TAXONOMIC, ECOLOGICAL AND BIOGEOGRAPHIC RE-EVALUATION OF TEMPERATE STREAM FISHES IN SOUTHERN AFRICA: A CASE STUDY OF THE *ENTEROMIUS ANOPLUS* COMPLEX

A thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

By

MANDA JULIET KAMBIKAMBI

ORCID ID

https://orcid.org/0000-0003-3735-3208

December 2020

ABSTRACT

Worldwide, cryptic species, which comprise two or more distinct species that are classified as one due to their morphological similarity, are more common than previously thought. In South Africa, the chubbyhead barb, *Enteromius anoplus* (Weber, 1897), is the most widespread freshwater fish species. The widespread occurrence of this species across different river basins raised questions on its taxonomic status due to the likely occurrence of hidden diversity. This further raised the need to evaluate the ecological patterns associated with likely occurrence of such hidden diversity. Phylogenetic analyses of the cytochrome *b* gene of *E. anoplus* populations across South Africa revealed the occurrence of four genetically distinct species. These findings supported the restoration *Enteromius cernuus* (Barnard 1943) **stat. rev.** from the Olifants River system; the restoration of *Enteromius anoplus sensu stricto* (Weber 1897) **stat. rev.** from the Gouritz River system; the redescription of *Enteromius oraniensis* (Barnard 1943) **stat. rev.** from the Orange River system; and the description of a new species, *Enteromius mandelai* **sp. nov.** from the Eastern Cape rivers. Analyses of morphometric and meristic data revealed that these species were differentiated based on barbel length and number of lateral line scales.

Ecological studies on *Enteromius mandelai* were conducted within headwater streams of major river systems in the Eastern Cape region. These headwater streams had different catchment characteristics ranging from near-natural to anthropogenically-perturbed mainly due to agriculture-related disturbances and invasion of riparian zones by non-native plants. Stable isotope analysis provided evidence of trophic plasticity for *E. mandelai* across the different headwater streams. Specifically, *E. mandelai* exhibited large isotopic niches in agriculture-disturbed headwater streams, whereas small isotopic niches were observed in headwater streams invaded by non-native plants. Because of the evidence of trophic plasticity for *E. mandelai*, this study further hypothesized that this species would exhibit variable environmental niche patterns related to its distribution, abundance and the presence of contra-specifics due to different abiotic factors across its range. *Enteromius mandelai* was generally most abundant at sites with high total dissolved solids and sand substratum, which was characteristic of large pool habitats, and least abundant at sites with high turbidity. In the Great Fish River, *E. mandelai* occurred as the only species in most headwater sites, whereas in the Keiskamma and Buffalo River systems, it co-occurred with *Amatolacypris trevelyani* and *Sandelia bainsii*. *Enteromius mandelai* was, nevertheless, found to be ubiquitous together with

having wide environmental niches, and exhibited no discernible patterns in its habitat associations.

The taxonomic re-evaluation of the *E. anoplus* complex, which provided evidence of the existence of new species, has implications for the biogeographic zonation of the ichthyofauna of the Cape Fold Ecoregion (CFE), Amathole-Winterberg Highlands Ecoregion (AWHE) and the Southern Temperate Highveld Ecoregion (STHE). For example, the currently recognised southern limit of the STHE, which encompasses the AWHE, is based on the occurrence of *E. anoplus*, *Enteromius pallidus* and *Labeo umbratus*. However, evidence from this and other recent studies indicate the occurrence of either new species or distinct lineages in the southern-most section of the STHE, which is consistent with the patterns observed in the CFE. Comparison of taxonomic composition showed that species in the eastern part of the CFE were similar to species in the AWHE and the southern-most section of the STHE. Because of the close affinities among most of the new taxa in these regions, this study proposes that the current delimitation of the CFE should be reviewed by extending it to include the AWHE and sections of the STHE.

TABLE OF CONTENTS

ABSTRACT ..... **i**

ACKNOWLEDGEMENTS ..... **xiii**

CHAPTER 1: **General introduction** **1**

Unique fish communities of headwater streams 1

Distribution and threats to the freshwater fish fauna in southern Africa 2

Ecological attributes of headwater streams 7

Historical biogeography of Cape fauna 8

Taxonomic history of the Cape fauna 12

Thesis outline 15

CHAPTER 2: Allopatric differentiation in the *Enteromius anoplus* complex in South Africa, with the revalidation of *E. cernuus* and *E. oraniensis*, and description of a new species, *E. mandelai* (Teleostei: Cyprinidae) **18**

Abstract 18

Materials and methods 22

Results 31

Taxonomic accounts 42

Enteromius anoplus (Weber, 1897) 42

Enteromius cernuus (Barnard, 1938) 46

Enteromius oraniensis (Barnard, 1943) 52

Enteromius mandelai sp. n. 58

Discussion 66

CHAPTER 3: Trophic relationships and food web dynamics of *Enteromius mandelai* sp. nov in the Great Fish, Keiskamma and Buffalo river systems **70**

Introduction 70

Materials and Methods 75

Results 87

Discussion 106

CHAPTER 4: Environmental factors and fish species associations influencing the distribution patterns and habitat associations of *Enteromius mandelai* sp. nov. (Cyprinidae) **111**

Introduction 111

Materials and methods 113

Results 121

Discussion 136

CHAPTER 5140Southern temperate freshwater ecoregions revisited: a proposal to extend the Cape Fold Ecoregion to encompass the Amathole-Winterberg Highland Ecoregion 140

Introduction 140

Materials and Methods 145

Results 153

Discussion 157

CHAPTER 6: General discussion 160

REFERENCES 165

APPENDICES 195

Appendix A: Physical-chemical, substrate types and land-use patterns of all sampled sites where fishes were captured in the Great Fish, Keiskamma and Buffalo River systems. 195

Appendix B: Fish species collected during the current survey at sites in the Great Fish, Keiskamma and Buffalo River systems. The ✓ symbol represents present and × symbol represents absent. * represents non-native fish species. 199

Appendix C: Scientific and common names of fish species mentioned in the thesis. 203

LIST OF FIGURES

Figure 1.1: Biogeographical zones of southern African freshwater fish fauna recognised by Skelton (2001). Red and pink denote Tropical fish fauna. Red denotes a higher diversity of tropical fish fauna while the pink colour denotes a lower diversity of tropical fish fauna. Light blue denotes Karroid fauna whereas dark blue denotes Cape fauna. Cross-banded are denote overlap between Tropical and Temperate fish fauna. 3
Figure 1.2: The Cape and Karroid group of the Temperate Biogeographical zone in South Africa. The Cape group includes the Cape Fold Ecoregion, Amathole-Winterberg Highlands Ecoregion and the Drakensberg-Maloti Highlands Ecoregion. Quaternary catchments present in the Temperate Biogeographical zone: 1 = Olifants (Clanwilliam), 2 = Berg, 3 = Breede, 4 = Gouritz, 5 = Coastal rivers, 6 = Gamtoos, 7 = Swartkops, 8 = Sundays, 9 = Bushmans, 10 = Great Fish, 11 = Keiskamma, 12 = Kei, 13 = Mzimvubu, 14 = Mkomazi, 15 = Tugela, 16 = Mfolozi, 17 = Komati, 18 = Olifants, 19 = Limpopo, 20 = Vaal, 21 = Orange and 22 = Buffels. 4
Figure 1.3: Palaeogeographies of the Orange River drainage systems from Dingle and Hendry, 1984. (a) late Cretaceous, (b) Palaeogene, (c) Neogene. 1, 2 and 3 are successive positions of the Middle Orange channel in Neogene reconstructions. Lower, Middle and Upper refer to courses of the Orange River. Abbreviations: BP = Bosluispan; BV = Brandvlei; VV = Van Wyks Vlei; PK = Preiska; AM = Asbestos Mountains; AD = Arris Drift; VD = Vanrhynsdorp; K = Kangnas; G = Gamoep; P = Pofadder; B = Britstown; H = Hopetown; HK = Henkries. 10
Figure 1.4: Enteromius anoplus s.l. from the (a) Olifants River (Clanwilliam division) (b) Gouritz River (c) Great Fish River and (d) Orange River. 15
Figure 2.1: Map showing distribution of the chubbyhead barb, *Enteromius anoplus* sensu lato in South Africa, based on data from the NRF-South African Institute for Aquatic Biodiversity database (NRF-SAIAB). The 21 hydrobasins of South Africa are shown: 1 = Buffels, 2 = Olifants (drains to South Atlantic Ocean), 3 = Berg, 4 = Breede, 5 = Gouritz, 6 = Coastal Drainages, 7 = Gamtoos, 8 = Swartkops, 9 = Sundays, 10 = Bushmans, 11 = Great Fish River, 12 = Keiskamma, 13 = Swart-Kei, 14 = Mzimvubu, 15 = Umgeni, 16 = Tukela, 17 = Mfolozi, 18 = Komati, 19 = Olifants (drains to Limpopo River), 20 = Limpopo, 21 = Vaal, 22 = Orange. The Olifants, Buffels and Karkloof River systems are shown in dark blue. 21
Figure 2.2: Map showing sampling localities for the present study. South African river basins: 1 = Buffels, 2 = Olifants (drains to South Atlantic Ocean), 3 = Berg, 4 = Breede, 5 = Gouritz, 6 = Coastal Drainages, 7 = Gamtoos, 8 = Swartkops, 9 = Sundays, 10 = Bushmans, 11 = Great

Fish River, 12 = Keiskamma, 13 = Swart-Kei, 14 = Mzimvubu, 15 = Umgeni, 16 = Tukela, 17 = Mfolozi, 18 = Komati, 19 = Olifants (drains to Limpopo River), 20 = Limpopo, 21 = Vaal, 22 = Orange. 26
Figure 2.3: Map showing sampling localities for the present study. South African river basins: 1 = Buffels, 2 = Olifants (drains to South Atlantic Ocean), 3 = Berg, 4 = Breede, 5 = Gouritz, 6 = Coastal Drainages, 7 = Gamtoos, 8 = Swartkops, 9 = Sundays, 10 = Bushmans, 11 = Great Fish River, 12 = Keiskamma, 13 = Swart-Kei, 14 = Mzimvubu, 15 = Umgeni, 16 = Tukela, 17 = Mfolozi, 18 = Komati, 19 = Olifants (drains to Limpopo River), 20 = Limpopo, 21 = Vaal, 22 = Orange. 32
Figure 2.4: Scatter plot of PC1 against PC2 for a PCA carried out on 18 morphometric characters for 175 specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* 34
Figure 2.5: The morphometric character (barbel length) identified by the second principal components axis as contributing the most to variation among *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* 36
Figure 2.6: The positive morphometric characters identified by the second and third principal component axis (a) predorsal fin length (b) pectoral to pelvic fin length (c) body depth as contributing the most to variation among *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* 37
Figure 2.7: Scatter plot of PC1 against PC2 for a PCA carried out on nine raw meristic characters for specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* The four species show considerable overlap in meristic characters. 39
Figure 2.8: Scatter plot of number of lateral line scales against standard length for specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* 40
Figure 2.9: Scatter plot of number of circumpendicular scales against standard length for specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* 41
Figure 2.10: General body features and alcohol preserved coloration of *Enteromius anoplus* s.s. (a) lectotype (ZMA 100995), 95 mm standard length, from the Buffels River by Laingsburg, Gouritz River system (b) paralectotype (ZMA 100995a), 75 mm standard length, from the Buffels River by Laingsburg, Gouritz River system (c) unsexed sub-adult topotype (SAIAB 200353), 37.8 mm standard length, from the Buffels River, Gouritz River system..42
Figure 2.11: General body features and alcohol preserved coloration of *Enteromius cernuus* (a) lectotype (SAIAB 209738), 52.6 mm standard length, from the Olifants River, Clanwilliam

Division (b) paralectotype (SAIAB 135553), 54.4 mm standard length, from Troetroe River, Olifants River system (c) unsexed adult topotype (RUSI 65417), 42.5 mm standard length, from the Breekrans River, Olifants River, Olifants River system. 47
Figure 2.12: General body features and alcohol preserved colouration of *Enteromius oraniensis* (a) neotype (SAIAB 209735), 53 mm standard length, Bossieslangspruit River, Orange River system (b) paratype (SAIAB 209736), live coloration of mature breeding male, Bossieslangspruit River, Orange River system (not examined) (c) unsexed adult paratype (SAIAB 209737), 50.1 mm standard length, Stormberg River, Orange River system.....52
Figure 2.13: General body features and alcohol preserved colouration of *Enteromius mandelai* n. sp (a) the holotype (SAIAB 209731) adult female, 63.7 mm standard length, Glen Owen Farm, Great Fish River mainstem (b) Adult specimen (SAIAB 209738) 56.4 mm standard length, from the Keiskamma River (c) Adult specimen (SAIAB 209733), 69.5 mm standard length, from the Zeleni River, Buffalo River system.....58
Figure 2.14: Lateral view of live *Enteromius mandelai* n. sp showing live coloration of mature breeding (a) female paratype (SAIAB 209732), Glen Owen Farm, Great Fish River mainstem (not examined) (b) male specimen (SAIAB 209734), Tarka River, Great Fish River system (not examined) (c) female specimen (SAIAB 209733), Buffalo River (not examined). Scale bar 10mm. 59
Figure 3.1: Map of South Africa (top centre pane) showing the Great Fish, Keiskamma and Buffalo rivers. Right and Left Panes show sampled localities in the (a) Great Fish and (b) Keiskamma and Buffalo river systems. 77
Figure 3.2: Streams characterised by native vegetation in the (a) Great Fish (b) Keiskamma and (c) Buffalo River systems.....80
Figure 3.3: Streams impacted by agricultural activities (a) Great Fish (b) Keiskamma and (c) Buffalo River systems.....81
Figure 3.4: Streams with riparian zone invaded by non-native plant species in the (a) Great Fish and (b) Keiskamma River systems. 82
Figure 3.5: Stable isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ for *Enteromius mandelai* using muscle tissue (represented by sample-size corrected standard ellipse area (SEAc)), macroinvertebrates (red diamonds) and basal sources (green triangles) for all sampled sites in the Great Fish Rivers. The abbreviations are as follows: ALG = Algae; C_3 = C_3 plants; C_4 = C_4 plants; CPOM = Course particulate organic matter; FPOM = Fine particulate organic matter; Che = *Chematopsyche*; TRI = *Tricorythus*; SIM = *Simulium*; AES = *Aeshna*; LYC = Lycosidae; TET = *Tetragnatha*; LIB = Libellulidae; LES = *Lestes*; GER = Gerridae; BAE = Baetidae; APP = *Appasus*; POT =

Potamonautes; AFR = *Afronurus*; CAS = *Castanophlebia*; CAE = *Caenis*; TAB = Tabanidae; SYN = Synlestidae; GOM =Gomphidae; SER = *Seratogomphus*; ZYG =Zygoptera; COE = Coenagroinidae; ORE = *Orectogyrus*; DYT = *Dytiscidae*; CHI = *Chimarra*; LEP = *Leptosalis*; COR = Corixidae; NOT = Notonectidae; BUR = *Burnupia*; APH = Aphanicella. 99
Figure 3.6: Stable isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ for *Enteromius mandelai* (black ellipses), *Amatoloacypris trevelyani* (red ellipses) using muscle tissue, macroinvertebrates (red diamonds) and basal sources (green triangles) for all sampled sites in the Keiskamma and Buffalo Rivers. The abbreviations are as follows; ALG = Algae; C_3 = C_3 plants; C_4 = C_4 plants; CPOM = Course particulate organic matter; FPOM = Fine particulate organic matter; TAD = Anura(tad-pole); TRI = *Tricorythus*; SUG = *Sugarina*; SIM = *Simulium*; HYD = *Hydropsyche*; AES = *Aeshna*; LES = *Lestes*; PLE = *Plea*; GER = Gerridae; BAE = Baetidae; APP = *Appasus*; RHA = *Rhagovelia*; POT = *Potamonautes*; AFR = *Afronurus*; CAS = *Castanophlebia*; CAE = *Caenis*; TAB = Tabanidae; GOM =Gomphidae; LEP = *Leptosalis*; NOT = Notonectidae; BUR = *Burnupia*; APH = *Aphanicella*; OLI = Oligochaeta. 103
Figure 3.7: Isotopic niche size for *Enteromius mandelai* using sample size corrected standard ellipse areas (SEAc) based on muscle tissue for the Great Fish River system. 104
Figure 3.8: Isotopic niche size for *Enteromius mandelai* and *Amatolacypris trevelyani* using sample size corrected standard ellipse areas (SEAc) based on muscle tissue for the Keiskamma River system.....105
Figure 3.9: Isotopic niche size for *Enteromius mandelai* and *Amatolacypris trevelyani* using sample size corrected standard ellipse areas (SEAc) based on muscle tissue for the Buffalo River system.....105
Figure 4.1: Map of all the sampled sites where *Enteromius mandelai* was present (green circles) and absent (white circles) in the Great Fish River and the two major tributaries Koonap and Kat River systems. 117
Figure 4.2: Non-metric multidimensional scaling (NMDS) ordination showing the spatial patterns of distribution of *Enteromius mandelai* together with both native (green circles) and non-native (red circles) fishes in the (a) Great Fish River, (b) Keiskamma River and (c) Buffalo River.....124
Figure 4.3: Outlying mean index analysis indicating the species and environmental variable vectors for the Great Fish River. (a) Projection of the sampling sites and the mean of the distribution of the utilization weights of fishes; (b) projection of the environmental variable scores; (c) Eigenvalues showing how much variation can be explained by the different axes (d)

projection of the individual sampling sites from Appendix B; (e) the individual niches for the different species. 128
Figure 4.4: Outlying mean index analysis indicating the species and environmental variable vectors for the Keiskamma River. (a) Projection of the sampling sites and the mean of the distribution of the utilization weights of fishes; (b) projection of the environmental variable scores; (c) Eigenvalues showing how much variation can be explained by the different axes (d) projection of the individual sampling sites from Appendix B; (e) the individual niches for the different species. 132
Figure 4.5: Outlying mean index analysis indicating the species and environmental variable vectors for the Buffalo River. (a) Projection of the sampling sites and the mean of the distribution of the utilization weights of fishes; (b) projection of the environmental variable scores; (c) Eigenvalues showing how much variation can be explained by the different axes (d) projection of the individual sampling sites; (e) the individual niches for the different species. 135
Figure 5.1: Freshwater bioregions of Africa and Madagascar (Thieme et al., 2005). 141
Figure 5.2: Freshwater ecoregions of Africa and Madagascar (Thieme et al., 2005).....142
Figure 5.3: Map of freshwater ecoregions of South Africa. Adapted from Abell et al. (2008). 143
Figure 5.4: the Cape Fold Ecoregion represented by dots and the Amathole-Winterberg Highlands Ecoregion represented by slashed lines. River drainages: 1 = Olifants, 2 = Berg, 3 = Breede, 4 = Gouritz, 5 = Gamtoos, 6 = Sundays, 7 = Coastal drainages 8 = Swartkops, 9 = Bushmans, 10 = Great Fish, 11 = Keiskamma. 144
Figure 5.5: Main River systems in the Cape Fold Ecoregion 147
Figure 5.6: Results of cluster analysis showing grouping of river systems based on the distribution of freshwater fishes. The current ecoregion designations are shown in red (Cape Fold Ecoregion), green (Amatolo-Winterberg Highlands Ecoregion) and black (Southern Temperate Highveld Ecoregion).....154

LIST OF TABLES

Table 2.1: River systems and GPS coordinates of localities where the samples used in the present study were collected. The numbers of individuals (n) that were used for molecular and morphological analyses per locality are indicated. 24
Table 2.2: Morphometric and meristic characters of *Enteromius* used in the current study (reproduced from Chakona et al., 2014). 30
Table 2.3: Ranges of model-corrected genetic divergences (%) between species of *Enteromius anoplus* s.s., *Enteromius cernuus*, *Enteromius oraniensis*, and *E. mandelai*. 33
Table 2.4: Summary of Principal Components and their percentage variance and factor loadings for the first two principal component (PC) axes carried out on morphometric characters from specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *Enteromius mandelai*. The most important factor loadings are in bold. 35
Table 2.5: Factor loadings for the first two principal component axes of a PCA carried out on nine meristic characters from 71 specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* The most important factor loadings are in bold. 39
Table 2.6: Morphometric and meristic data for *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *Enteromius mandelai* sp. nov. 64
Table 3.1: Land-use patterns of all sampled sites where *Enteromius mandelai* and other fish species were captured in the Great Fish, Keiskamma and Buffalo River systems. 78
Table 3.2: The ranges of physico-chemical variables measured in 18 localities in headwater tributaries of the Great Fish (n = 12), Keiskamma (n = 3) and Buffalo (n = 2) river systems, Eastern Cape, South Africa. 87
Table 3.3: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for basal sources in the Great Fish, Keiskamma and Buffalo river systems. 89
Table 3.4: Results of permutational analysis of variance (PERMANOVA) for effect of river system and land-use type on basal sources and macroinvertebrate $\delta^{13}C$ and $\delta^{15}N$ values. 91
Table 3.5: Mean values of carbon and nitrogen stable isotopes (± standard deviation) and functional feeding groups (FFG) for aquatic macroinvertebrates that were collected from the headwaters of the Great Fish, Keiskamma and Buffalo river systems. Land-use types were indicated as A for agriculture, I for invaded and N for near natural riparian zones. 92
Table 3.6: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for consumer groups including macroinvertebrate functional feeding groups and fish species that occurred in the Great Fish, Keiskamma and Buffalo River systems. 93

Table 3.7: Results of permutational multivariate analysis of variance (PERMANOVA) for effect of river system (Great Fish, Keiskamma and Buffalo rivers) and land-use pattern (near natural, agriculture and invaded) on *Enteromius mandelai* $\delta^{13}C$ and $\delta^{15}N$. A one-way PERMANOVA was conducted for each independent variable (river system and landscape type) separately. For significant one-way PERMANOVAs, pairwise comparisons with the PERMANOVA t statistic were reported for each independent variable. All tests were significant at $P < 0.05$. Significant *P* values are indicated in bold type. 94
Table 3.8: Stable isotope community metrics comparing the trophic structure of *E. mandelai* communities sampled from the Great Fish, Keiskamma and Buffalo river systems. SEAc, core isotopic niche area of the community ($‰^2$); NR, $\delta^{15}N$ range; CR, $\delta^{13}C$ range; CD, mean distance to centroid; MNND, mean nearest neighbour distance; SDNND, standard deviation of nearest neighbour distance and Land-use type.....97
Table 4.1: Models comparison for *Enteromius mandelai* abundance in the Great Fish, Keiskamma and Buffalo River Systems. The models were Poisson, Quasi-Poisson, negative binomial (NB) and zero-inflated negative binomial (ZINB). These were compared based on the log-likelihood, degrees of freedom (df) and Akaike's information criterion (AIC). 121
Table 4.2: Parameter estimates, standard errors (SE) and the associated *P*-values for zero-inflated negative binomial models (ZINB) for the abundance of *Enteromius mandelai* in the Great Fish, Keiskamma and Buffalo River systems. 122
Table 4.3: Niche parameters for fish species collected in the Great Fish, Keiskamma and Buffalo River system. 129
Table 5.1: Zoogeography of freshwater fishes in the Cape Fold Ecoregion, Amathole-Winterberg Highland Ecoregion and river systems in between.....149
Table 5.2: The depicted percentage contribution, cumulative percentage and mean contribution of fish assemblage structure for the Olifants River system and the other river systems, according to similarity percentages SIMPER analysis. The first column identifies the speices explained by that row, the second column shows the percentage dissimilarity explained by that species, the third column tallies the cumulative Bray Curtis metric for the species thus far represented in the table, and the last two columns show mean abundance in the Olifants River and mean abundance in the rest of the river systems examined. 155
Table 5.3: The depicted percentage contribution, cumulative percentage and mean contribution for the south-western and south-eastern clusters according to SIMPER analysis. 156

Table 5.4: Total number of fish species, number of endemic fish and percentage endemism of fish from the Olifants River system in the west coast to the Buffalo River in the east coast of South Africa. 157

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my supervisors Dr Wilbert Kadye and Dr Albert Chakona for their guidance and support during this study. Thank you for getting me through this.

This study was funded by the British Ecological Society, University of Zambia and the National Research Foundation (NRF) of South Africa under the Foundational Biodiversity Information Programme: Biodiversity surveys in priority inland areas (FBIP) grants (grant reference no. IBIP-BS13100251309), and I am thankful for their support. Permission for this study was granted by the Rhodes University Ethical Standards Committee (approval number 2019-0314-776) and research permits were granted by the Eastern Cape Department of Economic Development and Environmental Affairs (through permit numbers CRO 130/19CR and CRO 44/18CR).

This study involved assistance from many areas and I would like to express my gratitude to the following people in particular: Nonjabulo Matomela, Charles Teta, Edith Phala, and Pule Mpopetsi for their valuable field assistance. Thanks are also due to Tholoana Ntokoane and Taryn Bodill for their laboratory assistance. Thank you to Tadiwa Mutizwa for helping me with the basics of genetic analysis. Special thanks are also due to Nkosinathi Mazungula, Amanda Gura, Nonkoliso Mgibantaka, Vuyani Hanisi and Siphamandla Mceleli from the South African Institute for Aquatic Biodiversity Collections Division Platform for providing me with specimens, specimen pictures, DNA samples, storage and work space whenever needed.

Thanks are also due to my fellow students and postdocs at the Department of Ichthyology and Fisheries Science and the South African Institute for Aquatic Biodiversity with whom I have shared this journey with. Thank you for all the merriment and commiserations.

Last but not least, I would like to thank my family for their love and support. My father Joseph, thanks for the check-ups. My sister Musonda, as far as you were, you still made me believe in myself. My mother Tamala, who has always led by example and has given me far more than life and opportunity. I dedicate this thesis to them.

CHAPTER 1

General introduction

Unique fish communities of headwater streams

Headwater streams constitute unique and dynamic freshwater ecosystems at the origins of river networks (Gomi et al., 2002; Richardson & Danehy, 2007). Because headwater catchments are predominantly small, they are easily influenced by both small- and large- scale differences in local conditions, making them the most variable of all running water habitats. They offer a wide array of habitats for unique fish species, particularly rheophilic taxa, including mountain and suckermouth catfishes, small-bodied cyprinids, kneriids, mormyrids, darters, sculpins and characins (Skelton, 2001; Thieme et al., 2005; Meyer et al., 2007; Richardson, 2019). Growing evidence from disparate regions indicate that, contrary to previous perceptions, headwater streams harbour a higher number of fish species than previously documented (e.g. Stiassny et al., 2007; Súarez et al., 2007; Khaefi et al., 2017; Van Ginneken et al., 2017; Chakona et al., 2018; Schmidt et al., 2019). Thus, these systems represent the next frontiers for biodiversity exploration and discovery of new species.

Genetic structure refers to DNA sequences as well as the various ways that combinations of alleles become packaged within individuals (Herbers, 2010). In a population, the genetic structure is characterised by the number of subpopulations within it, the frequencies of different genetic variants (alleles) in each subpopulation, and the degree of genetic isolation of the subpopulations (Chakraborty, 1993). Traditionally, population genetic structure assessments provide information on the delimitation of species and population boundaries, geographic patterns of genetic variation and divergence into major evolutionary lineages. Fishes that inhabit headwater streams often show high levels of genetic structuring due to limited gene flow (Finn & Poff, 2011; Sterling et al., 2012; Schanke et al., 2017). This is because, in addition to the dendritic nature of river networks and the presence of natural physical barriers such as waterfalls and rapids, many of the mountain ranges drained by these headwater streams are separated by large geographic distances which limit dispersal and range expansion of freshwater restricted taxa (Fagan, 2002; Stegmann et al., 2019).

In many regions, headwater systems have been identified and demarcated as important fish sanctuaries or key biodiversity areas due to the occurrence of narrow range endemic

species and unique genetic lineages (Skelton et al., 1996; Abell et al., 2007; Nel et al., 2007). These fragile habitats and the unique biodiversity they contain are however facing increasing threats from human activities. Among other impacts, human activities have caused rapid land transformations through clearing of indigenous vegetation for agricultural purposes and growing of monoculture plantations; hydrological modifications due to excessive water abstraction and construction of impoundments; and introduction of non-native piscivores, such as rainbow trout *Onchorhynchus mykiss,* brown trout *Salmo trutta,* largemouth bass *Micropterus salmoides,* smallmouth bass *Micropterus dolomieu* and sharptooth catfish *Clarias gariepinus* (Allan, 2004; Dudgeon et al., 2006; Wang et al., 2006; Vitule et al., 2009; Cucherousset & Olden, 2011; Weyl et al., 2014; 2020). In southern Africa, the vulnerability of headwater streams and their unique faunas to human induced threats have been well documented, particularly for the Cape Fold Ecoregion, where studies have provided evidence of several localised extinctions of populations of species belonging to the genera *Pseudobarbus* (Clark et al., 2009; Ellender et al., 2011; Chakona et al., 2020a), *Galaxias* (Clark et al., 2009; Chakona et al., 2020a) and *Sandelia* (Clark et al., 2009; Ellender et al., 2011).

Distribution and threats to the freshwater fish fauna in southern Africa

Freshwater fishes of southern Africa have been divided into tropical and temperate assemblages based on their geographic ranges (Skelton, 1986; 1994; 2001) (Figure 1.1). The diversity of the tropical fish fauna substantially declines from north to south. The Orange River represents the southern boundary for this fauna on the west coast whereas the Pongolo River system to the Bushmans River system represents their southern-most distribution limit on the east coast in South Africa (Skelton, 1986; 2001; Skelton et al., 1991). The dominant components of the tropical fauna are cyprinids, cichlids and siluroid catfishes. The temperate fauna is dominated by cyprinids, with three austroglanid catfishes, two anabantid species and a single galaxiid species.

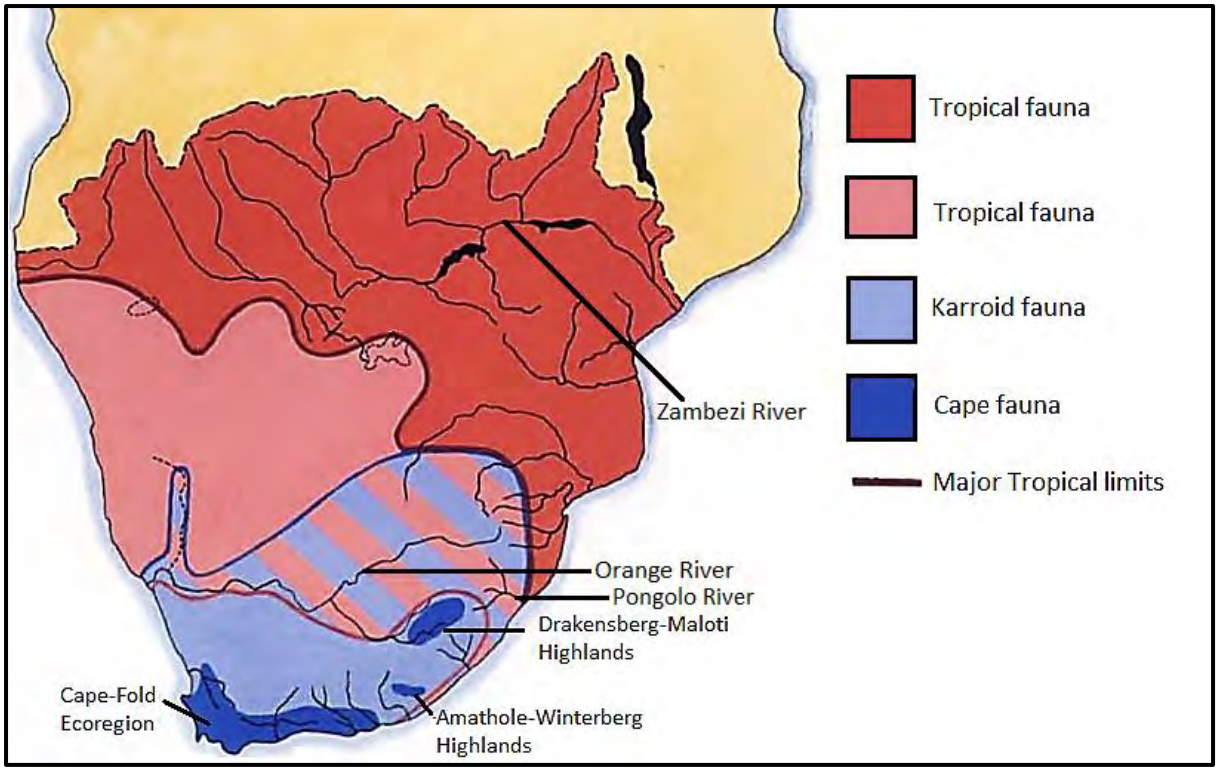


Figure 1.1: Biogeographical zones of southern African freshwater fish fauna recognised by Skelton (2001). Red and pink denote Tropical fish fauna. Red denotes a higher diversity of tropical fish fauna while the pink colour denotes a lower diversity of tropical fish fauna. Light blue denotes Karroid fauna whereas dark blue denotes Cape fauna. Cross-banded are denote overlap between Tropical and Temperate fish fauna.

The temperate fauna has been further divided into two groups according to their respective distributions, the Karroid and Cape fauna (Skelton, 1986) (Figure 1.1, 1.2). The distribution pattern of the Karroid fauna is centered around the lower sections of the Orange River system and largely coincided with the Karoo sequence of geological strata (Skelton, 1986; 2001) (Figure 1.2). The Cape group is confined to river systems associated with the Cape Fold belt, the Amathole Mountains and the Drakensberg Mountains (Skelton, 1986; 2001; Figure 1.2). The distributions of species in the Cape and Karoo groups are however not mutually exclusive and some overlap is found. Based on the distribution ranges of species Abell et al. (2008) recognised three freshwater ecoregions within the Cape group: the Cape Fold Ecoregion (CFE), the Amathole-Winterberg Highlands Ecoregion (AWHE) and the Drakensberg-Maloti Highlands Ecoregion (DMHE) (Figure 1.2). An ecoregion is "a relatively large unit of land or water containing geographically distinct assemblage of species, natural communities, and

environmental conditions" (Olson et al., 2001). Factors that may contribute to defining such regions for the aquatic systems of southern Africa include climate, vegetation, geology, altitude and the nature of the biological communities (Skelton, 2001). Because ecoregions are defined by their shared biotic and abiotic characters, they represent practical units on which to base conservation planning. Of the 20 species currently recognised for the Cape group, 16 of them are endemic to the CFE, with the AWHE only represented by three species and the DMHE represented by a single species (Skelton, 2001; Thieme et al., 2005).

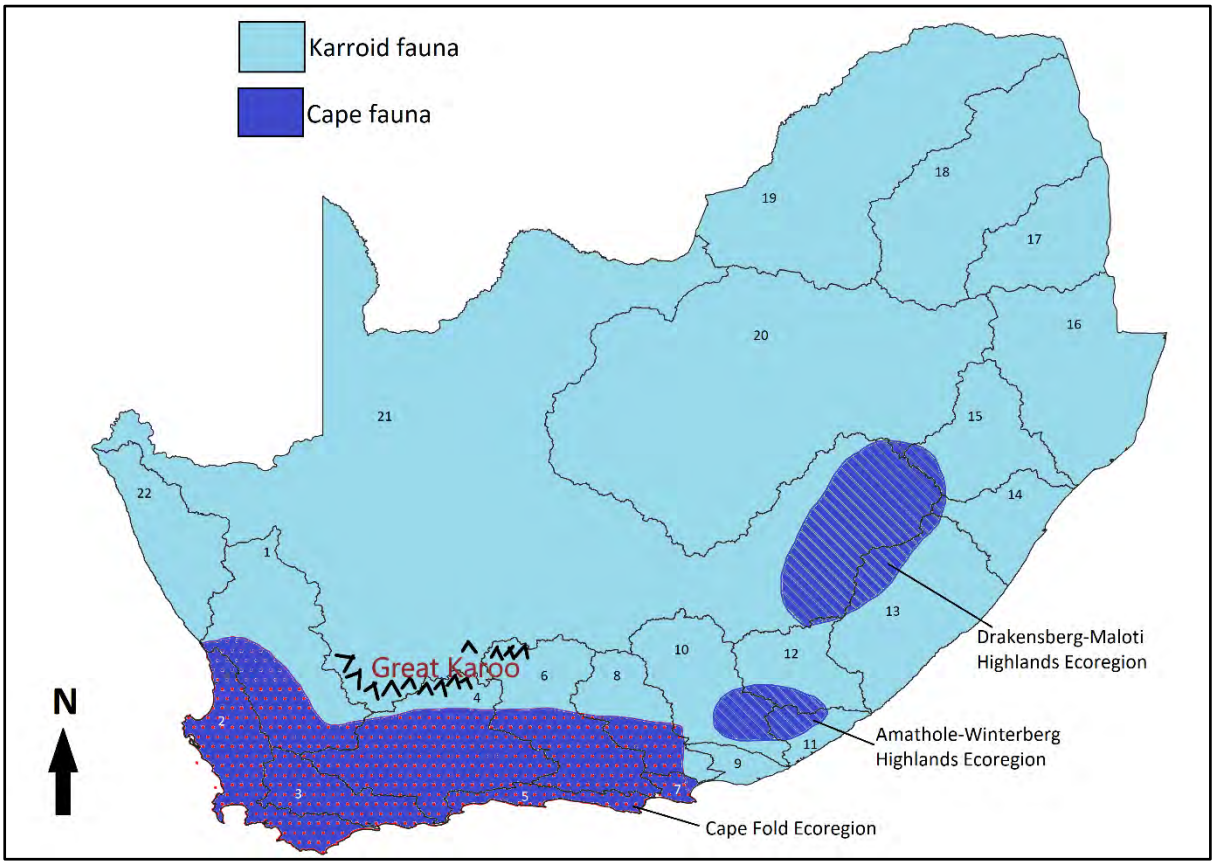


Figure 1.2: The Cape and Karroid group of the Temperate Biogeographical zone in South Africa. The Cape group includes the Cape Fold Ecoregion, Amathole-Winterberg Highlands Ecoregion and the Drakensberg-Maloti Highlands Ecoregion. Quaternary catchments present in the Temperate Biogeographical zone: 1 = Olifants (Clanwilliam), 2 = Berg, 3 = Breede, 4 = Gouritz, 5 = Coastal rivers, 6 = Gamtoos, 7 = Swartkops, 8 = Sundays, 9 = Bushmans, 10 = Great Fish, 11 = Keiskamma, 12 = Kei, 13 = Mzimvubu, 14 = Mkomazi, 15 = Tugela, 16 = Mfolozi, 17 = Komati, 18 = Olifants, 19 = Limpopo, 20 = Vaal, 21 = Orange and 22 = Buffels.

The CFE, located at the southern-most part of the African continent, covers the Mediterranean climatic region and extends from the south-western to the south-eastern parts of South Africa (Thieme et al., 2005; Abell et al., 2008; Figure 1.2). 20 out of the 23 formally described and valid fish species in the CFE are endemic (Barnard, 1943; Skelton, 2001; Tweddle et al., 2009; Ellender et al., 2017). The number of endemic fishes in the CFE is considerably higher than currently recognised, as many newly discovered genetic lineages await formal description (Wishart et al., 2006; Swartz et al., 2007, 2009; Chakona & Swartz, 2013; Chakona & Skelton, 2017). This high level of endemism has been attributed to the long period of isolation of the CFE from adjacent regions following the formation of the Cape Fold Mountains (Skelton, 1980; 1988), and also the role of sea-level transgressions that are hypothesized to have promoted diversification of stream fishes on the south coast (Chakona et al., 2013a). The diversity per river system is however low, with most systems having no more than four species (Skelton, 1986; 2001; Ellender et al., 2017). The low fish diversity in the CFE could be related to the fact that most river systems originate from the Cape Fold Belt and flow for only short distances before discharging into the sea, which potentially limits the available niches to accommodate diverse fish fauna (Skelton, 1986; 1994). Most of the river systems in the CFE are also characterised by clear flowing oligotrophic water with low conductivity and low suspended solids as they drain predominantly over erosion-resistant quartz-rich sandstones of the Table Mountain Group (Allanson et al., 1990; Day et al., 1998). Generally, ecoregions with moist forest and large lakes support significantly more fish species than other ecoregions and fish richness tends to decrease outward from the equator. In Africa, ecoregions with the highest fish richness are all in the Great Lakes bioregion (Lakes Malawi, Tanganyika and Victoria) and several ecoregions in the West Coastal Equatorial and Congo River bioregions (e.g., the Central West Coastal Equatorial, Southern West Coastal Equatorial, Cuvette Centrale, Malebo Pool, Lower Congo, and Kasai) (Thieme et al., 2005).

Recent DNA-based studies have shown that the taxonomic status of almost all fishes in the CFE require revision (Wishart et al., 2006; Chakona & Swartz, 2013; Chakona et al., 2013a, 2014, 2015; Chakona & Skelton, 2017; Skelton et al., 2018; Bronaugh et al., 2020). These studies have uncovered genetic differentiation within traditionally recognised species, indicating that the diversity of stream fishes in this region had been previously underestimated (e.g. Chakona & Skelton, 2017; Ellender et al., 2017). Additionally, the most recent IUCN Red List assessment of the conservation status of freshwater fishes of South Africa classified a significant proportion of the fishes of the CFE as highly threatened (Tweddle et al., 2009;

Ellender et al., 2017). Studies have also documented that mainstem populations of native fishes in this region have been extirpated, mainly due to human-induced impacts such as habitat destruction, pollution and predation from, and competition with non-native fishes, including the largemouth bass, smallmouth bass, rainbow trout, brown trout, common carp *Cyprinus carpio*, and African sharptooth catfish (Clark et al., 2009; Tweddle et al., 2009; Ellender & Weyl, 2014; Ellender et al., 2017; Chakona et al., 2020; Luger et al., 2020). Headwater tributaries therefore represent important refugia for remnant populations of native fishes in the CFE (Chakona et al., 2020b). There are however serious conservation concerns as most of the headwater tributaries that harbor highly threatened stream fishes on the CFE remain poorly protected (Jordaan et al., 2020).

