). Mutualism, facilitation, and the structure of ecological communities. *BioScience* **51**, 235. https://doi.org/10.1641/0006-3568(2001)051[0235:MFATSO]2.0.CO;2

Stephens D. & Krebs J. (1986). *Foraging Theory*. Princeton University Press, Princeton, NJ.

Svanbäck R. & Bolnick D.I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences* **274**, 839–844. https://doi.org/10.1098/rspb.2006.0198

Swartz E.R. (2000). *Population geneties of* Pseudobarbus phlegethon*,* Barbus calidus *and* Barbus erubescens *(Teleostei: Cyprinidae) of the Olifants River System, Western Cape Province*. University of Stellenbosch, Stellenbosch.

Swartz E.R., Flemming A.F. & Mouton P. le F.N. (2004). Contrasting genetic patterns and population histories in three threatened redfin species (Cyprinidae) from the Olifants River system, western South Africa. *Journal of Fish Biology* **64**, 1153–1167. https://doi.org/10.1111/j.0022-1112.2004.00375.x

Swartz E.R., Skelton P.H. & Bloomer P. (2009). Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): Shedding light on the drainage history of rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution* **51**, 75–84. https://doi.org/10.1016/j.ympev.2008.10.017

Swartz E.R., Skelton P.H. & Bloomer P. (2007). Sea-level changes, river capture and the evolution of populations of the Eastern Cape and fiery redfins (*Pseudobarbus afer* and *Pseudobarbus phlegethon*, Cyprinidae) across multiple river systems in South Africa. *Journal of Biogeography* **34**, 2086–2099. https://doi.org/10.1111/j.1365-2699.2007.01768.x

Swisher B.J., Soluk D.A. & Wahl D.H. (1998). Non-Additive Predation in Littoral Habitats: Influences of Habitat Complexity. *Oikos* **81**, 30. https://doi.org/10.2307/3546464

Therneau T. & Atkinson B. (2019). *rpart: Recursive Partitioning and Regression Trees*.

Tickner D., Opperman J.J., Abell R., Acreman M., Arthington A.H., Bunn S.E., *et al.* (2020). Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. *BioScience* **70**, 330–342. https://doi.org/10.1093/biosci/biaa002

Tweddle D., Bills R., Swartz E., Coetzer W., da Costa L., Engelbrecht J., *et al.* (2009). The status and distribution of freshwater fishes. In: *The status and distribution of freshwater biodiversity in southern Africa*. p. 37.

Uiterwaal S.F. & DeLong J.P. (2020). Functional responses are maximized at intermediate temperatures. *Ecology* **101**. https://doi.org/10.1002/ecy.2975

Uno H. & Power M.E. (2015). Mainstem-tributary linkages by mayfly migration help sustain salmonids in a warming river network. *Ecology Letters* **18**, 1012–1020. https://doi.org/10.1111/ele.12483

Usio N. & Nakano S. (1998). Influences of microhabitat use and foraging mode similarities on intra- and interspecific aggressive interactions in a size-structured stream fish assemblage. *Ichthyological Research* **45**, 19–28. https://doi.org/10.1007/BF02678571

Uszko W., Diehl S., Englund G. & Amarasekare P. (2017). Effects of warming on predator-prey interactions - a resource-based approach and a theoretical synthesis. *Ecology Letters* **20**, 513–523. https://doi.org/10.1111/ele.12755

Van Liefferinge C., Dillen A., Vught I., Auwerx J. & Meire P. (2019). Microhabitat suitability as a tool to improve the success rate of a translocation or reintroduction, case-study of the bullhead (Cottus perifretum) in Flanders, Belgium. *Restoration Ecology* **27**, 504–512. https://doi.org/10.1111/rec.12907

Vasseur D.A., DeLong J.P., Gilbert B., Greig H.S., Harley C.D.G., McCann K.S., *et al.* (2014). Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20132612. https://doi.org/10.1098/rspb.2013.2612

Vaughan I.P. & Ormerod S.J. (2010). Linking ecological and hydromorphological data: approaches, challenges and future prospects for riverine science. *Aquatic Conservation: Marine and Freshwater Ecosystems* **20**, S125–S130. https://doi.org/10.1002/aqc.1104

Villéger S., Brosse S., Mouchet M., Mouillot D. & Vanni M.J. (2017). Functional ecology of fish: current approaches and future challenges. *Aquatic Sciences* **79**, 783–801. https://doi.org/10.1007/s00027-017-0546-z

Voeten C.C. (2019). *buildmer: Stepwise Elimination and Term Reordering for Mixed-Effects Regression*.

Vörösmarty C.J., McIntyre P.B., Gessner M.O., Dudgeon D., Prusevich A., Green P., *et al.* (2010). Global threats to human water security and river biodiversity. *Nature* **467**, 555–561

van der Walt J.A. (2014). *Black bass (Micropterus spp.) in the Olifants-Doorn river system: distribution, distribution barriers, predatory impact and management*. Cape Peninsula University of Technology, Cape Town, South Africa. https://core.ac.uk/download/pdf/148364784.pdf.

van der Walt J.A., Impson D.N. & Jordaan M.S. (2017a). *Pseudobarbus phlegethon. The IUCN Red List of Threatened Species* **e.T107649522A107654419**. https://doi.org/10.2305/iucn.uk.2017-3.rlts.t107649522a107654419.en

van der Walt J.A., Jordaan M.S., Bills R. & Impson D.N. (2017b). *Austroglanis gilli. The IUCN Red List of Threatened Species* **e.T2427A99448983**. http://dx.doi.org/10.2305/IUCN.UK.2017- 3.RLTS.T2427A99448983.en

van der Walt J.A., Jordaan M.S. & Impson D.N. (2017c). *Pseudobarbus calidus. The IUCN Red List of Threatened Species* **e.T2562A100139530**. http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T2562A100139530.en

van der Walt J.A., Marr S.M., Wheeler M.J., Impson N.D., Garrow C. & Weyl O.L.F. (2019). Successful mechanical eradication of spotted bass (*Micropterus punctulatus* (Rafinesque, 1819)) from a South African river. *Aquatic Conservation: Marine and Freshwater Ecosystems* **29**, 303–311. https://doi.org/10.1002/aqc.3035

van der Walt J.A., Weyl O.L.F., Woodford D.J. & Radloff F.G.T. (2016). Spatial extent and consequences of black bass (*Micropterus* spp.) invasion in a Cape Floristic Region river basin. *Aquatic Conservation: Marine and Freshwater Ecosystems* **26**, 736–748. https://doi.org/10.1002/aqc.2589

Warburton K., Retif S. & Hume D. (1998). Generalists as sequential specialists: diets and prey switching in juvenile silver perch. *Environmental Biology of Fishes* **51**, 445–454. https://doi.org/10.1023/A:1007489100809

Ward A.J., Webster M.M. & Hart P.J. (2006). Intraspecific food competition in fishes. *Fish and Fisheries* **7**, 231–261. https://doi.org/10.1111/j.1467-2979.2006.00224.x

Ward M., Tulloch A.I., Radford J.Q., Williams B.A., Reside A.E., Macdonald S.L., *et al.* (2020). Impact of 2019–2020 mega-fires on Australian fauna habitat. *Nature Ecology & Evolution* **4**, 1321–1326. https://doi.org/10.1038/s41559-020-1251-1

Wasserman R.J., Alexander M.E., Dalu T., Ellender B.R., Kaiser H. & Weyl O.L.F. (2016). Using functional responses to quantify interaction effects among predators. *Functional Ecology* **30**, 1988–1998. https://doi.org/10.1111/1365-2435.12682

Weiher E., Freund D., Bunton T., Stefanski A., Lee T. & Bentivenga S. (2011). Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**, 2403–2413. https://doi.org/10.1098/rstb.2011.0056

Weyl O.L.F., Barrow S., Bellingan T.A., Dalu T., Ellender B.R., Esler K., *et al.* (2016). *Monitoring of invertebrate and fish recovery following river rehabilitation using rotenone in the Rondegat River: report to the Water Research Commission*. Pretoria, South Africa.

Weyl O.L.F., Ellender B.R., Wassermann R.J., Truter M., Dalu T., Zengeya T.A., *et al.* (2020). Alien Freshwater Fauna in South Africa. In: *Biological Invasions in South Africa*. (Eds B.W. van Wilgen, J. Measey, D.M. Richardson, J.R. Wilson & T.A. Zengeya), pp. 153–183. Springer International Publishing, Cham.

Weyl O.L.F., Ellender B.R., Woodford D.J. & Jordaan M.S. (2013). Fish distributions in the Rondegat River, Cape Floristic Region, South Africa, and the immediate impact of rotenone treatment in an invaded reach. *African Journal of Aquatic Science* **38**, 201–209. https://doi.org/10.2989/16085914.2012.753401

Weyl O.L.F., Finlayson B., Impson N.D., Woodford D.J. & Steinkjer J. (2014). Threatened endemic fishes in South Africa's Cape Floristic Region: a new beginning for the Rondegat River. *Fisheries* **39**, 270–279. https://doi.org/10.1080/03632415.2014.914924

Whitehead A., Weyl O. & Bills I. (2007). Reproductive and feeding biology of the endangered fiery redfin, *Pseudobarbus phlegethon* (Barnard 1938) (Teleostei: Cyprinidae), in the Noordhoeks River, South Africa. *African Journal of Aquatic Science* **32**, 281–290. https://doi.org/10.2989/AJAS.2007.32.3.8.307

Wikramanayake E.D. (1990). Ecomorphology and Biogeography of a Tropical Stream Fish Assemblage: Evolution of Assemblage Structure. *Ecology* **71**, 1756–1764. https://doi.org/10.2307/1937583

van Wilgen B.W., Measey J., Richardson D.M., Wilson J.R. & Zengeya T.A. eds (2020). *Biological Invasions in South Africa.* Springer International Publishing, Cham.

Winemiller K.O., Fitzgerald D.B., Bower L.M. & Pianka E.R. (2015). Functional traits, convergent evolution, and periodic tables of niches. *Ecology Letters* **18**, 737–751. https://doi.org/10.1111/ele.12462

Winemiller K.O., Kelso-Winemiller L.C. & Brenkert A.L. (1995). Ecomorphological diversification and convergence in fluvial cichlid fishes. *Environmental Biology of Fishes* **44**, 235–261. https://doi.org/10.1007/BF00005919

Wishart M., Hughes J., Stewart B. & Impson D. (2006). Extreme levels of intra-specific divergence among Cape Peninsula populations of the Cape galaxias, *Galaxias zebratus* Castelnau 1861, reveals a possible species complex. *African Journal of Aquatic Science* **31**, 99–106. https://doi.org/10.2989/16085910609503876

Woodford D.J. (2005). *The impact of alien invasive smallmouth bass (*Micropterus dolomieu*) on the indigenous fish of the Rondegat River: A quantitative assessment with implications for rehabilitation*. University of Cape Town, Cape Town, South Africa. http://hdl.handle.net/11427/6739.

Woodford D.J., Barber-James H.M., Bellingan T.A., Day J.A., de Moor F.C., Gouws J., *et al.* (2013). Immediate impact of piscicide operations on a Cape Floristic Region aquatic insect assemblage: a lesser of two evils? *Journal of Insect Conservation* **17**, 959–973. https://doi.org/10.1007/s10841-013-9578-4

Woodford D.J., Impson N.D., Day J.A. & Bills I.R. (2005). The predatory impact of invasive alien smallmouth bass, *Micropterus dolomieu* (Teleostei: Centrarchidae), on indigenous fishes in a Cape Floristic Region mountain stream. *African Journal of Aquatic Science* **30**, 167–173. https://doi.org/10.2989/16085910509503852

APPENDICES

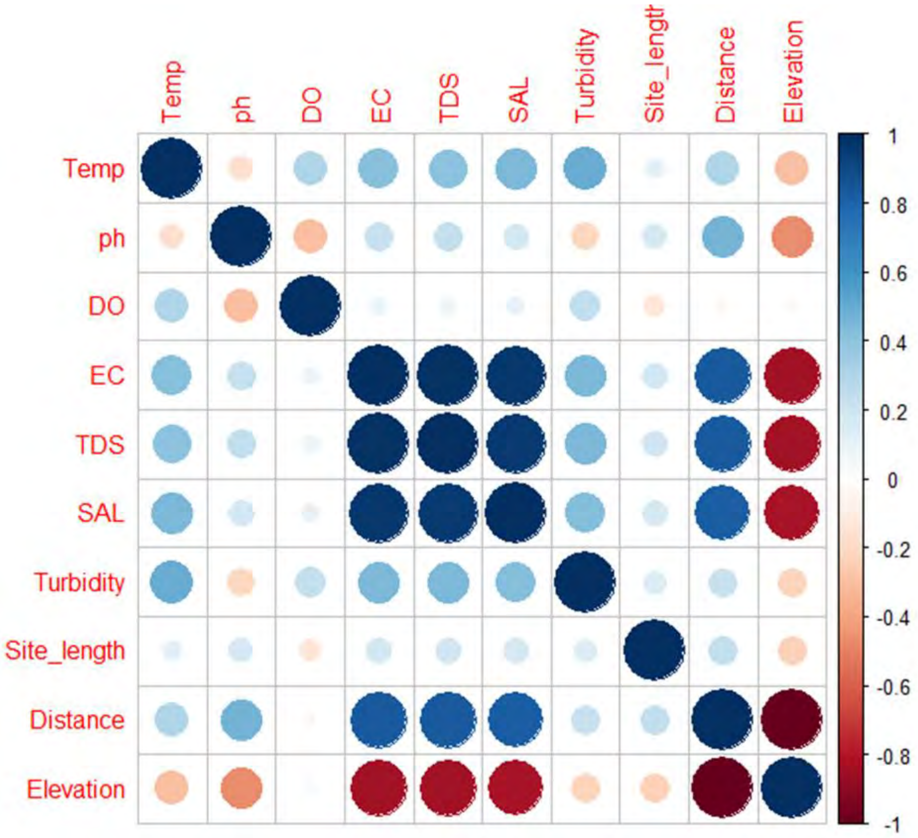

Figure S1: Correlation matrix of the continuous physicochemical variables used in habitat association modelling. Blue circles indicate positive correlations (r), while red circles denote negative correlations. Increasing size and depth of colour in circles on intersecting axes indicate strong correlations between variables. The cut-off point for autocorrelation was set at 0.8 (positive or negative).

Table S1: List of sites, their habitat features and measured physicochemical parameters across all remote underwater video surveys carried out between April 2018 and October 2019.

Month	Site	Latitude	Longitude	Distance (m)	Habitat	Substrate	Temp (°C)	pH	DO (mg/l)	EC (µS)	TDS (ppm)	Salinity (ppm)	Turbidity (ntu)	Site length (cm)
Apr 18	CB01	-32.2585	18.94736	21012	Stream	Sand	23.3	7.2	7.32	335	217	0.1	11.3	1550
Apr 18	CB02	-32.2587	18.94828	20923	Pool	Sand	22.3	7	8.03	314	204	0.1	5.8	470
Apr 18	CB03	-32.2578	18.95018	20708	Pool	Sand	21.6	6.4	5.84	320	206	0.1	8.7	4400
Apr 18	CB04	-32.2559	18.95256	20349	Pool	Sand	21.6	6.6	7.15	306	199	0.1	9.6	1480
Apr 18	CB05	-32.2606	18.9655	18362	Pool	Sand	19.7	6.63	5.02	293	189	0.09	4.84	920
Apr 18	CB06	-32.2611	18.96603	18281	Stream	Cobble	19.8	6.41	3.8	283	183	0.09	9.1	970
Apr 18	CB07	-32.2633	18.96797	17976	Pool	Sand	19.9	6.66	6.78	271	178	0.09	6.4	1470
Apr 18	CB08	-32.264	18.9684	17879	Stream	Sand	20.2	6.7	7.96	269	174	0.09	8.1	830
Apr 18	CB09	-32.266	18.96959	17622	Pool	Sand	21.35	6.55	6.28	260	169	0.08	7.7	190
Apr 18	CB10	-32.2665	18.9702	17540	Pool	Sand	21.4	6.45	9.15	250	163	0.08	5.5	710
Apr 18	CB11	-32.2677	18.97105	17394	Stream	Sand	22.8	6.31	6.13	227	149	0.07	7.1	490
Apr 18	CB12	-32.2684	18.97146	17302	Stream	Silt	22.2	6.69	8.72	214	140	0.07	1.42	1100
Apr 18	CB13	-32.2763	18.97501	16337	Pool	Sand	17.3	7.57	7.53	198	118	0.06	8.2	1600
Apr 18	CB14	-32.2767	18.9754	16273	Stream	Cobble	17.58	6.35	8.87	202	207	0.13	0	500
Apr 18	CB16	-32.2775	18.97639	16141	Pool	Sand	16.9	7.29	5.19	299	184	0.07	4.5	1020
Apr 18	CB17	-32.2776	18.97666	16104	Stream	Sand	16.93	7.17	10	195	131	0.06	1	700