Similar to the CFE, the unique fish fauna of the AWHE and the DMHE ecoregions have been found to contain hidden diversity comprising a number of historically isolated genetic lineages (Swartz et al., 2009; Chakona et al., 2015a; 2020b;). Aquatic biodiversity in these ecoregions is also threatened by anthropogenic activities, including the spread of invasive species, inter-basin water transfer schemes and construction of large impoundments (Thieme et al., 2005; Tweddle et al., 2009; Kadye & Booth 2012; 2013; Kubheka et al., 2017; Chakona et al., 2019). The AWHE covers a series of high altitude Afromontane forests and is situated on a relict arm of the Great Escarpment's southern rim (Hughes & Hughes, 1992; Thieme et al., 2005). Of the eight native freshwater fish species known from this ecoregion, the Eastern Cape rocky *Sandelia bainsii*, the border barb *Amatolacypris trevelyani* and the Amatola barb *Enteromius amatolicus* are endemic. Recent genetic analysis suggests that *Sandelia bainsii* comprises three allopatrically distributed lineages that have been informally designated as *Sandelia* sp. "Kowie", *Sandelia* sp. "Keiskamma" and *Sandelia* sp. "Buffalo" (Chakona et al., 2020b). Genetic analysis on *Amatolacypris trevelyani* also uncovered species level genetic divergences between the populations in the Buffalo and the Keiskamma river systems (Ellender, 2013). Field observations suggest that remaining populations of these endemic fishes are fragmented due to the impact of invasive alien species such as African sharptooth catfish largemouth bass, smallmouth bass and rainbow trout (Kadye and Booth 2012; 2013; 2020). Additional threats include water pollution and habitat degradation caused by forestry activities in the upper catchments (Henderson, 1992; Thieme et al., 2005).

The DMHE encompasses the whole of Lesotho, excluding the westernmost lowland areas and includes small parts of South Africa just north and south of Lesotho (Thieme et al., 2005; Abell et al., 2008) (Figure 1.2). The delineation of this ecoregion follows the boundaries

of the Drakensberg-Maloti Highlands and comprises the middle sub-region of the Montane-escarpment aquatic region (Skelton 1993). The Drakensberg-Maloti Highlands are a residual portion of the peripheral uplands of the Great Escarpment that has progressively been eroded and dissected northward and westward by the rivers on the southern and eastern southern African coasts (Skelton 1986). This ecoregion is dissected by many small streams, most of which make up the headwaters of the extremity of the Orange River, also known as the Senqu River in Lesotho. Only one species, the Maloti minnow *Pseudobarbus quathlambae* with relatives in the CFE (Swartz et al., 2009) is endemic to this ecoregion. Swartz et al., (2009) showed that *P. quathlambae* is a complex of at least two distinct and allopatrically distributed genetic lineages that have been designated as evolutionary significant units (ESUs), pending findings from ongoing studies that aim to determine their taxonomic distinctiveness. The two ESUs are the 'Mohale' ESU found in the Mohale catchment and an 'eastern' ESU east of the Mohale catchment (Skelton, 2001; Swartz et al., 2009). The future persistence of these lineages is uncertain due to the large-scale water development schemes in the Lesotho Highlands which will likely lead to the mixing of these lineages and promote spread of other species. For example, the building of the Katse-Mohale inter-basin water transfer that linked the Mohale Reservoir to the Katse Reservoir led to the transfer of large native fish species such as the smallmouth yellowfish *Labeobarbus aeneus* (Rall & Sephaka, 2008). Additionally, a recent survey by Shelton et al. (2017) only recorded five specimens for the Mohale lineage of *P. quathlambae*, and the authors attributed the crash in this population to the invasion of the Mohale catchment by *L. aeneus* and the Orange River mudfish *L. capensis* following the construction of the Katse-Mohale inter-basin water transfer scheme. These fishes had previously been prevented from invading the Upper Mohale catchment by the 20 m high Semongkoaneng waterfall (Rall, 1999).

Findings from these previous and ongoing studies highlight the need for reviewing aspects related to the taxonomy, ecology, biogeography and conservation of stream fishes in the CFE and other biodiversity hotspots in southern Africa. This is particularly the case for species that are still perceived to have broad geographic ranges across the region.

Ecological attributes of headwater streams

Ecological attributes are features of taxon such as its range size, life history, geographical distribution, climatic tolerances, habitat types, environmental indicator values, edaphic

preferences, and so on, that describe the overall ecology of the taxon (Hill et al., 2004; Hill et al., 2007). An understanding of the ecological attributes of the fish fauna associated with headwater streams is crucial for identifying effective measures to facilitate persistence of the unique biota of these systems (e.g. Kadye et al., 2016; Chakona et al., 2019; Sifundza et al., 2020). Ecological aspects that shape aquatic communities are divided into abiotic and biotic factors (Southwood, 1977; Poff, 1997; Jackson et al., 2001; Richardson, 2019). Abiotic factors include habitat characteristics at micro- (e.g., substratum size, type, depth and flow), meso- (e.g., stream's hydrological and nutrient regime) and macro-scales (e.g., catchment vegetation, stream order and size). Among the most frequently explored abiotic factors in lotic ecosystems are those related to physical habitat (e.g. stream channel width and depth, substrate type, riparian vegetation) and chemical water quality (e.g. dissolved oxygen, pH, turbidity, conductivity) which directly impose physiological limits to fish survival (Gomi et al., 2002; Dudgeon et al., 2006; Arthington et al., 2016). These factors influence the distribution and abundance patterns of freshwater fish species (Carvalho & Tejerina-Garro, 2015; De Castro et al., 2016; Chakona et al., 2019; Kadye & Booth, 2014; 2020; Carvalho et al., 2020; Sifundza et al., 2020). Biotic factors include intra- and inter-specific interactions such as competition and predation that influence the persistence of both individual species and communities in freshwater ecosystems (Gilliam & Fraser, 2001; Butler & Wooden, 2012; Hammerschlag et al., 2019). Despite the importance of biotic and abiotic factors for understanding the persistence of species in streams, only a few studies have specifically focused on documenting the ecological attributes of freshwater fishes in the CFE and AWHE (Chakona & Swartz, 2012; Chakona et al., 2019; Kadye & Booth, 2014; 2020; Sifundza et al., 2020). Given the discovery of new species and unique genetic lineages, there is need for reviewing existing and generating new information on the biology and ecology of the fishes of this region.

Historical biogeography of Cape fauna

Tectonic and climatic events as well as ecological factors have been used to explain the patterns of freshwater fish distributions in southern Africa. The occurrence of closely related fish species in this region has intrigued evolutionary biologists for a long time (e.g. Gilchrist & Thompson, 1913; Barnard, 1943; Jubb & Farquharson, 1965; Greenwood & Jubb, 1967; Jubb, 1967; Gaigher & Pott, 1973). Many hypotheses have been presented to explain these patterns. With the exception of *Galaxias zebratus*, the freshwater fishes of this region were seen as being the products of ancestors from further north in Africa that dispersed southwards in a series of

invasions to populate the subcontinent (Jubb, 1964; Gabie, 1965; Jubb & Farquharson, 1965). The first invasion is thought to have been in the mid-Pliocene (2–3 million years ago) and resulted in what is now the Cape and Karroid fauna reaching the Orange and Cape coastal rivers (Jubb & Farquharson, 1965). A general absence of Tertiary fish fossils creates a problem with the "waves of invasion" theory because it limits the ability to date the timing and thus determine the mechanisms that would have facilitated these invasion events.

The "waves of invasion" theory was first challenged by the discovery of the phylogenetic relationship between the Maloti minnow *P. quathlambae* from the Drakensberg Mountain drainages and other redfin minnows from the Cape Fold Mountain drainages (Skelton, 1980; 1986; 1988). The phylogenetic relationship of the redfin minnows of the genus *Pseudobarbus* and the diversity and distribution of other Cape freshwater fishes were proposed to have been influenced and shaped by isolation following formation of mountain ranges, sea level changes and drainage rearrangements (Skelton, 1986; 1988; 1996). The evolution of the Orange River, in particular, was hypothesised to have played a central role in shaping the distribution patterns of temperate freshwater fishes in southern Africa. The complementary species pairs (e.g. *Austroglanis sclateri* (Orange) – *A. gilli/barnardi* (Olifants), *Labeobarbus aeneus/L. kimberlyensis – L. seeberi* (Olifants)) between the Orange and the Olifants river systems were presented as likely evidence for potential historical ichthyofaunal connection between the two river systems (Barnard, 1943; Jubb, 1967; Skelton, 1980; 1986). Indeed, there is geological evidence to support repeated shifts in the exit points (river mouths) for rivers carrying runoff from the upper-Vaal Orange catchment areas over geological times (Dingle & Hendry, 1984) (Figure 1.3a-c).

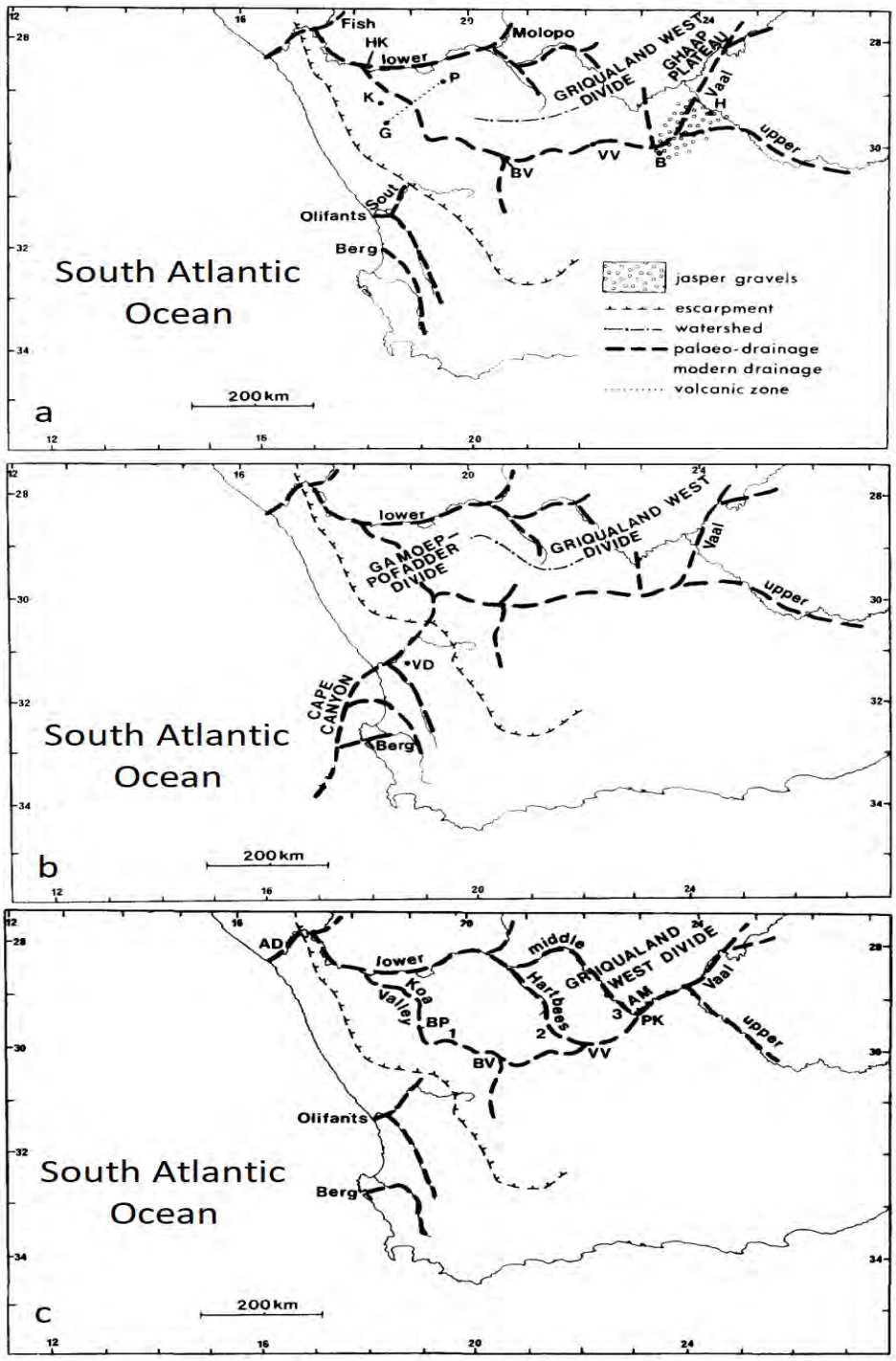


Figure 1.3: Palaeogeographies of the Orange River drainage systems from Dingle and Hendry, 1984. (a) late Cretaceous, (b) Palaeogene, (c) Neogene. 1, 2 and 3 are successive positions of the Middle Orange channel in Neogene reconstructions. Lower, Middle and Upper refer to courses of the Orange River. Abbreviations: BP = Bosluispan; BV = Brandvlei; VV = Van Wyks Vlei; PK = Preiska; AM = Asbestos Mountains; AD = Arris Drift; VD = Vanrhynsdorp; K = Kangnas; G = Gamoep; P = Pofadder; B = Britstown; H = Hopetown; HK = Henkries.

The initial exit during the late Cretaceous (between 100.5 and 66 million years ago) time was, at the present date, the mouth of the Orange River (Figure 1.3a). This exit shifted to the mouth of the present day Olifants River during the Paleocene (66 to 56 million years ago) (Figure 1.3b). This arrangement lasted until late Miocene (11.6 to 5.3 million years ago) when the upper-Vaal-Orange River shifted back to the present day mouth of the Orange system (Dingle & Hendry, 1984) (Figure 1.3c). Skelton (1986) attributed the existence of highest diversity of the Cape fauna compared to the other river systems in the CFE to the historical connection of the Olifants and the Orange River systems as this would have facilitated range expansion of the tropical elements. Drainage rearrangements are also documented to have occurred between the Orange and the Gouritz river systems during the Oligocene (38 to 26 million years ago) (Dingle & Hendry, 1984). For example, *Pseudobarbus tenuis,* the sister species of *P. quathlambae* from the Orange River occurs in the Gouritz River system. Skelton (1994) hypothesized that drainage capture of tributaries of the Orange River system by an expanding Gouritz River system could potentially explain the relationship of *P. tenuis,* which, based on morphological evidence available at the time, was considered to be more closely related to *P. quathlambae* from the upper Orange River system. The complex drainage evolution of southern Africa, which was associated with river diversions and river capture events had for a long time been considered to provide the most plausible explanations for the biogeographic patterns of freshwater fishes in this region (Skelton, 1994).

In addition to facilitating more accurate documentation of biological diversity, genetic studies are providing further clarity on the evolutionary relationships of the fishes of this region and are shedding more light on the processes that shaped the diversity and distribution patterns of the Cape fauna (Swartz et al., 2007; 2009; 2014; Chakona et al., 2013a, 2013b; Chakona et al., 2015a). For example, Swartz et al (2007, 2009) showed that *P. tenuis* slender redfin from the Gouritz River system was closely related to *P. phlegethon* fiery redfin from the Olifants-Doring river system. Additionally, *P. quathlambae* maloti minnow from the DMHE was distantly related to all single barbelled redfin minnows from the CFE. A river capture between the Olifants and Gouritz river systems was considered to be the most reasonable explanation for the close relationship between *P. tenuis* on the south coast and *P. phlegethon* on the west coast of South Africa (Swartz et al., 2007). Genetic studies also support the role of mechanisms such as inter-drainage dispersal through intermittent freshwater connections (Chakona et al., 2013b, 2018) and connectivity associated with sea level changes (Swartz et al., 2007; 2009, 2014; Chakona et al., 2013a). For example, the distribution patterns of redfins (*Pseudobarbus*

species) were explained by Swartz et al. (2007; 2009) with a combination of isolation within coastal drainages and dispersal by river captures and confluences of currently isolated coastal rivers during periods of lower sea levels. Chakona et al. (2013a) attributed the diversification of *Pseudobarbus*, *Sandelia*, and *Galaxias* to allopatric isolation within upland tributaries where the confluences were drowned during the Miocene-Pliocene major sea level transgression. Furthermore, for some taxa (e.g. *Sandelia capensis*, *Galaxias zebratus*, *Pseudobarbus burchelli*), there is some inference based on observed patterns that their distribution patterns are consistent with human-assisted translocation (Ellender & Weyl, 2014; Swartz et al., 2014; Chakona et al., 2015b; Bronaugh et al., 2020; Chakona et al., 2020a).

The above studies indicate that the diversification and distribution patterns of the Cape fauna was shaped by complex mechanisms. Similar to the southern temperate fish fauna, Australian freshwater fish communities were considered highly unique but species poor, largely due to Australia's arid climate and long isolation from other land masses (Unmack, 2001; Unmack and Hammer, et al., 2013). Since the last attempt to analyse freshwater fish composition patterns and determine a bioregionalisation scheme (Unmack, 2001), the number of described and candidate freshwater fish species in Australia has increased (e.g. Cook et al., 2014; González-Orozco et al., 2015; Hammer et al., 2019). This increase led to a reconsideration of the formation and patterns of biodiversity on the continent (Shelley et al., 2019) where substantial differences from the former bioregionalisation scheme were found. Similar increases in the number of described and candidate species of Cape fauna raises the need for increased biogeographic research in this region. New insights into freshwater biodiversity across the southern temperate region will also enable approaches to river management that account for unique biodiversity features, such as areas with high endemism or taxonomic richness.

Taxonomic history of the Cape fauna

Despite a long history of taxonomic research in this region (e.g. Boulenger, 1905; Barnard, 1943; Jubb, 1965; 1967; Skelton, 2001), growing evidence indicates that the taxonomic status of many of the fish species in this region remains unresolved. Cyprinid fishes of the genera *Labeo, Enteromius* and *Pseudobarbus* typify examples of this general pattern. For example, the moggel *Labeo umbratus* (Smith, 1841) was considered to be widespread in South Africa and encompassed the Orange River system, extended south of the Great Escarpment to the

southward-flowing river systems, including the Gouritz, Gamtoos, Sundays, Bushmans, Great Fish and Keiskamma rivers. Genetic analysis of *L. umbratus* found that this species was composed of two unique lineages from the west flowing Orange River and southern flowing drainages respectively (Ramoejane et al., 2020). The southern lineage was further divided into three sub-lineages, one restricted to the Gouritz River, the second largely confined to the Gamtoos River and the third distributed across river systems on the east (the Sundays, Bushmans, Great Fish, Keiskamma, Buffalo and Nahoon rivers). The Great Escarpment and deep incisions along the south coast were considered to have potentially played a role in shaping the patterns of genetic and geographic structuring in this species (Ramoejane et al., 2020).

A similar scenario was revealed for the goldie barb *Enteromius pallidus*. *E. pallidus* was considered to be widely distributed across the westward-flowing Orange River in the interior of South Africa and the southward flowing coastal streams, separated from the Orange River by the Great Escarpment (Skelton, 2001). Chakona et al. (2015b) documented two distinct lineages within *E. pallidus*, reflecting this separation. The revisions by Martin & Chakona (2019) revealed that *E. pallidus* is confined to the eastern CFE where it is distributed from the Krom River to the Great Fish River system. The northern lineage is thought to represent a previously unrecognised species or may belong to a different, but known species or species complex not associated with 'true' *E. pallidus*.

The Eastern Cape redfin *Pseudobarbus afer* was considered to be a single widespread and variable species occurring in multiple isolated river systems in the CFE at the southern tip of Africa. A molecular study by Swartz et al. (2007) identified four distinct lineages within *P. afer* which were named as the Forest, Krom, St Francis and Mandela lineages that are separated by deep mitochondrial cytochrome *b* genetic divergences (3.3–8.3%). This mitochondrial divergence is consistent with interspecific distances observed in other closely related cyprinids (Van Ginneken et al., 2017; Mamonekene et al., 2019; Schmidt et al., 2018; 2019; Shen et al., 2016). Chakona & Skelton (2017) found that in addition to their deep genetic divergence, the Krom, St Francis and Mandela lineages can be separated based on scale counts, length of oral barbels and consistent differences in body colour pattern, supporting their status as distinct species. The Mandela lineage from the Baakens, Swartkops and Sundays rivers were considered to represent *P. afer* s.s. The Krom lineage, restricted to the Krom River system, has been named *P. senticeps* (Chakona & Skelton, 2017). Finally, the St Francis lineage found in the Gamtoos, Kabeljous and Swart River system forms a new species *Pseudobarbus swartzi*

Chakona & Skelton, 2017. The geographical distributions of these lineages were largely consistent with the subgroupings identified by Skelton (1988). Distinct lineages have also been found in other primary freshwater fish species such as *Sandelia capensis* Cuvier 1829 (Bronaugh et al., 2020), *Sandelia bainsii* Castelanu 1861 (Chakona et al., 2020), and *Galaxias zebratus* Castelanu 1861 (Chakona et al., 2013a; Wishart et al., 2006) within the southern draining river systems. Some of these lineages have already been formally described as new species and the distributions of the original species revised (Swartz et al 2007; Chakona et al., 2013b; Chakona and Skelton 2017; Chakona and Swartz 2014).

These patterns challenge the notion of the existence of widely distributed freshwater fishes in the region. For example, the chubbyhead barb *Enteromius anoplus* (Weber, 1897), which is the focal species for the present study (Figure 1.2), is the most widely distributed freshwater fish species in South Africa with a range extending from the Highveld Limpopo to upland KwaZulu Natal, Transkei and middle and upper Orange River. It also occurs in large coastal rivers in Eastern and Western Cape, such as Olifants, Gouritz, Gamtoos, Sundays and Great Fish rivers (Skelton, 2001). Barnard (1938, 1943) was one of the first to detect potential taxonomic differences in geographically subdivided *E. anoplus* populations. Barnard (1943), divided the chubbyhead barbs into two species, namely *Barbus karkensis* (Gilchrist & Thompson, 1913) from KwaZulu Natal and *Barbus anoplus* for the rest of its distribution. *Barbus anoplus* was further subdivided by Barnard (1943) into three geographically isolated forms from the Orange, Olifants and Gouritz river systems supported by morphological differences among the three forms. In subsequent studies using molecular analysis, Engelbrecht (1996) found that *E. anoplus* could be subdivided into at least two groups consisting of a Vaal River group and a north-eastern escarpment (Crocodile and Tugela Rivers) group. Furthermore, Da Costa (unpublished) found that *E. anoplus* had significant genetic variation and potentially represented multiple species. Although *E. anoplus* has, for a long time, been known to potentially represent a species complex harbouring undocumented diversity, there has not been any dedicated studies that were done in an attempt to test this hypothesis through integration of multiple sources of evidence, including genetics, morphology and meristics. This is perhaps due to the fact that chubbyhead barbs are found in highly variable ecological environments, and thus the variation in morphology identified by Barnard (1943) was thought to reflect adaptation to these variable habitats.

The habitat and ecology of *E. anoplus* sensu lato have been previously assessed (Cambray, 1983a; 1983b; Cambray & Bruton, 1984; 1985). The species has been found to

occur in a wide variety of habitats from small streams to large rivers and lakes, and associates with cover such as fallen logs, brushwood or marginal vegetation (Skelton, 2001). *Enteromius anoplus* is omnivorous and feeds on insects, zooplankton, seeds, green algae and diatoms (Cambray, 1983a; Skelton, 2001). It has both an early maturity, one year at 40mm FL, and a high reproductive rate which enables it to quickly colonize new areas (Cambray & Bruton, 1984; 1985). Most of these studies were carried out in the Orange River system. However, previous studies (e.g. Engelbrecht 1996; Da Costa, unpublished) have revealed existence of unique lineages, some that show strong geographic affinities. These unique lineages could have a different ecology and thus conservation of these lineages might require other circumstances than those in the Orange River. This raises the need to provide more accurate information on the ecology, distribution patterns and population status of these lineages for effective management strategies.

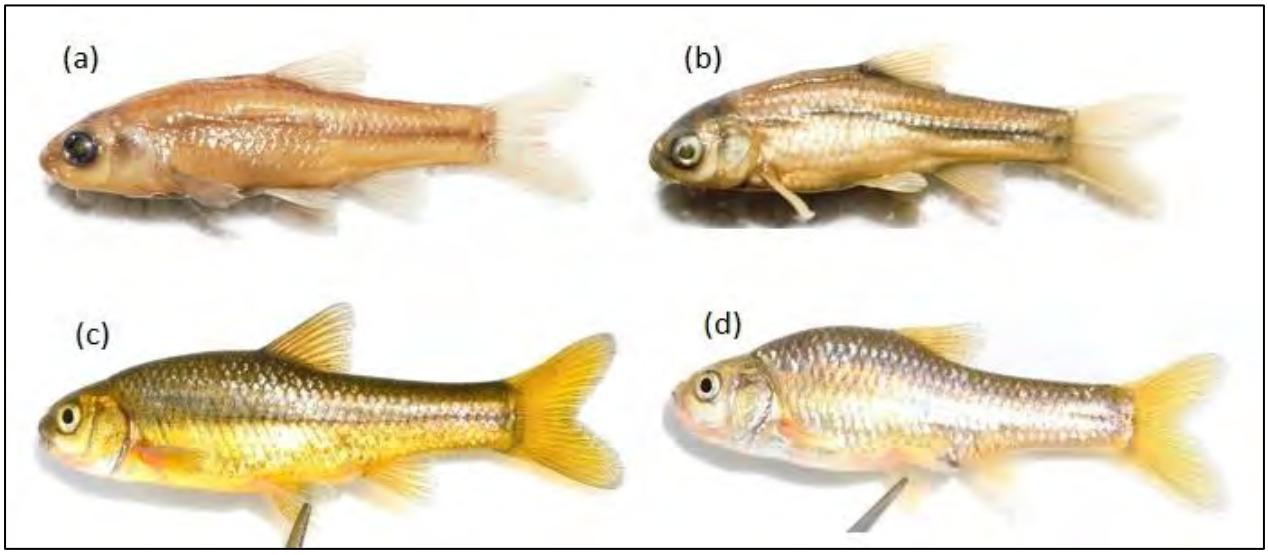


Figure 1.4: Enteromius anoplus s.l. from the (a) Olifants River (Clanwilliam division) (b) Gouritz River (c) Great Fish River and (d) Orange River.

Thesis outline

Animals bound to complex and disjunct habitats are often represented by multiple discrete conservation units or even unrealised cryptic species (Beheregaray & Caccone, 2007). These cryptic species represent an emerging challenge to how we view and manage landscapes, gather and apply ecological information, and design conservation programs (e.g. Beheregaray &

Caccone, 2007; Bickford et al., 2007). The status or perception of a particular freshwater species as 'common' and widespread should thus be challenged whenever possible. The present study therefore aimed to contribute towards the knowledge base by providing information on the taxonomy, ecology and biogeography of the chubbyhead barb *Enteromius anoplus* complex. The present study had four main objectives which represent four main data chapters.

The first objective was to review the taxonomy of *E. anoplus*, focusing on populations in the southern temperate region, which encompasses the Orange, Olifants, Gouritz, Great fish and adjacent river systems (Figure 1.2). Targeted sampling was done to collect DNA tissue samples and voucher specimens from the type localities of *E. anoplus* (Gouritz River system) and two of its synonyms *E. cernuus* (Olifants River system) and *E. oraniensis* (Orange River system) to determine the taxonomic distinctiveness of these species. The study provides genetic, morphological and biogeographical evidence for the separation and recognition of three species that were previously included under *E. anoplus* from the southern temperate region, and the description of a new species *Enteromius mandelai*, which is endemic to the Eastern Cape Province of South Africa. This constitutes the first data chapter (Chapter 2) of the thesis. Chapter 2 is presented as a scientific paper is published in the Journal of Fish Biology. The details of this paper are as follows: Kambikambi, M.J., Kadye, W.T., & Chakona, A. (2021) Allopatric differentiation in the *Enteromius anoplus* complex in South Africa, with the revalidation of *E. cernuus* and *E. oraniensis*, and description of a new species, *E. mandelai* (Teleostei: Cyprinidae). *Journal of Fish Biology:* N/A. https://doi.org/10.1111/jfb.14780. Data collection was carried out by Kambikambi, M., Chakona, A. and Kadye, W. Laboratory work and data analysis was carried out by Kambikambi, M. The manuscript was written by Kambikambi, M. with input from Chakona, A. and Kadye, W.

The distribution and abundance of freshwater fish species across different spatial scales is influenced by abiotic and biotic factors. The second objective was to evaluate trophic niche patterns of *E. mandelai.* Dual stable isotope tracers of carbon and nitrogen were used in Chapter 3 to examine the trophic niche patterns of *Enteromius mandelai* across headwater streams with riparian zones impacted by agricultural activities, non-native plant species and near natural streams in the Great Fish, Keiskamma and Buffalo rivers. Parametric and non-parametric analysis was used to determine if there were any differences in $\delta^{13}C$ and $\delta^{15}N$ values for basal sources, invertebrate consumer groups and fish. *Enteromius mandelai* showed isotopic niche differentiation in the differently impacted headwater streams. The third objective was to

determine the species-environment relationships of *E. mandelai* and Chapter 4 assessed the environmental factors affecting the distribution and abundance patterns of *E. mandelai* within headwater streams of the Great Fish, Keiskamma and Buffalo rivers in the Eastern Cape Province. The study showed that *E. mandelai* exhibited differences in its spatial patterns across the different river systems, but it did not exhibit any clear environmental niche patterns.

The fourth objective was to integrate the newly generated information from the present study and existing information from previous studies to test the "fidelity" of the biogeographic zones previously proposed for the fishes of the CFE. Chapter 5 therefore explored the current distribution patterns of southern temperate fish to propose extension of the CFE to encompass the AHWE. Lastly in Chapter 6, the results of the present study are summarised and its implications on the temperate bioregion are broadly discussed. This chapter also evaluates the extent to which the original goals were achieved.

CHAPTER 2

Kambikambi, M.J., Kadye, W.T., & Chakona, A. (2021) Allopatric differentiation in the *Enteromius anoplus* complex in South Africa, with the revalidation of *E. cernuus* and *E. oraniensis*, and description of a new species, *E. mandelai* (Teleostei: Cyprinidae). *Journal of Fish Biology:* N/A. https://doi.org/10.1111/jfb.14780.

Allopatric differentiation in the *Enteromius anoplus* complex in South Africa, with the revalidation of *E. cernuus* and *E. oraniensis*, and description of a new species, *E. mandelai* (Teleostei: Cyprinidae)

Abstract

The chubbyhead barb, *Enteromius anoplus*, as currently described, is the most widely distributed freshwater fish in South Africa. The species occurs in almost all the major river systems across the country, with the exception of the small coastal drainages on the south coast. The use of a comprehensive data set of mitochondrial (mtDNA) cytochrome *b* (cyt *b*) sequences uncovered the presence of four distinct lineages or operational taxonomic units (OTUs) within *E. anoplus*: (i) the Gouritz lineage endemic to the Gouritz River system, (ii) the Olifants lineage endemic to the Olifants-Doring River system, (iii) the Orange lineage endemic to the Orange River system, and (iv) the Eastern Cape lineage which is distributed across six river systems from the Great Fish to the Buffalo rivers in the Eastern Cape Province of South Africa. The present study provides a new diagnosis for *E. anoplus* s.s. (the Gouritz lineage), revalidates *E. cernuus* (the Olifants lineage), and *E. oraniensis* (the Orange lineage) as distinct species. The study also provides a description for a new species, *E. mandelai* sp. nov (the Eastern Cape lineage). *Enteromius cernuus* and *E. mandelai* differ from both *E. anoplus* and *E. oraniensis* in having a complete lateral line (vs an incomplete lateral line in the latter species). *Enteromius cernuus* further differs from the other three species in having long maxillary barbels which reach or exceed the vertical through the middle of the eye and the lowest number of circumpenducular scales (10–12 vs 12–16 for the other three species). *Enteromius oraniensis* is distinctive from the other three species by having inconspicuous barbels. These findings add to the growing body of literature that shows that the freshwater fishes of the Cape Fold and adjacent freshwater ecoregions have narrow geographic ranges.

This has ramifications for conservation planning and management, as well as our understanding of the evolutionary history of the stream fishes in these global endemic hotspots.

KEYWORDS

hidden diversity, freshwater fish, Cape Fold Ecoregion, Amatolo-Winterberg Highlands Ecoregion, Southern Temperate Highveld Ecoregion

Introduction

The freshwater fish family Cyprinidae contains over 3000 species in about 375 genera (Froese & Pauly, 2019). It is the most widely distributed freshwater fish family with representatives in Europe, Asia, Africa and North America (Froese & Pauly, 2019). In southern Africa, *Enteromius* Cope, 1867 is the most widely distributed cyprinid genus, with representatives in almost all the major river systems in this region. Of the 38 currently recognised species of this genus in southern Africa, 20 are endemic to South Africa (Froese & Pauly, 2019; Skelton, 2001). Skelton (2001) divided some of these species into two broad groups, the chubby head and the goldie barb groups, based on unique morphological characteristics and the development of distinctive golden coloration pattern during the breeding season. The chubbyhead barb group is represented by four species, the chubbyhead barb *E. anoplus* (Weber, 1897), the redtail barb *E. gurneyi* (Günther, 1868), the Amatola barb *E. amatolicus* (Skelton, 1990) and the Marico barb *E. motebensis* (Steindachner, 1894). Three species belonging to this group have narrow geographic ranges, with *E. anoplus* being the only exception as its range extends from coastal systems in the south to the Limpopo River system in the north, making it the most widely distributed freshwater fish in South Africa (Skelton, 2001; Figure 2.1).

Enteromius anoplus was originally described in the genus *Barbus*, based on 48 syntypes that were collected from the Buffels River in the Gouritz River system on the south coast of South Africa (Weber, 1897; Figure 2.1). Gilchrist & Thompson (1913), described *E. karkensis* based on a single specimen (the holotype) that was collected from the Karkloof River, a tributary of the uMngeni River system, which discharges into the Indian Ocean close to the town of Durban in South Africa (Figure 2.1). Almost three decades later, Barnard (1938) described *E. cernuus* following examination of 572 syntypes that were collected from three

localities in the Clanwilliam-Olifants River system on the west coast of South Africa (Figure 2.1). In his revision of the fishes of the south-western Cape, Barnard (1943) recognised three subspecies of *E. anoplus*; namely *E. anoplus typica* from the Gouritz River system, *E. anoplus cernuus* from the Clanwilliam-Olifants River system and *E. anoplus oraniensis* from the Orange River system. Barnard (1943) also recognised *E. karkensis* as a separate species from *E. anoplus* which he considered to be confined to river systems in the KwaZulu Natal Province (Figure 2.1). According to Barnard (1943), the only character differentiating *E. karkensis* from *E. anoplus typica* was the possession of a complete lateral line in *E. karkensis* versus the possession of an incomplete lateral line in *E. anoplus typica*. Barnard (1943) identified possession of very short, inconspicuous barbels as the single most important character that separated *E. anoplus oraniensis* from both *E. anoplus typica* and *E. anoplus cernuus* which have well developed and conspicuous barbels. Similarly, Barnard (1943) separated the Olifants River system and Gouritz River system subspecies based on the lateral line which was nearly complete in *E. anoplus cernuus* but incomplete and often reduced in *E. anoplus typica*. However, when Lévêque & Daget (1984) published a checklist of the freshwater fishes of Africa, *E. karkensis* and the three subspecies recognised by Barnard (1943) were placed into synonymy with *E. anoplus*. Although no justification was given for this decision, it was accepted by subsequent workers, making *E. anoplus* the most widely distributed freshwater fish in South Africa (Cambray 1983, 1984, 1985; Skelton 1993, 2001).

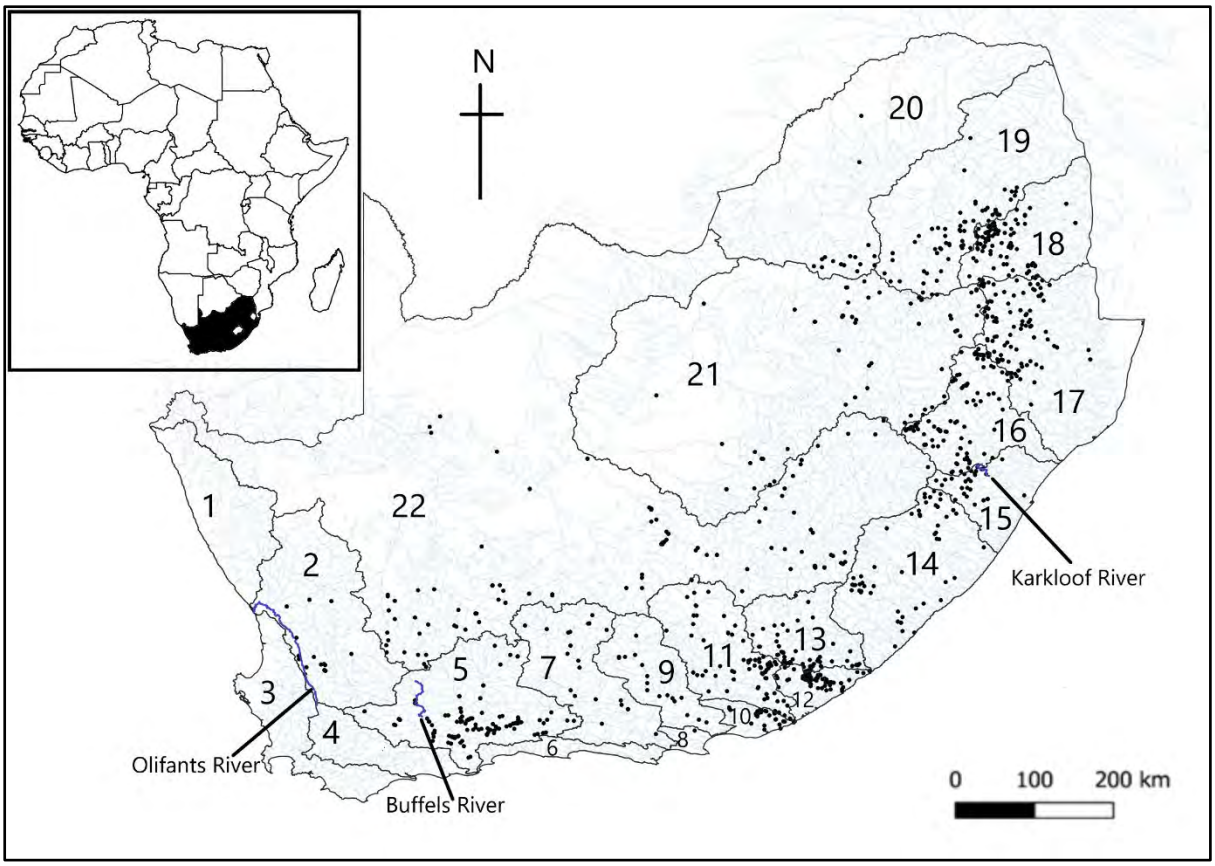


Figure 2.1: Map showing distribution of the chubbyhead barb, *Enteromius anoplus* sensu lato in South Africa, based on data from the NRF-South African Institute for Aquatic Biodiversity database (NRF-SAIAB). The 21 hydrobasins of South Africa are shown: 1 = Buffels, 2 = Olifants (drains to South Atlantic Ocean), 3 = Berg, 4 = Breede, 5 = Gouritz, 6 = Coastal Drainages, 7 = Gamtoos, 8 = Swartkops, 9 = Sundays, 10 = Bushmans, 11 = Great Fish River, 12 = Keiskamma, 13 = Swart-Kei, 14 = Mzimvubu, 15 = Umgeni, 16 = Tukela, 17 = Mfolozi, 18 = Komati, 19 = Olifants (drains to Limpopo River), 20 = Limpopo, 21 = Vaal, 22 = Orange. The Olifants, Buffels and Karkloof River systems are shown in dark blue.

Taxonomic interest in the chubby head barb was revived when Engelbrecht (1996) found allozyme differentiation among populations of *E. anoplus* from four adjacent river systems in eastern and central South Africa. This study identified two genetically distinct groups, the "Vaal River group" comprising samples from the westward-draining Orange River system, and a "North Eastern Escarpment group" which comprised samples from the eastward draining Inkomati River System, Pongola and Tukela river systems (Engelbrecht, 1996). Morphological examination indicated that these two groups could be consistently separated based on possession of an incomplete lateral line in the Vaal River group in contrast to presence of a

complete lateral line in the North Eastern Escarpment group (Engelbrecht, 1996). Da Costa (unpublished) provided further evidence that corroborated existence of substantial levels of genetic differentiation within *E. anoplus*, highlighting that this species requires taxonomic revision to provide more accurate species boundaries and map their geographic ranges.

In recent years, the application of multiple lines of evidence or integrative taxonomy has provided improved taxonomic rigour and facilitated more accurate inventory of biodiversity, particularly for morphologically conservative species complexes with only subtle differences (Englmaier *et al*., 2020; Schmidt *et al*., 2018; Van Ginneken *et al*., 2017). The purpose of the present study was to integrate molecular and morphological data to critically evaluate the nomenclatural status of the three subspecies of *E. anoplus*, namely *E. anoplus anoplus* *E. anoplus oraniensis* and *E. anoplus cernuus* (Barnard, 1943) by sequencing the mitochondrial cytochrome b gene (cyt *b*) and examining a comprehensive number of specimens from the Olifants, Orange, Gouritz and various river systems across the chubby head barb's range in the Eastern Cape Province of South Africa (Figure 2.1). The study found consistent genetic and morphological evidence supporting the revalidation of *E. cernuus* from the Clanwilliam Olifants River system, *E. oraniensis* from the Orange River system and the description of a new species, *E. mandelai* **sp. nov**. which is endemic to the Eastern Cape Province of South Africa. Information from the present study will facilitate reevaluation of the ecology, conservation status and understanding of the evolutionary and biogeographic history of the species within this complex.

Materials and methods

Sample collection

This study used fresh samples that were collected during field surveys of river systems in the Cape Fold Ecoregion, Amatolhe-Winterberg and Southern Temperate Highveld Ecoregions between April 2018 and August 2019. Additional samples were sourced from the Biomaterials Bank at the National Research Foundation-South African Institute for Aquatic Biodiversity (NRF-SAIAB; Table 2.1; Figure 2.2). Field collections followed ethical standards that were approved by the Rhodes University Ethical Standards Committee (approval number 2019-0314-776) and research permits were granted by the Eastern Cape Department of Economic Development and Environmental Affairs (through permit numbers CRO 130/19CR and CRO 44/18CR).