Month	**Site**	**Latitude**	**Longitude**	**Distance (m)**	**Habitat**	**Substrate**	**Temp (°C)**	**pH**	**DO (mg/l)**	**EC (µS)**	**TDS (ppm)**	**Salinity (ppm)**	**Turbidity (ntu)**	**Site length (cm)**
Apr 18	CB18	-32.278	18.97738	16021	Pool	Cobble	24	6.95	7.64	196	126	0.06	6	1200
Apr 18	CB19	-32.2791	18.97908	15826	Stream	Bedrock	24.5	7.24	5.93	195	125	0.06	6.5	900
Apr 18	CB20	-32.2795	18.97938	15762	Pool	Silt	24.5	7.21	6.64	194	129	0.06	8.9	2400
Apr 18	CB21	-32.2808	18.98085	15566	Pool	Silt	24.1	7	5.97	196	128	0.06	5	780
Apr 18	CB22	-32.2813	18.98119	15497	Stream	Bedrock	24	6.91	8.27	192	122	0.06	2.5	300
Apr 18	CB23	-32.3191	19.01596	9927	Pool	Cobble	23.6	6.68	10.95	157	101	0.05	6	720
Apr 18	CB24	-32.3194	19.01687	9828	Pool	Sand	23.9	6.6	7.55	158	103	0.05	4.69	2900
Apr 18	CB25	-32.3239	19.01863	9290	Stream	Cobble	23.4	6.5	7.38	149	98	0.05	12.7	930
Apr 18	CB26	-32.3262	19.01914	9016	Pool	Sand	23.8	6.73	7.78	141	93	0.05	7.5	1500
Apr 18	CB27	-32.3268	19.01927	8955	Stream	Sand	23.3	6.5	9.07	143	92	0.04	9.2	470
Apr 18	CB28	-32.3273	19.01937	8889	Pool	Silt	23.9	6.38	6.71	150	150	0.05	17	1300
Apr 18	CB29	-32.3296	19.01943	8714	Pool	Boulders	22.4	6.63	8.63	131	85	0.04	11.1	600
Apr 18	CB30	-32.3289	19.01944	8634	Stream	Cobble	22.8	6.55	8.39	133	87	0.04	14	900
Apr 18	CB31	-32.3407	19.02096	7374	Stream	Cobble	16.8	6.68	7.24	115	75	0.04	10.4	500
Apr 18	CB32	-32.3412	19.02106	7322	Pool	Boulders	17.1	6.81	7.37	117	75	0.04	13	1300
Apr 18	CB33	-32.3465	19.0269	6465	Stream	Silt	15.7	6.8	9.25	103	66	0.03	8.4	420
Apr 18	CB34	-32.3471	19.02719	6390	Pool	Cobble	16.32	7.4	5.72	140	91	0.04	22.6	800
Apr 18	CB35	-32.3481	19.02856	6219	Pool	Sand	15.8	6.99	6.81	107	69	0.03	8.5	750
Apr 18	CB36	-32.3483	19.02899	6176	Stream	Cobble	15.9	6.98	8.63	100	64	0.03	7.5	300
Apr 18	CB37	-32.37	19.05279	2740	Pool	Boulders	17.7	6.82	9.83	55	35	0.02	3.4	1000

Month	**Site**	**Latitude**	**Longitude**	**Distance (m)**	**Habitat**	**Substrate**	**Temp (°C)**	**pH**	**DO (mg/l)**	**EC (µS)**	**TDS (ppm)**	**Salinity (ppm)**	**Turbidity (ntu)**	**Site length (cm)**
Apr 18	CB38	-32.3697	19.05347	2710	Stream	Cobble	18.08	7.03	9.95	61	40	0.02	3.4	6000
Apr 18	CB39	-32.372	19.05729	2250	Pool	Boulders	18.6	7.05	7.84	53	34	0.02	3.2	2600
Apr 18	CB40	-32.3722	19.05781	2197	Stream	Cobble	18	6.47	9.29	49	32	0.02	3.3	200
Apr 18	CB41	-32.3755	19.06319	1572	Stream	Bedrock	16.8	6.56	7.42	41	26	0.01	2.9	590
Apr 18	CB42	-32.3756	19.06341	1549	Pool	Boulders	16.8	6.8	8.35	46	29	0.01	4.7	350
Apr 18	CB43	-32.3756	19.06565	1331	Pool	Boulders	16.1	6.51	8.4	46	29	0.01	0	1600
Apr 18	CB44	-32.3759	19.06637	1250	Pool	Vegetation	16.4	7.1	7.05	45	29	0.01	2.7	1000
Apr 18	CB45	-32.3762	19.0667	1204	Pool	Cobble	15.7	6.58	8.05	48	31	0.02	3.1	1800
Apr 18	CB46	-32.3764	19.06683	1174	Stream	Boulders	15.38	6.59	9.37	46	29	0.01	0.1	920
Apr 18	CB47	-32.3775	19.06748	1045	Stream	Bedrock	15	6.23	10.11	44	28	0.01	4.2	600
Apr 18	CB48	-32.378	19.06762	989	Pool	Bedrock	15.3	6.02	6.68	47	30	0.02	1.4	550
Apr 18	CB49	-32.384	19.07254	155	Stream	Cobble	15.1	6.64	9.17	45	27	0.01	2.3	300
Apr 18	CB50	-32.3841	19.07268	139	Pool	Cobble	15.2	6.66	8.87	43	26	0.01	2.6	350
Apr 18	CB51	-32.3844	19.07337	64.5	Stream	Cobble	15	6.86	8.38	40	27	0.01	1.8	400
Apr 18	CB52	-32.3847	19.07387	6.43	Pool	Boulders	15.6	6.9	8.58	44	27	0.01	0.7	800
Oct 18	CB01	-32.2585	18.94736	21012	Stream	Boulders	16	7.2	8.22	108	68	0.03	1	1550
Oct 18	CB02	-32.2587	18.94828	20923	Pool	Sand	16.5	7.48	8.2	113	75	0.04	1.2	470
Oct 18	CB03	-32.2578	18.95018	20708	Pool	Sand	16.8	7.52	8.36	112	72	0.04	0.6	4400
Oct 18	CB04	-32.2559	18.95256	20349	Pool	Sand	16.95	8.09	7.41	116	75	0.04	1.2	1480
Oct 18	CB05	-32.2606	18.9655	18362	Pool	Sand	17.6	8.25	7.5	110	74	0.04	1.4	920

Month	**Site**	**Latitude**	**Longitude**	**Distance (m)**	**Habitat**	**Substrate**	**Temp (°C)**	**pH**	**DO (mg/l)**	**EC (µS)**	**TDS (ppm)**	**Salinity (ppm)**	**Turbidity (ntu)**	**Site length (cm)**
Oct 18	CB06	-32.2611	18.96603	18281	Stream	Sand	17.3	7.15	7.43	110	70	0.03	0.2	970
Oct 18	CB07	-32.2633	18.96797	17976	Pool	Sand	17.3	7.33	7.43	98	65	0.03	3.4	1470
Oct 18	CB08	-32.264	18.9684	17879	Stream	Sand	17.53	7.49	7.48	107	70	0.04	3.2	830
Oct 18	CB09	-32.266	18.96959	17622	Pool	Sand	19.53	7.01	11.52	103	67	0.03	2.1	190
Oct 18	CB10	-32.2665	18.9702	17540	Pool	Sand	19.38	7.7	18.54	102	66	0.03	2.3	710
Oct 18	CB11	-32.2677	18.97105	17394	Stream	Cobble	18.18	7.12	9.82	102	65	0.03	0.4	490
Oct 18	CB12	-32.2684	18.97146	17302	Stream	Sand	18.4	7.32	14.08	105	67	0.03	0.3	1100
Oct 18	CB13	-32.2763	18.97501	16337	Pool	Sand	21.8	6.85	9.02	101	61	0.03	4.4	1600
Oct 18	CB14	-32.2767	18.9754	16273	Stream	Boulders	22.55	7.54	8.08	102	63	0.03	2.4	500
Oct 18	CB16	-32.2775	18.97639	16141	Pool	Sand	22.8	7.87	7.98	93	62	0.03	4.2	1020
Oct 18	CB17	-32.2776	18.97666	16104	Stream	Sand	22.03	6.88	8.06	99	64	0.03	4.2	700
Oct 18	CB18	-32.278	18.97738	16021	Pool	Sand	17.6	7.78	14.7	100	63	0.03	5.2	1200
Oct 18	CB19	-32.2791	18.97908	15826	Stream	Bedrock	17.4	7.14	18.12	85	59	0.03	2.1	900
Oct 18	CB20	-32.2795	18.97938	15762	Pool	Sand	17.98	7.74	7.46	99	64	0.03	4.2	2400
Oct 18	CB21	-32.2808	18.98085	15566	Pool	Silt	17.98	8.12	16.12	98	63	0.03	4.1	780
Oct 18	CB22	-32.2813	18.98119	15497	Stream	Bedrock	18.2	7.07	18.12	93	61	0.03	3.8	300
Oct 18	CB23	-32.3191	19.01596	9927	Pool	Sand	19.44	7.13	8.12	85	53	0.03	3.3	720
Oct 18	CB24	-32.3194	19.01687	9828	Pool	Sand	19.3	6.58	8.11	81	53	0.03	2.1	2900
Oct 18	CB25	-32.3239	19.01863	9290	Stream	Sand	21.03	6.75	8.09	82	50	0.03	0.4	930
Oct 18	CB26	-32.3262	19.01914	9016	Pool	Sand	20.38	7.1	8.44	83	54	0.03	0.1	1500

Month	Site	Latitude	Longitude	Distance (m)	Habitat	Substrate	Temp (°C)	pH	DO (mg/l)	EC (µS)	TDS (ppm)	Salinity (ppm)	Turbidity (ntu)	Site length (cm)
Oct 18	CB27	-32.3268	19.01927	8955	Stream	Sand	21.08	7.78	8.8	76	50	0.02	4.3	470
Oct 18	CB28	-32.3273	19.01937	8889	Pool	Sand	21.4	6.72	7.66	78	56	0.03	4.3	1300
Oct 18	CB29	-32.3296	19.01943	8714	Pool	Cobble	21.13	6.31	8.06	78	30	0.03	0.4	600
Oct 18	CB30	-32.3289	19.01944	8634	Stream	Boulders	21.2	6.58	8.5	76	50	0.02	0.4	900
Oct 18	CB31	-32.3407	19.02096	7374	Stream	Sand	22.11	7.22	7.44	62	39	0.02	4.4	500
Oct 18	CB32	-32.3412	19.02106	7322	Pool	Cobble	21.8	6.99	7.8	65	42	0.02	4.1	1300
Oct 18	CB33	-32.3465	19.0269	6465	Stream	Sand	21.8	7.02	7.51	61	38	0.02	4	420
Oct 18	CB34	-32.3471	19.02719	6390	Pool	Sand	21.23	6.87	7.53	72	39	0.02	3.4	800
Oct 18	CB35	-32.3481	19.02856	6219	Pool	Boulders	21.2	6.07	7.3	89	39	0.02	4.12	750
Oct 18	CB36	-32.3483	19.02899	6176	Stream	Sand	21.2	7.02	7.21	62	42	0.02	3.8	300
Oct 18	CB37	-32.37	19.05279	2740	Pool	Cobble	21.4	6.49	6.72	48	31	0.02	5.1	1000
Oct 18	CB38	-32.3697	19.05347	2710	Stream	Cobble	21.3	6.68	6.38	49	31	0.02	5.3	6000
Oct 18	CB39	-32.372	19.05729	2250	Pool	Boulders	20.18	6.6	6.49	41	26	0.01	4.1	2600
Oct 18	CB40	-32.3722	19.05781	2197	Stream	Cobble	20.18	6.6	6.49	41	26	0.01	4.1	200
Oct 18	CB41	-32.3755	19.06319	1572	Stream	Boulders	17.6	6.64	8.5	43	29	0.01	2.1	590
Oct 18	CB42	-32.3756	19.06341	1549	Pool	Boulders	17.5	6.52	7.88	40	27	0.01	2.1	350
Oct 18	CB43	-32.3756	19.06565	1331	Pool	Cobble	17.1	6.09	7.78	42	27	0.01	5.2	1600
Oct 18	CB44	-32.3759	19.06637	1250	Pool	Cobble	17.53	6.38	7.72	41	27	0.01	3.1	1000
Oct 18	CB45	-32.3762	19.0667	1204	Pool	Cobble	18	6.29	6.94	42	26	0.01	5.2	1800
Oct 18	CB46	-32.3764	19.06683	1174	Stream	Cobble	17.9	6.19	7.12	42	27	0.01	3.8	920

Month	Site	Latitude	Longitude	Distance (m)	Habitat	Substrate	Temp (°C)	pH	DO (mg/l)	EC (µS)	TDS (ppm)	Salinity (ppm)	Turbidity (ntu)	Site length (cm)
Oct 18	CB47	-32.3775	19.06748	1045	Stream	Cobble	19.2	6.48	8.98	40	26	0.01	3.1	600
Oct 18	CB48	-32.378	19.06762	989	Pool	Bedrock	19.28	6.19	6.98	49	33	0.02	4	550
Oct 18	CB49	-32.384	19.07254	155	Stream	Vegetation	18.75	6.54	9.35	39	25	0.01	1.3	300
Oct 18	CB50	-32.3841	19.07268	139	Pool	Boulders	18.75	6.54	9.35	39	25	0.01	1.3	350
Oct 18	CB51	-32.3844	19.07337	64.5	Stream	Cobble	18.3	6.5	8.6	37	22	0.01	2.1	400
Oct 18	CB52	-32.3847	19.07387	6.43	Pool	Boulders	19.5	6.36	8.16	38	24	0.01	0.12	800
Oct 19	CB01	-32.2585	18.94736	21012	Stream	Sand	15.2	7.15	3.57	111	73	0.03	3.3	1550
Oct 19	CB02	-32.2587	18.94828	20923	Stream	Bedrock	15.3	7.18	3.6	109	70	0.03	4.8	470
Oct 19	CB03	-32.2578	18.95018	20708	Pool	Sand	16	7.57	3.47	119	74	0.03	9.8	4400
Oct 19	CB04	-32.2559	18.95256	20349	Pool	Sand	17.5	7.83	3.45	113	72	0.03	17.7	1480
Oct 19	CB05	-32.2606	18.9655	18362	Stream	Sand	15	7.15	3.51	87	56	0.03	1.8	920
Oct 19	CB06	-32.2611	18.96603	18281	Stream	Sand	15.5	7.63	3.39	100	65	0.03	0	970
Oct 19	CB07	-32.2633	18.96797	17976	Stream	Sand	16.35	7.25	3.38	134	88	0.03	0	1470
Oct 19	CB08	-32.264	18.9684	17879	Stream	Sand	16.3	7.28	4.65	104	67	0.03	0	830
Oct 19	CB09	-32.266	18.96959	17622	Stream	Sand	15.8	7.29	3.38	93	60	0.03	3.9	190
Oct 19	CB10	-32.2665	18.9702	17540	Stream	Sand	16.6	7.64	3.47	91	58	0.03	1.9	710
Oct 19	CB11	-32.2677	18.97105	17394	Stream	Cobble	17	7.42	3.38	88	57	0.03	3.2	490
Oct 19	CB12	-32.2684	18.97146	17302	Stream	Sand	17.25	7.27	2.91	87	57	0.03	1.7	1100
Oct 19	CB13	-32.2763	18.97501	16337	Pool	Sand	21.2	7.59	3.93	89	58	0.03	1.5	1600
Oct 19	CB14	-32.2767	18.9754	16273	Stream	Bedrock	21.7	7.33	3.68	90	58	0.03	1.7	500