Samples were collected by either electric fishing or seine netting. At each locality, up to 10 specimens were selected for processing. Clove oil (0.1ml/l) was used to anaesthetise the fish. To capture the live colour patterns of the specimens, a Nikon D3100 7.4/9V camera was used.l A small piece of fin clip or muscle tissue was collected from each specimen using a pair of scissors or scalpel blade and placed into a 1.5ml vial containing 95% ethanol for DNA analysis. Tissue samples were deposited into the NRF-SAIAB Biomaterials Bank and kept at -80 ℃ for long term storage. In the field, the source specimens and additional voucher specimens were fixed in 10% formalin. In the laboratory, these specimens were transferred to 10% and 50% ethanol to wash off the formalin. Finally, the specimens were transferred to 70% ethanol for long term storage. All voucher specimens were deposited into the National Fish Collection at the NRF-SAIAB in Makhanda (Grahamstown). The rest of the collected fish were released back to their habitat alive.

Table 2.1: River systems and GPS coordinates of localities where the samples used in the present study were collected. The numbers of individuals (n) that were used for molecular and morphological analyses per locality are indicated.

System	River	lineage	Catalogue & field number	Latitude	Longitude	Morphology sample size (n)	DNA sample size (n)
Orange	Stormbergspruit	*E. oraniensis*	MK19AL17	-30.776	26.422	13	1
Orange	Stormbergspruit	*E. oraniensis*	SAIAB 209737	-30.858	26.418	20	1
Orange	Bossielaagtespruit	*E. oraniensis*	SAIAB 209736	-30.799	27.077	20	2
Olifants	Olifants	*E. cernuus*	SAIAB 135553	-31.610	18.746	5	
Olifants	Olifants	*E. cernuus*	SAIAB 135483	-32.564	18.996	5	
Olifants	Olifants	*E. cernuus*	SAIAB 200351	-32.441	18.959		2
Olifants	Olifants	*E. cernuus*	SAIAB 135479	-32.144	18.873	5	
Olifants	Breekrans	*E. cernuus*	RUSI 65417	-32.559	19.299	8	
Gouritz	Buffels	*E. anoplus* ss	SAIAB 200353	-33.189	20.858		2
Gouritz	Gamka	*E. anoplus* ss	SAIAB 186167	-33.469	21.734		2
Gouritz	Nels	*E. anoplus* ss	SAIAB 186161	-33.467	21.735		2
Gouritz	Buffels	*E. anoplus* ss	SAIAB 200353	-33.189	20.858	10	
Gouritz	Buffels	*E. anoplus* ss	SAIAB 130195	-33.432	21.416	10	
Great Fish River	Tarka	*E. mandelai* sp. n	SAIAB 209734	-32.218	25.929	5	4
Great Fish River	Pauls	*E. mandelai* sp. n	MK19AL03	-32.035	25.521	10	4
Great Fish River	Great Fish River	*E. mandelai* sp. n	SAIAB 209732	-31.899	25.210	20	4
Great Fish River	Koonap	*E. mandelai* sp. n	Koonap b	-32.395	26.512		4

Great Fish River	Eyre	*E. mandelai* sp. n	Eyre 1	-32.507	26.792		4
Great Fish River	Eyre	*E. mandelai* sp. n	Eyre 3	-32.531	26.790		2
Great Fish River	Balfour	*E. mandelai* sp. n	Balfour 2	-32.575	26.655		1
Great Fish River	Balfour	*E. mandelai* sp. n	Balfour 3	-32.567	26.620		2
Great Fish River	Balfour	*E. mandelai* sp. n	Balfour 4	-32.535	26.677		2
Great Fish River	Fairbairn	*E. mandelai* sp. n	Fairbairn 1	-32.509	26.709		3
Keiskamma	Rabula	*E. mandelai* sp. n	Rabula 1	-32.749	27.097		3
Keiskamma	Rabula	*E. mandelai* sp. n	Rabula 2	-32.758	27.134		2
Keiskamma	Rabula	*E. mandelai* sp. n	Rabula 3	-32.749	27.132		2
Keiskamma	Keiskamma	*E. mandelai* sp. n	MK19AL09	-32.718	27.010	13	
Keiskamma	Amatele	*E. mandelai* sp. n	MK19AL10	-32.717	27.009	6	4
Buffalo	Yellowwoods	*E. mandelai* sp. n	MK19AL12	-32.807	27.470	7	2
Buffalo	Yellowwoods	*E. mandelai* sp. n	MK19AL15	-32.725	27.489	11	2
Buffalo	Yellowwoods	*E. mandelai* sp. n	MK19AL21	-32.758	27.488	6	2
Buffalo	Izele	*E. mandelai* sp. n	SAIAB 209733	-32.771	27.389	12	3
Buffalo	Izele	*E. mandelai* sp. n	MK19AL20	-32.732	27.432	11	3
Buffalo	Mgqakwebe	*E. mandelai* sp. n	Buffalo3	-32.822	27.343		3
Tyolomqa	Tyolomqa	*E. mandelai* sp. n	MK19AL22	-33.072	27.445	9	4

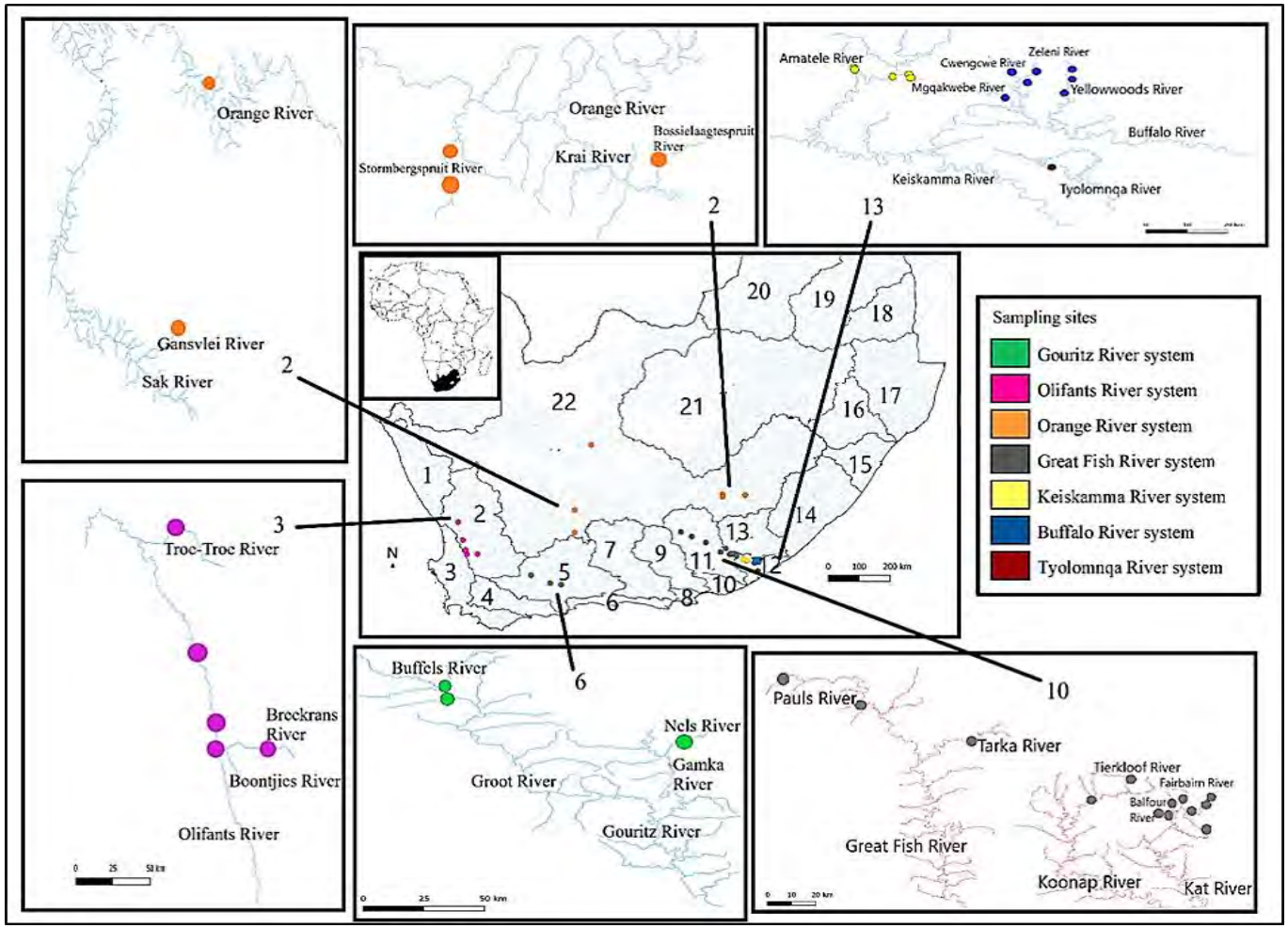


Figure 2.2: Map showing sampling localities for the present study. South African river basins: 1 = Buffels, 2 = Olifants (drains to South Atlantic Ocean), 3 = Berg, 4 = Breede, 5 = Gouritz, 6 = Coastal Drainages, 7 = Gamtoos, 8 = Swartkops, 9 = Sundays, 10 = Bushmans, 11 = Great Fish River, 12 = Keiskamma, 13 = Swart-Kei, 14 = Mzimvubu, 15 = Umgeni, 16 = Tukela, 17 = Mfolozi, 18 = Komati, 19 = Olifants (drains to Limpopo River), 20 = Limpopo, 21 = Vaal, 22 = Orange.

DNA extraction, amplification and sequencing

DNA extraction was done at the NRF-SAIAB using the salting out method (Sunnucks & Hales, 1996). All newly generated sequences were submitted to GenBank (accession numbers: MW465845–MW465901). The mitochondrial cytochrome *b* (cyt *b*) gene was amplified using the primers M172F (3'- AAC CAC CGT TGT TCA ACT A - 5') designed by the SAIAB genetics laboratory and ThrR (5'-ACC TCC GAT CTT CGG ATT ACA AGA CCG-3') from Machordom and Doadrio (2001). The polymerase chain reactions (PCRs) were performed with a Veriti 96-well thermal cycler (Applied Biosystems, Waltham, Middlesex County, Massachusetts, United States of America). Every reaction mixture (20 µl) contained 0.5 µl (10 µM stock) of each primer, 10 µl of Taq Ready Mix (Taq Polymerase) (Southern Cross Biotechnology, Cape Town, South Africa), 5 µl of template DNA and 4 µl molecular grade water. The amplification profile was as follows: 94 °C for 2 min, followed by 35 cycles of 94 °C for 45 s, 52 °C for 60 s and 72 °C for 60 s, with a final extension at 72 °C for 5 min. PCR products were purified using the ExoSAP method with a reaction mixture containing 0.5 µl exonuclease I, 1 µl FastAP with 5 µl PCR product. Cycle Sequencing was performed in 10 µl volumes with 1 ul BigDye Terminator v3.1 Ready Reaction Mix (Applied Biosystems), 1.5 µl molecular grade water, 1 µl of 3.2 µM stock of single primer (M172F) and 5 µl of purified DNA as template. Automated sequencing was performed at the South African Institute for Aquatic Biodiversity molecular laboratory using a 3500 genetic analyser (Applied Biosystems).

Molecular analysis

Newly generated sequences were cleaned and trimmed to equal lengths manually using the program SeqMan Pro 15.3 (DNASTAR, Madison,WI, USA) and were aligned using the program ClustalX 2.1 (Larkin *et al*., 2007). The program DnaSP 5.10 (Librado & Rozas, 2009) was used to translate nucleotide sequences to amino acid sequences to check for stop codons and errors. The program DnaSP v6 (Librado & Rozas, 2009) was also used to determine the number of polymorphic sites and unique haplotypes. Prior to analyses, jMODELTEST 2.1.7 (Darriba et al., 2012) was used to select an appropriate model for sequence evolution using the Akaike information criterion (AIC) (Burnham & Anderson, 2002). Model-corrected genetic distances were calculated using PAUP* 4.b10 (Swofford, 2002). Maximum likelihood analysis was done using heuristic tree searches and applying the tree-bisection reconnection (TBR) branch-swapping algorithm with 100 random taxon addition replicates using PAUP* 4.b10

(Swofford, 2002). Bayesian phylogenetic analysis was performed with MrBayes 3.2.7 (Ronquist *et al*., 2012). Two parallel analyses of four Markov chains were run for 50 million generations and trees were sampled every 1000 generations. The first tree was randomly initiated, and Bayesian posterior probabilities were estimated through pseudo-replication to determine support for relationships represented on the tree. The first 25% of the sampled trees for each analysis was discarded as burn-in, and the remaining trees were used to calculate a majority rule consensus tree. The programme Tracer v1.7.1 was used to diagnose convergence of Markov Chain Monte Carlo analyses, and get estimates of the posterior distributions of parameter values (Rambaut *et al*., 2018).

Species delimitation

Two tree-based species delimitation methods, the General Mixed Yule Coalescent (GMYC) (Fujisiwa & Barraclough, 2013) and the Bayesian implementation of the Poisson Tree Processes (bPTP) (Zhang *et al*., 2013) were used to identify operational taxonomic units (OTUs) within *Enteromius anoplus* s.l. The GMYC model relies on branch lengths to delimit OTUs, and requires an ultrametric tree to define intraspecific (coalescent) and interspecific (speciation) threshold patterns (Fujisiwa & Barraclough, 2013). The bPTP is a coalescent phylogeny-based species delimitation method intended to delimit species based on single locus molecular data (Zhang *et al*., 2013). The method relies on the number of substitutions between species between haplotypes and assumes that more molecular variability is expected between species than within a species (Zhang *et al*., 2013). The ultrametric trees for GMYC analyses were estimated in BEAST (Drummond and Rambaut, 2007) using two priors (Yule model and Coalescent model with constant population size) and two rates of molecular evolution (constant and relaxed clock). For each of the four genera considered in the present study, we compared three trees which were built based on the following combinations of priors and rates of molecular evolution: (i) yule model and a constant clock, (ii) yule model and a relaxed clock, and (iii) coalescent model with constant population size and a constant clock. The GMYC analyses were conducted with the package 'splits' (Species Limits by Threshold Statistics (http://r-forge.r- project.org/projects/splits) using R 4.0.4 (R Core Team, 2021). The ultrametric tree for GMYC was estimated in BEAST. The bPTP analysis was performed in the Exelixis Lab's web server http://species.h-its.org/ptp/, following the default parameters except for a 20% burn-in. The results are presented in Figure 2.3.

Morphometric and meristic analysis

Methods used to obtain morphometric and meristic data (Table 2.2) followed Chakona *et al*., (2014) and Skelton (1988). A total of 11 meristic and 20 morphometric characters were obtained for 175 specimens: Olifants-Doring River system (n = 23), Gouritz River system (n = 20), Orange River system (n = 51), and a number of rivers in the Eastern Cape Province (n = 81) (Table 2.1). Measurements were taken to the nearest 0.1 mm using digital callipers. Specimens were placed into groups established by the genetic results: *E. anoplus sensu stricto* (specimens from the Gouritz River system; *n* = 20), *E. cernuus* (specimens from the Olifants River system, *n* = 23), *E. oraniensis* (specimens from the Orange River system; *n* = 51), *E. mandelai* sp. nov. (specimens from the Great Fish, Keiskamma and Buffalo River systems; *n* = 81). Principal Component Analysis (PCA) was executed on log-transformed morphometric data and raw meristic variables separately to find the variables that influence differences among the groupings. The statistical program Past (Hammer et al., 1999) was used to analyse morphometric and meristic data.

Table 2.2: Morphometric and meristic characters of *Enteromius* used in the current study (reproduced from Chakona et al., 2014).

Character	Description	Acronym
Morphometric measurements		
Standard length	Tip of the snout to the point of flexure of the caudal fin	SL
Pre-dorsal length	Tip of the snout to the origin of the dorsal fin	PDL
Head length	Tip of the snout to the posterior body margin of the operculum	HL
Snout length	Tip of the snout to the anterior bony edge of the orbit	S
Orbit diameter	The greatest bony diameter of the orbit	OD
Post orbit length	Distance between the posterior bony edge of orbit to the posterior bony edge of operculum	PO
Head depth	Maximum depth measured from nape	HD
Body depth	Maximum depth measured from the anterior base of the dorsal fin	BD
Barbel length	From base to tip of barbel	B
Dorsal fin base	Distance between anterior and posterior base of dorsal fin	DB
Dorsal fin height	From anterior base to tip of dorsal fin	DH
Pectoral fin length	From anterior base to tip of pectoral fin	PtL
Pelvic fin length	From anterior base to tip of pelvic fin	PvL
Anal fin base	Distance between anterior and posterior base of anal fin	AfB
Anal fin height	From anterior base to tip of anal fin	AfH
Caudal peduncle length	Distance from posterior base of anal fin the point of flexure of the caudal fin	CPL
Caudal peduncle depth	The least depth of the caudal peduncle	CPD
Pectoral to pelvic fin length	Distance between the posterior margins of the fin bases	PP
Pelvic to anal fin length	Distance between the posterior base of the pelvic fin to the anterior base of the anal fin	PA
Body width	The greatest width just anterior to the origin of the dorsal fin	BW
Meristic counts		
Lateral line scales	Number of scale rows along the lateral line	LL
Lateral line to dorsal fin scales	Number of scale rows between lateral line scale row and anterior base of dorsal fin	LD
Lateral line to pelvic fin scales	Number of scale rows between lateral line scale row and base of pelvic fin	LP
Lateral line to anal fin scales	Number of scale rows between lateral line scale row and anterior base of anal fin	LA
Caudal peduncle scales	Number of scale rows around the caudal peduncle	CP
Predorsal scales	Number of scale rows from the edge of the nape to the anterior base of the dorsal fin	PDS
Unbranched dorsal fin rays	Number of unbranched dorsal fin rays	UdR

Branched dorsal fin rays	Number of branched dorsal fin rays	bDr
Anal fin rays	Includes both simple and branched rays; two last rays counted as one	AR
Pectoral fin rays	Includes both simple and branched rays	PlR
Pelvic fin rays	Includes both simple and branched rays	PcR

Results

Molecular data

The alignment of 65 sequences for 583 nucleotides of cytochrome *b* resulted in 20 haplotypes defined by 87 variable sites. A transitional model (GTR + I) was selected as the most appropriate model for sequence evolution. The results from Bayesian analysis indicated that *E. anoplus* s.l. was monophyletic and the haplotypes were split into two major clades whose relationship was well resolved (Figure 2.3). The first major clade comprised samples from the Gouritz, Olifants-Doring and Orange River systems. There is clear genetic and geographic structuring within this clade as all samples from the Gouritz River system, including the topotypes of *E. anoplus*, were recovered as a distinct clade, with no haplotypes shared with adjacent river systems (Figure 2.3). Similarly, all samples from the Olifants-Doring River system, including the topotypes of *E. cernuus*, formed a distinct clade, with no sharing of haplotypes with any other system. Samples from the Orange River system, which is the type system for *E. oraniensis* also formed a distinct clade comprising samples from two southern draining tributaries, the Stormberg and the Bossielaagtespruit Rivers (Figure 2.3). The second major clade in *E. anoplus* sensu lato comprised samples from coastal draining systems on the south and east coast of South Africa from the Kowie to the Buffalo river system (Figure 2.3). Estimates of the number of candidate species using GMYC ranged from 4 to 5, excluding outgroups (Figure 3). The coalescent model with constant population size and constant clock (CONC), and the Yule model and a relaxed clock (RELA) gave the most conservative estimate of the number of candidate species. The candidate species presented by the constant population size and constant clock (CONC) model were consistent with the clades inferred using the Bayesian analysis (Figure 3). The candidate species presented by the Yule model and a relaxed clock were not in line with the clades inferred using the Bayesian analysis. The first candidate species in this model was distributed in the Gouritz, Olifants and Orange River systems. The second candidate species was from the Great Fish River and Tyolomqa Rivers. The third candidate species was from the Great Fish, Keiskamma, Buffalo and Tyolomqa Rivers. The

fourth candidate species from the Yule model and a relaxed clock was from the Buffalo River. The Yule model and a constant clock model results were largely consistent with the candidate species inferred using the constant population size and constant clock, with the exception of *E. mandelai* clade which was separated into two OTUs (Figure 3). The first OTU contained specimens from the Great Fish River and Tyolomqa Rivers. The second OTU contained specimens from the Great Fish, Keiskamma, Buffalo and Tyolomqa Rivers. The bPTP method gave similar results to the GMYC coalescent model with constant population size and constant clock (CONC) (Figure 2.3). The inter-lineage genetic divergences were 1.97–8.80 % and intra-lineage divergences were 0.0–0.23 % (Table 2.3).

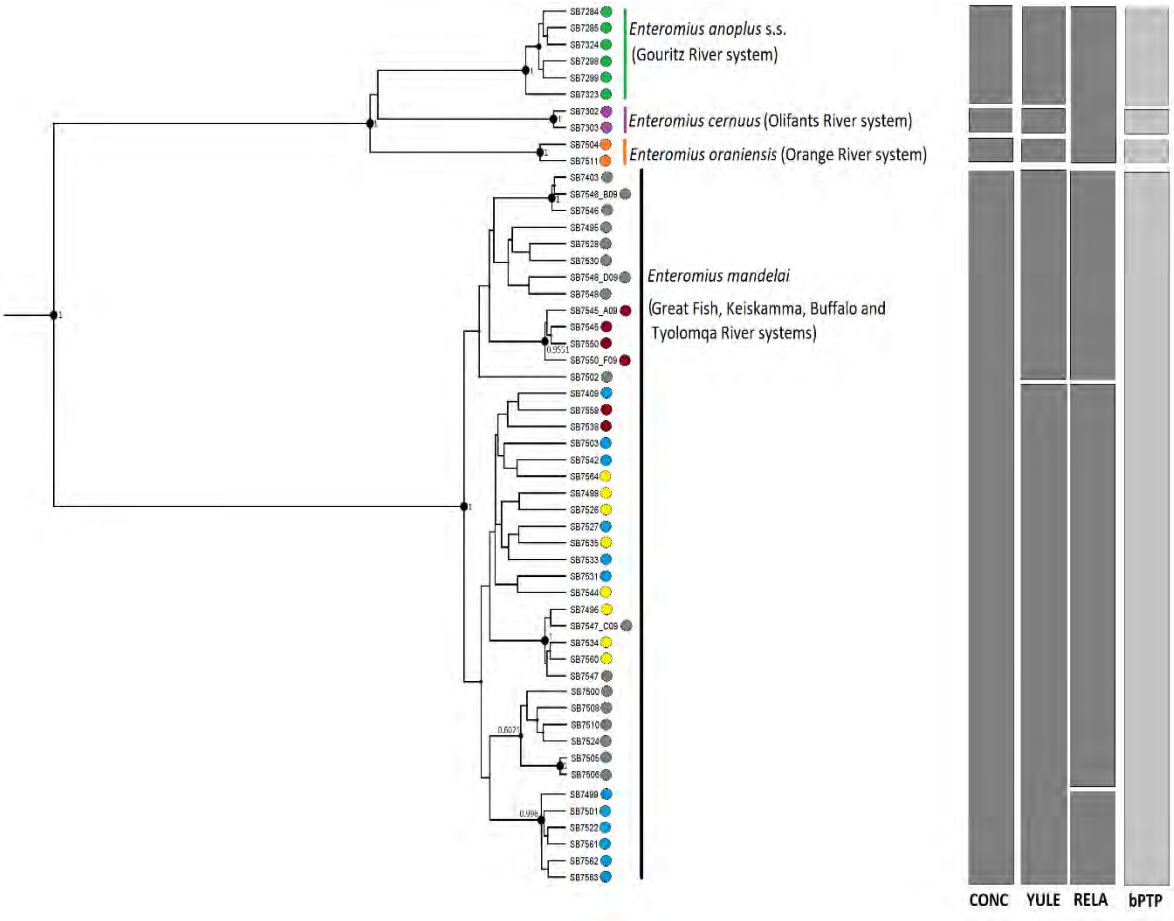


Figure 2.3: Map showing sampling localities for the present study. South African river basins: 1 = Buffels, 2 = Olifants (drains to South Atlantic Ocean), 3 = Berg, 4 = Breede, 5 = Gouritz, 6 = Coastal Drainages, 7 = Gamtoos, 8 = Swartkops, 9 = Sundays, 10 = Bushmans, 11 = Great Fish River, 12 = Keiskamma, 13 = Swart-Kei, 14 = Mzimvubu, 15 = Umgeni, 16 = Tukela, 17 = Mfolozi, 18 = Komati, 19 = Olifants (drains to Limpopo River), 20 = Limpopo, 21 = Vaal, 22 = Orange.

Table 2.3: Ranges of model-corrected genetic divergences (%) between species of *Enteromius anoplus* s.s., *Enteromius cernuus*, *Enteromius oraniensis*, and *E. mandelai*.

	***E. anoplus* s.s.**	***E. cernuus***	***E. oraniensis***	***E. mandelai***
***E. anoplus* s.s.**	0.06 (0 - 0.20)			
E. cernuus	1.97 (1.81 - 2.01)	0.0		
E. oraniensis	3.05 (2.59 - 3.24)	2.24 (2.00 - 2.40)	0.23 (0.20 -0.39)	
E. mandelai	8.13 (7.47 - 8.88)	7.45 (7.04 - 8.90)	8.8 (8.13 - 9.80)	0.51 (0 - 4.01)

Morphological data

The present study determined whether there were consistent morphological differences between the MOTUs identified from the genetic analyses above. As the lineages are allopatrically distributed, the specimens were divided into four groups consistent with the geographic ranges of these lineages. The first PCA analysis performed on 18 morphometric characters showed broadoverlap among the four MOTUs, indicating that they could not be separated based on the morphological characters that were considered for the present study (Figure 2.4). The first principal component axis (PCI) explained 89.80%, PCII accounted for 5.92% of the observed variation among the lineages, and PCIII accounted for 4.28% of the observed variation among the lineages (Table 2.4). The first PCA was not included as it is indicative of size differences. The most strongly loading positive variables on PCII are barbel length and predorsal length (Table 2.4). The most strongly loading negative values on this axis are pectoral to pelvic fin length. The most strongly loading positive variables in PCIII are barbel length, body depth and pectoral to pelvic fin length whereas, the most strongly loading negative values on this axis are caudal peduncle length, pectoral to pelvic fin length, body width and post orbit length. These lineages could be separated based on barbel length (Figure 2.5). Specimens from the Orange River system have inconspicuous barbels (range 0.41–1.16 mm; average 0.73 mm), whilst specimens from the Olifants River systems have distinctly long barbels (Figure 2.5). However, scatter plots for pre dorsal length, pectoral to pelvic fin length

and body depth revealed that these morphometric characters are taxonomically uninformative to differentiate among the four lineages (Figure 2.6).

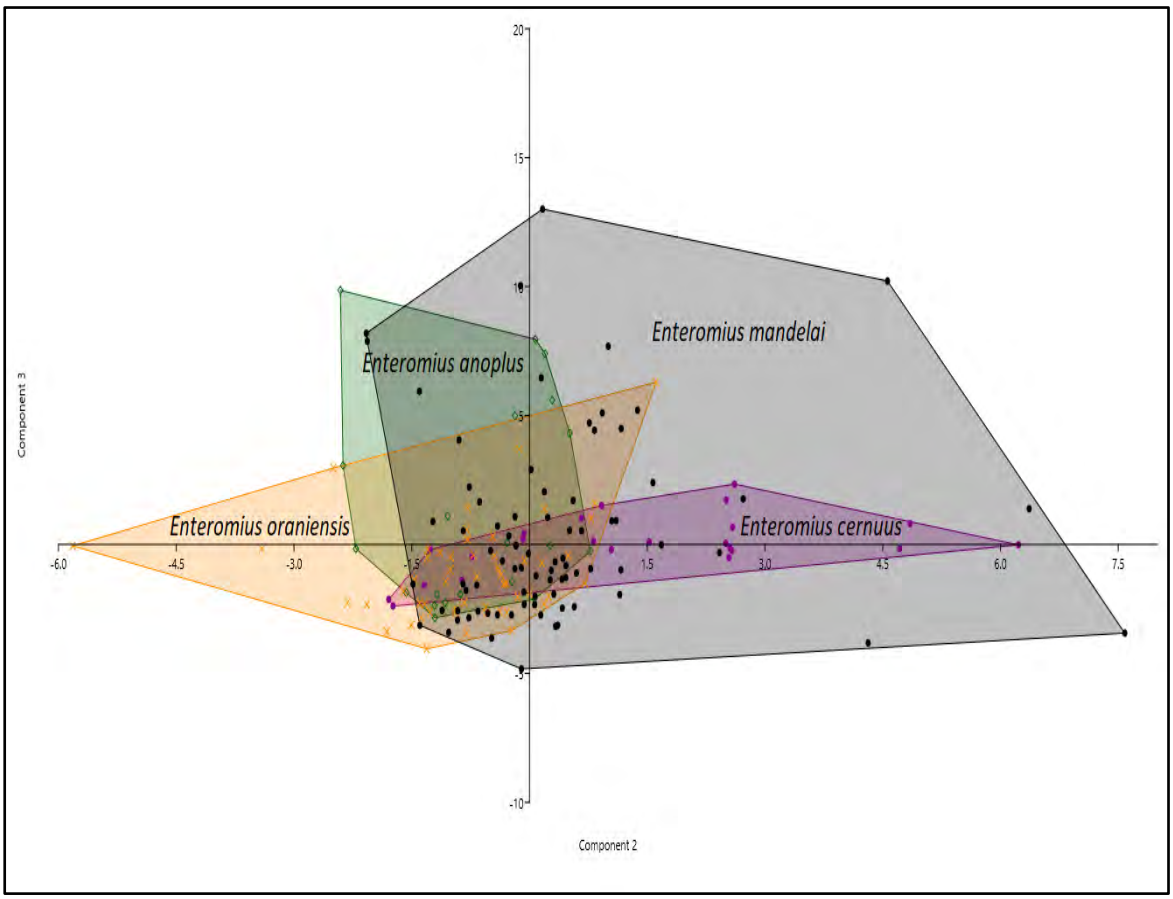


Figure 2.4: Scatter plot of PC1 against PC2 for a PCA carried out on 18 morphometric characters for 175 specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.*

Table 2.4: Summary of Principal Components and their percentage variance and factor loadings for the first two principal component (PC) axes carried out on morphometric characters from specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *Enteromius mandelai*. The most important factor loadings are in bold.

Principal component	**Eigenvalue**	**% Variance**	
1	7.97	89.80	
2	0.53	5.92	
3	0.38	4.28	
Morphometric characters	**Factor loadings**		
	PC 1	PC 2	PC 3
head length	**0.31128**	-0.05926	0.03838
head depth	0.098464	0.28706	0.056421
snout length	0.1558	0.16835	-0.00386
orbit diameter	0.099428	-0.04421	-0.02058
post orbit length	0.11635	0.29125	**-0.33568**
barbel length	-0.01307	**0.51737**	**0.3184**
pre dorsal length	**0.53287**	0.37205	0.13488
body depth	0.23118	-0.28003	**0.43893**
dorsal fin base	0.12092	-0.12669	-0.05751
dorsal fin height	0.21994	-0.03711	-0.05262
pectoral fin length	**0.33528**	0.082227	-0.0056
pelvic fin length	0.15924	0.11954	-0.00223
anal fin base	0.085862	0.096221	0.17548
anal fin height	0.1948	-0.06841	0.048224
caudal peduncle length	0.25741	-0.14488	**-0.54542**
caudal peduncle depth	0.13623	0.040036	-0.00236
pectoral to pelvic fin length	0.26227	-0.44554	**0.33553**
pelvic to anal fin length	0.24078	-0.18923	-0.08399
body width	0.22469	-0.07167	**-0.3371**

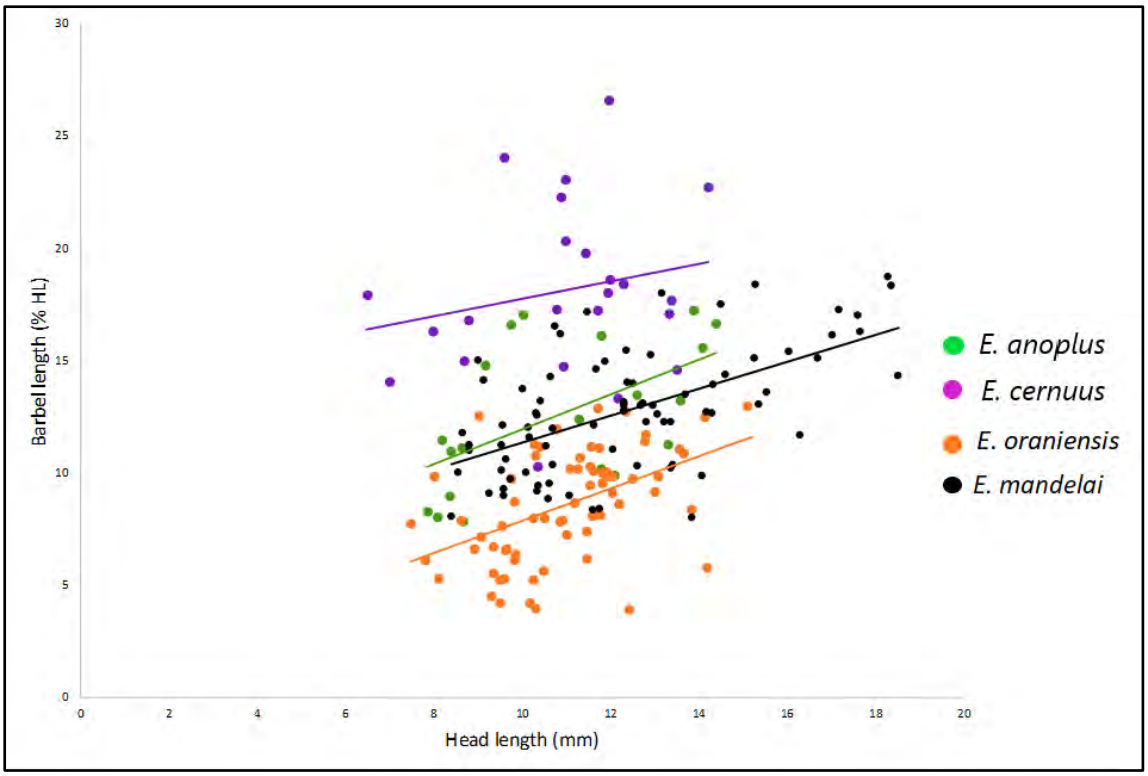


Figure 2.5: The morphometric character (barbel length) identified by the second principal components axis as contributing the most to variation among *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.*

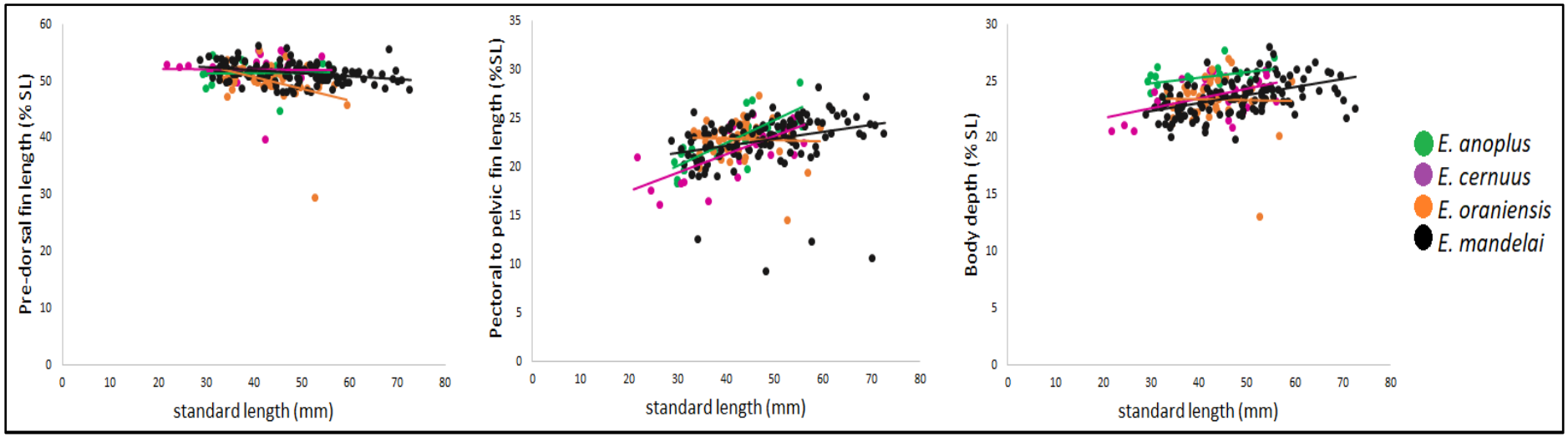


Figure 2.6: The positive morphometric characters identified by the second and third principal component axis (a) predorsal fin length (b) pectoral to pelvic fin length (c) body depth as contributing the most to variation among *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.*

The second PCA performed on nine meristic characters also showed considerable overlap among the four MOTUs (Figure 7). PCI, primarily contrasting differences in the number of pored lateral line scales, explained 98% of the total variation (Table 2.5). The scatterplot of the number of lateral line scales against standard length show that the Eastern Cape and the Olifants lineages can be clearly separated from the Gouritz and the Orange lineages based on differences in the number of pored lateral line scales (Figure 8). Specimens of the Eastern Cape lineage have a complete lateral line with 20–37 (mode 35) pored scales, with the exception of only three specimens from the 81 that had less than 15 pored lateral line scales (Figure 2.8). Specimens from the Olifants River system also have a complete lateral line with 27–35 (mode 33) pored scales. In contrast, both the Gouritz and Orange lineages have incomplete lateral lines, with 2–24 (mode 8) pored scales. Of the 20 specimens examined from the Gouritz River system, 17 of them had 3–24 pored lateral line scales, with only three individuals having 32–35 pored lateral line scales. Also, a high proportion (90%) of the 52 examined specimens from the Orange River system had 2–15 (mode 12) pored lateral line scales, with only two specimens having 31 and 32 pored lateral line scales (Figure 2.8). The lineages could also be separated based on the number of circumpenducular scales (Figure 2.9). Specimens from the Olifants system have fewer lower number of circumpenducular scales (range 10–12, mode 12) compared to the other three lineages; Gouritz (range 12–16, mode 16), Eastern Cape (range 14–16, mode14) and Orange (range 12–16, mode 14) (Figure 2.9).

The present study revealed allopatric genetic divergence and consistent differences in barbel length and scale counts, supporting the designation of the four lineages identified within *E. anoplus* s.l. as distinct taxonomic entities. In the present study, *E. anoplus* s.s. (the Gouritz lineage) is redescribed, *E. cernuus* (the Olifants lineage) and *E. oraniensis* (the Orange lineage) are revalidated and redescribed, and a new species to science, *E. mandelai* sp. nov. (the Eastern Cape lineage) is formally described.

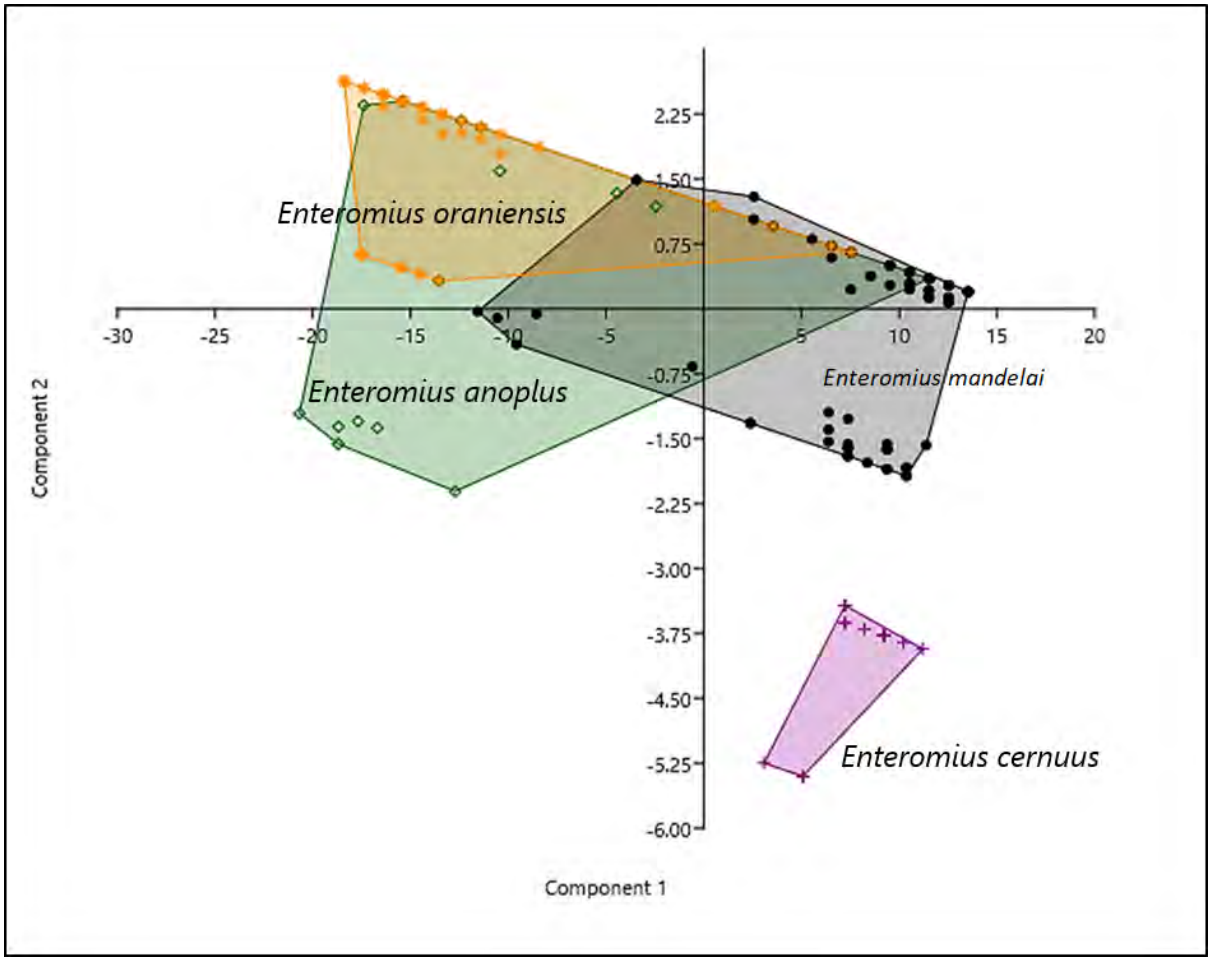


Figure 2.7: Scatter plot of PC1 against PC2 for a PCA carried out on nine raw meristic characters for specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* The four species show considerable overlap in meristic characters.

Table 2.5: Factor loadings for the first two principal component axes of a PCA carried out on nine meristic characters from 71 specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.* The most important factor loadings are in bold.

	PC 1	**PC 2**
Lateral line scales	**0.99**	-0.08
lateral line to dorsal fin origin	0.01	0.20
lateral line to pelvic fin origin	0.00	0.09
lateral line to anal fin origin	0.01	0.13
circumpenducular scales	0.08	**0.96**

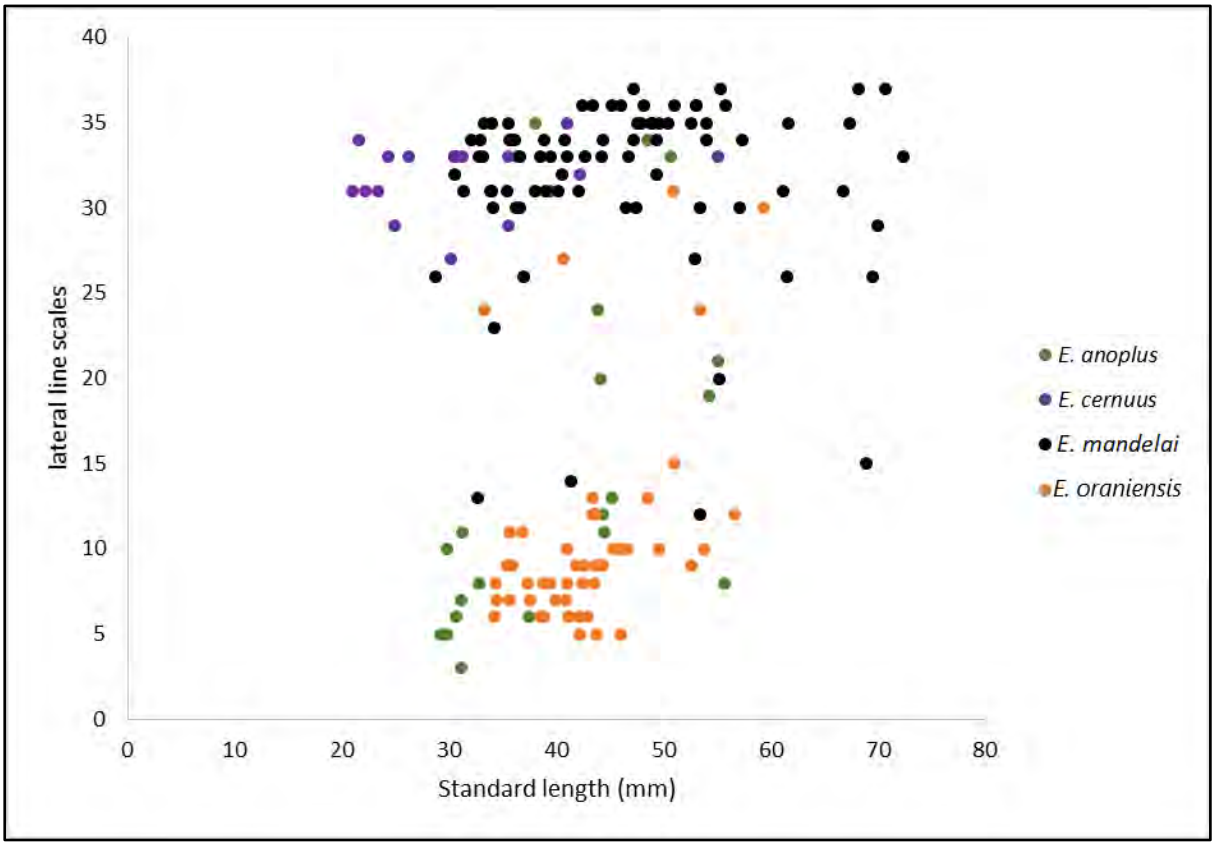


Figure 2.8: Scatter plot of number of lateral line scales against standard length for specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.*

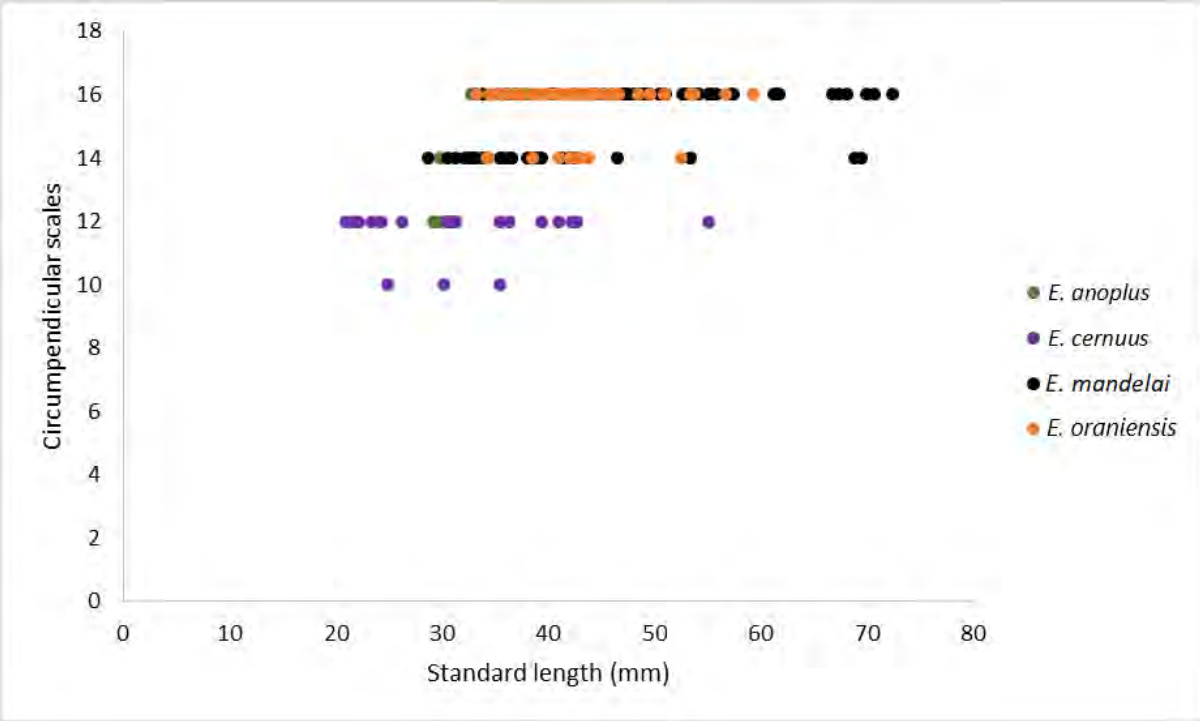


Figure 2.9: Scatter plot of number of circumpendicular scales against standard length for specimens of *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *E. mandelai.*

The present study revealed allopatric genetic divergence and consistent differences in barbel length and scale counts, supporting the designation of the four lineages identified within *E. anoplus* s.l. as distinct taxonomic entities. In the present study, *E. anoplus* s.s. (the Gouritz lineage) is redescribed, *E. cernuus* (the Olifants lineage) and *E. oraniensis* (the Orange lineage) are revalidated and redescribed, and a new species to science, *E. mandelai* sp. nov. (the Eastern Cape lineage) is formally described.

Taxonomic accounts

***Enteromius anoplus* (Weber, 1897)**

Figures 2.10a-c

Chubbyhead barb (English), Dikkop-ghieliemientjie (Afrikaans)

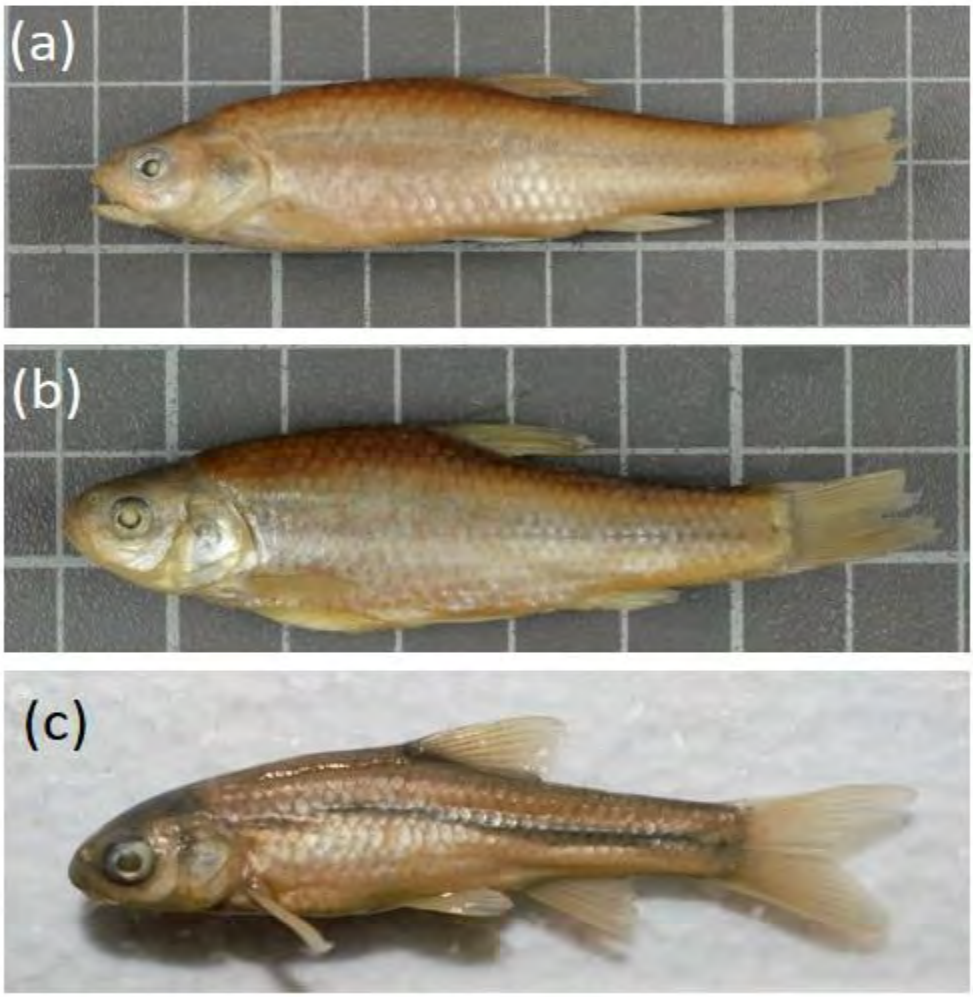


Figure 2.10: General body features and alcohol preserved coloration of *Enteromius anoplus* s.s. (a) lectotype (ZMA 100995), 95 mm standard length, from the Buffels River by Laingsburg, Gouritz River system (b) paralectotype (ZMA 100995a), 75 mm standard length, from the Buffels River by Laingsburg, Gouritz River system (c) unsexed sub-adult topotype (SAIAB 200353), 37.8 mm standard length, from the Buffels River, Gouritz River system.

Barbus anoplus Weber, 1897; Boulenger, 1905; Boulenger, 1911; Gilchrist & Thompson, 1913; Boulenger, 1916; Barnard, 1938; Barnard 1943; Barnard, 1947; Groenewald, 1958; Crass, 1960; Farquharson, 1962; Jubb, 1963; Matthes, 1964; Jubb, 1965; Jubb, 1966; Jubb, 1967; Greenwood & Jubb, 1967; Jackson, 1973; Gaigher & Pott, 1973; Gaigher, 1975; Fowler, 1976; Skelton, 1976; Cambray & Jubb, 1977; Skelton & Cambray, 1981; Nijssen et al., 1982; Lévêque and Daget, 1984; Ibarra & Stewart, 1987; Skelton, 1990; Skelton, 1993; Farm, 2000; Skelton, 2001; Valid as *Enteromius anoplus* -- Yang et al., 2015; Hayes & Armbruster, 2017.

Barbus karkensis Gilchrist and Thompson, 1913; Barnard 1943; Jubb 1963 (synonymy with *Barbus anoplus*) Jubb, 1967; Fowler, 1976

Barbus cernuus Barnard, 1938; Fowler, 1976

Barbus anoplus typica Barnard, 1943

Barbus anoplus cernuus Barnard, 1943

Barbus anoplus oraniensis Barnard, 1943; Fowler, 1976

Barbus anoplus anoplus Fowler, 1976

Materials examined

Lectotype. Morphometric and meristic data for the lectotype (ZMA 100995) of *Enteromius anoplus* were obtained from the Institute of Taxonomic Zoology (Zoologisch Museum), University of Amsterdam, Netherlands (now the Naturalis Biodiversity Centre (NCB) Naturalis, *Nederlands Centrum voor Biodiversiteit Naturalis*). ZMA 100995 95 mm SL.

Paralectotypes: Morphometric and meristic data for paralectotypes (ZMA 100995a, n = 148) of *Enteromius anoplus* were obtained from the Institute of Taxonomic Zoology (Zoologisch Museum), University of Amsterdam, Netherlands (now the Naturalis Biodiversity Centre (NCB) Naturalis, *Nederlands Centrum voor Biodiversiteit Naturalis*). 75 mm SL unsexed, measurements and pictures provided by Esther Dondorp.

Topotypic specimens. SAIAB 130195, 10 unsexed (43.9–55.6 mm SL), Laingsburg, Seweweeeksport Road, Buffels River, Gouritz River system, -33.955, 20.35917, collected by S. Thorne, 3 November 1986; SAIAB 200353, 10 unsexed (29.2–38.1 mm SL), Laingsburg, about 1km above the N1 bridge, Buffels River, Gouritz River system, -33.18994, 20.8585, collected by Roger Bills, V. Bills, 20 August 2014.

Etymology. *anoplus* – unarmed, allusion not explained, perhaps referring to unserrated last dorsal fin ray.

Diagnosis. Soft-rayed minnow with incomplete lateral line, rarely more than 24 pored scales (mode 8), barbels well developed, reaching but not surpassing 1.8 mm.

Comparison with soft-rayed congeners in southern Africa. *Enteromius anoplus* is readily differentiated from *E. mandelai* and *E. cernuus* by having an incomplete lateral line. Possession of a longer pair of barbels separates *E. anoplus* (average 1.61 mm) from *E. oraniensis* (average 0.92 mm) which has inconspicuous barbels. Separated from *E. amatolicus* (Skelton, 1990), *E. motebensis* (Steindachner, 1894) and *E. gurneyi* (Gunther, 1868) by absence of tubercles on the head during breeding season (*vs* development of nuptial tubercles on either snout, lower jaw, forehead or sides of head in the other species). Further separated from both *E. gurneyi* and *E. motebensis* by possession of a single pair of barbels (*vs* two pairs of barbels in the other two species) and from *E. amatolicus* by possession of short barbels that do not surpass the vertical through the middle of the eye (*vs* distinctly long barbels that surpass the vertical through the posterior edge of the eye). Differentiated from *E. unitaeniatus* (Gunther, 1867), *E. viviparus* (Weber, 1897), *E. lineomaculatus* (Boulenger, 1903), and *E. barotseensis* (Pellegrin, 1920) by the lack of distinct lateral line chevron markings and possession of a single pair of relatively short maxillary barbels not exceeding middle portion of eye (*vs* the existence on the lateral line of conspicuous chevron markings and the existence of two pairs of long and prominent barbels exceeding middle portion of the eye in the other four species). Distinguished from *E. treurensis* (Groenewald, 1958), *E. breviceps* (Trewavas, 1936), *E. annectens* (Gilchrist & Thompson, 1913), *E. pallidus* (Smith, 1841), *E. brevipinnis* (Jubb, 1966), *E. radiatus* (Peters, 1853) and *E. bifrenatus* (Fowler, 1935) by the possession of one pair of oral barbels (compared to the presence in the other species of two pairs of long barbels). Differentiated from *E. neefi* (Greenwood, 1962) and *E. pallidus* (Smith, 1841) by the absence of two pairs of oral barbels and the absence of dispersed black spots on the body (vs the presence of two pairs of oral barbels and black spots on the body in these three species). Differentiated from *E. fasciolatus* (Gunther, 1868) by the absence of prominent vertical bars along the body (*vs*. the presence in *E. fasciolatus*).

Description. Morphometric and meristic data summarised in Table 2.6 are based on the lectotype, 1 paralectotype and 20 recently collected specimens (29.17–55.64 mm SL) from the Buffels River, Gouritz River System. General body shape and coloration are shown in Figure 2.10.

Body fusiform, moderately laterally compressed. Dorsal profile convex, ventral profile from tip of the snout to origin of pelvic fins straight. Greatest body depth at the anterior base of dorsal fin. Post-dorsal profile gently tapers to base of caudal fin. Head relatively small, length subequal to body depth. Snout short, bluntly rounded, equal to or slightly longer than eye diameter. Mouth small, terminal, with single pair of barbels. Barbels do not surpass vertical through centre of eye. Eyes relatively large, round, situated dorsolaterally, nearer to tip of snout than distal margin of operculum.

Scales. Scales moderately large, radiately striated; rarely greater than 24 pored lateral line scales (mode 8). Lateral line incomplete, only three of the 20 specimens examined had a complete lateral line. Circumpenducular scales 12–16 (mode 16); pre-dorsal scale rows 13–14 (mode 13); scale rows between the origin of the dorsal fin and the lateral line 3–4 (mode 4); scale rows between the origin of the pelvic fin and the lateral line 3–4 (mode 4); scale rows between lateral line and anal fin origin 3–4 (mode 4). Scales between posterior edge of head and dorsal fin embedded and smaller than flank scales.

Fins. Dorsal fin situated almost in the centre of the body, with 3 unbranched rays and 7 branched rays. Pectoral fins fan-shaped, posterior edges gently rounded, not reaching pelvic fin origin. Pelvic fin with 8 rays, origin slightly in front of dorsal fin origin. Tip of adepressed pelvic fin does not reach anal fin origin. Anal fin with 3 simple rays and 5 branched rays; distal margin almost straight; origin closer to pelvic fin origin than to caudal fin base. Caudal fin forked, lobes gently rounded with moderately pointed tips.

Colouration (live and fresh specimens). Metallic silvery, greenish or greyish above, with a more or less pronounced yellow or golden tinge in males, more greyish in females. Inconspicuous caudal spot present.

Colouration (preserved). Greyish-brown on the flank, white ventrally. An indistinctive lateral stripe present in most specimens, terminating in a dark caudal spot.

Reproduction. No specific studies have focussed on the breeding biology of *Enteromius anoplus* s.s. from the Gouritz River system, but it is likely to breed in summer following the onset of the rains as reported for the other congeners (Cambray & Bruton, 1984; Cambray & Bruton, 1985; Cambray et al., 1978).

Distribution. Endemic to the Gouritz River system where it occurs in the foothill and mainstem sections of the rivers as well as man-made impoundments (Figure 2.1).

Habitat and ecology. Tolerant of turbid and eutrophic habitats from foothill streams to mainstem sections of major rivers and man-made impoundments. This species does not penetrate into upper reaches of mountain tributary streams with oligotrophic water. Although no studies have specifically focussed on the feeding ecology of this species, it is likely to be omnivorous, as reported for other congeners, mainly feeding on insects, zooplankton, seeds, green algae and diatoms (Cambray & Bruton, 1985; Cambray, 1983a).

***Enteromius cernuus* (Barnard, 1938)**

Figures 2.11a-c

Barbus cernuus Barnard, 1938; Fowler, 1976; *Barbus anoplus cernuus* Barnard, 1943; Synonym of *Barbus anoplus* Weber 1897- Lévêque and Daget 1894; Synonym of *Enteromius anoplus* (Weber 1897) –Hayes and Armbruster 2017.

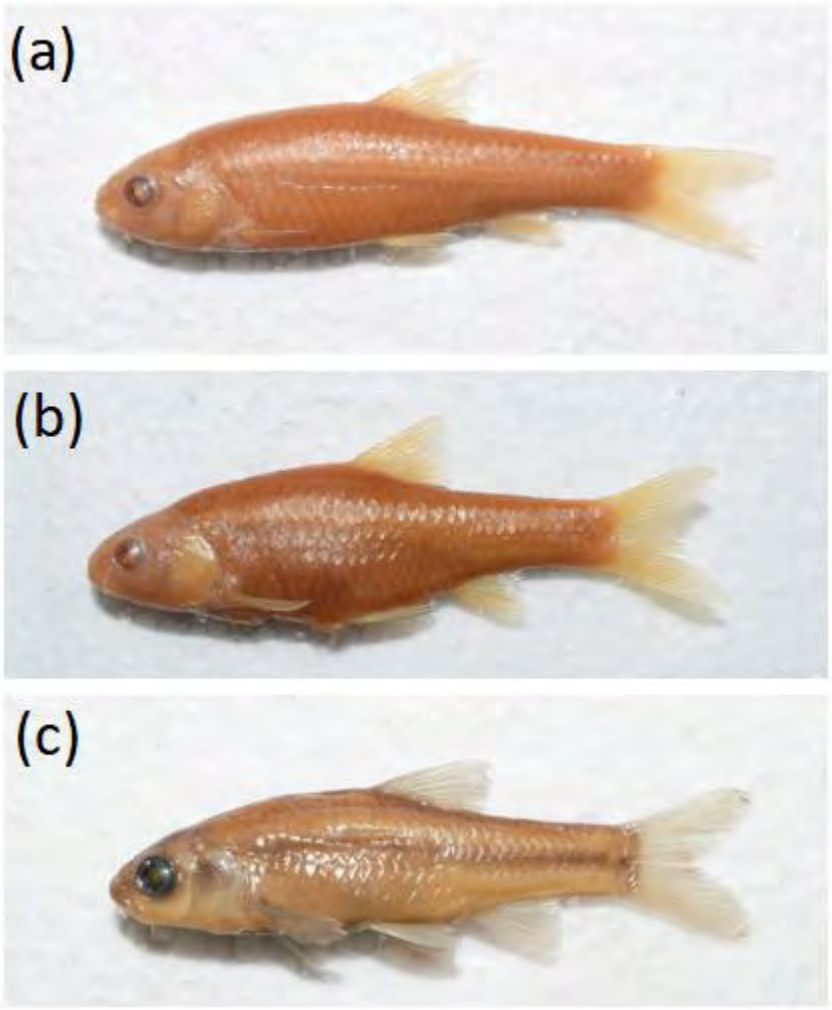


Figure 2.11: General body features and alcohol preserved coloration of *Enteromius cernuus* (a) lectotype (SAIAB 209738), 52.6 mm standard length, from the Olifants River, Clanwilliam Division (b) paralectotype (SAIAB 135553), 54.4 mm standard length, from Troetroe River, Olifants River system (c) unsexed adult topotype (RUSI 65417), 42.5 mm standard length, from the Breekrans River, Olifants River, Olifants River system.

Material examined

Lectotype (see below)**.** (Figures 2.11a): In compliance with article 74.7.1-3, a lectotype is hereby designated from syntypes to become the unique bearer of the name *E. cernuus*. The lectotype was chosen from syntypes identified by Barnard (1938) stored at the South African Institute for Aquatic Biodiversity (SAIAB 135479, SAIAB 135483, SAIAB 135553). The lectotype was deposited at

the South African Institute for Aquatic Biodiversity (catalogue no SAIAB 209738) for future reference. The lectotype is an adult, 52.6 mm standard length (SL), collected on April 1937 by Barnard, K.H., Thorne, C.W. and Harrison, A.C. at Olifants River, Clanwilliam in the Western Cape Province of South Africa.

Paralectotypes. In compliance with article 74.1.3 the designation of a lectotype permanently deprives all other specimens that were formerly syntypes of that nominal taxon of the status syntype; those specimens then become paralectotypes. Z18759/SAIAB 135479 (n = 268), collected on April 1937 by Barnard, K.H., Thorne, C.W. and Harrison, A.C. at Olifants River, Clanwilliam in the Western Cape Province of South Africa. Z18765/SAIAB 135483 (n = 64), collected on 11 April 1937 by Barnard, K.H., Thorne, C.W. and Harrison, A.C. at Boontjies River, Citrusdal, Olifants River System, in the Western Cape Province of South Africa. Z19019 /SAIAB 135553 (n = 240) collected on April 1937 by Barnard, K.H. and Thorne, C.W. at Vanrynsdorp, Troetroe River, Olifants River System, in the Western Cape Province of South Africa.

Additional material. RUSI 65417, 8 unsexed (21.6–42.7 mm SL), Breekrans River, below hut, Olifants River, Olifants River system, 32.5594, 19.2992, collected by Bills R, Cunningham M.M., Swartz. E, 23 March 2002.

Etymology. With the face turned towards the earth, inclined forwards, stooping or bowing forwards.

Lectotype designation for *Enteromius cernuus* (Barnard, 1938). The small African barbs are abundant across Africa. With nearly 350 recognised species in the genus *Enteromius* (Eschmeyer, 2019), the recent works suggest that the actual number of species is higher (Mipounga *et al*., 2019; Schmidt *et al*., 2018, 2019). Taxonomic revision is needed for a number of species in the genus *Enteromius*. This taxonomic revision is hindered by factors such as the similar body morphology of species and many of the described species have only a few specimens available for study. It is difficult to answer the broader questions of the generic status and relationships within the genus *Enteromius* without type specimens. Taxonomic revision is required for species with wide geographic ranges, such as the case of *E. anoplus*. There are currently 572 syntypes for *E. cernuus*.

In order to facilitate the ongoing taxonomic revision of the *E. anoplus* complex in South Africa, the designation of a lectotype (Art. 74.1 of ICZN) is therefore necessary.

Diagnosis. Lateral line complete (28–33 pored scales, mode 31), circumpendicular scales (10–12, mode 12), single pair of conspicuous oral barbels which surpass the vertical through the middle of the eye.

Comparison with congeners in southern Africa. Possession of a complete lateral line separates *E. cernuus* from *E. oraniensis* and *E. anoplus*. *Enteromius cernuus* can be differentiated from *E. mandelai* by the possession of relatively long barbels (average 2.1 mm vs 1.66 mm) and a lower number of circumpendicular scales (10–12, mode 12 vs 12–16, mode 14). Separated from *E. amatolicus* (Skelton, 1990), *E. motebensis* (Steindachner, 1894), and *E. gurneyi* (Gunther, 1868) by absence of tubercles on the head during breeding season (*vs* development nuptial tubercles on either snout, lower jaw, forehead or sides of head in the other species). Further separated from both *E. gurneyi* and *E. motebensis* by possession of a single pair of barbels (vs two pairs of barbels in the other two species) and from *E. amatolicus* by possession of short barbels that do not surpass vertical through middle of eye (vs distinctly long barbels that surpass vertical through posterior edge of the eye). Differentiated from *E. unitaeniatus* (Gunther, 1867)*, E. viviparus* (Weber, 1897), *E. lineomaculatus* (Boulenger, 1903)*,* and *E. barotseensis* (Pellegrin, 1920) by lack of distinct chevron markings on lateral line and possession of a single pair of relatively short maxillary barbels not exceeding middle portion of eye (*vs* conspicuous chevron markings on lateral line and possession of two pairs of long and prominent barbels exceeding middle portion of eye). Distinguished from *E. treurensis* (Groenewald, 1958), *E. breviceps* (Trewavas, 1936), *E. annectens* (Gilchrist & Thompson, 1913), *E. pallidus* (Smith, 1841), *E. brevipinnis* (Jubb, 1966), *E. radiatus* (Peters, 1853) and *E. bifrenatus* (Fowler, 1935) by the possession of one pair of oral barbels (vs the presence in other species of two pairs of long barbels). Differentiated from *E. neefi* (Greenwood, 1962) and *E. pallidus* (Smith, 1841) by the absence of two pairs of oral barbels and the absence of dispersed black spots on the body (vs the presence of two pairs of oral barbels and black spots on the body in these three species). Differentiated from *E. fasciolatus* (Gunther, 1868) by the absence of prominent vertical bars along the body (*vs*. the presence in *E. fasciolatus*).

Description. Morphometric and meristic data summarised in Table 2.6 are based on the lectotype and 15 paralectotypes (21–35 mm SL) and 8 topotypes (21.62–42.70 mm SL) from the Olifants River system. General body shape and coloration are shown in Figure 2.11.

Body fusiform, moderately laterally compressed. Dorsal profile convex, ventral profile from tip of the snout to origin of pelvic fins straight. Greatest body depth at the anterior base of dorsal fin. Post-dorsal profile gently tapers to base of caudal fin. Head relatively small, length subequal to body depth. Snout short, bluntly rounded, equal to or slightly longer than eye diameter. Mouth small, terminal, with single pair of posterior barbels. Barbels do not surpass middle portion of the eye. Eyes relatively large, round, situated dorsolaterally, nearer to tip of snout than distal margin of operculum.

Scales. Scales moderately large, radiately striated; 27–35 (mode 33) scale rows along the lateral line to end of hypural plate. Circumpendicular scales 10–12 (mode 12); pre-dorsal scale rows, 5–6 (mode 6); scale rows between the origin of the dorsal fin and the lateral line 3–4 (mode 4); scale rows between the origin of the pelvic fin and the lateral line 3–4 (mode 4); scale rows between lateral line and anal fin origin 3–4 (mode 4). Scales between posterior edge of head and dorsal fin embedded and smaller than flank scales.

Fins. Dorsal fin situated almost in the centre of the body, with 3 unbranched and 7 branched rays. Pectoral fins fan-shaped, with 14-17 rays, not reaching pelvic fin origin. Pelvic fin with 8 rays, origin slightly in front of dorsal fin origin. Tip of adepressed pelvic fin does not reach anal fin origin. Anal fin with 3 simple rays and 5 branched rays; distal margin almost straight; origin closer to pelvic fin origin than to caudal fin base. Caudal fin forked; lobes gently rounded with moderately pointed tips.

Colouration (live and fresh specimens). Silvery, slightly brownish above, with bright yellow or golden tinge in males, and a pinkish violet sheen in females

Colouration (preserved). More or less conspicuous dark lateral stripe, ending usually in a small spot at the end of caudal peduncle. Fins pale, without any red patches or any pinkish suffusion.

Distribution. Endemic to the Olifants River system where it has been recorded from the mainstem sections of the Olifants River and three of its tributaries, the Boontjes, Doorn and Troetroe rivers. There are no records of this species from the Doring catchment of the Olifants-Doring River system (Figure 2.1).

Habitat and ecology. Inhabits perennial streams with oligotrophic water as they mostly drain over Table Mountain sandstone.

Reproduction: Unknown, but spawning possibly occurs around November-December, based on observations of redfin minnows (*Pseudobarbus*) that are also endemic to the Cape Fold Ecoregion which is a winter rainfall period. Further studies are however required to determine the breeding biology of *E. cernuus*, because studies of *E. oraniensis* which is endemic to the Orange River system, within a summer rainfall region, indicate that spawning coincides with the onset of the rains in summer (Cambray & Bruton, 1984;Skelton, 2001).

***Enteromius oraniensis* (Barnard, 1943)**

Figures 2.12a-c.

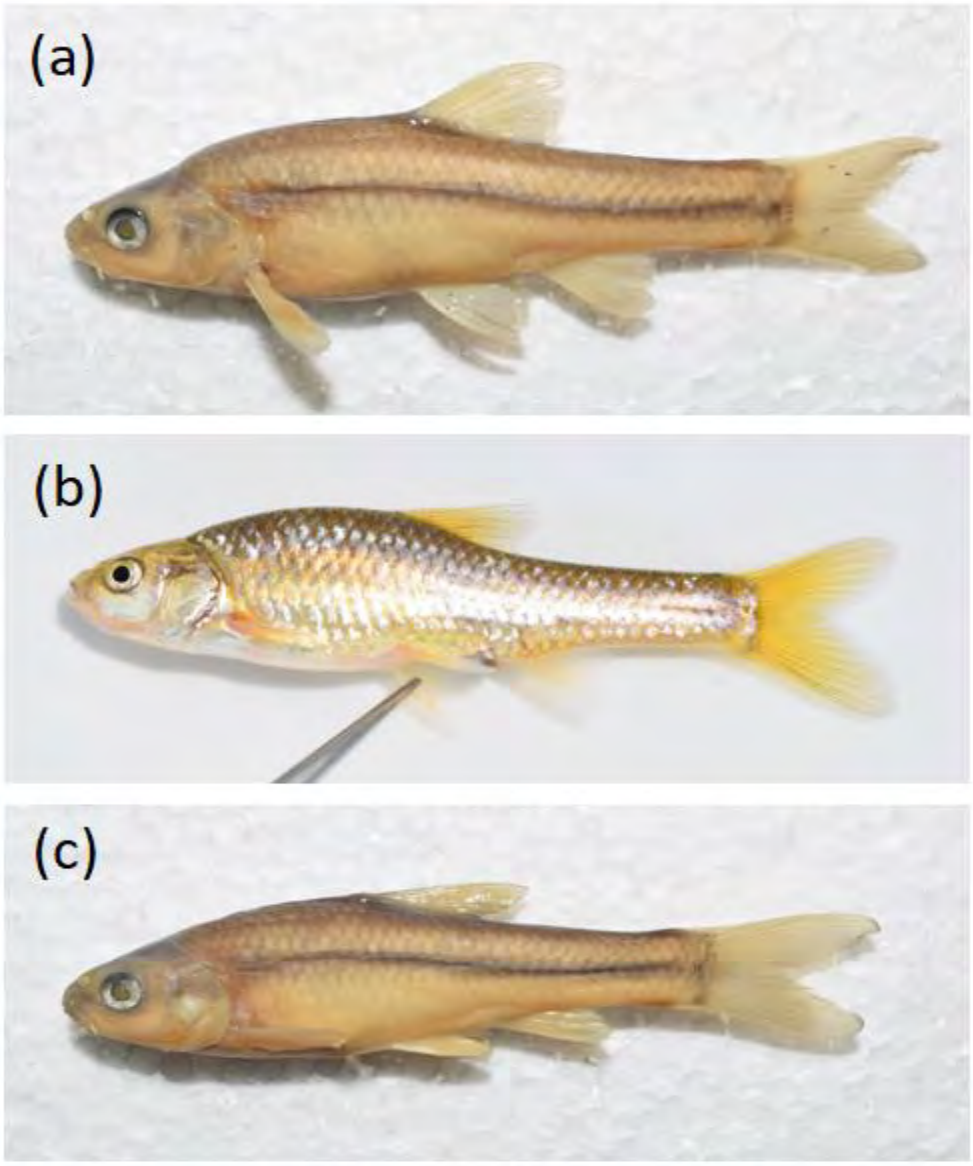


Figure 2.12: General body features and alcohol preserved colouration of *Enteromius oraniensis* (a) neotype (SAIAB 209735), 53 mm standard length, Bossieslangspruit River, Orange River system (b) paratype (SAIAB 209736), live coloration of mature breeding male, Bossieslangspruit River, Orange River system (not examined) (c) unsexed adult paratype (SAIAB 209737), 50.1 mm standard length, Stormberg River, Orange River system.

Barbus anoplus oraniensis Barnard, 1943;

Synonym of *Barbus anoplus* Weber 1897- Lévêque and Daget 1894;

Synonym of *Enteromius anoplus* (Weber 1897) – Hayes and Armbruster 2017

Material examined. Neotype. Figures 12a-c, 13a-c. A neotype was deposited at the South African Institute for Aquatic Biodiversity (catalogue no. SAIAB 209735) in accordance with Article 75.3.7 for future reference. The neotype is 53 mm SL, collected on 30 August 2019 by A Chakona, W Kadye, M Kambikambi and N Matomela using an electrofisher, Bossieslaagtespruit River, Orange River system, -32.7763, 26.422.

Additional material. RUSI 65398, 13 unsexed (27.4–49.1 mm SL), Sak River near Loxton, Sak River, Orange River System, - 31.2544, 22.1286, collected by Bills R, Naran D, 12 May 2002; SAIAB 129093, 5 unsexed (36.9–55.3 mm SL), Karoo National Park, Beaufort West District, Sak River, Orange River system, -32.25, 22.16667, collected by Skelton Paul, Cecil Nonqane; SAIAB 209735, Field no: MK19AL16, 20 unsexed (41.2–59.3 mm SL), Bossieslaagtespruit River, Orange River system, -32.79982, 27.07691, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 30 August 2019; Field no: MK19AL17, 13 unsexed (33.2–56.7 mm SL), Stormberg River, Orange River system, -32.7763, 26.422, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 30 August 2019; SAIAB 209737, Field no: MK19AL18, 20 unsexed (34.5–53.4 mm SL), Stormberg River, Orange River system, -30.8583, 26.4184, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 30 August 2019.

Etymology. The specific epithet, *oraniensis*, is a noun in apposition, in reference to the Orange River system to which the species is endemic.

Neotype designation for *Enteromius oraniensis* (Barnard, 1943).

Taxonomic revision is needed for a number of species in the genus *Enteromius* (Van Ginneken *et al*., 2017; Schmidt *et al*., 2019). This taxonomic revision is hindered by factors such as the absence of extant type material for species and unclear type locality details. It is difficult to answer the broader questions of the generic status and relationships within the genus *Enteromius* without

primary type specimens. Barnard (1943) based his description on 1300 syntypes, all of which are lost. As no extant types exist for this species, to facilitate ongoing taxonomic revision of the *E. anoplus* complex, there is an unequivocal need for a neotype to be designated (Art. 75.3 of ICZN).

All qualifying requirements (Art. 75.3 of ICZN) are satisfied. The neotype is designated as part of a revisionary work to enhance the stability of nomenclature and clarify the species taxonomic status (Art. 75.3.1). In 1943, Barnard described *Enteromius oraniensis.* He based his description on 1300 specimens from various rivers in the Orange and Vaal River system. These rivers included the Laken Vlei River, Merriman (Richmond Division), a tributary of the Orange River, collected by Barnard K.H., Boonstra L.D., and Thorne C.W., April 1939; Zak River, Williston, Calvinia Division, collected by R Smithers, March 1939 and by Barnard K.H., Boonstra L.D., and Thorne C.W., April 1939. All the following collected by Thorne C.W., Boonstra L.D., Hesse A.J., October – November 1939, Dry Hartz River at Taungs, Vaal River at Warrenton, Modder River and Tributaries near Boshof, Bloemfontein, and Dewetsdorp, Tributary of Caledon River at Smithfield, Stormberg River near Burghersdorp, Brandspruit north of Steynsburg, Orlogspoort River south-east of Colesberg and Sea-Cow (Seekoe) River near Hanover. No mention of a holotype or equivalent was provided in the text. In accordance with Article 75.3.4 of the ICZN, the authors made a thorough search for the types where it was determined that extant types for *E. oraniensis* are unlikely to be in existence. The syntypes could not be located within the SAIAB collection. Therefore, a neotype for *Enteromius oraniensis* was designated from recently collected specimens from the Bossielagtesptruit River, a tributary of the Orange River system. A diagnosis, redescription, and comparison of *E. oraniensis* and the other congeners in southern Africa are presented below in accordance with Articles 75.3.2 and 75.3.3.

Diagnosis. Lateral line incomplete (2 – 33 (mode 15)), oral barbels inconspicuous.

Comparison with soft-rayed congeners in southern Africa. *Enteromius oraniensis* differs from all the other three species in the complex by possession of inconspicuous barbels. Further separated from *E. cernuus* and *E. mandelai* in having an incomplete lateral line (2–33 (mode 12) pored scales vs 15–37 (mode 35) pored scales). Separated from *E. amatolicus* (Skelton, 1990), *E. motebensis* (Steindachner, 1894), and *E. gurneyi* (Gunther, 1868) by absence of tubercles on the head during

breeding season (*vs* development nuptial tubercles on either snout, lower jaw, forehead or sides of head in the other species). Further separated from both *E. gurneyi* and *E. motebensis* by possession of a single pair of barbels (vs two pairs of barbels in the other two species) and from *E. amatolicus* by possession of short barbels that do not surpass vertical through middle of eye (vs distinctly long barbels that surpass vertical through posterior edge of the eye). Differentiated from *E. unitaeniatus* (Gunther, 1867)*, E. viviparus* (Weber, 1897), *E. lineomaculatus* (Boulenger, 1903)*,* and *E. barotseensis* (Pellegrin, 1920) by the lack of distinct lateral line chevron markings and possession of a single pair of relatively short maxillary barbels not exceeding middle portion of eye (*vs* the existence on the lateral line of conspicuous chevron markings and the existence of two pairs of long and prominent barbels exceeding middle portion of the eye in the other four species). Distinguished from *E. treurensis* (Groenewald, 1958), *E. breviceps* (Trewavas, 1936), *E. annectens* (Gilchrist & Thompson, 1913), *E. pallidus* (Smith, 1841), *E. brevipinnis* (Jubb, 1966), *E. radiatus* (Peters, 1853) and *E. bifrenatus* (Fowler, 1935) by the possession of one pair of oral barbels (vs the presence in other species of two pairs of long barbels). Differentiated from *E. neefi* (Greenwood, 1962) and *E. pallidus* (Smith, 1841) by the absence of two pairs of oral barbels and the absence of dispersed black spots on the body (vs the presence of two pairs of oral barbels and black spots on the body in these three species). Differentiated from *E. fasciolatus* (Gunther, 1868) by the absence of prominent vertical bars along the body (*vs*. the presence in *E. fasciolatus*).

Description. Body fusiform, moderately laterally compressed. Dorsal profile convex, ventral profile from tip of the snout to origin of pelvic fins straight. Greatest body depth at the anterior base of dorsal fin. Post-dorsal profile gently tapers to base of caudal fin. Head relatively small, length subequal to body depth. Snout short, bluntly rounded, equal to or slightly longer than eye diameter. Mouth small, terminal, with single pair of posterior barbels. Barbels do not surpass the middle portion of the eye. Eyes relatively large, round, situated dorsolaterally, nearer to tip of snout than distal margin of operculum.