Month	**Site**	**Latitude**	**Longitude**	**Distance (m)**	**Habitat**	**Substrate**	**Temp (°C)**	**pH**	**DO (mg/l)**	**EC (µS)**	**TDS (ppm)**	**Salinity (ppm)**	**Turbidity (ntu)**	**Site length (cm)**
Oct 19	CB16	-32.2775	18.97639	16141	Pool	Sand	22.4	7.37	3.6	93	59	0.03	1.3	1020
Oct 19	CB17	-32.2776	18.97666	16104	Stream	Sand	22.4	7.37	3.6	93	59	0.03	1.3	700
Oct 19	CB18	-32.278	18.97738	16021	Stream	Sand	15.5	7.1	3.21	87	59	0.03	0	1200
Oct 19	CB19	-32.2791	18.97908	15826	Stream	Bedrock	16.2	7.19	3.18	85	55	0.03	0	900
Oct 19	CB20	-32.2795	18.97938	15762	Pool	Sand	16.6	7.23	3.05	90	57	0.03	1.7	2400
Oct 19	CB21	-32.2808	18.98085	15566	Stream	Sand	17.2	7.65	3.32	90	58	0.03	0	780
Oct 19	CB22	-32.2813	18.98119	15497	Stream	Bedrock	17.7	7.33	3.33	89	57	0.03	0	300
Oct 19	CB23	-32.3191	19.01596	9927	Stream	Sand	18.9	7.43	3.33	78	50	0.02	0	720
Oct 19	CB24	-32.3194	19.01687	9828	Pool	Sand	19.2	7.48	3.21	78	50	0.02	3.5	2900
Oct 19	CB25	-32.3239	19.01863	9290	Stream	Sand	20.1	7.8	7.61	77	50	0.02	2.4	930
Oct 19	CB26	-32.3262	19.01914	9016	Pool	Silt	19.8	7.26	8	73	47	0.02	10.1	1500
Oct 19	CB27	-32.3268	19.01927	8955	Stream	Sand	19.3	7.15	7.13	75	48	0.02	3.1	470
Oct 19	CB28	-32.3273	19.01937	8889	Pool	Silt	19	7.21	6.98	72	46	0.02	9.8	1300
Oct 19	CB29	-32.3296	19.01943	8714	Stream	Cobble	18.3	7.06	5.34	74	46	0.02	8.5	600
Oct 19	CB30	-32.3289	19.01944	8634	Stream	Cobble	18.6	7.09	6.71	71	46	0.02	8.7	900
Oct 19	CB31	-32.3407	19.02096	7374	Pool	Boulders	14.9	6.86	3.81	68	44	0.02	0.8	500
Oct 19	CB32	-32.3412	19.02106	7322	Pool	Boulders	14.83	7.01	3.8	68	44	0.02	1.6	1300
Oct 19	CB33	-32.3465	19.0269	6465	Stream	Sand	14.9	7.1	3.74	61	39	0.02	0	420
Oct 19	CB34	-32.3471	19.02719	6390	Stream	Cobble	15.3	6.98	3.72	55	39	0.02	0.9	800
Oct 19	CB35	-32.3481	19.02856	6219	Stream	Sand	15.3	7.23	3.71	58	36	0.02	0	750

Month	Site	Latitude	Longitude	Distance (m)	Habitat	Substrate	Temp (°C)	pH	DO (mg/l)	EC (µS)	TDS (ppm)	Salinity (ppm)	Turbidity (ntu)	Site length (cm)
Oct 19	CB36	-32.3483	19.02899	6176	Stream	Cobble	15.2	6.98	3.71	61	36	0.02	0	300
Oct 19	CB37	-32.37	19.05279	2740	Pool	Boulders	15.1	7.41	3.83	46	29	0.02	0.1	1000
Oct 19	CB38	-32.3697	19.05347	2710	Pool	Boulders	15.6	7.14	3.56	42	27	0.02	0	6000
Oct 19	CB39	-32.372	19.05729	2250	Pool	Boulders	16.8	7.19	3.84	48	31	0.01	0	2600
Oct 19	CB40	-32.3722	19.05781	2197	Stream	Boulders	17.1	7.11	3.77	50	31	0.02	0	200
Oct 19	CB41	-32.3755	19.06319	1572	Stream	Boulders	17.6	7.09	3.38	45	28	0.01	0	590
Oct 19	CB42	-32.3756	19.06341	1549	Pool	Boulders	17.65	7.34	3.24	45	29	0.01	0	350
Oct 19	CB43	-32.3756	19.06565	1331	Pool	Boulders	16.9	7.24	3.23	46	28	0.01	0	1600
Oct 19	CB44	-32.3759	19.06637	1250	Pool	Vegetation	16.5	7.23	3.23	39	25	0.01	0	1000
Oct 19	CB45	-32.3762	19.0667	1204	Pool	Boulders	16.2	7.03	3.08	46	30	0.01	1.2	1800
Oct 19	CB46	-32.3764	19.06683	1174	Stream	Cobble	15.5	7.08	3.2	42	41	0.01	0	920
Oct 19	CB47	-32.3775	19.06748	1045	Stream	Boulders	14.9	7.04	3.21	43	30	0.01	0	600
Oct 19	CB48	-32.378	19.06762	989	Pool	Bedrock	15.1	6.99	2.73	59	41	0.01	1.9	550
Oct 19	CB49	-32.384	19.07254	155	Stream	Vegetation	13.4	6.79	3.35	43	24	0.01	0.3	300
Oct 19	CB50	-32.3841	19.07268	139	Stream	Vegetation	13.4	6.79	3.35	43	24	0.01	0.3	350
Oct 19	CB51	-32.3844	19.07337	64.5	Stream	Boulders	13.1	6.89	3.38	44	28	0.01	0	400
Oct 19	CB52	-32.3847	19.07387	6.43	Pool	Boulders	13.05	6.98	4.5	45	27	0.01	0	800

Table S2a: A set of ten candidate models for *S. calidus* derived from the model selection process, showing the top model and nine other candidates ranked by AICc (from best-fit model to least fit model in the subset). Values in the model term columns indicate the averaged value at which that continuous term was held during the model selection process; + or – symbols indicate the inclusion or exclusion of a categorical variable, respectively.

Intercept	**Distance**	**EC**	**Habitat**	**Reach**	**Site length**	**Turbidity**	**Habitat x Reach**	**AICc**	**weight**
1.98	-0.78	-	-	+	0.31	0.45	-	746.14	0.37
2.01	-0.82	0.11	-	+	0.31	0.41	-	748.22	0.13
2.24	-0.99	-	+	+	0.26	0.30	-	748.65	0.11
1.88	-0.93	-	-	+	-	0.45	-	748.67	0.10
2.12	-1.06	-	+	+	-	0.28	-	749.31	0.08
2.23	-0.98	-	+	+	0.25	-	-	749.61	0.07
2.14	-1.02	-	+	+	-	-	-	749.92	0.06
1.88	-0.94	0.02	-	+	-	0.44	-	750.89	0.03
2.24	-0.99	0.02	+	+	0.26	0.29	-	751.07	0.03
2.24	-1.06	0.16	+	+	0.26	-	-	751.36	0.03

Table S2b: A set of ten candidate models for *P. phlegethon* derived from the model selection process, showing the top model and nine other candidates ranked by AICc (from best-fit model to least fit model in the subset). Values in the model term columns indicate the averaged value at which that continuous term was held during the model selection process; + or – symbols indicate the inclusion or exclusion of a categorical variable, respectively.