Scales. Scales moderately large, radiately striated; 2–15 (mode 12) scale rows along the lateral line to end of hypural plate. Lateral line incomplete, or pored lateral line scales variously interrupted (only 2 of the 52 specimens examined had a complete lateral line). Circumpendicular scales 12–16 (mode 16); pre-dorsal scale rows 13–14 (mode 13); scale rows between the origin of the dorsal

fin and the lateral line 3–4 (mode 4); scale rows between the origin of the pelvic fin and the lateral line 3–4 (mode 4); scale rows between lateral line and anal fin origin 3–4 (mode 4). Scales between posterior edge of head and dorsal fin embedded and smaller than flank scales.

Fins. Dorsal fin situated almost in the centre of the body, with 3 unbranched and 7 branched rays. Pectoral fins fan-shaped, posterior edges gently rounded, not reaching pelvic fin origin. Pelvic fin with 8 rays, origin slightly in front of dorsal fin origin. Tip of adepressed pelvic fin does not reach anal fin origin. Anal fin with 3 simple rays and 5 branched rays; distal margin almost straight; origin closer to pelvic fin origin than to caudal fin base. Caudal fin forked; lobes gently rounded with moderately pointed tips.

Colouration (live and fresh specimens). Pale silver colour, with a small dot at mid base of caudal fin.

Colouration (preserved). Brownish colour, with a relatively inconspicuous lateral stripe that ends in an indistinct dark spot at the base of the caudal fin.

Distribution. *Enteromius oraniensis* is endemic to the Orange River system, but the extent of its distribution in this extensive river system needs to be established through dedicated surveys.

Habitat and ecology. Cambray (1983a, b) and Cambray & Bruton (1984, 1985) did extensive studies of the biology and ecology of this species, mainly focussing on populations from Lake Le Roux in the Orange River system. The species occurs in a number of habitats ranging from tributaries to mainstem sections of rivers and man-made impoundments where it is usually associated with vegetated areas. Early maturation in the first year of life, high reproductive effort, having multiple clutches per year and rapid growth rate are important traits that make this species highly adaptable to habitats that are prone to regular input of high silt loads and experience extreme variation in flow regimes, ranging from devastating floods to erratic flows (Cambray & Bruton, 1984).

Reproduction: The reproductive biology of this species has been studied in detail (Cambray, 1983a, b) and Cambray & Bruton, (1984, 1985). Spawning occurs following onset of rainfall in summer. Fish spawn locally, although spawning migration has been occasionally observed, with adhesive eggs laid in flooded marginal vegetation (Cambray et al., 1978; Cambray & Bruton, 1984). *E. oraniensis* has early maturity (attains 40mm FL in first year), high reproductive rate (a female of 63 mm can have up to 3250 eggs) and a protracted reproductive season (up to six months) and an individual fish can have multiple clutches (Cambray & Bruton, 1984). These traits allow survival of this species in the dryland southern tributaries of the Orange River system that drain the Great Karoo.

***Enteromius mandelai* sp. n.**

Figures 2.13a-c, 2.14a-c.

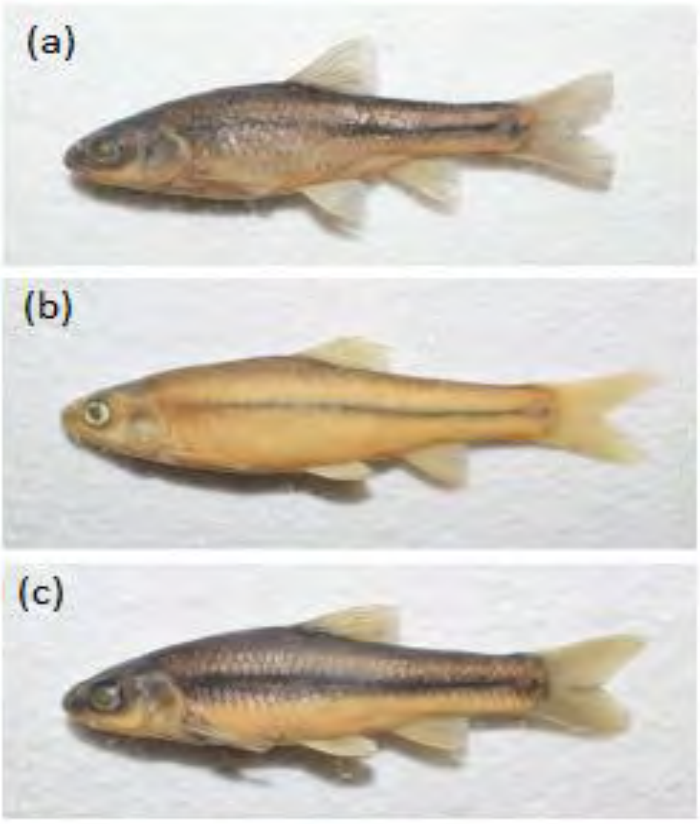


Figure 2.13: General body features and alcohol preserved colouration of *Enteromius mandelai* n. sp (a) the holotype (SAIAB 209731) adult female, 63.7 mm standard length, Glen Owen Farm, Great Fish River mainstem (b) Adult specimen (SAIAB 209738) 56.4 mm standard length, from the Keiskamma River (c) Adult specimen (SAIAB 209733), 69.5 mm standard length, from the Zeleni River, Buffalo River system.

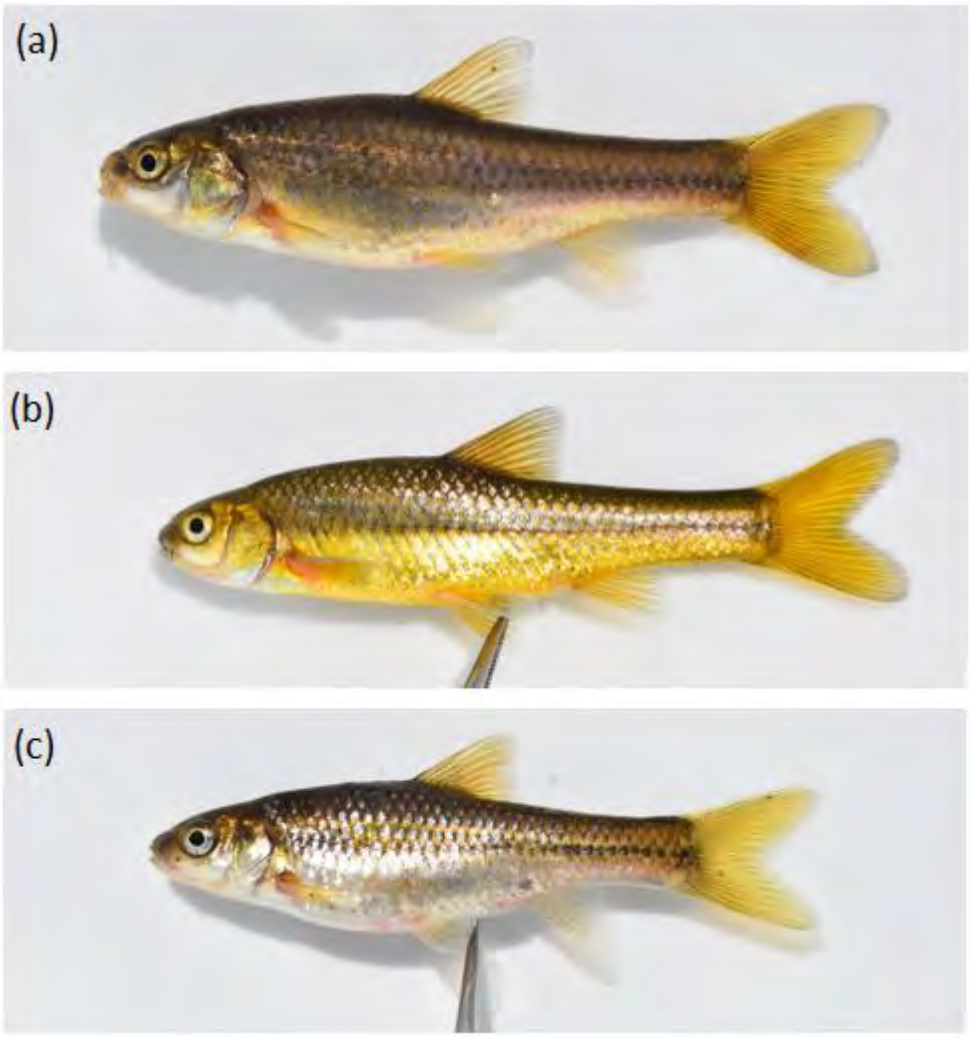


Figure 2.14: Lateral view of live *Enteromius mandelai* n. sp showing live coloration of mature breeding (a) female paratype (SAIAB 209732), Glen Owen Farm, Great Fish River mainstem (not examined) (b) male specimen (SAIAB 209734), Tarka River, Great Fish River system (not examined) (c) female specimen (SAIAB 209733), Buffalo River (not examined). Scale bar 10mm.

Proposed common name. Eastern Cape chubbyhead.

Holotype. SAIAB 209731, Field no: MK19AL06, Female, 65.03 mm SL, Glen Owen Farm, Great Fish River mainstem, -31.89859, 25.21005, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 28 August 2019.

Paratypes. SAIAB 209732, Field no: MK19AL06, 19 unsexed, 41.24–65.03 mm SL, same locality information and collectors as holotype.

Additional material. Field no: MK19AL01, 13 unsexed (38.8 – 67.4 mm S), Eyre River, Kat River branch, Great Fish River system, -32.50729, 26.79237, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 27 August 2019; SAIAB 209734, Field no: MK19AL02, 5 unsexed (40.7–72.4 mm SL), Tarka River, Great Fish River system, -32.21801, 25.92899, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 28th August 2019; Field no: MK19AL03, 10 unsexed (61.7–32.7 mm SL), Pauls River, Great Fish River system, -32.03481, 25.52129, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 28 August 2019; Field no: MK19AL09, 13 unsexed (59.0–33.1 mm SL), Keiskamma River, Keiskamma River system, -32.73249, 27.02731, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 29 August 2019; Field no: MK19AL10, 6 unsexed (57.2–69.5 mm SL), Amatele River, Keiskamma River system, -32.716944, 27.00944, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 29 August 2019; Field no: MK19AL12, 7 unsexed (36.2–52.9 mm SL), Yellowwoods River, Buffalo River system, -32.80778 S, 27.47003 E, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 29 August 2019; Field no: MK19AL15, 11 unsexed (31.3–46.5 mm SL), Yellowwoods River, Buffalo River system, -32.72511, 27.48864, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 29 August 2019; SAIAB 209733 Field no: MK19AL19, 12 unsexed (32.7–68.9 mm SL), Zeleni River, Buffalo River system, -32.77092, 27.38987, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 31 August 2019; Field no: MK19AL20, 11 unsexed (28.7–53.4 mm SL), Zeleni River, Buffalo River system, -32.73244, 27.43155, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 31 August 2019; Field no: MK19AL21, 6 unsexed (32.1–41.4 mm SL), Yellowwoods River, Buffalo River system, -32.75836, 27.488149, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 31 August 2019; Field no: MK19AL22, 9 unsexed (32.0–52.0 mm SL), Tyolomqa River, -33,07186, 27.44545, collected by A Chakona, W Kadye, M Kambikambi and N Matomela, 31 August 2019.

Etymology. *Enteromius mandelai* is named after South Africa's first democratically elected head of state, President Nelson Rolihlahla Mandela, who was from the Eastern Cape Province where this species is endemic. The species is named in honour and recognition of his legacy and selfless contribution towards promotion of peace, democracy, human rights, equality, social justice and sustainable development.

Diagnosis. Lateral line usually complete (15–37 pored scales, mode 35), barbels conspicuous but do not surpass vertical through middle of eye, circumpendicular scales (14–16, mode 14).

Comparison with soft-rayed congeners in southern Africa. Possession of a complete lateral line in the majority of specimens separates *E. mandelai* sp. nov. from *E. oraniensis* and *E. anoplus* s.s. 15 scales would not be complete unless its interrupted. You need to include this information here. It is further differentiated from *E. oraniensis* by possession of conspicuous oral barbels (*vs* inconspicuous barbels). *Enteromius mandelai* has relatively shorter barbels than *E. cernuus* (Table 6) and a higher number of circumpendicular scales (14–16 (mode 14) *vs* 10–12 (mode 12). Separated from *E. amatolicus* (Skelton, 1990), *E. motebensis* (Steindachner, 1894), and *E. gurneyi* (Gunther, 1868) by absence of tubercles on the head during breeding season (*vs* development nuptial tubercles on either snout, lower jaw, forehead or sides of head in the other species). Further separated from both *E. gurneyi* and *E. motebensis* by possession of a single pair of barbels (vs two pairs of barbels in the other two species) and from *E. amatolicus* by possession of short barbels that do not surpass vertical through middle of eye (vs distinctly long barbels that surpass vertical through posterior edge of the eye). Differentiated from *E. unitaeniatus* (Gunther, 1867)*, E. viviparus* (Weber, 1897), *E. lineomaculatus* (Boulenger, 1903)*,* and *E. barotseensis* (Pellegrin, 1920) by the lack of distinct lateral line chevron markings and possession of a single pair of relatively short maxillary barbels not exceeding middle portion of eye (*vs* the existence on the lateral line of conspicuous chevron markings and the existence of two pairs of long and prominent barbels exceeding middle portion of the eye in the other four species). Distinguished from *E. treurensis* (Groenewald, 1958), *E. breviceps* (Trewavas, 1936), *E. annectens* (Gilchrist & Thompson, 1913), *E. pallidus* (Smith, 1841), *E. brevipinnis* (Jubb, 1966), *E. radiatus* (Peters, 1853) and *E. bifrenatus* (Fowler, 1935) by the possession of one pair of oral barbels (vs the presence in other species of two pairs of long barbels). Differentiated from *E. neefi* (Greenwood,

1962) and *E. pallidus* (Smith, 1841) by the absence of two pairs of oral barbels and the absence of dispersed black spots on the body (vs the presence of two pairs of oral barbels and black spots on the body in these three species). Differentiated from *E. fasciolatus* (Gunther, 1868) by the absence of prominent vertical bars along the body (*vs*. the presence in *E. fasciolatus*).

Description. Morphometric and meristic data summarised in Table 6 are based on one holotype and 80 specimens from the Great Fish, Keiskamma and Buffalo River systems. General body shape and coloration are shown in Figure 13a-e. A fusiform minnow with body slightly compressed laterally. Dorsal profile more convex than ventral profile from tip of the snout to origin of dorsal fin. Post dorsal profile generally straight and descending from origin of dorsal fin to caudal fin insertion. Body depth greatest around the anterior bases of the dorsal and pelvic fins, tapering from posterior margin of dorsal fin base to base of caudal fin. Head relatively short, length almost equal to body depth. Snout short and bluntly rounded, mouth small and terminal with a single pair of posterior barbels. Barbels do not surpass the middle portion of the eye. Eyes round and relatively large: situated dorsolaterally, nearer to the tip of the snout than the distal margin of the operculum. Tubercles absent.

Scales. Scales moderately large, radiately striated; Lateral line usually complete (mode 35), (only three of the 81 specimens examined had lateral line with less than 15 pored scales). Circumpendicular scales 12–16 (mode 16); pre-dorsal scale rows 14–16; scale rows between the origin of the dorsal fin and the lateral line 3–4 (mode 4); scale rows between the origin of the pelvic fin and the lateral line 3–4 (mode 4); scale rows between lateral line and anal fin origin 3–4 (mode 4). Scales between posterior edge of head and dorsal fin embedded and smaller than flank scales.

Fins. Dorsal fin situated almost in the centre of the body, with 3 unbranched and 7 branched rays. Pectoral fins fan-shaped, posterior edges gently rounded, not reaching pelvic fin origin. Pelvic fin with 8 rays, origin slightly in front of dorsal fin origin. Tip of adepressed pelvic fin does not reach anal fin origin. Anal fin with 3 simple rays and 5 branched rays; distal margin almost straight; origin closer to pelvic fin origin than to caudal fin base. Caudal fin forked; lobes gently rounded with moderately pointed tips.

Colouration (live and fresh specimens). Metallic silvery, greenish or greyish above, with a more or less pronounced yellow or golden tinge in males, more greyish in females. A black spot is usually present at the base of the caudal fin.

Colouration (preserved). More or less conspicuous dark lateral stripe, not expanding at end, but ending in a more or less indistinct spot.

Distribution. Widely distributed in the Eastern Cape Province, from the Kowie to the Buffalo River systems.

Habitat and ecology. This species has been recorded in a wide range of habitats, from clear flowing mountain streams with rocky substrates to mainstem sections of rivers and man-made impoundments with high water turbidity and fine substrates. Although no studies have specifically focussed on the feeding ecology of this species, it is likely to be omnivorous, as reported for other congeners, mainly feeding on insects, zooplankton, seeds, green algae and diatoms (Cambray, 1983a, b; Cambray & Bruton, 1984, 1985).

Reproduction. No specific studies have focussed on the breeding biology of *Enteromius mandelai*, but it is likely to breed in summer following the onset of the rains as reported for the other congeners (Cambray et al., 1978; Skelton, 2001).

Table 2.6: Morphometric and meristic data for *Enteromius anoplus, Enteromius oraniensis, Enteromius cernuus* and *Enteromius mandelai* sp. nov.

	E. anoplus		***E. cernuus***			***E. oraniensis***		***E. mandelai* sp. nov.**	
	Lectotype n = 1	**Other specimens including topotypes n = 20**	**Lectotype n = 1**	**Syntypes n = 15**	**Other specimens including topotypes n = 8**	**Neotype n = 1**	**Other specimens including topotypes n = 50**	**Holotype n = 1**	**Other specimens n = 80**
Standard length (SL) (mm)	95	29.17-55.64	52.6	21-56	21.62-42.70	53.37	24.28 – 59.33	63.70	28.68-72.44
Head length (HL) (mm)	20	7.86-14.4	13.39	10.35-14.21	6.5-11	13.83	5.31 – 20.38	17.51	5.26-28.66
Percent of SL (%)									
Head length	22.22	25.34-30.07	53.56	31.28-64.33	24.78-37	25.91	18.83 – 39.76	26.93	18.36 – 39.57
Pre-dorsal length	42.11	44.95-54.78	111.08	64.69-131	39.79-53	49.86	30.10 -82.92	49.45	34.36 – 80.21
Dorsal fin base	10.53	11.44-17.49	27.4	14.8-33	6.60-14.69	12.67	6.21 – 36.81	12.12	7.80 – 19.95
Dorsal fin height	15.79	21.25-26.17	48.4	28.89-57.32	18.96-25.92	23.40	15.84 – 58.62	22.22	14.51 – 33.35
Body depth	21.05	23.29-27.76	55.84	28.24-62.52	20.62-25.97	24.56	13.75 – 62.30	24.23	12.56 – 41.34
Body width	12.63	11.31-15.07	32.44	14.73-39.05	9.61-14.91	14.02	7.29 – 32.21	16.99	7.06 – 27.52
Caudal peduncle length	21.05	17.46-26.18	42.56	24.86-53.14	18.14-22.21	24.26	15.91 – 45.83	20.59	13.23 – 27.52
Percentage of HL (%)									
Head depth	75	62.25-76.21	66.32	64.18-78.57	45.5-80.77	67.02	42.92 – 82.96	66.99	33.85 – 86.74
Snout length	20	21.98-29.33	27.78	25.04-31.89	16.38-27.69	28.77	19.17 – 35.43	29.69	19.64 – 36.56
Post Orbit length	65	39.01-52.62	51.83	47.75-60.54	39.28-57.81	49.31	32.73 – 59.62	52.03	42.71 – 59.89

Barbel length	N/A	7.84-17.27	17.92	13.33-24.01	10.31-26.61	14.67	3.69 – 35.81	11.99	5.77 – 21.45
Orbit diameter	25	18.81-31.26	20.16	20.16-26.96	23.75-30.15	22.27	18.69 – 38.23	23.59	16.71 – 40.73
Percentage of caudal peduncle length (%)									
Caudal peduncle depth	50	50.15-76.21	58.27	52.49-68.59	55.86-70.16	53.51	36.36 – 74.64	62.73	39.95 – 77.28
Unbranched dorsal fin rays	iii	iii	iii	iii	iii	iii	iii	iii	iii
Branched dorsal fin rays	7	7	7	7	7	7	7	7	7
Unbranched anal fin rays	iii	iii	iii	iii	iii	iii	iii	iii	iii
Branched anal fin rays	5	5	5	5	5	5	5	5	5
Pelvic fin rays	8	8	8	8	8	8	8	8	8
Lateral line scales	29	8 (3-35)		33(31-33)	31(28-33)	15	12 (2-33)	36	35 (15-37)
Lateral line to dorsal fin scales	5	6 (5-6)	6	5(5-6)	5	6	5(5-6)	6	6(5-6)
Lateral line to pelvic fin scales	3	4(3-4)	3	3	3	4	3(3-4)	4	3(3-4)
Lateral line to anal fin scales	3	4(3-4)	3	3	3	4	3(3-4)	4	3(3-4)
Caudal peduncle scales	14	16(12-16)	12	12	12 (10-12)	16	14(12-16)	16	14(14-16)
Pre-dorsal scales	14	13-14	13	13-14	13-14	14	13-14	14	13-14

Discussion

Since the original descriptions of *E. karkensis*, *E. cernuus* and *E. oraniensis* between 1913 and 1943, and their subsequent synonymisation with *E. anoplus* by Lévêque and Daget (1984), there has been no detailed taxonomic evaluation to determine the taxonomic distinctiveness of these species. All subsequent mentions in the literature were based on Skelton (1993, 2001) who adopted the synonymy proposed by Lévêque and Daget (1984), making *E. anoplus* the most widely distributed freshwater fish in South Africa. The present study found both genetic and morphological evidence to separate *E. anoplus* s.l. into four distinct and allopatrically distributed species. These were *E. anoplus* s.s., endemic to the Gouritz River system, *E. oraniensis*, endemic to the Orange River system, *E. cernuus*, endemic to the Olifants River system and *E. mandelai*, which is widely distributed in several coastal river systems in the Eastern Cape province of South Africa. These findings agree with Barnard's (1943) separation of these species, with the key distinguishing characters among them being differences in barbel length and number of pored lateral line scales. *Enteromius oraniensis* is readily separated from the other three species by having inconspicuous barbels and an incomplete lateral line. *Enteromius cernuus* has long barbels, a complete lateral line and fewer circumpendicular scales (10–12; mode 12). *Enteromius anoplus* has an incomplete lateral line and relatively short barbels that do not surpass vertical through middle of eye. *Enteromius mandelai* has a complete lateral line, relatively short barbels and higher number of circumpendicular scales (14–16; mode 14).

The geographic ranges of these four species largely resemble the patterns observed in two other cyprinid genera, *Pseudobarbus* and *Labeo*, which both have representative species or lineages endemic to the Orange, Olifants and Gouritz river systems, as well as representative species or lineages with broad distribution ranges in the Eastern Cape province (Chakona *et al*., 2015b; Chakona & Skelton, 2017; Martin & Chakona, 2019; Ramoejane *et al*., 2020). The close relationship between *E. oraniensis*, *E. cernuus* and *E. anoplus* supports the conclusion of Swartz *et al*. (2007) hat the Gouritz River system played an important role in the diversification and shaping the distribution patterns of redfin minnows of the genus *Pseudobarbus* in the CFE. The Gouritz River system represents a logical link between the Olifants-Doring system on the west coast and the southern tributaries of the Orange River system, through either river capture events or intermittent freshwater connections through drainage overtopping during periods of heavy rainfall. The comparable pattern in the distribution of the four species within the *E.*

anoplus complex and the single barbeled redfins (i.e. *P. phlegethon*, *P. tenuis*, *P. quathlambae* and the *P. afer* complex) suggest that these species were affected by similar evolutionary mechanisms. There is thus scope for future studies to reexamine the biogeographic hypotheses presented by Swartz *et al*., (2007, 2009) and Skelton (1986, 1994) in a comparative approach to shed more light on the mechanisms that drove the diversification and shaped contemporary distribution patterns of endemic stream fishes in the CFE.

The widespread sharing of haplotypes across several isolated river systems observed in *E. mandelai* is consistent with patterns found for a number of other stream fishes in the CFE, including *Galaxias* and *Sandelia* (Bronaugh *et al*., 2020; Chakona *et al*., 2018). These patterns suggest that range expansion occurred relatively recently in these fishes, potentially facilitated by intermittent freshwater connections through drainage overtopping during periods of heavy rainfall or human mediated translocations through either intentional introductions or inadvertently through interbasin water transfers (Chakona *et al*., 2013a; Chakona *et al*., 2018; Laurenson & Hocutt, 1986). Anecdotal evidence suggests that *E. mandelai* could have been potentially introduced into some of the rivers in the Eastern Cape as fodder for non-native trout *Oncorhynchus mykiss*, which were introduced into a number of tributary catchments of the Buffalo, Keiskamma and Kei river systems (Rivers-Moore *et al*., 2019). This could explain the broad distribution range and widespread sharing of haplotypes across isolated river systems where this species was recorded. The question on how *E. mandelai* attained its current widespread distribution range can be addressed through use of highly polymorphic markers such as SNPs or microsatellites to test the relative roles of natural and anthropogenic factors in shaping the species' genetic patterns (e.g. Bartáková *et al*., 2018). If the species was recently translocated through human intervention, it would be possible to identify source populations and map routes of spread (e.g. Cristescu, 2015; Hargrove *et al*., 2017; Oficialdegui *et al*., 2019).

One of the major concerns that were raised by several researchers following the commissioning of the Orange-Fish inter-basin water transfer (IBWT) scheme that transferred water from the Orange River to various systems in the water scarce Eastern Cape Province was the risk of biotic homogenisation through inter-basin dispersal of species. Interestingly, although Laurenson and Hocutt (1986) predicted that the Orange River chubbyhead barb was among the fish species that were likely to be translocated into the Eastern Cape rivers through the Orange-Fish inter-basin water transfer (IBWT) scheme, the present study did not find haplotypes of *E. oraniensis*, despite extensive sampling of the Great Fish and other river systems in the Eastern Cape province. Although this species has been recorded in the Gariep Dam (Ellender et al., 2010), which is the intake of the Orange-Fish IBWT, it is possible that

both intrinsic (e.g. life-history, ecological, morphological and behavioural traits) and extrinsic (e.g. unsuitable habitat) factors could have hindered *E. oraniensis* from exploiting the IBWT tunnel to expand its range into the Eastern Cape province. The species is generally associated with shallow vegetated marginal habitats, is a local spawner which lays adhesive eggs that attach onto the grass (Cambray & Bruton, 1984), and its small body size makes it highly susceptible to predation in the open waters of the lake. Thus, the deeper and open water section of the lake is likely to represent a barrier for this habitat specialist that is also presumed to be dispersal-limited (Cambray & Bruton, 1984). Contrary to the original predictions by Laurenson and Hocutt (1986), we conclude that the interplay between these intrinsic and extrinsic factors is the likely explanation for the failure of *E. oraniensis* to exploit the Orange-Fish IBWT tunnel to expand its range into the Great Fish and other adjacent river systems. Indeed, the four species that successfully utilised Orange-Fish IBWT to expand their ranges are the large-sized and benthivorous species, *Clarias gariepinus*, *Labeobarbus aeneus*, *Labeo capensis* and *L. umbratus*, which are capable of exploiting the deeper sections of the lake where the off-take tunnel is situated.

Conservation implications

The revalidation of *E. cernuus* further affirms the Olifants-Doring River system on the west coast of South Africa as an endemic hotspot, because 10% of the 11 currently described species and 4 genetic lineages of freshwater fishes from this system are not shared with any of the adjacent systems (Bronaugh et al., 2020; Chakona et al., 2013b; Ramoejane et al., 2020; Skelton, 2001; Swartz et al., 2007). The redescription of *E. anoplus*, revalidation of *E. oraniensis*, *E. cernuus*, and description of *E. mandelai* and the provision of evidence that these species have much narrower distribution ranges is critical for providing more accurate assessments of species extinction risk. These results render the current IUCN Red List assessment of *E. anoplus* s.l. obsolete, because despite containing four genetically and morphologically distinct species, three of which are narrow range endemics confined to single river systems, this complex was recently classified as Least Concern (Woodford, 2017).

In light of the results from the present study, there is need for undertaking ecological and biological assessments to determine the vulnerability *E. anoplus* s.s., *E. oraniensis*, *E. cernuus* and *E. mandelai* to the multiple anthropogenic impacts across their distribution ranges. The current documented ecology of *E. anoplus* s.l. may not be representative of each of these four species, because it is exclusively based on populations from the Orange River system (see Cambray & Bruton, 1984), which essentially represents *E. oraniensis*. Given the occurrence in

basins with different climatic regions, it is likely that *E. oraniensis*, *E. cernuus*, *E. anoplus* and *E. mandelai* show different life history and ecological traits such as habitat associations, spawning habitat selection and breeding time and duration. Vulnerability to impacts, for example predation may also differ between *E. cernuus* and the other three species, because the former evolved with large piscivores, in particular, *Clarias gariepinus* in the Orange system. This is in contrast to the CFE where vulnerability of stream fishes of this region to predation pressure from introduced piscivores has been attributed to their naivety due to the fact that they evolved in the absence of superior predators (Kadye & Booth, 2014). The two narrow range endemics, *E. anoplus* and *E. cernuus*, are likely to be listed under highly threatened categories of the IUCN as the systems where they occur are under threat from multiple impacts, particularly predation pressure from non-native piscivores and habitat degradation (Tweddle et al., 2009; Weyl et al., 2014). These impacts have also resulted in severe fragmentation of the remnant populations of *E. mandelai*, while *E. cernuus* is under threat from excessive pollution of the Orange River system from mine, industrial and domestic effluent discharge. There is thus need for generating baseline information, including knowledge of ecological requirements, habitat utilization, distribution, life history and feeding ecology to support conservation and protection of these species.

CHAPTER 3

Trophic relationships and food web dynamics of *Enteromius mandelai* sp. nov in the Great Fish, Keiskamma and Buffalo river systems

Introduction

The distribution and abundance of freshwater fish species across different spatial scales is influenced by biotic and abiotic factors (Grossman et al., 1998; Rahel & Nibbelink, 1999; Richardson, 2019). Biotic factors that affect freshwater fish distribution and abundance include food availability, predation, inter- and intra-specific interactions (Gilliam & Fraser, 2001; Jackson et al., 2001; Richardson & Danehy, 2007). The food web is an integrative conceptual tool that has been used in understanding the dynamics of energy transfer in an ecosystem, illustrating the feeding relationships among species within a community and revealing species interactions and community structure (Krebs, 2009; Smith & Smith, 2009). In headwater streams, understanding food web dynamics and trophic relationships is important because these ecosystems exhibit ecological dynamics that reflect both natural and anthropogenic changes (Brett & Goldman, 1997).

In southern Africa, headwater streams are mainly inhabited by small-bodied fish species such as minnows and catfishes (e.g. Skelton, 2001; Kadye et al., 2008; Van Oosterhout et al., 2009; Chakona et al., 2018). These headwater stream fishes are mostly composed of invertivores that have high dietary plasticity in response to available prey sources (Gaigher, 1975; Cambray, 1983a; Kambikambi et al., 2019; Matomela, 2020). Small-bodied minnows of the genera *Pseudobarbus* and *Enteromius* represent the main piscine elements for headwater streams in the Cape Fold Ecoregion, Amathole-Winterberg Highveld Ecoregion and Drakensberg-Maloti Highlands Ecoregion (Skelton, 2001; Tweddle et al., 2009; Ellender et al., 2017). These species have been found to be unspecialised facultative feeders, an attribute that has possibly played an important role in their successful utilisation of headwater streams (Gaigher, 1975; Cambray, 1983a). Recently, there has been increasing interest in studying the food web dynamics of these species (e.g. Kadye & Booth, 2012; Chakona et al., 2019; Kambikambi et al., 2019; Kadye & Booth, 2020) in order to understand their trophic inter-relationships as this information is critical for informing better conservation and management

of threatened ecosystems and their species. This is particularly being driven by the recent discovery of several new species and genetic lineages (Chakona & Skelton, 2017; Skelton et al., 2018; Chakona et al., 2020b), raising the need for information on the ecological attributes for these newly discovered species.

The present study (Chapter 2) has delimited a new species, *Enteromius mandelai* sp. nov., and as with many newly discovered or described species, published knowledge of the ecology of this species is lacking. *Enteromius mandelai* has been recorded from the Great Fish to the Buffalo River systems in the Eastern Cape Province of South Africa (Chapter 2). Previous information available for the chubbyhead barb *E. anoplus* s.l listed a broad range of feeding items, including insects, zooplankton, seeds, green algae and diatoms (Cambray, 1983a; Skelton, 2001). This trophic adaptability is important for fish species that occur in headwater habitats that are subject to high environmental variability. Furthermore, a wide range of feeding habits may reduce dietary overlap and interspecific competition among headwater fishes (Colwell & Futuyma, 1971; Odum & Barrett, 1971). The current knowledge of *E. mandelai'*s feeding habits is, however, based on observations and studies conducted across the distribution range of *E. anoplus'* sensu lato, which encompassed perennial to seasonal systems in the Orange River and southern draining river systems such as the Olifants, Gouritz, Gamtoos and Sundays rivers.

These river systems have varying environmental attributes (Allanson et al., 1990; South Africa. Department of Water Affairs and Forestry, 2004) leading to differences in the biotic communities occurring in these systems (Skelton, 2001; Day & de Moor, 2002; de Moor & Day, 2013). For example, the Orange River ranks within the four most turbid rivers in the world (Bremner et al., 1990). The macroinvertebrate fauna is dominated by filter-feeders, highlighting the importance of fine particulate material (bacteria, phytoplankton and detritus) in the ecology of the river (Palmer, 1996). Climatically and hydrologically, the Orange River is subject to striking contrasts in different sections (Allanson et al., 1990). The climate at the source is cool and temperate and becomes more arid as the river moves towards the west (Tooth & McCarthy, 2004). The average daily temperature varies from 12 ºC in the Drakensberg-Maloti Highlands to 22 ºC near the mouth. Rainfall varies from more than 1000 mm per year at the headwaters to about 300 mm per year at the Orange/Vaal confluence, and less than 100 mm per year in the lower sections (Allanson et al., 1990). In contrast, most of the drainage of the Gouritz River system falls within the rain shadow of the coastal arc of the Fold Mountains and is thus relatively poorly watered (Skelton, 1980). Habitats in the mountain tributaries of

the Gouritz River system are clear and acidic whereas those from the mainstem tributaries have, turbid, alkaline mineralised waters. Organisms in these reaches are able to tolerate significant variation in water quality. Organisms in the Gouritz River system, may be more resilient than organisms in many of the coastal rivers, which are less naturally variable.

Cryptic species, though being morphologically indistinguishable, may differ in their ecological and behavioural requirements (e.g. Schluter, 1996; Prochazka, 1998; Wellborn & Cothran, 2007). As the present study has separated this complex into four distinct species, with *E. mandelai* occurring in river systems from the Eastern Cape Province (Chapter 2), it is possible that their trophic ecologies may also vary. Within headwater streams of the Eastern Cape Province, *E. mandelai* was the only species occurring in most headwater streams at high altitudes (e.g. Kambikambi et al., 2019; Matomela, 2020). In some sections, it co-occurred with other native species such as the anabantid *Sandelia bainsii*, the small-bodied cyprinid *Amatolacypris trevelyani,* the river goby *Glossogobius callidus* and the non-native cichlid *Tilapia sparrmanii* (Kadye & Booth, 2020; Sifundza et al., 2020). Generally, co-occurring species are assumed to coexist in a community based on environmental processes, evolutionary processes, or a combination of both (Kraft et al., 2015). Niche-based approaches postulate that co-occurring species are required to exhibit ecological differences in at least one niche dimension, such as food preferences or space use (Jackson et al., 2001). From a trophic dimension, the coexistence of species is a result of trophic partitioning. Trophic partitioning is one of the most common mechanisms by which animals divide resources and partition the flow of key elements in foodwebs (Schoener 1986). This results in lessening the niche overlap and the intensity of interspecific competition (Hutchinson, 1961). Understanding trophic resource patterns of *E. mandelai* in the presence and absence of other fish species is important in the conservation and management of this newly discovered fish species.

In addition to the natural ecological attributes, the headwater streams inhabited by *E. mandelai* are characterised by multiple anthropogenic impacts, including invasion by non-native piscivores, flow regulation, invasion of riparian zones by non-native plant species and subsistence agriculture (Thieme et al., 2005; van Rensburg et al., 2011; Kadye & Booth, 2012; Weyl et al., 2020). Introduction of predatory fishes in pristine ecosystems can cause cascading changes within recipient freshwater ecosystems (Cucherousset & Olden, 2011; Koel et al., 2019). In the Eastern Cape Province, native fish species are often subject to predation by non-native piscivores such as the African sharptooth catfish *Clarias gariepinus,* rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, and largemouth bass *Micropterus salmoides*

(Skelton, 2001; Cambray, 2003; Kadye & Booth, 2020) which have effectively reduced the distribution of native fish. River catchments influence the physicochemical and biological characteristics of running water systems such as trophic processes in streams (Schlosser, 1995; Wang et al., 2006), changes in land-use patterns affect trophic relationships in headwater streams (Allan, 2004; Wanyama et al., 2018; Barbosa et al., 2019). Non-native plant species have invaded riparian zones of many headwater streams in this region. These invasive plants, which have been largely propagated through silviculture, include wattles (*Acacia* sp), gumtrees (*Eucalyptu*s sp), pines (*Pinus* sp) and tickberry (*Lantana camara*) (Thieme et al., 2005). These non-native plant species are known to cause erosion, siltation, alteration of the water table due to high rates of evapotranspiration (Thieme et al., 2005) and alteration of thermal regimes due to shading (Baltz & Moyle, 1984; Thiébaut et al., 2018). The headwaters of the Great Fish, Buffalo and Keiskamma rivers are also used for both crop and livestock farming (Geldenhuys & van der Merwe, 1988). Crop farming in particular is associated with the alteration of the physical and chemical properties of the soil (Wanyama et al., 2018), and is associated with nutrient input due to runoff. This alteration has negative effects on adjacent streams and rivers, which in turn affect the distribution and abundance of aquatic organisms (Dodds & Smith, 2016). Recently, Matomela (2020) found that the food web structure of three differently impacted headwater streams in the Kat River, a tributary of the Great Fish River, South Africa, differed substantially, which indicated that different land use patterns had a role in the structuring of trophic dynamics. Although the effects that invasive piscivores, agricultural activities and invasion of the riparian zone by non-native plants have on food web dynamics and species distribution have been widely documented (e.g. Hanchet, 1990; Hicks, 1997; Walser & Bart, 1999; De Castro et al., 2016; García et al., 2017; Tronquart et al., 2018; Barbosa et al., 2019), these are region specific effects and there is need to undertake studies in poorly studied regions such as those of southern Africa.

The aim of this chapter is to provide ecological information on the food web structure and trophic interactions of *E. mandelai* in its current known distribution range. This information can thus be complementary to studies on abiotic factors influencing *E. mandelai* distribution in order to have a holistic view of its ecological patterns. In stream ecology, the application of stable isotope analysis (SIA) is a common tool for evaluating food web dynamics, potential food sources and processes that drive stream ecosystems (Gannes et al., 1997; Whitledge & Rabeni, 1997; Rybczynski et al., 2008; Junker & Cross, 2014; Kambikambi et al., 2019). In trophic ecology, SIA is used to reconstruct diets, elucidate patterns of resource

acquisition and allocation and characterise niche properties (Fry, 1991; Allen et al., 2009; Bouillon et al., 2011). More recent studies have expanded the knowledge on the use of SIA by estimating the relative contributions of different food sources to consumer diets (Moore & Semmens, 2008; Phillips et al., 2014; Kadye et al., 2020) and evaluating the trophic niche structure of communities and populations (Program et al., 2007; Jackson et al., 2011). In ecology, the term "niche" describes the role a species plays in a community. This niche encompasses both the physical and environmental conditions a species requires, and the interactions it has with other species. The "trophic niche" of a species is given by whom it is eaten by. It is widely regarded as a key factor in the ecological segregation of species.

Stable isotope analysis also provides a relatively long-term and temporally-integrated measurement of trophic dynamics because it is less influenced by temporal bias (Vizzini & Mazzola, 2003; Boecklen et al., 2011). In general, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes are the most commonly used isotopes in trophic ecology studies. The carbon stable isotope is used to infer the energy sources of consumers and the nitrogen stable isotope is used to infer their trophic positions (DeNiro & Epstein, 1978; Vander Zanden & Rasmussen, 2001; Post, 2002).This study therefore examined the isotopic niche patterns as a proxy for trophic niche patterns for *E. mandelai* across different headwater streams of the Amathole-Winterberg Highland Ecoregion (AWHE) and Southern Temperate Highveld Ecoregion (STHE), South Africa.

In this study, SIA was used to examine the spatial trophic dynamics associated with *E. mandelai.* It was hypothesised that the trophic niche patterns of *E. mandelai* would vary in relation to differences in the food web characteristics of the headwaters and that different land-use characteristics among the headwater streams would influence the quality and quantity of trophic resources. Therefore, the headwater streams would be characterised by a wide variation in the isotopic values of basal food sources and the associated consumer groups, which would be reflected by the spatial differences in the trophic dynamics of *E. mandelai*. It was further hypothesized that *E. mandelai* would display trophic niche differentiation in relation to riparian land-use patterns of headwater streams and to other co-occurring species. To assess whether *E. mandelai* showed trophic plasticity among environments with different levels of riparian disturbance and in the presence or absence of other species, this study evaluated isotopic niche breadth and population-wide isotopic niche metrics (e.g., Jackson et al., 2011) of this species in different headwater streams. The objectives of the study were to (1) determine the isotope-based food webs across the different headwater streams across the distribution range of *E.*

mandelia within the AWHE and STHE, and (2) evaluate the isotopic niche patterns of *E. mandelai* across the different spatial scales.

Materials and Methods

Research permits for this study were granted by the Eastern Cape Department of Economic Development and Environmental Affairs (through permit numbers CRO 130/19CR and CRO 44/18CR). The Rhodes University Ethical Standards Committee approved the ethical standards used for field collections (approval number 2019-0314-776).