Int	**Dist**	**EC**	**Hab**	**Reach**	**Site length**	**Turb**	**Hab x Reach**	**Hab x Site**	**Hab x Turb**	**Reach x Site**	**Reach x Turb**	**AICc**	**weight**
-3.51	-1.76	-	+	+	-3.70	-	-	+	-	+	-	344.48	0.21
-1.52	-2.31	0.51	+	+	-	-	+	-	-	-	-	345.32	0.14
-1.64	-1.86	-	+	+	-	-	-	-	-	-	-	345.46	0.13
-1.49	-2.40	0.58	+	+	0.17	-	+	-	-	-	-	345.49	0.13
-4.34	-1.86	-	+	+	-5.09	0.06	-	+	-	-	-	346.07	0.09
-1.59	-1.88	-	+	+	0.14	-	-	-	-	-	-	346.49	0.08
-4.02	-2.29	0.63	+	+	-4.28	-0.04	-	+	-	+	-	347.03	0.06
-1.65	-1.88	-	+	+	-0.04	-	-	-	-	+	-	347.08	0.06
-1.53	-2.37	0.54	+	+	0.06	-	+	-	-	+	-	347.10	0.06
-1.02	-1.81	-	-	+	-	-0.19	-	-	-	-	-	347.30	0.05

Table S2c: A set of ten candidate models for *L. seeberi* derived from the model selection process, showing the top model and nine other candidates ranked by AICc (from best-fit model to least fit model in the subset). Values in the model term columns indicate the averaged value at which that continuous term was held during the model selection process; + or – symbols indicate the inclusion or exclusion of a categorical variable, respectively.

Int	**Dist**	**Hab**	**Reach**	**Site**	**Turb**	**Dist x Hab**	**Dist x Rch**	**Dist x Site**	**Hab x Rch**	**Hab x Site**	**Reach x Site**	**Dist x Hab x Reach**	**Dist x Hab x Site**	**Dist x Reach x Site**	**Hab x Reach x Site**	**AICc**	**weight**
0.95	0.83	-	+	-0.02	-	-	-	-0.67	-	-	-	-	-	-	-	494.64	0.24
1.17	0.86	-	+	0.45	-	-	-	-	-	-	-	-	-	-	-	495.74	0.14
0.98	1.04	-	+	-0.06	-	-	+	-0.74	-	-	-	-	-	-	-	495.99	0.12
0.87	0.83	-	+	0.16	-	-	-	-0.78	-	-	+	-	-	-	-	496.03	0.12
0.94	1.12	-	+	0.16	-	-	+	-0.89	-	-	+	-	-	-	-	496.61	0.09
1.00	0.83	-	+	-0.04	0.14	-	-	-0.66	-	-	-	-	-	-	-	496.62	0.09
1.14	0.87	-	+	0.56	-	-	-	-	-	-	+	-	-	-	-	497.55	0.06
1.18	0.85	-	+	0.45	0.14	-	-	-	-	-	-	-	-	-	-	497.58	0.06
1.17	0.92	-	+	0.45	-	-	+	-	-	-	-	-	-	-	-	497.91	0.05
0.95	0.83	-	+	0.12	0.17	-	-	-0.77	-	-	+	-	-	-	-	497.92	0.05

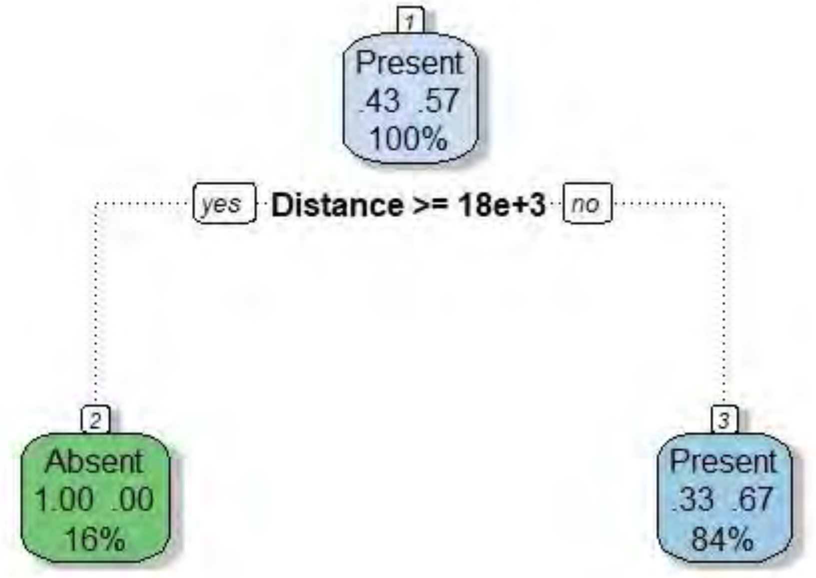

Figure S2a: Classification tree of *Sedercypris calidus* probability of presence / absence. Node 1 shows that this species had a .57 likelihood of presence across 100% of the samples. The first rule of the tree (distance greater than or equal to 18 km) then branches into Node 2, where there was a 1.0 likelihood of absence (i.e 100%), making up 16% of the sites in the lowest reaches of the river. If distance from uppermost site was less than 18 km (Node 3), making up 84% of cases, this species is likely to be present (0.67 likelihood).

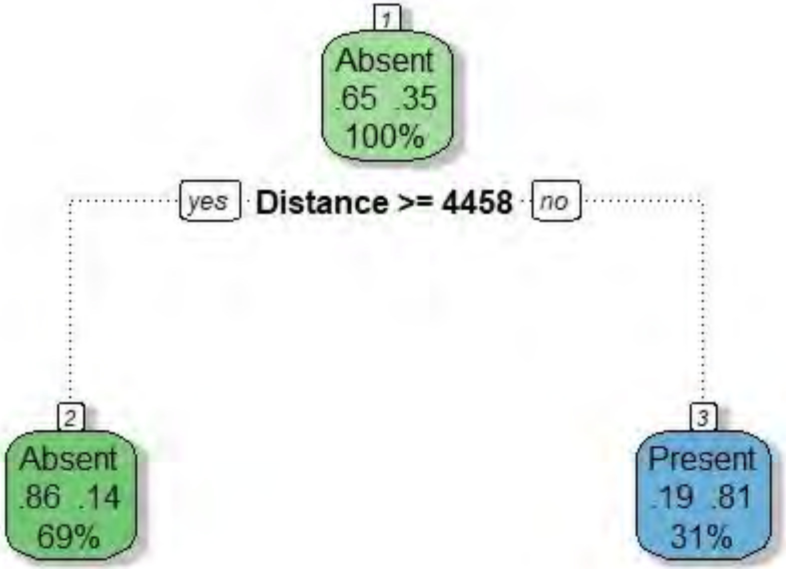

Figure S2b: Classification tree of *Pseudobarbus phlegethon* probability of presence / absence. Node 1 shows that this species had a .65 likelihood of presence across 100% of the samples. The first rule of the tree (distance greater than or equal to 4.5 km) then branches into Node 2, where there was a 0.86 likelihood of absence, making up 69% of the sampled sites in the river. If distance from uppermost site was less than 4.5 km (Node 3), making up 31% of cases, this species is likely to be present (0.81 likelihood).