Study area

This study focussed on the headwater streams of the Great Fish River, the Keiskamma River and the Buffalo River (Figure 3.1). The area drained by these rivers encompasses the Amathole-Winterberg Highlands Ecoregion (AWHE) and Southern Temperate Highveld Ecoregion (STHE). The AWHE covers a series of high-altitude Afromontane forests. The headwaters of a large number of small streams drain this ecoregion (Thieme et al., 2005). These include the headwaters of the Buffalo River, the lower Great Fish River, the Keiskamma River, and the Swart Kei River. The headwater streams are typically narrow, fast flowing, turbulent with rocky substrata. Many have sponges or bogs at their source and small areas of seasonally flooded marsh in flatter, low-altitude valleys (Hughes & Hughes, 1992). The climate of the AWHE is temperate, characterised by high rainfall, cool winters and moderately warm summers (Story, 1952). Most rainfall occurs in summer (December to February) and autumn (March to May) (Thieme et al., 2005; Hoare et al., 2006). The AWHE area as a whole receives between 800 mm and 1,000 mm per annum (Hoare & Bredenkamp, 1999; Clark et al., 2011). The region has an average daily maximum air temperature of 19 °C in winter and 29 °C in summer (van Zyl, 1994). Snow fall may occur at high altitudes on the Winterberg Mountains, with frost occurring regularly between April and September.

On the other hand, the STHE forms a rolling, grassy plateau 900–1900 m above sea level and gradually slopes down to the coast in the south-eastern Cape (Thieme et al., 2005). The dominant limnological features are rivers and seasonal pans (Thieme et al., 2005). In the Eastern Cape, mainstem sections of large river systems such as the Great Fish River and moderately-sized rivers such as the Keiskamma and Buffalo rivers form part of the STHE. The

STHE (Figure 3.1) has a temperate climate. Summer rainfall is most common, and varies from 1,400 mm in the east to 400 mm further west. Daily temperatures usually average 10–18°C with frost at night in winter to 15–40°C in summer (Thieme et al., 2005). Summers are hot, with long, dry, often windless periods broken by thunderstorms that generate both strong winds and local flooding.

Additionally, in the Great Fish River, the flow regime has been altered by the Orange-Great Fish Inter-Basin Water Transfer (IBWT) scheme from seasonal to perennial flow with little monthly fluctuation (Thieme et al., 2005). *Enteromius mandelai* has largely been undocumented in the sections that are altered by the IBWT. Most of the Great Fish River's tributaries are, however, uninfluenced by the IBWT and maintain natural flow regimes. The major tributaries include the Koonap and Kat rivers (Figure 3.1). The Koonap River has a dry catchment and has a high seasonal flow. In comparison, the Kat River drains wet catchments and has a perennial flow. *Enteromius mandelai* also occurs in narrow, fast flowing, turbulent streams such as those of the Keiskamma and Buffalo rivers that both have perennial flow, which is characterised by high seasonal discharge (Hughes & Hughes, 1992).

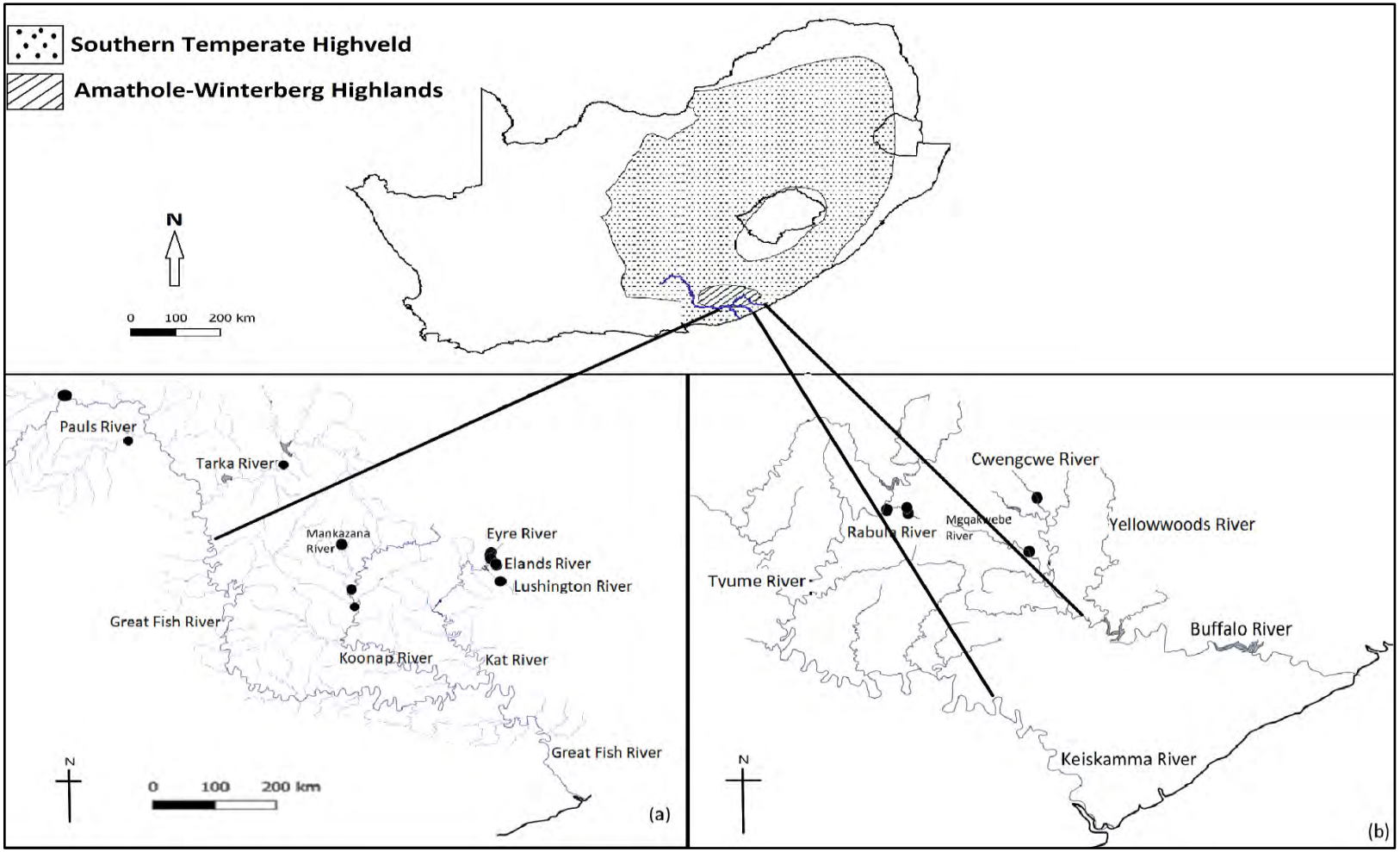


Figure 3.1: Map of South Africa (top centre pane) showing the Great Fish, Keiskamma and Buffalo rivers. Right and Left Panes show sampled localities in the (a) Great Fish and (b) Keiskamma and Buffalo river systems.

Table 3.1: Land-use patterns of all sampled sites where *Enteromius mandelai* and other fish species were captured in the Great Fish, Keiskamma and Buffalo River systems.

Site	System	River	Tributary	Land-use	Fish species	Latitude	Longitude
Lushington	Great Fish	Kat	Lushington	Invaded	*Enteromius mandelai*	26.8269	-32.6230
Eyre 1	Great Fish	Kat	Eyre	Near natural	*Enteromius mandelai*	26.7922	-32.5069
Eyre 2	Great Fish	Kat	Eyre	Near natural	*Enteromius mandelai*	26.7886	-32.5181
Eyre 3	Great Fish	Kat	Eyre	Near natural	*Enteromius mandelai*	26.7903	-32.5308
Elands 1	Great Fish	Kat	Elands	Agriculture	*Enteromius mandelai*	26.8125	-32.5589
Elands 2	Great Fish	Kat	Elands	Agriculture	*Enteromius mandelai*	26.8100	-32.5531
Elands 3	Great Fish	Kat	Elands	Agriculture	*Enteromius mandelai*	26.7969	32.5456
Mankazana	Great Fish	Kat	Mankazana	Near natural	*Enteromius mandelai*	26.2431	-32.4753
Braambos	Great Fish	Koonap	Braambos	Near natural	*Enteromius mandelai*	26.5939	-32.7575
Koonap	Great Fish	Koonap	Koonap	Near natural	*Enteromius mandelai*	26.5789	-32.7003
Tarka	Great Fish	Great Fish	Tarka	Agricultural	*Enteromius mandelai*	26.1331	-32.2317
Pauls	Great Fish	Great Fish	Pauls	Agricultural	*Enteromius mandelai*	25.5672	-32.2664
Upper Great Fish	Great Fish	Great Fish	Upper Great Fish	Near natural	*Enteromius mandelai*	25.4883	-31.1703
Rabula 1	Keiskamma	Keiskamma	Rabula	Near natural	*E. mandelai, Amatolacypris trevelyani*	27.0973	-32.7523
Rabula 2	Keiskamma	Keiskamma	Rabula	Near natural	*E. mandelai, Amatolacypris trevelyani*	27.1345	-32.7583
Rabula 3	Keiskamma	Keiskamma	Rabula	Near natural	*E. mandelai, Amatolacypris trevelyani*	27.1323	-32.7491
Cwengcwe	Buffalo	Buffalo	Cwengcwe	Near natural	*E. mandelai, A. trevelyani, Sandelia bainsii*	27.3566	-32.7329
Mgqakwebe	Buffalo	Buffalo	Mgqakwebe	Near natural	*Enteromius mandelai*	27.3432	-32.8221

Data collection

Field sampling for this study was conducted between August and October 2018. A total of 18 sites representative of the broad habitat types within each river system were selected from the Great Fish, Keiskamma and Buffalo River systems were sampled for the study (Figures 3.1–3.4, Table 3.1). The headwater streams were assigned to groups based on the land-use type of the riparian zone.

Three classes of riparian land-use were defined through visual estimation as near natural, agricultural and invaded landscape. A near natural (least impacted) land cover was categorised as having a riparian zone that was largely unaltered (Figure 3.2). The near natural vegetation in this region is dominated by deciduous broad-leafed forests and grasslands (Hoare et al., 2006). An agricultural land-use pattern was defined as having a riparian zone modified by agricultural activities. This was mainly small-scale crop farming and livestock farming (Figure 3.3). The invaded land-use pattern was categorised by the dominant presence of non-native invasive plants (Figure 3.4). The common non-native invasive plant species in this region include different species of wattles (*Acacia* sp), pine (*Pinus* sp), gumtree (*Eucalyptu*s sp), and tickberry (*Lantana camara*).

Thirteen sites were sampled in the Great Fish River, three in the Keiskamma River and two in the Buffalo River (Table 3.1). Additionally, the headwater streams were also differentiated by *E. mandelai* occurring in allopatry and sympatry with other fish species. Altitude and geographical co-ordinates were recorded using a global positioning system (GPS, Garmin etrex Ltd) unit to an accuracy of 5 m. A YSI Pro20 handheld dissolved oxygen meter (YSI, a Xylem brand, 1725 Brannum Lane, Yellow Springs, Ohio) was used to measure water temperature (°C) and dissolved oxygen (DO) (mg/l). A Hanna ISO Turbidity Portable Meter (HI98713; Hanna Instruments, Woonsocket, Rhode Island, United States of America) was used to measure turbidity (NTU). A Hanna HI98129 Combo meter was used to measure conductivity (µS/m) and total dissolved solids (TDS) (ppm) and pH (HANNA Instruments, Woonsocket, Rhode Island).

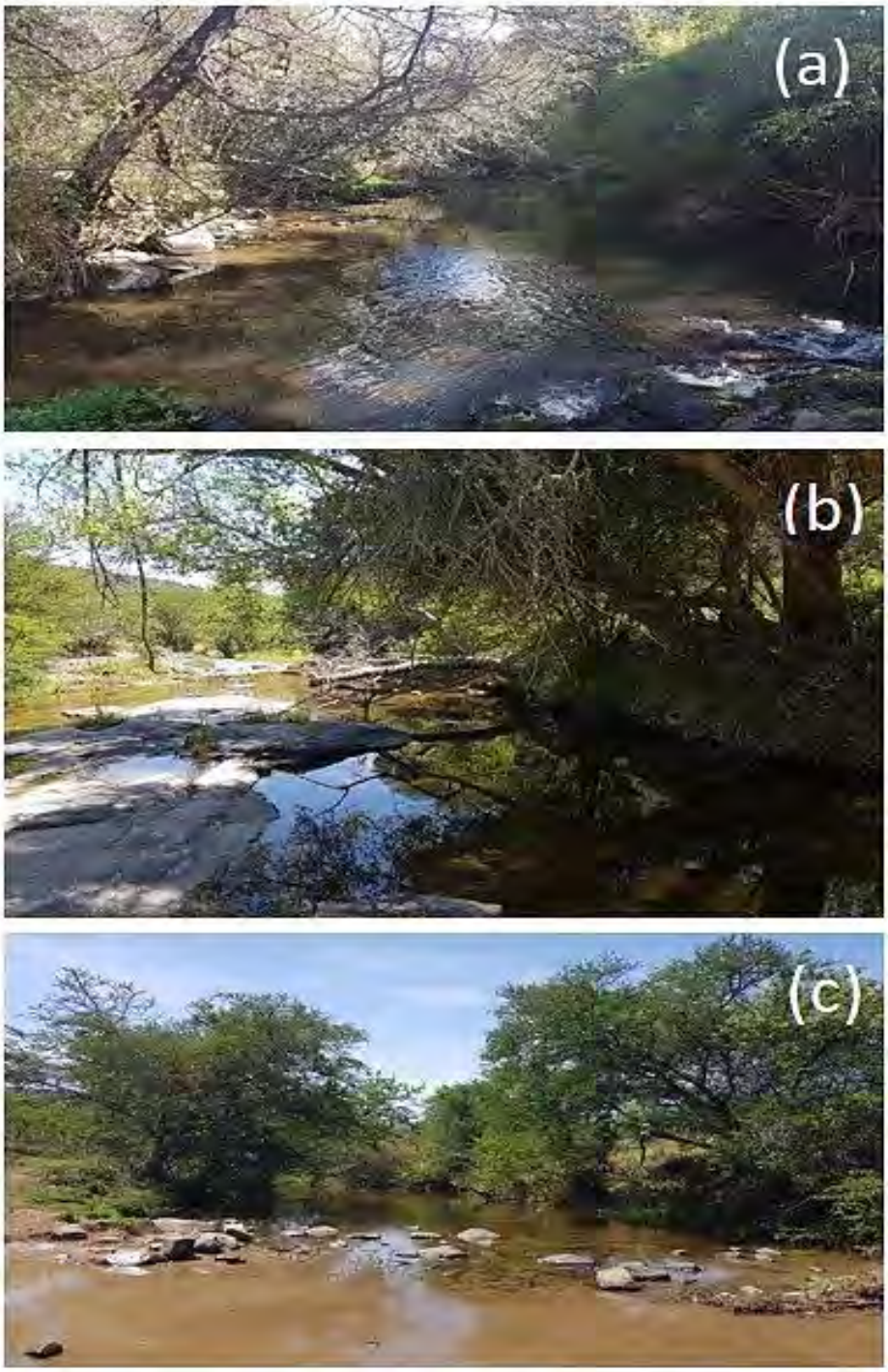


Figure 3.2: Streams characterised by native vegetation in the (a) Great Fish (b) Keiskamma and (c) Buffalo River systems.

Figure 3.3: Streams impacted by agricultural activities (a) Great Fish (b) Keiskamma and (c) Buffalo River systems.

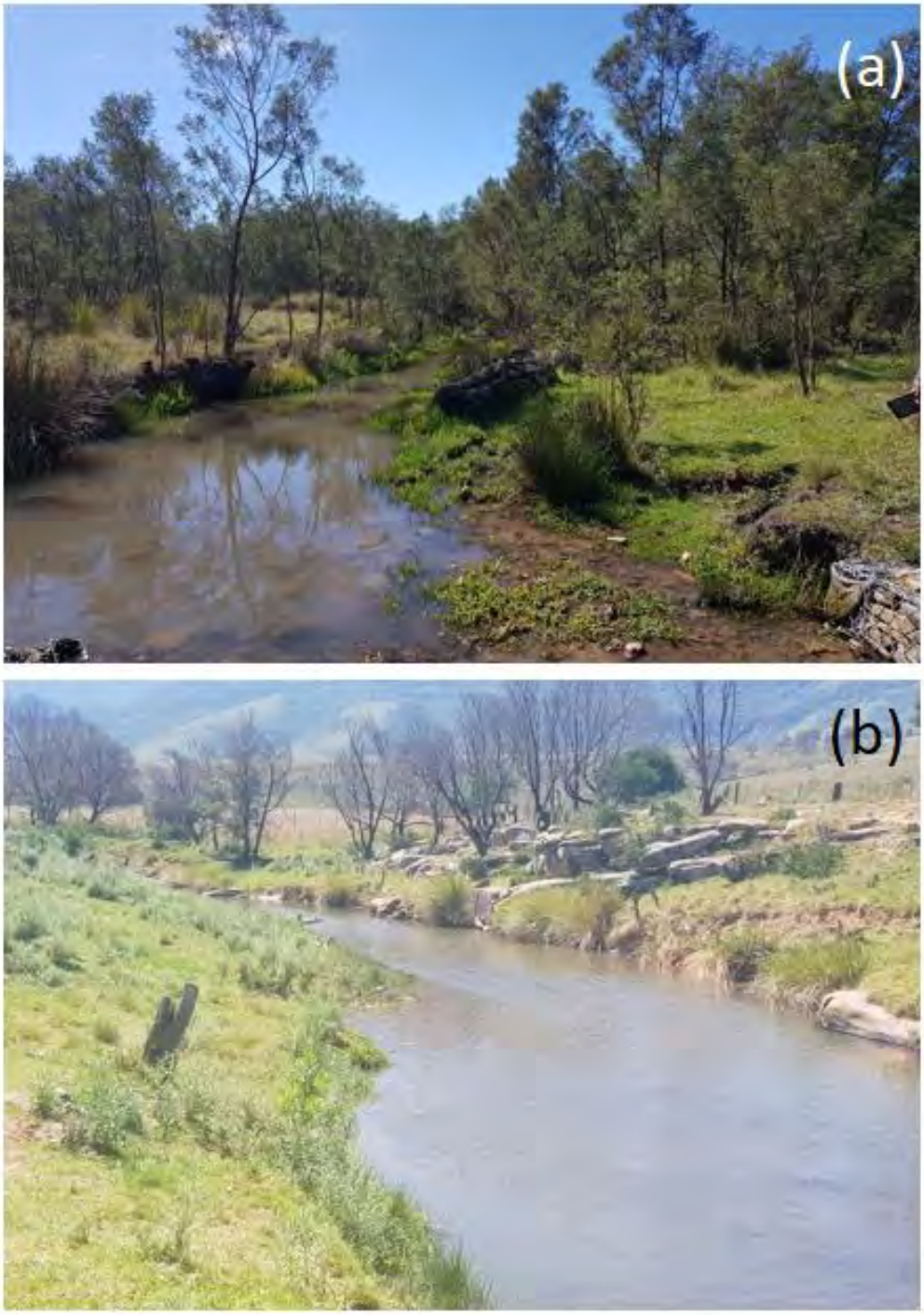


Figure 3.4: Streams with riparian zone invaded by non-native plant species in the (a) Great Fish and (b) Keiskamma River systems.

Potential basal food sources such as coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), epilithic algae, filamentous algae, and macrophytes (C_3 and C_4 aquatic plants) were collected. Coarse particulate organic matter is particulate organic matter > 1000 µm in size (Cummins, 1974). It is mostly of allochthonous origin, such as leaves, wood, bark, flowers, seeds and fruits, or of autochthonous origin such as macrophytes and filamentous algae. The CPOM of the studied river systems was primarily comprised of C_3 (leaves and plant debris) and C_4 plant matter (grasses) of terrestrial origin. It was collected by dislodging organic matter within an area of $1m^2$ and washing it through a 250 µm net. Organic particles in the size range of >0.45 to <1000 mm that were either suspended in the water column or deposited within lotic habitats were considered as FPOM. The FPOM was collected by filtering 25 litres of stream water through a 100 µm net. Three replicate samples were collected for both CPOM and FPOM at each site. Epilithic algae were scraped from the substratum using a scalpel blade, washed and stored in Eppendorf tubes. Filamentous algae and small bits of aquatic macrophytes were hand-picked and stored in Eppendorf tubes.

Macroinvertebrates were collected from instream and vegetated habitats in the headwater streams. In the instream habitats, macroinvertebrates were collected using a 250 µm mesh size net by disturbing the substrate through kicking for one minute. Vegetated sections were sampled by repeatedly sweeping a hand net through and over the vegetation for approximately one minute. The samples were sorted in the field using trays to isolate the macroinvertebrates from the detritus. The macroinvertebrates were sorted and identified to family level and stored on ice in the field. A reference sample of the macroinvertebrates was preserved in 70% ethanol (stored in two 180 ml translucent polypropylene containers with screw cups) to verify the identification using a stereomicroscope upon returning to the laboratory.

Fishes were sampled using a SAMUS backpack electrofisher, which was employed for 10 minutes, or by seine (3 m length × 3 mm mesh size net) netting. Current strength and setting for electrofishing gear were set to target species and conditions in the study area, such as conductivity, water depth and survey area. After multiple electrofishing passes were carried out, all fish captured were immediately removed from the net and placed in aerated buckets filled with ambient water to avoid prolonged exposure. *Enteromius mandelai* was the only fish species that occurred in most of the headwater sections in the AWHE and STHE. At each site where the species was found, 15 individuals were collected for SIA. These individuals were kept in a bucket containing stream water and then euthanased using clove oil. Total length and

standard length were measured of each individual fish. The caudal fin and white muscle tissues were excised and placed in Eppendorf tubes for stable isotope analysis. With the exception of the endangered Eastern Cape rocky, *Sandelia bainsii*, other fish species were processed in the same manner. Only a small piece of fin tissue was collected from *Sandelia bainsii* and the fish was returned alive to the stream. The basal food sources, aquatic invertebrates and fish were all sampled on the same day. All samples were kept on ice in the field. All the samples were then transported to the Freshwater Ecology Laboratory at the Department of Ichthyology and Fisheries Science at Rhodes University, Makhanda, South Africa.

In the laboratory, the ethanol preserved macroinvertebrates were identified to the lowest taxonomic unit possible using a light microscope. For stable isotope analysis, invertebrates of the same taxonomic group were pooled to achieve sufficient mass for analysis. The samples of fish, macroinvertebrates and basal food sources were oven-dried at 60 ℃ for 48 hours, after which they were ground into a fine powder with a mortar and pestle. The ground samples were packaged in Eppendorf tubes and sent for stable isotope analysis at the Stable Isotope Laboratory, Mammal Research Institute at University of Pretoria in South Africa. Aliquots of approximately 0.6–0.7 mg of muscle and fin tissue or 1–1.2 mg for plant materials were weighed into tin capsules that had been pre-cleaned with toluene. The ratios of the stable isotopes were measured against the reference standards, Pee Dee belemnite for $\delta^{13}C$ (Craig, 1957) and the atmospheric nitrogen gas for $\delta^{15}N$ (Ehleringer & Rundel, 1989) (Equation 1). A laboratory running standard (Merck Gel: $\delta^{13}C$ = -20.26‰, $\delta^{15}N$ = 7.89‰ and DL-Valine: $\delta^{13}C$ = -10.57‰, $\delta^{15}N$ = 6.15‰) and a blank sample were run after every 11 samples. Data corrections were done using the values obtained for the Merck Gel during each run, while the values for the DL-Valine standard provided the ± error for each run. These running standards were calibrated against international standards: National Institute of Standards and Technology (NIST): NIST 1557b (bovine liver), NIST 2976 (mussel tissue) and NIST 1547 (peach leaves). The delta notation (δ), the difference (‰, parts per thousand) between the isotopic ration of the sample and the standard was calculated as:

$$\delta X(‰) = [(\text{Rsample} - \text{Rstandard})/\text{Rstandard} - 1] \quad (1),$$

where X = $\delta^{15}N$ or $\delta^{13}C$ and R represents $^{15}N/^{14}N$ or $^{12}C/^{13}C$, respectively. Analytical precision was < 0.08‰ for $\delta^{13}C$ and <0.13‰ for $\delta^{15}N$. The isotopic analysis was done on a Flash SEA 1112 series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via ConFlo

IV system (ThermoFischer, Bremen, Germany), housed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria, South Africa.

Studies have shown that tissue differences in lipid content can potentially introduce a source of bias because lipids are generally depleted in $\delta^{13}C$ (Post et al., 2007; Michaud et al., 2013). This bias is removed either by chemical extraction to remove lipids from tissues or by mathematical corrections that are based on tissue C/N rations (Post et al., 2007). Previous studies on minnows in this region have shown that tissue C/N ratios are generally low (Kadye & Booth, 2013b; Kambikambi et al., 2019). In this study, both muscle and fin tissue C/N ratios were generally found to be low and comparable; hence, these tissues were analysed without modifications.

Data analysis

The macroinvertebrate groups were assigned to functional feeding groups following Palmer et al. (1993, 1996) and Cummins et al. (2005); scrapers/grazers, shredders, collector/gatherers, brushers, filterers, predators and omnivores. Grazers or scrapers are macroinvertebrates that feed on the biofilm layers. Biofilm consists of a complex community of algae, bacteria, fungi, and protozoans living in a matrix of secretions that adhere to solid surfaces like rocks and aquatic vegetation. They use rasping mouthparts to scrape the biofilm and algae off from the rocks and vegetation. Shredders are responsible for processing coarse particulate organic matter. Using their tearing mouthparts, they rip and shred the leaves as they feed. Collector/gatherers primarily roam the stream bottom scavenging for dead organisms, detritus, or other food particles that get lodged between rocks or in deep pools. Filterers either swim through the water column or sit sessile on the bottom filtering out particles that float by in the current. Often these are pieces of vegetation ripped up by shredders or tiny strips of biofilm dislodged by grazers, which flow downstream with the current. In addition, detritus from decaying plants and animals also provide a source of food. Predators feed on live prey and brushers are macroinvertebrates that feed on particles adhered to substrate and omnivores consume both live prey and plant material.

Food web structure

Differences in $\delta^{13}C$ and $\delta^{15}N$ values for basal food sources, invertebrate consumer groups and fish in the different river systems and land-use patterns were tested using permutational multivariate analysis of variance (PERMANOVA). PERMANOVA was performed with 9,999 unrestricted permutations and a significance level of $\alpha = 0.05$. Specifically, the study used one-way PERMANOVA to test the effect of riparian land use on stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values of basal sources (CPOM, FPOM, C_3 plants, C_4 plants and algae) as independent variables. One-way PERMANOVA was also used to test the effect of the differences among river systems on stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values of basal sources (CPOM, FPOM, C_3 plants, C_4 plants and algae). Then, two different one-way PERMANOVAs were used to test the differences among macroinvertebrate functional feeding groups (filterers, gatherers, predators, omnivores and herbivores) with regards to the factors "riparian land use" and "river systems". One-way PERMANOVA was also used to determine the effect of (1) of riparian land use and (2) river systems on *E. mandelai* $\delta^{13}C$ and $\delta^{15}N$ values. Analyses were performed on Euclidean distance measures of untransformed data. A statistically significant one-way PERMANOVA was followed by non-parametric pairwise comparisons with the PERMANOVA t statistic between group levels to determine which groups were different from each other. All statistical analyses were conducted using the community ecology package "*vegan"* (Oksanen et al., 2019) in the R statistical computing program (R Development Core Team 2020).

Isotope niche metrics

To investigate the trophic niche structure of *E. mandelai*, $\delta^{13}C$ and $\delta^{15}N$ values were used to derive quantitative isotope niche metrics for the different headwater streams (Layman et al., 2007; Jackson et al., 2011). These metrics included: $\delta^{13}C$ range (CR) indicating trophic diversity at the base of the food web; $\delta^{15}N$ range (NR), providing information on the trophic length; distance to the centroid (CD), giving an indication on the average trophic diversity. Two other metrics: mean nearest neighbour distance (MNND), which estimates the density and clustering of species within the community; and standard deviation of the nearest neighbour distance (SDNND), which gives a measure of the evenness of species packing in isotope biplot space. The standard ellipse area (SEA, expressed as $‰^2$) was used as a measure of trophic niche size for *E. mandelai* (Jackson et al., 2011). A small-sample size-corrected standard ellipse

(SEAc) was used to compare trophic niche sizes. All metrics were calculated using the Stable Isotope Bayesian Ellipses in R (Jackson et al., 2011) package in the R statistical computing programme (R Core Team, 2020).

Results

The physico-chemical variables showed variation in the three river systems. Water temperature was highest in the Buffalo River with a range of 22.0–23.7 °C and the lowest in the Great Fish River with a range of 7.5– 4.6 °C (Table 3.2). Dissolved oxygen and conductivity values were highest in the Buffalo River and lowest in the Great Fish River (Table 3.2). Turbidity was lower in the Buffalo River (4.5–4.6 NTU) than the Keiskamma and Great Fish river systems (4.9–16.0 NTU). The pH was alkaline in all three river systems (7.7–9.5).

Table 3.2: The ranges of physico-chemical variables measured in 18 localities in headwater tributaries of the Great Fish (n = 12), Keiskamma (n = 3) and Buffalo (n = 2) river systems, Eastern Cape, South Africa.

	Great Fish River (2 – 5/08/2018)	Keiskamma River (23/10/2018)	Buffalo River (23/10/2018)
Temperature (°C)	7.5 – 14.6	16.0 – 20.9	22 – 23.7
pH	7.8 – 9.5	7.7 – 8.0	7.9 – 8.0
Total dissolved solids (ppm)	72 – 99	102 – 138	268 – 289
Conductivity ($\mu S.m^{-1}$)	12.3 – 17.1	16.7 – 23.7	46.0 – 48.7
Turbidity (NTU)	4.9 – 11.7	6.1 – 16.0	4.5 – 4.6

Food web structure

Potential basal food sources included both autochthonous and allochthonous organic matter (Table 3.3). Across the three river systems and land-use (near natural, invaded and agriculture) types, the isotope values for basal sources did not differ significantly for $\delta^{13}C$ and $\delta^{15}N$ (Table 3.4). In all three river systems, macrophytes were generally most depleted in $\delta^{13}C$, with values ranging from -33.05±0.27 ‰ to -27.27±2.07 ‰ for C_3 plants and -31.58±0.03 ‰ to -22.11±0.00 ‰ for C_4 plants (Table 3.3). An exception was the Elands River site, which had enriched $\delta^{13}C$ values (-11.98±0.02 ‰) for C_4 plant matter. The FPOM and CPOM were most enriched in $\delta^{13}C$, with values ranging from -18.77±0.06 ‰ to -25.23±0.32 ‰ for FPOM and -23.05±0.60 ‰ to -28.58±0.20 ‰ for CPOM (Table 3.3). In comparison, algae had a wide range of $\delta^{13}C$ and $\delta^{15}N$ values in all three river systems and land-use types.

Thirty-one different invertebrate taxa and one anuran taxon were collected (Table 3.5). The PERMANOVA test showed that there were no significant differences for macroinvertebrate functional feeding groups among river systems and riparian land use for both $\delta^{13}C$ and $\delta^{15}N$ (Table 3.4). In the Great Fish River system, 10 aquatic invertebrate orders were collected (Table 3.4, 3.5). The taxonomic composition of macroinvertebrates was different among the different land-use regimes. From the 27 taxa collected from the Great Fish River system, 23 were collected from streams with a near natural riparian zone, 11 from agriculturally disturbed headwater streams and 9from streams with riparian zones invaded by non-native plants (Table 3.5). In the Keiskamma River system, seven aquatic invertebrate orders were collected (Table 3.5). These included Ephemeroptera with one family and four genera, Hemiptera with one family and two genera, Diptera and Odonata with one family and one genus each. The orders Trichoptera, Decapoda and Gastropoda had one genus each collected. In the Buffalo River system, seven aquatic invertebrate orders were collected (Table 3.5). These included Ephemeroptera with one family and three genera, Diptera and Hemiptera with two genera, Odonata, Trichoptera and Decapoda with one genus each and the order Oligochaeta.

Table 3.3: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for basal sources in the Great Fish, Keiskamma and Buffalo river systems.

Site	$\delta^{13}C$ (‰) CPOM	FPOM	C_3 macrophytes	C_4 macrophytes	Algae	$\delta^{15}N$ (‰) CPOM	FPOM	C_3 macrophytes	C_4 macrophytes	Algae
Great Fish River system										
Lushington	-24.23 ±0.19	-24.51±0.41	-29.62±0.04	-27.94±0.28		1.96±0.15	3.57±0.70	0.11±0.19	4.17±0.19	
Eyre 1		-25.10±0.89	-32.06±0.32	-30.75±0.05	-32.88±0.82		1.44±4.01	8.02±0.18	4.42±0.30	5.00±0.04
Eyre 2	-24.87±1.71	-26.18±0.15	-33.05±0.27	-31.58±0.03		2.94±0.32	4.34±0.94	4.78±1.59	7.91±0.28	
Eyre 3	-23.05±0.60	-23.55±0.37		-29.03±0.65	-26.21±0.00	2.51±0.21	2.12±0.32		4.81±0.32	3.73±0.00
Elands 1	-25.68±0.63	-23.19±0.03	-28.16±0.47	-22.86±8.87	-20.51±0.41	3.45±0.43	4.10±0.49	6.55±2.51	3.38±4.46	5.89±0.96
Elands 2	-25.95±0.29	-23.19±0.21	-31.78±0.10	-11.98±0.02	-14.77±0.46	7.15±0.68	5.74±0.57	0.64±2.18	2.93±0.27	8.76±0.23
Elands 3	-25.30±0.31	-20.59±0.73	-30.90±0.11	-30.99±0.12		4.15±0.75	3.39±1.04	3.76±0.51	5.11±0.59	
Mankazana					-17.17±0.00					1.55±0.00
Braambos				-22.11±0.00					9.29±0.00	
Koonap					-13.56±0.00					4.08±0.00
Tarka				-25.93±5.93	-32.35±0.00				12.68±1.29	12.18±0.00
Pauls					-17.11±2.36					2.60±1.34
Upper Great Fish				-27.45±0.00	-23.05±0.00				10.61±0.00	6.94±0.00
Keiskamma River system										
Rabula 1	-25.66±0.29	-25.71±0.62	-29.43±0.05		-30.63±0.15	5.26±0.56	5.43±0.62	5.70±3.02	0.64±0.02	7.34±0.22

	$\delta^{13}C$ (‰) CPOM	FPOM	C_3 macrophytes	C_4 macrophytes	Algae	$\delta^{15}N$ (‰) CPOM	FPOM	C_3 macrophytes	C_4 macrophytes	Algae
Rabula 2	-28.58±0.20	-24.53±0.35	-27.27±2.07	-29.58±0.85	-29.01±0.29	5.32±0.18	7.61±0.44	4.17±0.37	7.11±0.79	8.08±0.11
Keiskamma River system										
Rabula 3	-27.49±0.06	-25.23±0.32	-30.02±0.09	-31.53±0.12		4.89±0.67	0.59±0.15	5.53±0.03	5.77±0.12	
Buffalo River system										
Cwengcwe	-27.22±0.22	-21.29±1.38	-29.68±0.18	-29.78±0.03		2.91±0.58	3.35±0.95	1.34±0.24	7.78±0.03	
Mgqakwebe		-18.77±0.06	-29.17±0.13	-28.12±0.19	-26.40±0.23		6.25±0.63	2.99±0.03	5.88±2.20	9.40±0.27

Table 3.4: Results of permutational analysis of variance (PERMANOVA) for effect of river system and land-use type on basal sources and macroinvertebrate $\delta^{13}C$ and $\delta^{15}N$ values.

		PERMANOVA			
		$\delta^{13}C$		$\delta^{15}N$	
Independent variable	Dependent variables	*F*	*P*	*F*	*P*
	Basal sources				
River system		1.545	0.171	1.567	0.150
Land use type		0.337	0.964	0.321	0.972
	Macroinvertebrates				
River system		1.034	0.393	1.192	0.284
Land use type		0.549	0.887	0.549	0.903

For *E. mandelai*, there were statistically significant differences among the river systems for both $\delta^{13}C$ (PERMANOVA, $F = 3.452$, $P = 0.042$) and $\delta^{15}N$ (PERMANOVA, $F = 4.071$, $P = 0.016$) (Table 3.7). Non-parametric pairwise comparisons with the PERMANOVA t-statistic indicated that *E. mandelai's* $\delta^{13}C$ values varied significantly among river systems, particularly for the Keiskamma: Buffalo ($P = 0.008$) and Great Fish: Keiskamma ($P = 0.008$) river systems. Non-parametric pairwise comparisons also indicated significant differences for $\delta^{15}N$ values between the Buffalo: Great Fish ($P = <0.0001$) and the Great Fish: Keiskamma river systems ($P = <0.0001$) (Table 3.7). One-way PERMANOVA analysis also showed significant differences in *E. mandelai* $\delta^{15}N$ values (PERMANOVA, $F = 4.151$, $P = 0.021$) for the land-use factor (Table 3.7). Post-hoc pairwise comparisons revealed that these differences were significant for comparisons between Agriculture: Invaded ($P = <0.0001$) and Invaded: Near natural ($P = <0.0001$) riparian land-use types (Table 3.7). No significant differences for *E. mandelai* $\delta^{13}C$ values (PERMANOVA, $F = 2.207$, $P = 0.104$) were observed among land-use types.

Table 3.5: Mean values of carbon and nitrogen stable isotopes (± standard deviation) and functional feeding groups (FFG) for aquatic macroinvertebrates that were collected from the headwaters of the Great Fish, Keiskamma and Buffalo river systems. Land-use types were indicated as A for agriculture, I for invaded and N for near natural riparian zones.

			Great Fish River		Keiskamma River		Buffalo River		Land-use	
Order	Family	Genus	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	patterns	FFG
Ephemeroptera	Baetidae		-26.09±1.31	6.84±1.49	-28.28±2.17	8.06±0.78	-30.84±0.27	7.01±0.12	A, I, N	Gatherer
	Heptaginiidae	*Afronurus*	-26.18±1.46	7.94±0.54	-28.08±1.96	10.08±1.09			A, I, N	Brusher
	Trycorythidae	*Tricorythus*	-24.18±1.13	6.49±1.56	-28.17±1.52	7.77±0.61	-29.13±0.10	8.20±0.07	A, N	Gatherer
	Leptophlebidae	*Castanophlebia*	-24.89±0.59	7.15±0.47	-27.88±1.48	8.17±0.80	-29.25±0.00	7.95±0.00	A, I, N	Gatherer
	Caenidae	*Caenis*	-24.10±0.00	3.44±0.00	-27.12±0.00	6.91±0.00	-28.05±0.23	7.42±0.73	N	Gatherer
Diptera	Simuliidae	*Simulium*	-24.02±1.06	6.78±0.92			-27.09±0.13	8.10±0.02	A, I, N	Filterer
		Rhabdiomastix	-26.11±0.00	1.70±0.00					N	Shredder
	Tabanidae		-21.98±1.33	8.75±0.56	-25.41±0.70	11.00±0.11			A, I	Predator
	Athericidae	*Suragina*			-26.63±0.15	10.03±0.51	-31.34±0.00	8.44±0.00	N	Predator
Odonata	Synlestidae		-24.24±0.45	8.41±0.18					I, N	Predator
	Ashnidae	*Aeshna*	-25.15±1.38	8.88±2.35	-27.99±0.79	9.76±0.41			A, I, N	Predator
	Lestidae	*Lestes*	-24.47±±1.13	9.99±4.72	-26.89±0.42	10.22±0.61	-26.83±0.77	10.31±1.61	N	Predator
	Gomphidae	*Seratogomphus*	-26.77±4.71	11.15±3.45					A	Predator
	Coenagrionidae		-26.70±3.17	9.98±4.06					A, N	Predator
	Libellulidae		-25.94±2.26	10.39±4.39					N	Predator
Coleoptera	Gyrinidae	*Orectogyrus*	-24.04±0.07	6.52±0.59					N	Predator
		Dytiscidae	-26.01±1.04	9.83±0.00					N	Predator
Trichoptera	Hydropsychidae	*Cheumatopsyche*	-24.03±1.39	8.31±0.79					A, I, N	Gatherer
		Hydropsyche			-28.46±1.82	9.62±0.53	-30.47±1.19	11.11±0.10	N	Filterer
	Philopotamidae	*Chimarra*	-24.57±0.24	6.75±0.07					I	Filterer
Hemiptera	Sialidae	*Leptosialis*	-25.41±0.85	6.10±0.87					N	Predator
	Corixidae		-25.68±2.01	8.07±3.66					A, N	Predator
	Pleidae	*Plea*					-24.63±0.37	8.85±1.01	N	Predator
	Belostomatidae	*Appasus*	-26.14±0.03	11.57±5.22	-24.58±0.15	9.81±0.66	-23.68±1.75	10.68±0.63	N	Predator
	Notonectidae		-25.81±0.32	9.94±4.29	-25.27±8.25				N	Predator
	Gerridae		-23.00±±0.00	7.38±0.00					N	Predator
	Veliidae	*Rhagovelia*			-26.24±0.00	10.69±0.00			N	Predator
	Planorbidae	*Burnupia*	-20.52±0.00	6.00±0.00	-21.03±0.00	9.09±0.00			N	Scraper

Order	Family	Genus	Great Fish River		Keiskamma River		Buffalo River			FFG
			$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)		
Plecoptera	Notonemouridae	*Aphanicella*	-24.22±1.71	8.53±2.09					A, N	Shredder
Haplatoxida			-22.64±0.12	7.41±0.34	-25.94±0.00	10.90±0.00			A	Gatherer
Anura			-24.20±3.02	9.44±4.01	-26.03±0.03	10.22±0.10	-27.84±0.00	9.16±0.00	A, I, N	Herbivore

Table 3.6: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for consumer groups including macroinvertebrate functional feeding groups and fish species that occurred in the Great Fish, Keiskamma and Buffalo River systems.