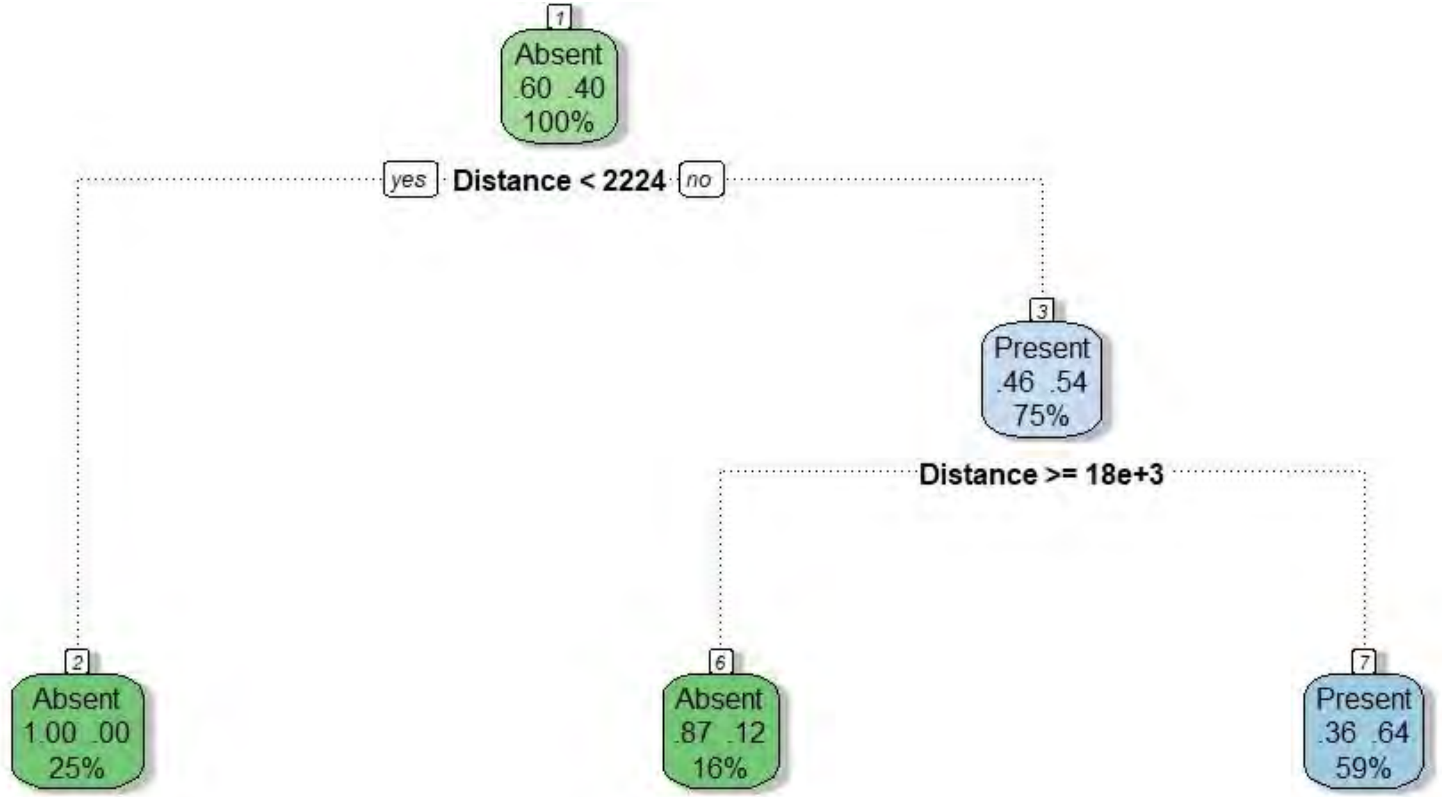

Figure S2c: Classification tree of *Labeobarbus seeberi* probability of presence / absence. Node 1 shows that this species had a .60 likelihood of absence across 100% of the samples. The first rule of the tree (distance less than 2.2 km) then branches into Node 2, where there was a 1.00 likelihood of absence, making up 25% of the sampled sites in the river. If distance from uppermost site was greater than 2.2 km (Node 3), making up 75% of cases, this species is likely to be present (0.54 likelihood). A second rule after Node 3 determines presence / absence likelihoods using 18 km from the uppermost site as the break point in classification of presence/ absence likelihoods.

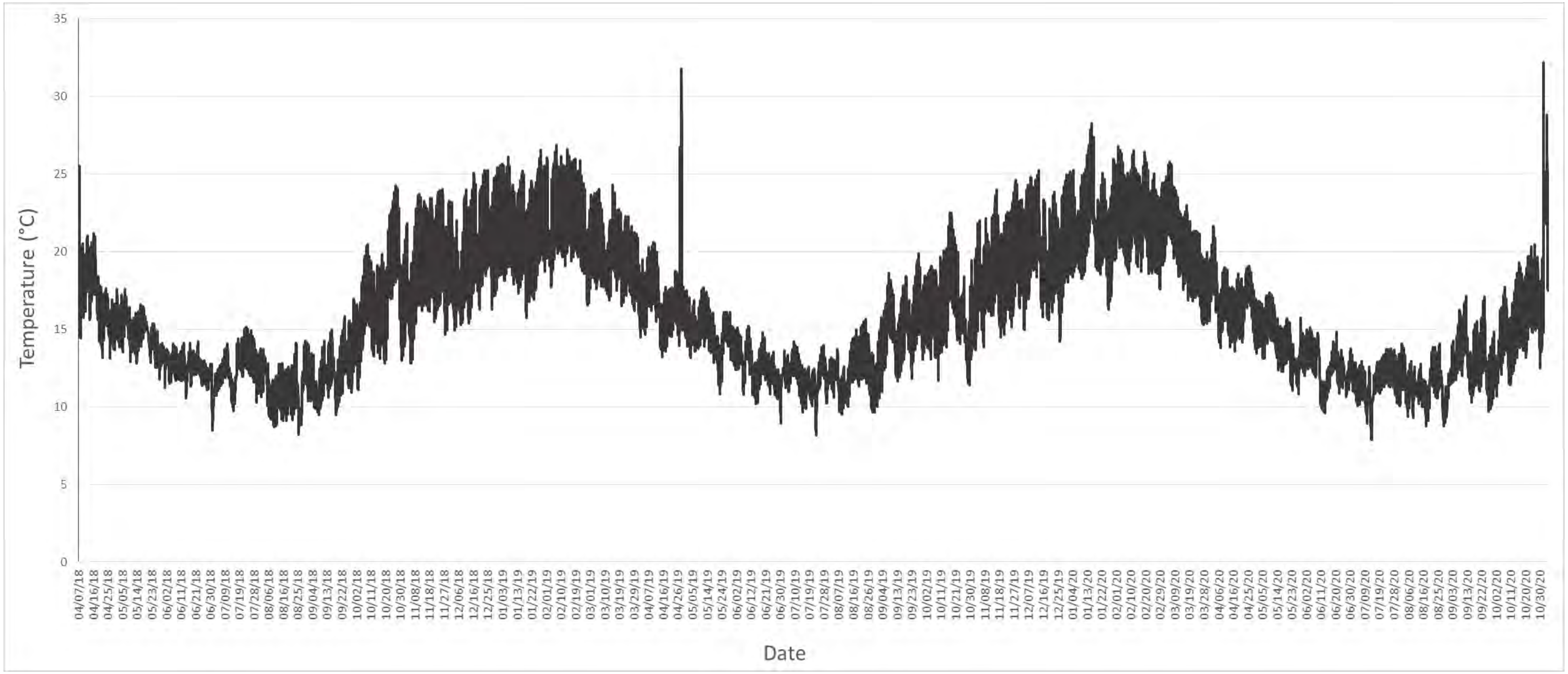

Figure S3: An example of the collected temperature data spanning April 2018 to October 2020. This particular readout was taken from a site in the middle reaches, downstream of the boundary of the CapeNature protected area (logger label CBTEMP_5, latitude -32.36999994, longitude 19.05279053, deployed on 7 April 2018).