	Great Fish River		Keiskamma River		Buffalo River	
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Filterers	-24.09±1.00	6.78±0.86	-28.46±1.82	9.62±0.53	-28.44±1.94	9.31±1.65
Scrapers/grazers	-20.52±0.00	6.00±0.00	-21.03±0.00	9.09±0.00		
Collectors/gatherers	-24.94±1.39	6.96±1.26	-27.94±1.61	8.09±0.97	-29.30±1.16	7.71±0.56
Shredders	-24.86±1.49	9.09±2.72				
Brushers	-26.18±1.46	7.94±0.54	-28.08±1.96	10.08±1.09		
Predators	-25.10±2.25	9.12±3.31	-26.39±1.35	10.16±0.83	-25.62±2.38	9.82±1.31
Omnivores	-20.30±1.51	7.13±1.32	-21.42±2.54	8.17±0.79	-22.48±0.49	11.61±0.15
Herbivore (tadpole)	-24.20±3.02	9.44±4.01	-26.03±0.03	10.22±0.20	-27.84±0.00	9.16±0.00
Enteromius mandelai	-23.26±1.92	11.69±2.30	-24.24±1.21	11.80±0.78	-23.21±1.06	12.95±1.12
Amatolacypris trevelyani			-24.33±1.17	11.75±0.63	-24.03±1.31	11.09±0.34
Sandelia bainsii					-24.58±1.84	8.99±2.86

Table 3.7: Results of permutational multivariate analysis of variance (PERMANOVA) for effect of river system (Great Fish, Keiskamma and Buffalo rivers) and land-use pattern (near natural, agriculture and invaded) on *Enteromius mandelai* $\delta^{13}C$ and $\delta^{15}N$. A one-way PERMANOVA was conducted for each independent variable (river system and landscape type) separately. For significant one-way PERMANOVAs, pairwise comparisons with the PERMANOVA t statistic were reported for each independent variable. All tests were significant at $P < 0.05$. Significant *P* values are indicated in bold type.

	One-way PERMANOVA				Pairwise comparisons			
	$\delta^{13}C$		$\delta^{15}N$		$\delta^{13}C$		$\delta^{15}N$	
Independent variable	*F*	*P*	*F*	*P*		*P*		*P*
River system	3.452	**0.042**	4.071	**0.016**	Buffalo: Great Fish	0.315	Buffalo: Great Fish	**<0.0001**
					Buffalo: Keiskamma	**0.008**	Buffalo: Keiskamma	0.130
					Great Fish: Keiskamma	**0.008**	Great Fish: Keiskamma	**<0.0001**
Land-use	2.207	0.104	4.151	**0.021**			Agriculture: Invaded	**<0.0001**
							Agricultural: Near natural	0.711
							Invaded: Near natural	**<0.0001**

Isotopic niche metrics

In the Great Fish River system, *E. mandelai* exhibited a wide variation in isotopic niche sizes (Table 3.8). Large isotopic niche sizes were observed in both agriculturally disturbed (Elands River site 3, SEAc = 11.01 ‰^{2}; Braambos River, SEAc = 2.52 ‰^{2}) and near natural (Eyre River site 1, SEAc = 2.09 ‰^{2}; site 3, SEAc = 2.18 ‰^{2}) sites (Figure 3.5, 3.7). At these sites, *E. mandelai* had wide CR and NR ranges, indicating a wide breadth in carbon sources and high trophic ranges. In addition, *E. mandelai* exhibited high values for CD, MNND and SDNND (Table 3.8) at these sites, which indicated low trophic redundancy (trophic redundancy refers to the phenomenon that species in the same ecosystem fill similar roles, which results in a sort of "insurance" in the ecosystem. Redundant species can easily do the job of a similar species from the same functional niche) and high intraspecific variability. By comparison, small isotope niche sizes were observed across different land use activities, including natural (Eyre River site 2, SEAc = 0.72 ‰^{2}), agriculture-disturbed (Pauls River, SEAc = 0.86 ‰^{2}; Elands River site 1, SEAc = 0.92 ‰^{2}), and plant-invaded (Lushington River, SEAc = 0.70 ‰) (Figure 3.5,3.7). With the exception of Elands River site 1 which had high CR values, *E. mandelai* had low CR and NR ranges at all sites, indicating a narrow breadth in carbon sources and low trophic ranges. Furthermore, *E. mandelai* exhibited low values for CD, MNND and SDNND at these sites (Table 3.8), which indicated higher trophic redundancy and lower intraspecific variation. Intermediate isotopic niche sizes were also observed within natural sites (Mankazana River, SEAc = 1.21 ‰^{2}; Koonap River, SEAc = 1.41 ‰^{2}; upper Great Fish River, SEAc = 1.01 ‰^{2}) and agriculture-disturbed sites (Elands River site 2, SEAc = 1.54 ‰; Tarka River, SEAc = 1.79 ‰) (Figure 3.5, 3.7). At these sites, *E. mandelai* also had high CR and NR ranges, indicating a wide breadth in carbon sources and high trophic ranges. Additionally, *E. mandelai* exhibited high values for CD, MNND and SDNND at these sites (Table 3.8), indicating low trophic redundancy and high intraspecific variation.

In the Keiskamma River system, *E. mandelai* occurred in sympatry with *Amatolacypris trevelyani* at the sampled sites. The three sites had a natural landscape. The isotopic niche size of *E. mandelai* varied among these three sites. *Enteromius mandelai* had the largest isotopic niche size at the Rabula River site 1 (SEAc = 2.53 ‰^{2}) and the lowest at the Rabula River site 2 (SEAc = 0.78 ‰^{2}) (Table 3.8, Figure 3.6, 3.8). Additionally, *E. mandelai* had the highest CR and NR ranges at the Rabula River site 1, indicating a greater range of basal resources and higher trophic length than the other sites. Conversely, the population at Rabula River site 2 had the lowest CR and NR values indicating a lower range of basal resources and shorter trophic

length. Furthermore, *E. mandelai* had the highest CD, MNND and SDNND at the Rabula River site 1, indicating low trophic redundancy and high intraspecific variation, whereas the other two sites had lower CD, MNND, and SDNND values indicating higher trophic redundancy and low intraspecific variation. In comparison to *A. trevelyani, E. mandelai* had a larger isotopic niche at Rabula River site 1 than did the populations at the other sites. *Enteromius mandelai* also had higher community metric (CR, NR, CD, MNND and SDNND) values at Rabula River site 1 indicating a narrower niche breadth in carbon sources, higher trophic ranges, higher trophic redundancy and lower intraspecific variation than *A. trevelyani* (Table 3.6). The opposite pattern was seen for *E. mandelai* in the Rabula River site 3. Conversely, in the Rabula River site 2, despite having a smaller isotopic niche size than *A. trevelyani*, *E. mandelai* had higher CR, NR, CD and SDNND values (Table 3.8).

In the Buffalo River system, *E. mandelai* occurred in sympatry with *Amatolacypris trevelyani* and *Sandelia bainsii* at the Cwengcwe River site and in allopatry at the Mgqakwebe River site. The isotopic niche size of *E. mandelai* varied between the two sites. *Enteromius mandelai* had the largest isotopic niche size at the Mgqakwebe River site (SEAc = 0.76 ‰^{2}) and smallest at the Cwengcwe River site (SEAc = 0.43 ‰^{2}) (Table 3.8, Figure 3.7, 3.9). Furthermore, *E. mandelai* had the highest CR and NR ranges at the Mgqakwebe River site, indicating a wider range of basal resources and higher trophic length than at the Cwengcwe River site (Table 3.8). With the exception of MNND, *E. mandelai* had the highest CD and SDNND at the Mgqakwebe River site indicating lower trophic redundancy than at the Cwengcwe River site (Table 3.8, Figure 3.9). Additionally, at the Cwengcwe River site, *E. mandelai* had lower community metric (CR, NR, CD and MNND) values indicating a narrower niche breadth in carbon sources, lower trophic ranges, lower trophic redundancy and lower intraspecific variation than *A. trevelyani* (Table 3.8).

Table 3.8: Stable isotope community metrics comparing the trophic structure of *E. mandelai* communities sampled from the Great Fish, Keiskamma and Buffalo river systems. SEAc, core isotopic niche area of the community (‰^{2}); NR, δ^{15}N range; CR, δ^{13}C range; CD, mean distance to centroid; MNND, mean nearest neighbour distance; SDNND, standard deviation of nearest neighbour distance and Land-use type.

Site	Species	SEAc	CR	NR	CD	MNND	SDNND	Land-use
Great Fish River system								
Lushington	*Enteromius mandelai*	0.71	1.89	1.39	0.54	0.35	0.39	Invaded
Eyre 1	*Enteromius mandelai*	2.09	2.60	3.60	0.96	0.43	0.57	Near natural
Eyre 2	*Enteromius mandelai*	0.73	1.82	2.31	0.56	0.27	0.32	Near natural
Eyre 3	*Enteromius mandelai*	2.18	2.47	2.88	0.94	0.58	0.43	Near natural
Elands 1	*Enteromius mandelai*	0.92	4.42	0.82	1.11	0.37	0.14	Agriculture
Elands 2	*Enteromius mandelai*	1.54	3.77	1.36	1.04	0.39	0.24	Agriculture
Elands 3	*Enteromius mandelai*	11.01	7.56	6.56	2.17	1.10	1.12	Agriculture
Mankazana	*Enteromius mandelai*	1.21	2.60	2.38	0.77	0.29	0.19	Near natural
Braambos	*Enteromius mandelai*	2.52	3.29	3.37	1.10	0.53	0.55	Near natural
Koonap	*Enteromius mandelai*	1.41	2.85	1.21	0.92	0.46	0.24	Near natural
Tarka	*Enteromius mandelai*	1.79	2.37	2.14	0.98	0.45	0.37	Agriculture
Pauls	*Enteromius mandelai*	0.86	1.22	2.19	0.61	0.32	0.31	Agriculture
Upper Great Fish	*Enteromius mandelai*	1.01	1.00	2.15	0.79	0.37	0.37	Near natural
Keiskamma River system								
Rabula 1	*Enteromius mandelai*	2.53	3.47	3.31	0.97	0.46	0.64	Near natural
	Amatolacypris trevelyani	0.66	1.77	1.12	0.69	0.44	0.19	
Rabula 2	*Enteromius mandelai*	0.78	3.05	1.34	0.81	0.22	0.21	Near natural
	Amatolacypris trevelyani	1.42	1.02	0.90	0.56	0.89	0.14	
Rabula 3	*Enteromius mandelai*	0.94	2.36	2.08	0.74	0.31	0.16	Near natural
	Amatolacypris trevelyani	1.23	3.21	1.26	1.02	0.48	0.16	
Buffalo River system								
Cwengcwe	*Enteromius mandelai*	0.43	2.50	0.48	0.65	0.39	0.18	Near natural
	Amatolacypris trevelyani	1.26	3.70	1.32	1.18	0.42	0.14	
Mgqakwebe	*Enteromius mandelai*	0.76	3.56	1.33	0.80	0.27	0.30	Near natural

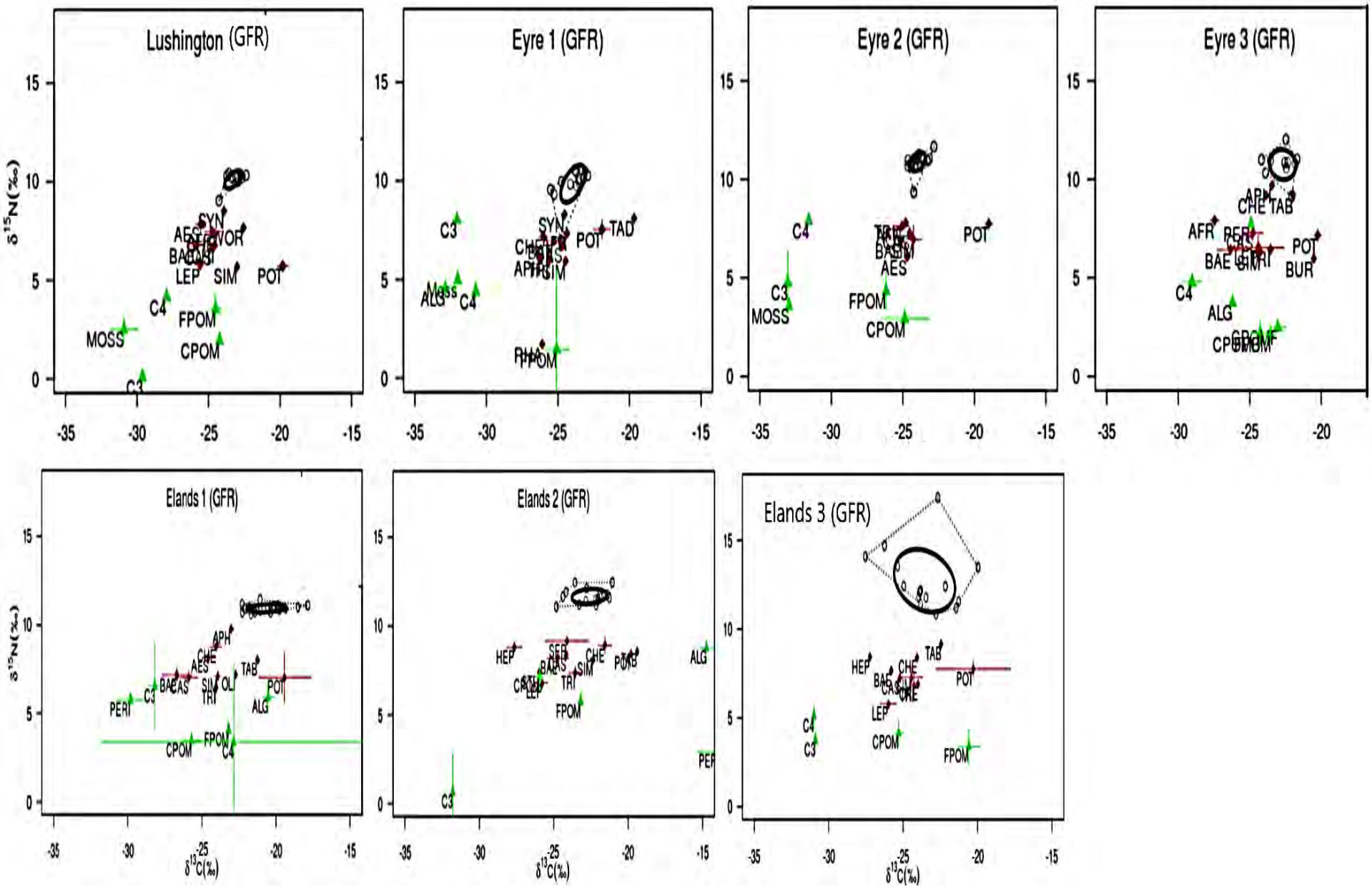

Lushington (GFR)
Eyre 1 (GFR)
Eyre 2 (GFR)
Eyre 3 (GFR)
Elands 1 (GFR)
Elands 2 (GFR)
Elands 3 (GFR)
δ15N(‰)
δ13C(‰)
SYN
AES
LEP
SIM
POT
C4
FPOM
MOSS
CPOM
C3
TAB
ALG
APH
CHE
TAB
PERI
TRI
HEP
BUR
AFR

Figure 3.5: Stable isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ for *Enteromius mandelai* using muscle tissue (represented by sample-size corrected standard ellipse area (SEAc)), macroinvertebrates (red diamonds) and basal sources (green triangles) for all sampled sites in the Great Fish Rivers. The abbreviations are as follows: ALG = Algae; C_3 = C_3 plants; C_4 = C_4 plants; CPOM = Course particulate organic matter; FPOM = Fine particulate organic matter; Che = *Chematopsyche*; TRI = *Tricorythus*; SIM = *Simulium*; AES = *Aeshna*; LYC = Lycosidae; TET = *Tetragnatha*; LIB = Libellulidae; LES = *Lestes*; GER = Gerridae; BAE = Baetidae; APP = *Appasus*; POT = *Potamonautes*; AFR = *Afronurus*; CAS = *Castanophlebia*; CAE = *Caenis*; TAB = Tabanidae; SYN = Synlestidae; GOM =Gomphidae; SER = *Seratogomphus*; ZYG =Zygoptera; COE = Coenagroinidae; ORE = *Orectogyrus*; DYT = *Dytiscidae*; CHI = *Chimarra*; LEP = *Leptosalis*; COR = Corixidae; NOT = Notonectidae; BUR = *Burnupia*; APH = Aphanicella.

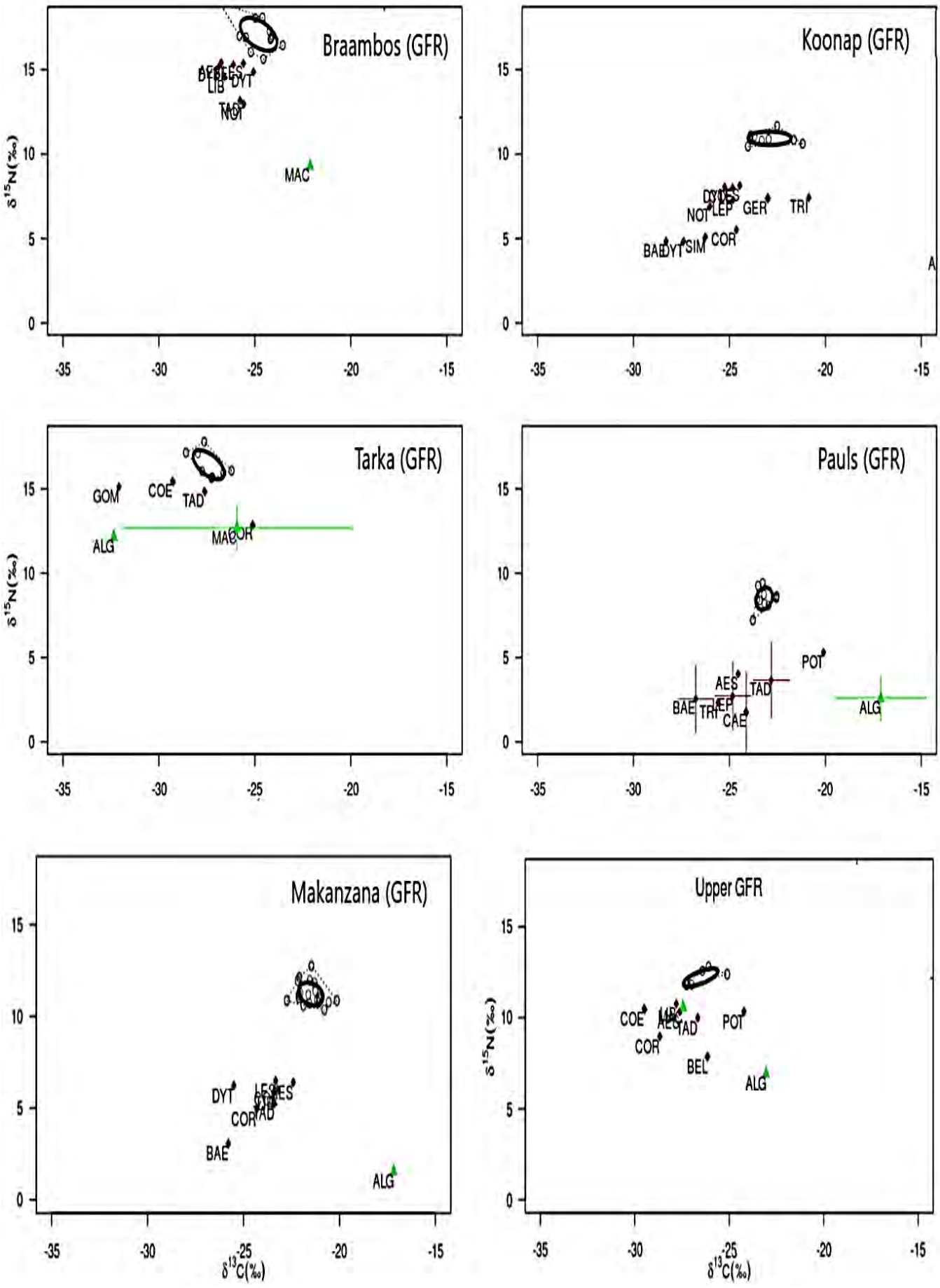

Braambos (GFR)
Koonap (GFR)
Tarka (GFR)
Pauls (GFR)
Makanzana (GFR)
Upper GFR
δ15N(‰)
δ13C(‰)
MAC
GER
TRI
SIM
COR
GOM
COE
TAD
ALG
POT
AES
BAE
CAE
DYT
BEL

Figure 3.5 continued: Stable isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ for Enteromius mandelai using muscle tissue (represented by sample-size corrected standard ellipse area (SEAc)), macroinvertebrates (red diamonds) and basal sources (green triangles) for all sampled sites in the Great Fish Rivers. Stable isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ for Enteromius mandelai using muscle tissue, macroinvertebrates and basal sources for all sampled sites in the Great Fish River. The abbreviations are as follows; ALG = Algae; C_3 = C_3 plants; C_4 = C_4 plants; CPOM = Course particulate organic matter; FPOM = Fine particulate organic matter; Che = Chematopsyche; TRI = Tricorythus; SIM = Simulium; AES = Aeshna; LYC = Lycosidae; TET = Tetragnatha; LIB = Libellulidae; LES = Lestes; GER = Gerridae; BAE = Baetidae; APP = Appasus; POT = Potamonautes; AFR = Afronurus; CAS = Castanophlebia; CAE = Caenis; TAB = Tabanidae; SYN = Synlestidae; GOM =Gomphidae; SER = Seratogomphus; ZYG =Zygoptera; COE = Coenagroinidae; ORE = Orectogyrus; DYT = Dytiscidae; CHI = Chimarra; LEP = Leptosalis; COR = Corixidae; NOT = Notonectidae; BUR = Burnupia; APH = Aphanicella.

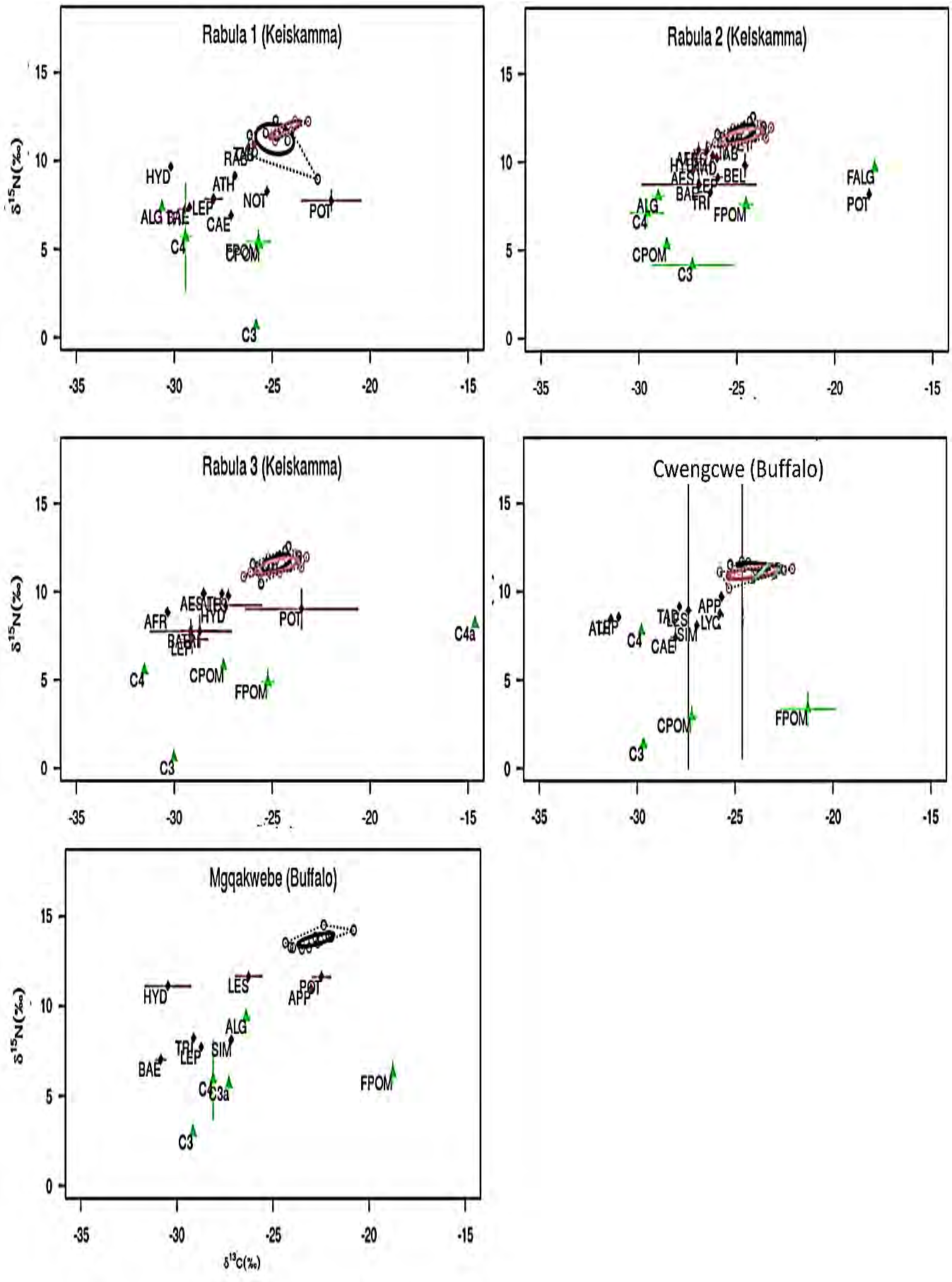

Rabula 1 (Keiskamma)
Rabula 2 (Keiskamma)
Rabula 3 (Keiskamma)
Cwengcwe (Buffalo)
Mgqakwebe (Buffalo)
δ15N(‰)
δ13C(‰)
HYD
ATH
NOT
POT
ALG
BAE
LEP
CAE
C4
CPOM
C3
FPOM
FALG
BEL
TRI
AFR
C4a
APP
LYC
SIM
LES
C3a

Figure 3.6: Stable isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ for *Enteromius mandelai* (black ellipses), *Amatoloacypris trevelyani* (red ellipses) using muscle tissue, macroinvertebrates (red diamonds) and basal sources (green triangles) for all sampled sites in the Keiskamma and Buffalo Rivers. The abbreviations are as follows; ALG = Algae; C_3 = C_3 plants; C_4 = C_4 plants; CPOM = Course particulate organic matter; FPOM = Fine particulate organic matter; TAD = Anura(tad-pole); TRI = *Tricorythus*; SUG = *Sugarina*; SIM = *Simulium*; HYD = *Hydropsyche*; AES = *Aeshna*; LES = *Lestes*; PLE = *Plea*; GER = Gerridae; BAE = Baetidae; APP = *Appasus*; RHA = *Rhagovelia*; POT = *Potamonautes*; AFR = *Afronurus*; CAS = *Castanophlebia*; CAE = *Caenis*; TAB = Tabanidae; GOM =Gomphidae; LEP = *Leptosalis*; NOT = Notonectidae; BUR = *Burnupia*; APH = *Aphanicella*; OLI = Oligochaeta.

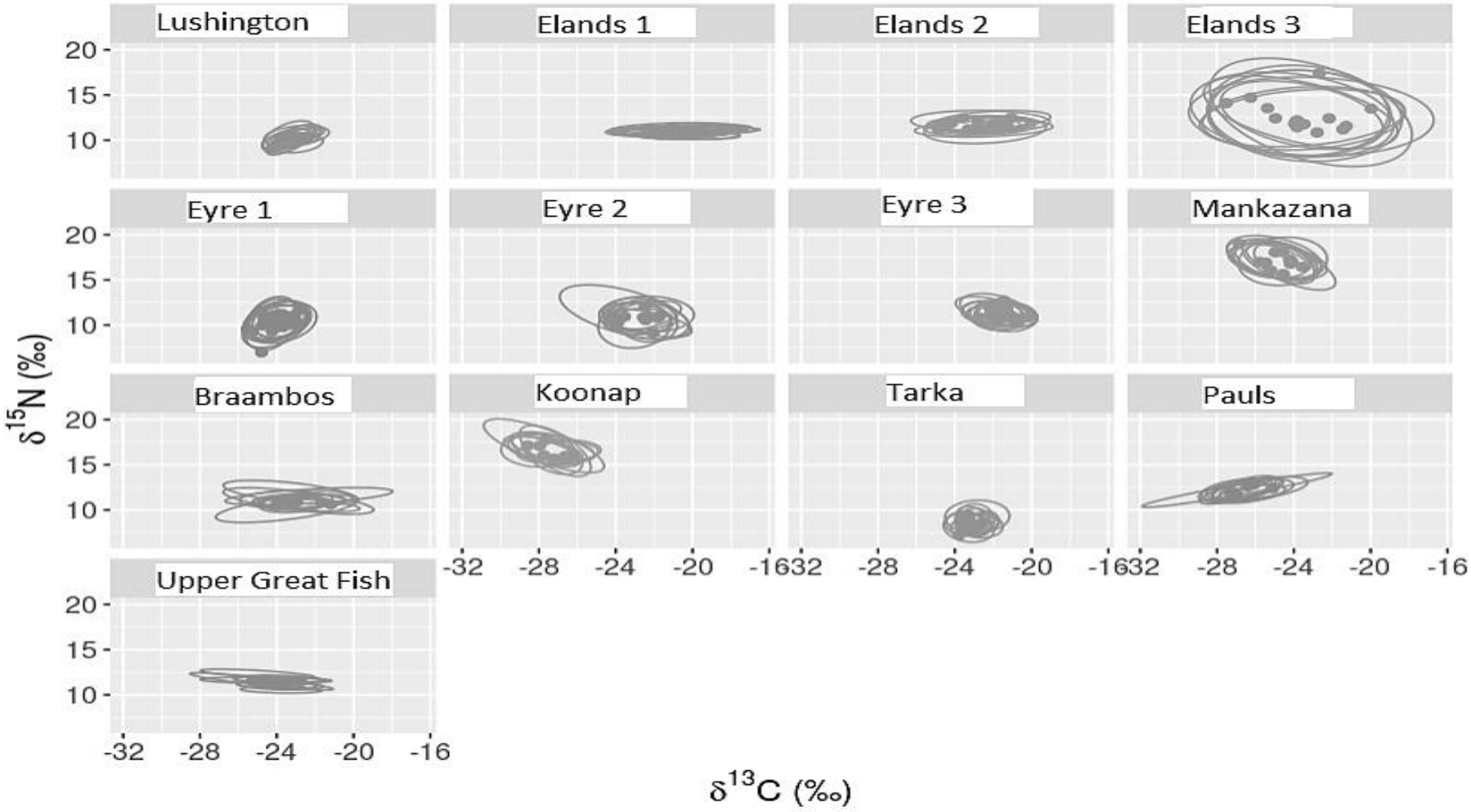


Figure 3.7: Isotopic niche size for *Enteromius mandelai* using sample size corrected standard ellipse areas (SEAc) based on muscle tissue for the Great Fish River system.

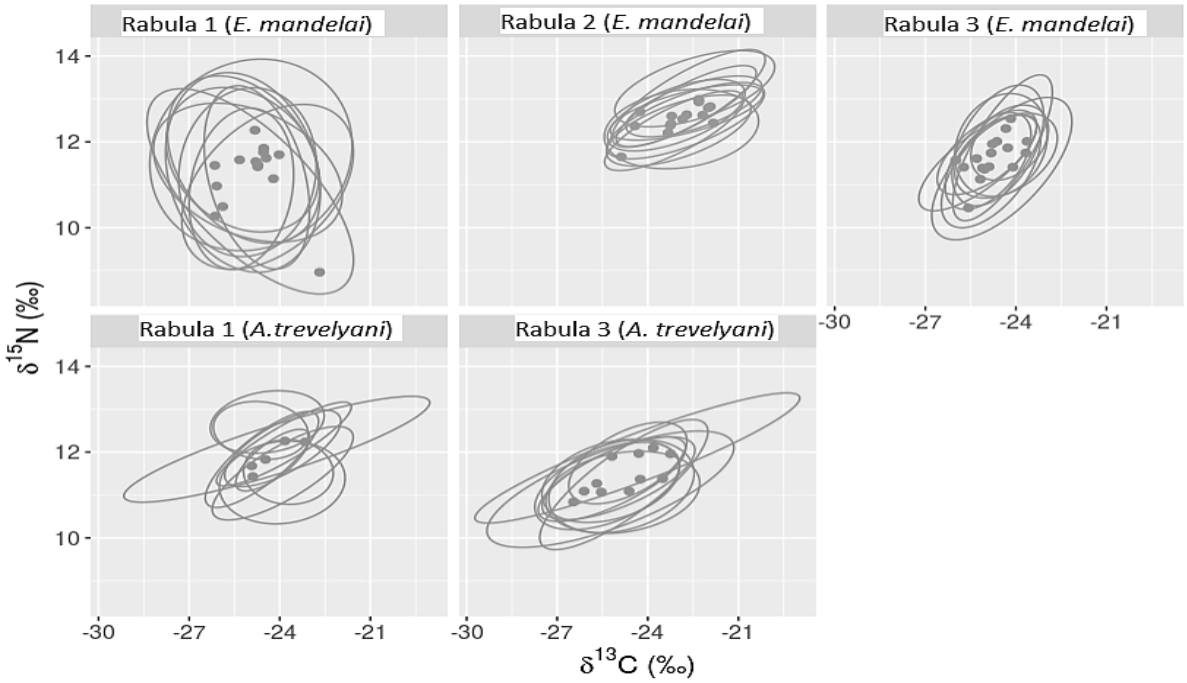


Figure 3.8: Isotopic niche size for *Enteromius mandelai* and *Amatolacypris trevelyani* using sample size corrected standard ellipse areas (SEAc) based on muscle tissue for the Keiskamma River system.

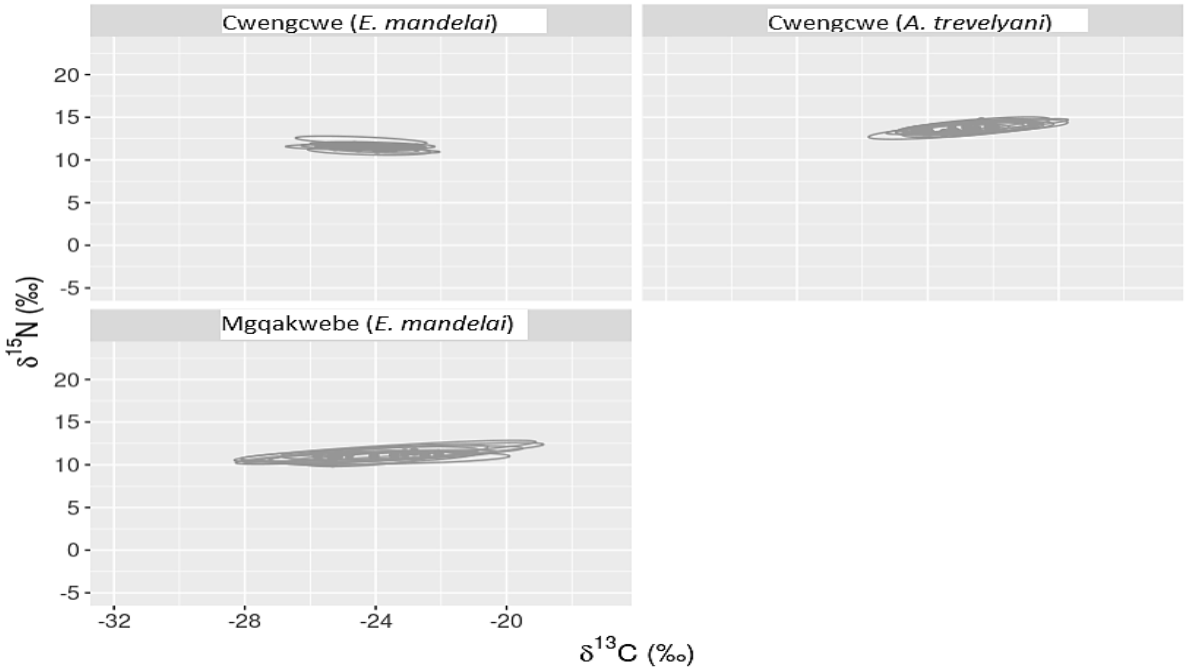


Figure 3.9: Isotopic niche size for *Enteromius mandelai* and *Amatolacypris trevelyani* using sample size corrected standard ellipse areas (SEAc) based on muscle tissue for the Buffalo River system.

Discussion

Understanding how *E. mandelai* is influenced by available trophic resources and how this is expressed through the food chain is critical to explaining the trophic dynamics of this species. The study hypothesised that different land-use characteristics among the the headwater streams would have an effect on the trophic resources. The isotopic results showed that basal sources and macroinvertebrate consumer group $\delta^{13}C$ and $\delta^{15}N$ values were not statistically significant, contrary to the expectation that the headwater streams would differ in their basal source and macroinvertebrate consumer group values due to differences in environmental and land-use characteristics. It was hypothesised that the trophic niche patterns of *E. mandelai* would vary in relation to differences in the food web charactersitics. This hypothesis was supported by the results which showed that *E. mandelai* exhibited variable isotopic values across different river systems and land-use types. This suggests that though *E. mandelai* is able to surve in streams with different characteristics, some trophic conditions are better than others. *Enteromius mandelai* was expected to show wider niche breadth in headwater streams with near natural vegetation as these habitats are expected to support diverce food sources. Although not statistically tested, *E. mandelai* generally had larger isotopic niches in agriculturally disturbed landscapes. This may be due to the increses nutrient content in these streams from agricultural activities which leads to and increase in consumer groups. The smallest isotopic niche for *E. mandelai* were found in in headwater streams invaded by non-native plants. These streams may therefore not be a preffered habitat for *E. mandelai*. There was a limited number of sites with riparian zones dominated by invasive plants included in this study. This is because most of the invasive plant dominated headwater stream sites sampled did not have any fish species, particularly *E. mandelai* occuring in them. These sites where therefore left out of the trophic analysis. The study also hypothesised that *E. mandelai* would display trophic niche differentiation in relation to the presence of other fish species. It was expected that *E. mandelai* would have smaller isotopic niches when occuring with other fish species and larger isotic niches in isolation. The results were in agreement with these expectations.

Despite the the lack of statisitical significance in stable isotope values among basal sources and macroinvertebrate functional feeding groups in the different headwater streams, there was considerable variation in stable isotope-based food web charactersistics. Among all sampled sites, macrophytes were generally the most depleted in $\delta^{13}C$ compared to CPOM and FPOM. An exception was the agriculture-disturbed Elands River site which had enriched $\delta^{13}C$

values for macrophytes. This is likely linked to the influence of agricultural activity which increases carbon ranges as a result of runoff of agrochemicals and manure associated with livestock grazing causing a wider variation in these basal sources. This enrichment is similar to that shown by studies elsewhere were agricultural land-use has had an effect on basal sources (Allen et al., 2009; Griffiths et al., 2013; Lee et al., 2018). However, there were no significant differences in particulate organic matter values among the least impacted and disturbed headwater streams. This is in contrast to previous studies which have shown that the composition of leaf litter influenced the structure and function of stream ecosystems by altering the nutrient content and energy transfer (Hicks, 1997; Milanovich et al., 2014; García et al., 2017). For example, in streams with catchments invaded by non-native plants such as black wattle, there is expected to be a reduction in primary production due to the dominance of invasive plants as the major source of allochthonous matter (Clulow et al., 2011) which leads to less variation in the stable isotope values of basal sources. Additional studies are required to explore why the present study did not show trophic deviations in streams invaded by non-native plants.

Among the macroinvertebrates, the general pattern was that higher $\delta^{13}C$ and $\delta^{15}N$ values for macroinvertebrates corresponded with basal sources that had higher $\delta^{13}C$ and $\delta^{15}N$ values. The Lushington River site which had a riparian zone impacted by the non-native black wattle, had enriched $\delta^{13}C$ values for macroinvertebrate consumer groups indicating that CPOM and FPOM were the likely source of elevated carbon stable isotopes. Moreover, this habitat had the lowest number of invertebrate species, the majority of which were composed of predators and filterers which fed on FPOM. These results are in line with other studies in the northern hemisphere which have found that headwater streams with an invaded landscape have a simplified food web with generalist macroinvertebrate groups and a narrow trophic niche breadth for consumer groups due to dominance of black wattle as the major source of allochthonous matter (Lowe & Likens, 2005). In streams with agriculturally disturbed catchments, the macroinvertebrate functional feeding groups predominantly had enriched $\delta^{13}C$ values, showing concordance with the range of values observed for CPOM and FPOM, suggesting that these basal sources could be the major carbon sources supporting these macroinvertebrates. Aquatic invertebrate family richness in agriculture disturbed streams was greater than in wattle invaded streams, and were mainly composed of predators and gatherers which primarily wander the stream bottom scavenging for dead organisms, detritus, or other food particles that get lodged between rocks or in deep pools. Streams with a natural landscape

had both enriched and depleted $\delta^{13}C$ values for macroinvertebrate consumer groups indicating that multiple basal sources including macrophytes and particulate organic matter influenced the food web structure, leading to increased trophic diversity of macroinvertebrate consumer groups. The increased richness could be due to higher amounts of allochthonous input from the terrestrial landscape than in agriculture disturbed streams, which would also account for the greater representation of the collector gatherers. The majority of macroinvertebrate consumer groups, however, as in the agriculture disturbed streams, was composed of predators and gatherers.

In contrast to the stable isotopic values of basal sources and macroinvertebrate communities, there were considerable variations in *E. mandelai's* isotope niches. *Enteromius mandelai* thus appeared to show trophic plasticity among the headwater streams with different riparian land use types. Generally, comparison of the SEAc for *E. mandelai* showed that it exhibited large isotopic niches at the sites with wider breadth in carbon sources (CR) and population spanning longer trophic levels (NR) compared with sites with lower CR and NR values. Specifically, *E. mandelai* had the largest isotopic niches (SEAc) in some headwater sites with agricultural land use that had wider breadth in carbon sources (CR) and longer trophic levels (NR). In the agriculture-disturbed Elands River sites, the isotopic niche sizes for *E. mandelai* was wider downstream, suggesting a more generalised feeding habit. Studies on streams that are influenced by agricultural land use have shown significant increases in primary production that in turn increases the trophic niche breadth of consumer groups (Allen et al., 2009; Griffiths et al., 2013; Lee et al., 2018). *Enteromius mandelai* had the lowest isotopic niche (SEAc) in the stream with a riparian zone invaded by non-native vegetation. This site had the lowest range of basal sources (CR), trophic niche length (NR) and the degree of trophic diversity (CD). These results are in line with studies that have found that streams with an invaded landscape are expected to have a simplified food web with a narrow niche breadth for consumer groups due to dominance of black wattle as the major source of allocthonous matter (Matomela., 2019). In headwater streams with a natural landscapes, *E. mandelai* showed intermediate isotopic niche sizes compared to those of invaded and agricultural landscapes. This may be due to the lack of influx of excess nutrients from agricultural activity which would increase primary production, and the lack of allochthonous matter input from non-native vegetation which would reduce the primary production in the system. The spatial variation observed for the niches in the natural, invaded and agriculture disturbed headwater streams for *E. mandelai* is consistent with patterns observed for generalist species that change their niches

according to available resources (e.g. Cambray, 1983a). For example, within the Eyre River, *E. mandelai* in the lower sections had a wide isotopic niche and low trophic redundancy due to the wider NR and CR values, suggesting a broad prey range and high intraspecific variability. Therefore one of the factors contributing to the wide distribution of *E. mandelai* in the river systems of the Eastern Cape Province may be its adaptability to a variety of trophic resources.