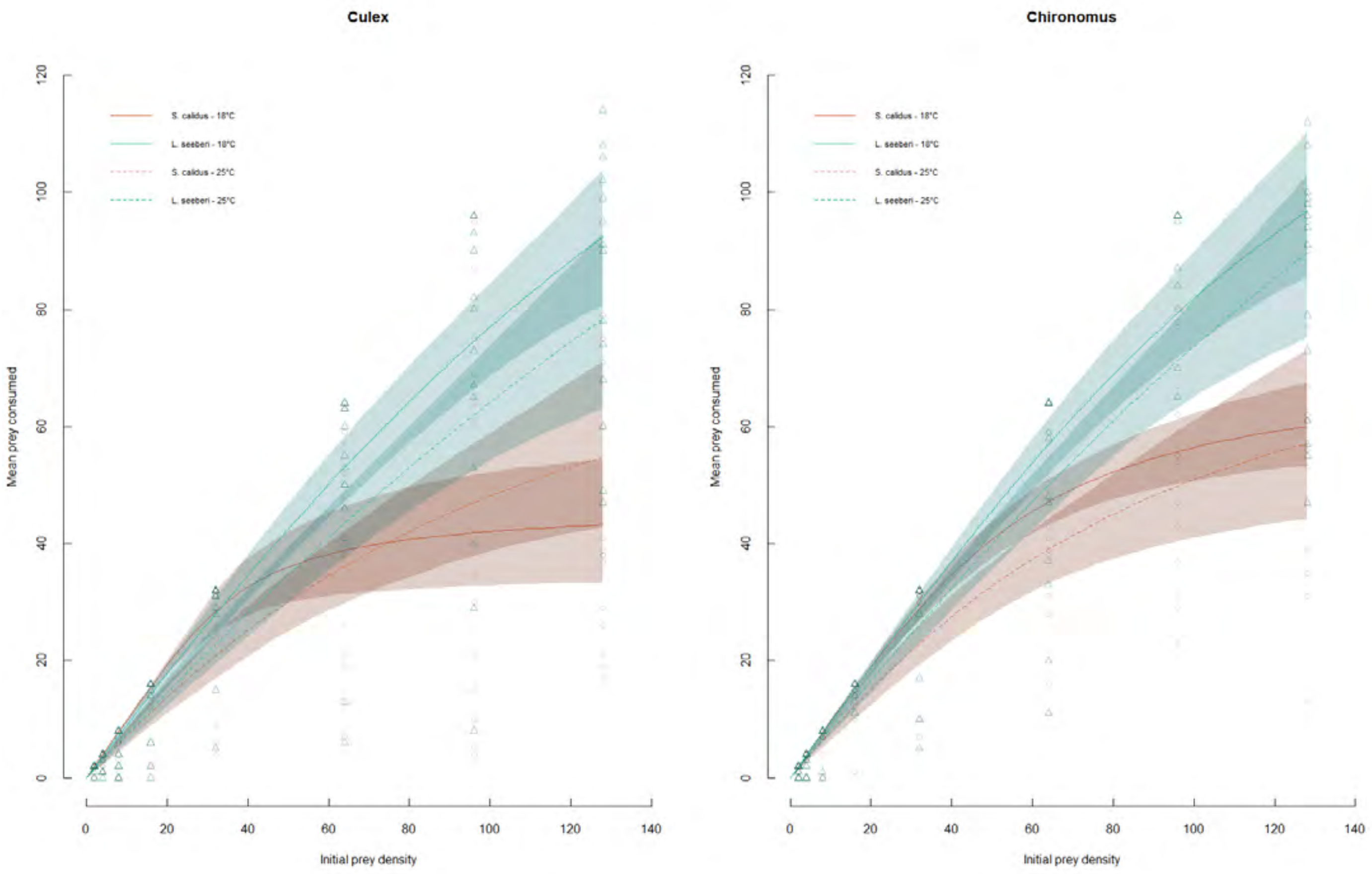

Figure S4: Functional response curves overlaid with individual prey consumption (red circles = *S. calidus*, cyan triangles = *L. seeberi*). Solid lines indicate 18°C temperature treatments, dashed lines indicate 25°C temperatures.

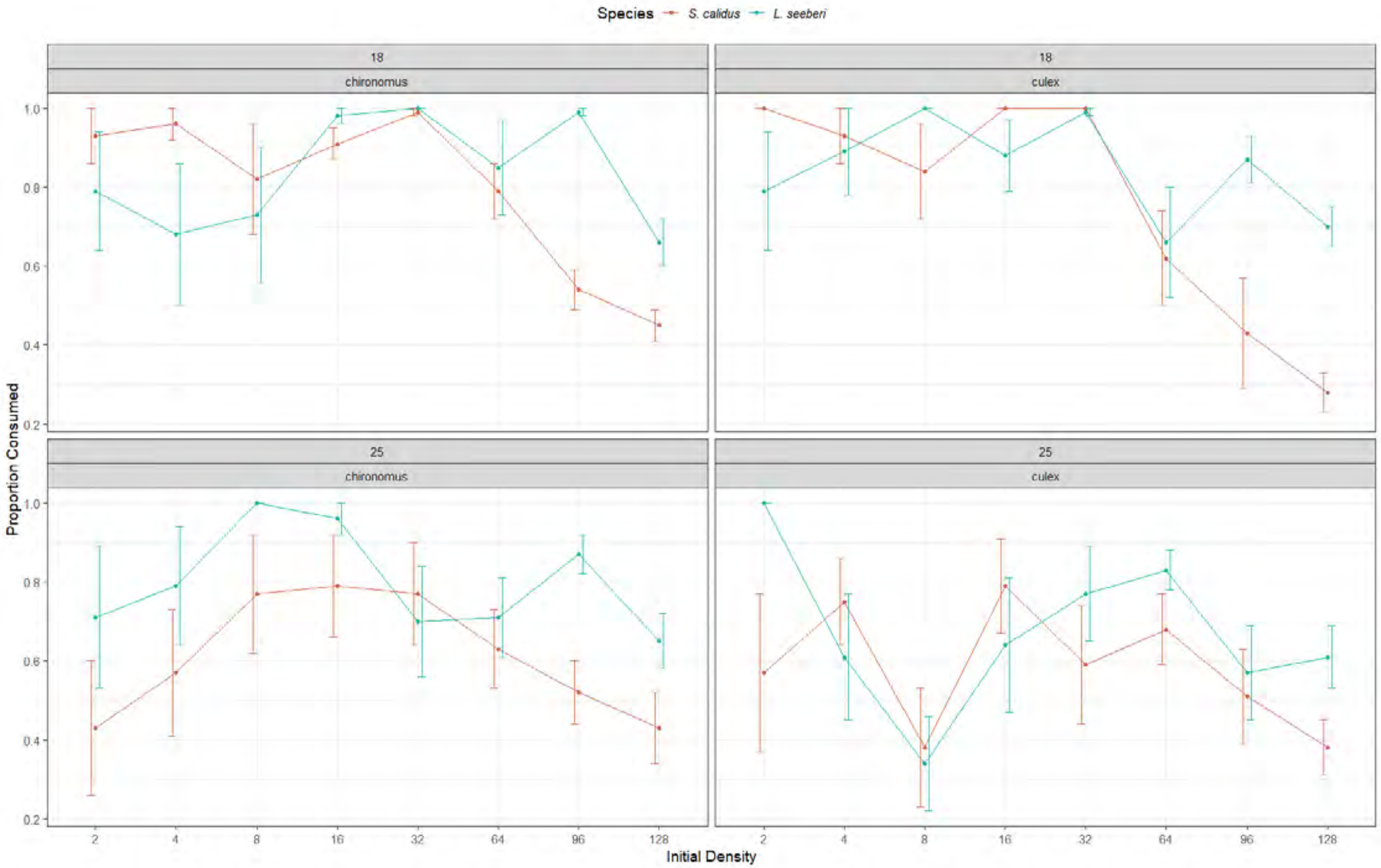

Figure S5: Proportion of prey consumed per prey density by both fish species (red lines = *S. calidus*; blue lines = *L. seeberi*), at each temperature treatment and for both prey types.

Table S3: Prey IDs, the assigned functional group for inclusion in proportional diet calculations and the assigned food groups to translate these diet groups into the closest match I the Food-Fish Model for comparison of observed diet with feeding capacities. Diet data was extracted from Woodford (2005) with permission.

Prey ID	Functional group	Food group: food-fish model
Corixidae	Aquatic insects	Insects
Elmidae	Aquatic insects	Insects
Baetidae	Aquatic nymphs	Insects
Ephemeroptera	Aquatic nymphs	Insects
Heptageniidae	Aquatic nymphs	Insects
Simulidae	Dipteran larvae	Larvae
Chironomidae	Dipteran larvae	Larvae
Diptera larvae	Dipteran larvae	Larvae
Chironomid pupa	Dipteran larvae	Larvae
Odonata	Large aquatic nymphs	Insects
Corydalidae	Large aquatic nymphs	Insects
Libellulidae	Large aquatic nymphs	Insects
Terrestrial Coleoptera	Surface insects	Insects
Gyrinidae	Surface insects	Insects
Coleoptera	Surface insects	Insects
Terr. Inverts	Surface insects	Insects
Terr. Diptera	Surface insects	Insects
Hymenoptera	Surface insects	Insects
Trichoptera	Trichopteran larvae	Larvae
Ecnomidae	Trichopteran larvae	Larvae
Leptoceridae	Trichopteran larvae	Larvae
Cladocera	Zooplankton	Zooplankton