Smaller isotopic niche sizes were observed for *E. mandelai* when occuring in allopatry than in symaptry with *A. trevelyani* and *S. bainsii* in the Keiskamma and Buffalo River systems. This suggests that *E. mandelai* displays trophic plasticity, which enables its coexistence with other native fish in small headwater systems where resources could otherwise be limiting. Several classical studies have also indicated that an absence of interspecific competition can lead to larger trophic niches (Hutchinson, 1957; Van Valen, 1965). Isotopic similarities were however observed in the headwater streams where *E. mandelai* co-occurred with other native species. In the Keiskamma River, *E. mandelai* occurred in sympatry with *A. trevelyani* and the similarities in isotopic compositition between these two species suggested potential diet resource overlap. The similarity in isotopic composition was also observed in the Buffalo River system where *E. mandelai* occurred with *A. trevelyani* and *S. bainsii* in the Cwengcwe River. These findings are not consistent with the niche partitioning hypothesis (*sensu* Hutchinson 1961), which predicts clear dietary differences among coexisting species. However, these findings are consistent with the results of a recent study that presented evidence for lack of trophic niche partitioning for the small-bodied cyprinids *Pseudobarbus skeltoni* and its congener *P. burchelli* in the Riviersonderend River of the Cape Fold Ecoregion (Kadye et al., 2016). Ecological niche theory predicts that co-existence is facilitated by resource partitioning mechanisms that are influenced by biotic and abiotic interactions. The lack of trophic differentiation documented in the current study implies that biotic interactions (resource partitioning) may not be as important as abiotic (environmental) selective pressures in these streams. The alternative hypothesis of environmental filtering is explored in Chapter 4.

To conclude, *E. mandelai* was found to be a generalist species able to adapt to headwater streams with different ecological characteristics. These findings are important because they suggest that *E. mandelai* is likely to respond to shifts in environment by changing its feeding behaviour. However, though *E. mandelai* exhibited trophic plasticity in both near natural and disturbed streams, the least suitable habitats for the species was the streams with non-native vegetation. Furthermore, *E. mandelai* isotopic niche size in the presence of other species is reduced suggestive of niche differentiation practices among the species to avoid

competition. General food web characteristics for headwater streams in this area indicate that enriched CPOM and FPOM are the main basal sources for consumers in these headwaters and that gatherer and predator macroinvertebrate functional feeding groups dominate in the headwater streams of the AWHE and STHE. This information can be used for the conservation and management of *E. mandelai* in its currently know distribution range.

CHAPTER 4

Environmental factors and fish species associations influencing the distribution patterns and habitat associations of *Enteromius mandelai* sp. nov. (Cyprinidae)

Introduction

Headwater streams are the dominant component of lotic habitats, constituting over 80% of river networks by length globally (Gomi et al., 2002; Meyer et al., 2007). Although the diversity is generally low compared to higher order systems (Minshall et al., 1985; Rahel & Hubert, 1991; Stegmann et al., 2019), headwater streams provide unique habitats that are utilised by narrow range endemic species, seasonal migrants and specialised taxa adapted to surviving or spawning in these highly variable environments (Meyer et al., 2007; Richardson, 2019). For example, endemic small-bodied cyprinids of the genera *Amatolacypris*, *Sedercypris*, *Enteromius* and *Pseudobarbus* are the dominant taxa associated with many headwater streams in the Cape Fold Ecoregion (CFE) of South Africa (Skelton, 2001; 2016; 2018). Similar to other headwater stream biota of the Mediterranean-climate regions , such as the Permanent Maghreb (Smith & Darwall, 2006), the Iberian Peninsula (Aparicio et al., 2000; Merciai et al., 2017), the Dalmatia (Smith & Darwall, 2006), the fishes of the CFE have been severely impacted by predation and competition from introduced non-native fish species, habitat destruction and water abstraction which have caused substantial declines in historical ranges and population sizes (Weyl et al., 2014; Benejam et al., 2016; Ellender et al., 2017; Merciai et al., 2017; Chakona, et al., 2020a). Given the discovery of undocumented diversity in these headwater taxa, as presented in Chapter 2, there is need to assess the ecology of the newly identified species in order to provide science-based decisions for their protection.

Enteromius mandelai sp. nov., a recently described species previously thought to be *E. anoplus* s.l, occurs in headwater streams and tributaries of several river systems in the Eastern Cape Province of South Africa (see Chapter 2). Previous studies on *E. anoplus* s.l. have shown that it easily adapts to different environmental conditions and is highly persistent in the face of environmental stochasticity, including seasonality (Cambray, 1983a; Cambray & Bruton, 1985; Kadye & Booth, 2012). The widespread occurrence of *E. anoplus* s.l. in headwater streams and major tributaries invokes the role of adaptive resilience of headwater fish species in habitats that are subject to high environmental variability, which has been reported in the

other studies (e.g. Ellender & Weyl, 2015). From an ecological perspective, resilience is the capacity of species within an ecosystem to respond to a perturbation or disturbance by resisting damage and recovering quickly. For example, Schlosser (1987) reported that headwater streams that are subject to high environmental stochasticity, such as variation in flow and temperature, are likely to be inhabited by fish species that show propensity to resilience, owing to factors such as the ability to recolonise and high reproductive rates. Similarly, other studies have shown that habitat variations that impose strong ecological constraints likely favour species that are eurytopic, and have adaptable behaviours to environmental extremes (Southwood, 1988; Dynesius & Jansson, 2000). Although *E. mandelai* is generally widespread in headwater streams of the Eastern Cape Province, there are no dedicated studies to evaluate the broad-scale distribution and abundance patterns of this new species. In addition, both empirical and anecdotal evidence indicate that this species is likely to be influenced by anthropogenic impacts, including invasions by piscivores and land use in different catchments (Kadye et al., 2020).

Among the most frequently explored environmental factors in lotic ecosystems are those related to physical habitat (e.g. substrate type, riparian vegetation, stream channel width and depth) and physico-chemical conditions (e.g. dissolved oxygen, pH, turbidity, conductivity) (Jackson et al., 2001; Richardson & Danehy, 2007). These environmental factors are assumed to structure communities through niche-based processes at local scales by influencing species-environment relationships and interspecific interactions (Tonn et al., 1990) Additionally, because stream ecosystems are intricately linked to their surrounding landscapes (Hynes, 1975; Vannote et al., 1980), anthropogenic modifications such as conversion of indigenous forest into pasture and invasions by non-native tree plantations often compromise the structure and function of stream ecosystems, which ultimately impacts fish distribution and abundance patterns (Allan, 2004).

This study therefore aimed to determine the environmental factors affecting the distribution and abundance patterns of *E. mandelai* within headwater streams of the Amathole-Winterberg Highlands and the Southern Temperate Highveld ecoregions, South Africa. This was achieved by comparing both distribution (presence/absence) and abundance (counts) of *E. mandelai* against micro- (physical-chemical variables), meso- (associated land-use patterns) and macro-scales (major river systems). Land-use patterns were categorised into near natural, agricultural and invaded types and three major river systems (Great Fish, Keiskamma and Buffalo River systems) were considered. The study hypothesised that *E. mandelai* would

exhibit differences in distribution and abundance patterns as a result of differences in the micro-, meso- and macro-scale variables. Furthermore, to better understand the patterns of coexistence associated with this species, *E. mandelai* and other co-occurring fish species within the study area were compared in relation to their abiotic environment. The objectives of this study were to (1) determine the magnitude of the environmental factors associated with *E. mandelai* abundance patterns, (2) evaluate the distribution patterns of *E. mandelai* in relation to other co-occurring species and (3) evaluate the spatial patterns together with the associated niches of *E. mandelai* and other fishes occurring in the study area. For niche breadth measurement, species with low tolerance values have narrow niche breadths and occur within a limited range of conditions (habitat specialists) whereas those with high tolerance values have wide niche breadths and occur across a wide range of environmental conditions (habitat generalists) (Dolédec et al., 2000).

Materials and methods

Field collections followed ethical standards that were approved by the Rhodes University Ethical Standards Committee (approval number 2019-0314-776). Research permits were granted by the Eastern Cape Department of Economic Development and Environmental Affairs (through permit numbers CRO 130/19CR and CRO 44/18CR).

Study area and species

Study area

This study focussed on the headwater streams of the Great Fish River, the Keiskamma River and the Buffalo River (Figure 3.1). The area drained by these rivers encompasses the Amathole-Winterberg Highlands Ecoregion (AWHE) and Southern Temperate Highveld Ecoregion (STHE). The AWHE covers a series of high-altitude Afromontane forests. The headwaters of a large number of small streams drain this ecoregion (Thieme et al., 2005). These include the headwaters of the Buffalo River, the lower Great Fish River, the Keiskamma River, and the Swart Kei River. The headwater streams are typically narrow, fast flowing, turbulent with rocky substrata. Many have sponges or bogs at their source and small areas of seasonally flooded marsh in flatter, low-altitude valleys (Hughes & Hughes, 1992). The climate of the

AWHE is temperate, characterised by high rainfall, cool winters and moderately warm summers (Story, 1952). Most rainfall occurs in summer (December to February) and autumn (March to May) (Thieme et al., 2005; Hoare et al., 2006). The AWHE area as a whole receives between 800 mm and 1,000 mm per annum (Hoare & Bredenkamp, 1999; Clark et al., 2011). The region has an average daily maximum air temperature of 19 °C in winter and 29 °C in summer (van Zyl, 1994). Snow fall may occur at high altitudes on the Winterberg Mountains, with frost occurring regularly between April and September.

On the other hand, the STHE forms a rolling, grassy plateau 900–1900 m above sea level and gradually slopes down to the coast in the south-eastern Cape (Thieme et al., 2005). The dominant limnological features are rivers and seasonal pans (Thieme et al., 2005). In the Eastern Cape, mainstem sections of large river systems such as the Great Fish River and moderately-sized rivers such as the Keiskamma and Buffalo rivers form part of the STHE. The STHE (Figure 3.1) has a temperate climate. Summer rainfall is most common, and varies from 1,400 mm in the east to 400 mm further west. Daily temperatures usually average 10–18°C with frost at night in winter to 15–40°C in summer (Thieme et al., 2005). Summers are hot, with long, dry, often windless periods broken by thunderstorms that generate both strong winds and local flooding.

Additionally, in the Great Fish River, the flow regime has been altered by the Orange-Great Fish Inter-Basin Water Transfer (IBWT) scheme from seasonal to perennial flow with little monthly fluctuation (Thieme et al., 2005). *Enteromius mandelai* has largely been undocumented in the sections that are altered by the IBWT. Most of the Great Fish River's tributaries are, however, uninfluenced by the IBWT and maintain natural flow regimes. The major tributaries include the Koonap and Kat rivers (Figure 3.1). The Koonap River has a dry catchment and has a high seasonal flow. In comparison, the Kat River drains wet catchments and has a perennial flow. *Enteromius mandelai* also occurs in narrow, fast flowing, turbulent streams such as those of the Keiskamma and Buffalo rivers that both have perennial flow, which is characterised by high seasonal discharge (Hughes & Hughes, 1992).

The distribution pattern of *E. mandelai* and other co-occurring species in the Great Fish, Keiskamma and Buffalo rivers were determined from 73 sites. Fifty-five sites were sampled in the Great Fish River, nine sites from the Keiskamma River and nine sites from the Buffalo River. Additionally, of the 73 sampled sites, 32 sites had a near natural riparian zone, 35 sites

had an agricultural land use pattern and six sites had a riparian zone invaded by non-native plants.

Data collection

Sampling was conducted between April 2018 and August 2019 at 77 sites in the Great Fish (59 sites), Keiskamma (9 sites) and Buffalo (9 sites) rivers in the Eastern Cape, South Africa (Figures 4.2, 4.3). Data were mainly collected by electrofishing (SAMUS-725MP powered by a 12 V battery) and seine netting (3 m length by 3 mm mesh size net). Electrofishing was the most commonly used method of sampling. Apart from voucher specimens and specimens used for molecular genetics (Chapter 2) and stable isotope analyses (Chapter 3), fish sampled were identified using keys in Skelton (2001) and released to the collection sites alive. Altitude and geographical co-ordinates were recorded using a global positioning system (GPS, Garmin etrex Ltd) unit to an accuracy of 5 m. A YSI Pro20 handheld dissolved oxygen meter (YSI, a Xylem brand, 1725 Brannum Lane, Yellow Springs, Ohio) was used to measure pH, water temperature (°C) and dissolved oxygen (DO) (mg/l). A Hanna ISO turbidity portable meter (HI98713) was used to measure turbidity (NTU). A Hanna HI98129 Combo meter was used to measure conductivity (µS/cm) and total dissolved solids (TDS) (ppm) (HANNA Instruments, Woonsocket, Rhode Island). Stream depth was measured using a graduated pole from 30 random measurements. To evaluate stream width, 5 to 10 transects were set across the sampled area perpendicular to flow, and each transect was measured using a graduated tape. The length of the entire sampled stretch was also measured from upstream to downstream. Substratum composition was visually assessed within a radius of 25 cm at each of the 30 random points within each sampling site. Substratum types were qualitatively assessed and classified following a modified Wentworth scale as bedrock (> 500 mm), boulder (250–500 mm), cobble (50–250 mm), gravel (2–50 mm), sand (1–2 mm) and silt (< 1 mm) (Cummins, 1962, Wentworth 1922). Substratum types were expressed as proportions (%) of the total sampled points. Three classes of riparian land-use were defined through visual estimation as near natural, agricultural and invaded landscape. A near natural (least impacted) land cover was categorised as having a riparian zone that was largely unaltered (Figure 3.2). The near natural vegetation in this region is dominated by deciduous broad-leafed forests and grasslands (Hoare et al., 2006). An agricultural land-use pattern was defined as having a riparian zone modified by agricultural activities. This was mainly small-scale crop farming and livestock farming

(Figure 3.3). The invaded land-use pattern was categorised by the dominant presence of non-native invasive plants (Figure 3.4). The common non-native invasive plant species in this region include different species of wattles (*Acacia* sp), pine (*Pinus* sp), gumtree (*Eucalyptu*s sp), and tickberry (*Lantana camara*).

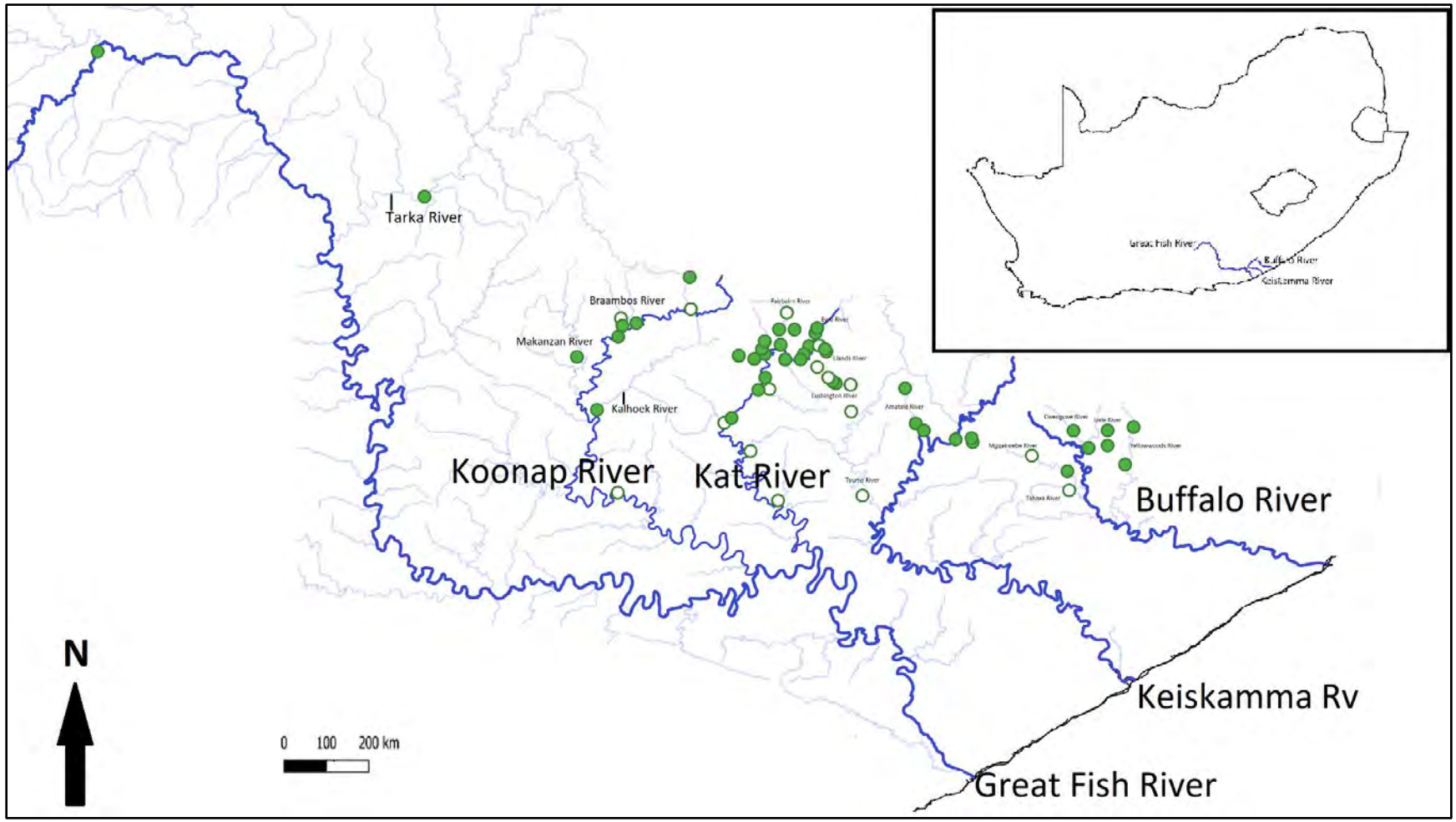


Figure 4.1: Map of all the sampled sites where *Enteromius mandelai* was present (green circles) and absent (white circles) in the Great Fish River and the two major tributaries Koonap and Kat River systems.

Data analysis

A generalized linear mixed effects model (GLMM) was used to determine the relationship between *E. mandelai* abundance and the environmental variables. Generalised linear mixed models provide a more flexible approach for analysing nonnormal data when random effects are present. The abundance data were fish counts based of electrofishing surveys from 68 sites. In the GLMM, *E. mandelai* count data were used as the response variable, whereas physical and chemical variables and landscape patterns were used as explanatory variables (Table 4.1). Prior to GLMM analysis, physical-chemical variables were standardised as z-scores in order to allow for comparisons of predictor variables measured in different units (Quinn & Keough, 2002), whereas microhabitat data were arcsine transformed. The arcsine square root transformation is standard procedure when analysing proportional data in ecology, with applications in data sets containing binomial and non-binomial response variables. Multicollinearity in predictor variables was evaluated based on the variance inflation factors (VIF) that were based on a generalized linear regression model. Variables with VIF values less than 5 were retained for subsequent analyses (Ringle et al., 2015). Four GLMMs were developed to assess the relationship between fish abundance and environmental variables. These models were Poisson, Quasi-Poisson, negative binomial (NB) and zero-inflated negative binomial (ZINB). Poisson regression is used to model count variables, and assumes that the variance is equal to the mean (Greene, 2005; O'Hara & Kotze, 2010). When the variance exceeds the mean, over-dispersion occurs. Because over-dispersion is so common, several models have been developed for these data, including the negative binomial, quasi-Poisson (Wedderburn, 1974) and zero-inflated (Lambert, 1992) models.

The Quasi-Poisson and negative binomial regression are a generalisation of Poisson regression where variance is a linear function of the mean in the Quasi-Poisson model and variance is a quadratic function of the mean in the negative binomial model (Wedderburn, 1974; Yau et al., 2003; O'Hara & Kotze, 2010). When there are excess zeros in the observed data, the Quasi-Poisson and NB models may be inadequate. The zero-inflated negative binomial (ZINB) regression is used for count data that exhibit over-dispersion and excess zeros (Mullahy, 1986; Saffari & Robiah, 2011). The ZINB data distribution combines the negative binomial distribution and the logit distribution. The GLMMs were conducted using the R statistical package *glmmADMB* (Skaug et al., 2014). The best predictor variables were evaluated using multi-model inference, which was conducted from a fully saturated model with all environmental variables (Bolker et al., 2009).

Multi-model inference was conducted in R using the package *MuMIn* (Bartón, 2014). To select the model that best fit the data, Akaike information criterion (AIC) was used to evaluate the goodness-of-fit of each model based on log-likelihood functions of the data. The most parsimonious model was selected based on the lowest Akaike weight (Burnham & Anderson, 2004). For the best model, the probability distribution of the residuals was evaluated using quantile-quantile plots.

The distribution patterns of *E. mandelai,* in relation to other species, associated environmental variables and land use patterns were analysed using a non-metric multidimensional scaling (NMDS). The NMDS is a means of visualising the level of similarity of individual cases of a dataset. Non metric multidimensional scaling is an indirect gradient analysis approach which produces an ordination based on a distance or dissimilarity matrix. The goal of NMDS is to collapse information from multiple dimensions (e.g., from multiple communities, sites, etc.) into just a few, so that they can be visualised and interpreted. Non-metric multidimensional scaling was performed based on a binomial distance matrix for the fish data based on presence/absence and presented as $n \times p$ (sample × species) data matrix. Environmental data were presented as $n \times q$ (sample × environmental variable) data matrix. Similar to the GLMMs, physical-chemical variables were standardised using z-scores, whereas microhabitat data were arcsine transformed prior to NMDS. The NMDS was performed using the function *meta-MDS* in the *vegan* package in R (Oksanen et al., 2019). The function *envfit* in the vegan package was used to visualize the joint fish distribution patterns and the environmental variables. The statistical significance of the environmental variables were evaluated based on randomisation permutation tests (× 1000). The NMDS stress values were used to evaluate how well the ordination summarised the data, with values < 0.1 indicating a good fit (Clarke et al., 2014).

To determine the spatial patterns and associated environmental niches for *E. mandelai* and other species found in headwater streams, environmental niche parameters along broad environmental gradients were tested using outlying mean index analysis (I_{OM}, Dolédec et al., 2000). The I_{OM} is an ordination technique designed to explicitly take into account the ecological niche within a community ((I_{OM}, Dolédec et al., 2000). The presence/absence data (Appendix B) were used to analyse species niche parameters. The I_{OM} describes a species based on two factors; a) niche position – the deviation of a species' mean habitat condition from a hypothetical species

that is uniformly distributed and tolerates average habitat conditions, and b) niche breadth – the dispersion of a species from the typically average environmental conditions that it uses, which is represented by its level of tolerance. For niche position measurement, habitat conditions were based on physical-chemical factors that were measured at each site. A high marginality (I_{OM}) indicates that a species is found under atypical environmental conditions (marginal niches), whereas a low marginality indicates that there is no difference between the overall environmental conditions and those where the species is found. An index of tolerance is used as a measure of niche breadth. High tolerance values indicate that the species is distributed along a variety of environmental conditions, while low values imply that the species is distributed along a more limited range of environmental conditions. A third niche parameter is residual tolerance which represents variance in species niche that is not explained by the measured variables (marginality axis). This index helps to determine the influence of the tested environmental conditions on the distribution of the species. Low values indicate that the relationship between the study's environmental conditions and the species distribution is high, whereas high values indicate that they are weakly related. Additionally, the outlying mean index analysis also provides an inertia estimate, and its value represents the total variance of the environmental table weighted by the species distribution profile. This variability is decomposed into OMI + tolerance + residual tolerance (expressed as percentages of inertia), so the individual proportion of this variability associated with the three indices may be directly related with the specific parameter of interest examined in this study (marginality or tolerance). Monte Carlo randomisations (999 permutations) were used to test the null hypothesis that individual species' niche positions were not constrained by environmental variables. The packages *ade4* and *adehabitatHS* for environmental niche assessment were used in the R statistical program. Prior to I_{OM} ordination, environmental variables were standardised as z-scores in order to meet the assumptions of multivariate normality. All analyses were carried out using the R statistical program (R Development Core Team, 2020).

Results

Relationship between E. mandelai abundance and environmental variables

Enteromius mandelai was captured at 49 out of 68 sampled sites (Appendix A). In the Great Fish River system, *E. mandelai* was captured from 43 of the 59 sampled sites. In the Keiskamma River, *E. mandelai* was captured from four out of six sites. In the Buffalo River, it was captured at two out of three sites.

Comparison of GLMMs using AIC showed that the ZINB (AIC = 608.7) was a better fitting model than the NB (AIC = 622.6), Poisson (AIC = 1399.5) and Quasi- Poisson models (Table 4.1). Consequently, the ZINB model was used to evaluate the relationship between *E. mandelai* and environmental factors. Two physical chemical variables (total dissolved solids and turbidity) and one substrate variable (sand) showed significant effects ($P < 0.05$) (Table 4.2). These three environmental factors (TDS, turbidity and sand) were important predictors of the abundance of *E. mandelai* in the Great Fish, Keiskamma and Buffalo rivers. The abundance of *E. mandelai* was higher at sites with higher total dissolved solids (GLMM, z-value = 2.70, $P = 0.0068$) and sand substrates (GLMM, z-value = 2.06, $P = 0.0392$). By contrast, the abundance of *E. mandelai* was lower at sites with higher turbidity values (GLMM, z-value = -3.89, $P = 0.0001$).

Table 4.1: Models comparison for *Enteromius mandelai* abundance in the Great Fish, Keiskamma and Buffalo River Systems. The models were Poisson, Quasi-Poisson, negative binomial (NB) and zero-inflated negative binomial (ZINB). These were compared based on the log-likelihood, degrees of freedom (df) and Akaike's information criterion (AIC).

	Poisson	Quasi-Poisson	NB	ZINB
Log-likelihood	-766.4	N/A	-293.754	-285.31
df	68	N/A	68	68
AIC	1566.8	N/A	623.5	608.6

Table 4.2: Parameter estimates, standard errors (SE) and the associated *P*-values for zero-inflated negative binomial models (ZINB) for the abundance of *Enteromius mandelai* in the Great Fish, Keiskamma and Buffalo River systems.

	Estimate	SE	z-value	*P*-value
Intercept	3.83	0.59	6.52	<0.0001***
Temperature	-0.30	0.27	-1.11	0.268
Conductivity	-0.03	0.15	-0.22	0.825
pH	0.09	0.24	0.41	0.683
Total dissolved solids	0.75	0.28	2.70	0.007 **
Turbidity	-0.79	0.20	-3.89	0.0001 ***
Dissolved oxygen	-0.00	0.13	0.00	0.997
Average depth	0.00	0.34	0.01	0.995
Average width	-0.40	0.24	-1.68	0.092
Boulder	-0.54	0.75	-0.72	0.4700
Sand	3.97	1.92	2.06	0.039 *
Cobble	-0.31	1.52	-0.20	0.840
Gravel	-0.38	0.94	-0.41	0.682
Vegetation	0.23	1.15	0.20	0.843
Invaded landscape	0.16	0.75	0.22	0.827
Natural landscape	0.21	0.37	0.56	0.575

Significance codes: 0 '***' 0.001 '**' 0.05 '*'

Distribution patterns of E. mandelai in relation to other co-occurring species

Enteromius mandelai was found in 53 of the 73 sampled sites. The other native species *Sandelia bainsii, Glossogobius callidus, Labeo umbratus, Anguilla mossambica, Amatolacypris trevelyani* and *Oreochromis mossambicus* were collected at 26, 14, nine, eight, seven and one site respectively (Appendix B).

In the Great Fish River, the NMDS ordination indicated an adequate goodness-of-fit (stress = 0.07). Six environmental variables (pH, temperature, conductivity, dissolved oxygen, turbidity and total dissolved solids) and three land-use types (agriculture, invaded and near natural) were

significant (randomisation permutation tests, $P < 0.05$) in explaining the spatial patterns in the distribution of fishes (Figure 4.2a). There appeared to be an upstream to downstream gradient in the spatial distribution of species on NMDS axis 1. *Enteromius mandelai* was the only species in most of the upper sections of headwater sites. These sites were characterised by high DO concentrations. In the middle sections of major tributaries, it co-occurred mostly with the native (*S. bainsii, G. callidus, L. umbratus* and *A. mossambica*) fishes and was generally absent in habitats with the non-native fish species (*T. sparrmanii, L. aeneus, C. gariepinus* and *O. mykiss*) (Figure 4.2a).

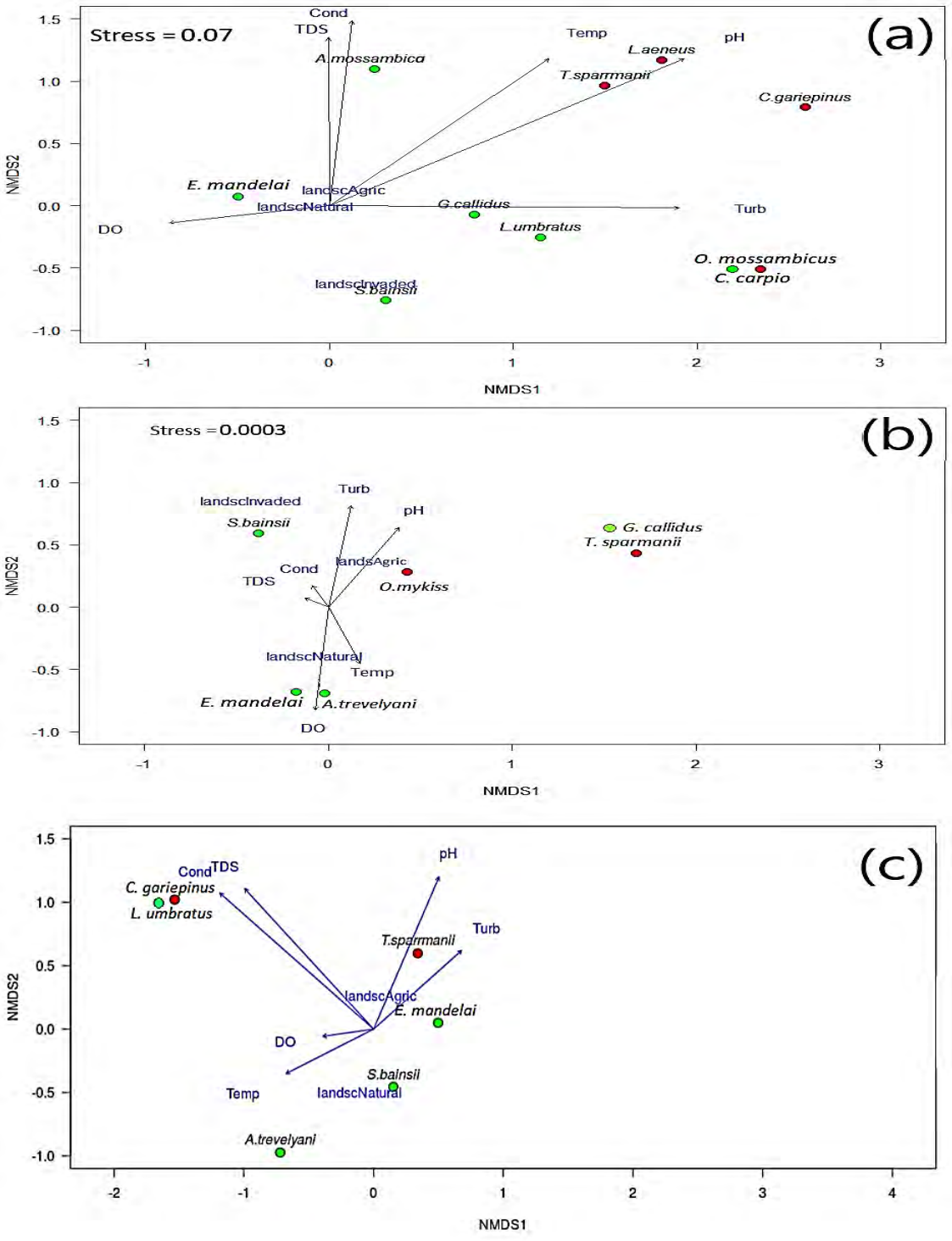


Figure 4.2: Non-metric multidimensional scaling (NMDS) ordination showing the spatial patterns of distribution of *Enteromius mandelai* together with both native (green circles) and non-native (red circles) fishes in the (a) Great Fish River, (b) Keiskamma River and (c) Buffalo River.

In the Keiskamma River, four native (*E. mandelai, S. bainsii, A. trevelyani* and *G. callidus*) and two non-native (*T. sparrmanii* and *O. mykiss)* fish species were collected. The NMDS ordination indicated a stress value of 0.0003, indicating a high goodness-of-fit. Six environmental variables (pH, temperature, conductivity, dissolved oxygen, turbidity and total dissolved solids) and three landscape patterns (agriculture, invaded and near natural) were significant (randomization permutation tests, $P < 0.05$) in explaining the spatial patterns in the distribution of fishes (Figure 4.2b). The NMDS axis 1 represented a gradient from headwater streams from higher to lower altitudes. The native fishes *E. mandelai, S. bainsii* and *A. trevelyani* and non-native *O. mykiss* were found occurring in the upstream sections of the headwater streams. *Enteromius mandelai* mostly co-occurred with *Amatolacypris trevelyani* at sites characterised by low temperature, high dissolved oxygen values and a near natural riparian zone. *Sandelia bainsii* was the only species to be found at sites with an invaded landscape. In contrast, the non-native *T. sparrmanii* and native *G. callidus* were found in the downstream sections. These downstream sites were characterised by high pH and turbidity.

In the Buffalo River, the NMDS ordination produced a stress value of <0.0005. Six environmental variables (pH, temperature, conductivity, dissolved oxygen, turbidity and total dissolved solids) and two land use types (agriculture, and near natural) were significant (randomisation permutation tests, $P < 0.05$) in explaining the spatial patterns in the distribution of fishes (Figure 4.2c). Four native (*E. mandelai, A. trevelyani, S. bainsii* and *L. umbratus*) and two non-native (*T. sparrmanii* and *C. gariepinus*) fish species were collected. The NMDS axis 1 did not reveal any clear patterns in fish distribution from the physical-chemical variables and land use. The NMDS axis 2 revealed a spatial gradient in the distribution of species from upstream to downstream. In contrast to other rivers, *E. mandelai* occurred mostly in the lower sections of the headwater streams, whereas *A. trevelyani* was found at the most upstream sites. *Enteromius mandelai* exhibited no clear associations with any environmental variables. *Amatolacypris trevelyani* occurred at sites with a near natural riparian zone and low temperatures. The native *L. umbratus* and non-native *C. gariepinus* occurred at sites with the highest conductivity and TDS values, whereas the non-native *T. sparrmanii* occurred at sites with high pH and turbidity values.

Environmental niches of Enteromius mandelai in relation to native and non-native fish species

For the Great Fish River, the first and second I_{OM} axes explained 88.3 % and 5.7 % of the variation in the data, respectively. The first axis was used to interpret the results. This axis was positively correlated with dissolved oxygen and negatively correlated with turbidity (Figure 4.3). With the exception of *T. sparrmanii* and *L. aeneus*, there was no significant deviation of environmental niches from the average habitat conditions (Monte Carlo randomisation test, $P > 0.05$) for the fish species, indicating no clear relationship between species niches and associated environmental factors within the headwater sections (Table 4.3). Similarly, the global test on marginality (the distance between the average environmental conditions used by a species and the mean environmental conditions of the sampling units of the study area) of all species was not significant (Monte Carlo randomisation test, $P > 0.05$). *Enteromius mandelai* had the lowest I_{OM} or marginality (0.07, Table 4.3) indicating that there was no difference between the overall environmental conditions and those were the species is found. With the exception of *C. gariepinus* ($I_{OM} = 4.23$), the marginality I_{OM} values were higher for non-native species (10.18 – 44.48) than native species (0.07 – 10.18). The higher marginality in non-native species indicated that the species were found under atypical conditions within the study area. The non-native *T. sparrmanii* had the highest tolerance (measure of niche breadth; 5.09) of all species (0.0 – 1.89) indicating that this species was distributed along a wider variety of environmental conditions compared to the other species. Residual tolerance (i.e., the variance in the species niche that is not taken into account by the marginality axis) was highest in the non-native species *T. sparrmanii* and *C. gariepinus* indicating that there was a weaker relationship between the study's environmental conditions the species distribution of these two species compared to the other species. The inertia values for *E. mandelai* showed that 90.1% of the variability was from residual tolerance. This was higher than for any other species. 8.2 % of the variability of *E. mandelai* data came from tolerance (i.e. variability in niche breadth) and only 1.8% of the variability came from marginality (I_{OM}).

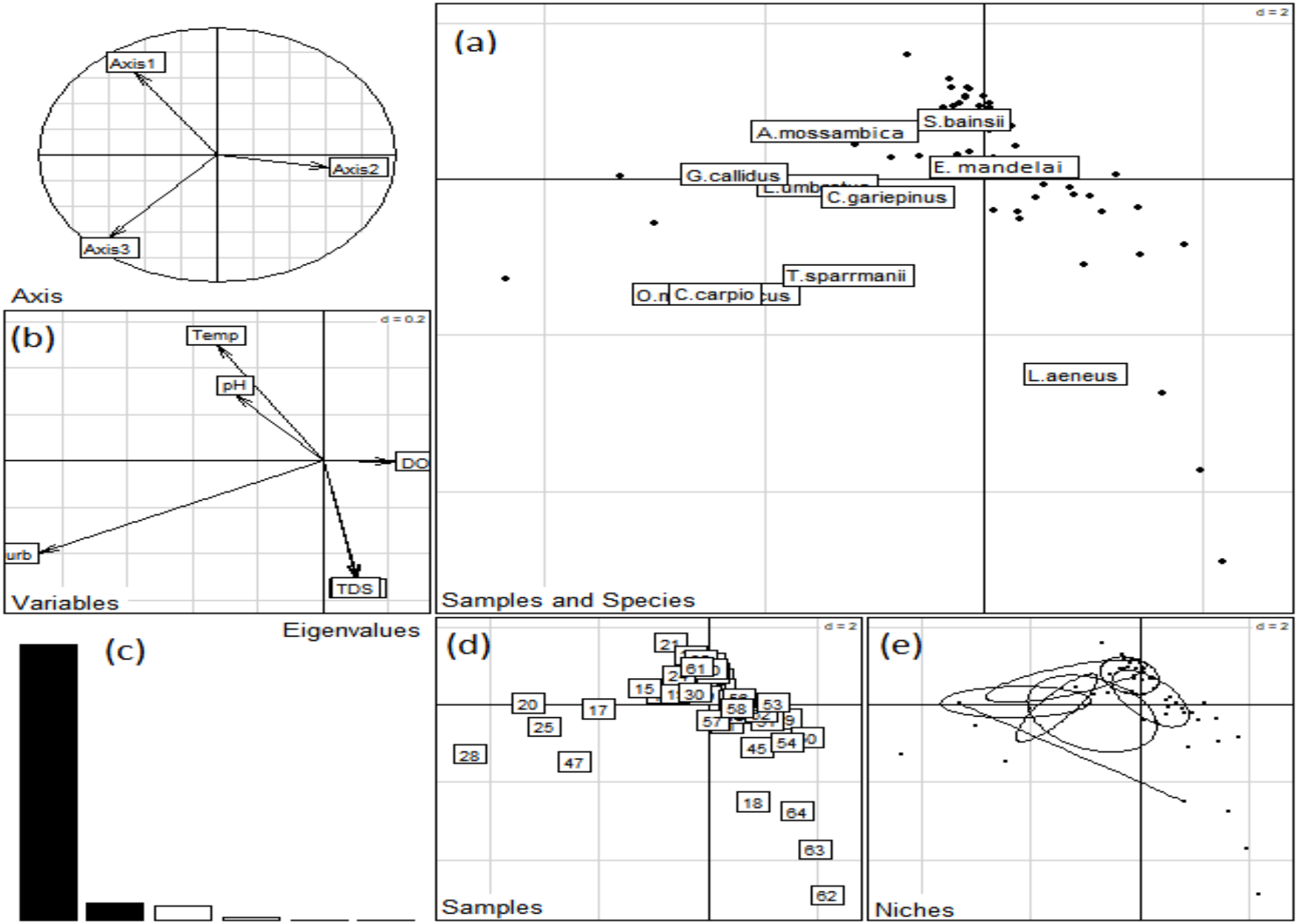

Axis1
Axis2
Axis3
Axis
(a)
d = 2
A.mossambica
S.bainsii
E. mandelai
G.callidus
C.gariepinus
T.sparrmanii
C.carpio
L.aeneus
Samples and Species
(b)
d = 0.2
Temp
pH
DO
urb
TDS
Variables
Eigenvalues
(c)
(d)
d = 2
Samples
(e)
d = 2
Niches

Figure 4.3: Outlying mean index analysis indicating the species and environmental variable vectors for the Great Fish River. (a) Projection of the sampling sites and the mean of the distribution of the utilization weights of fishes; (b) projection of the environmental variable scores; (c) Eigenvalues showing how much variation can be explained by the different axes (d) projection of the individual sampling sites from Appendix B; (e) the individual niches for the different species.

Table 4.3: Niche parameters for fish species collected in the Great Fish, Keiskamma and Buffalo River system.

Species	I_{OM}	Tol.	R_{Tol}	$I_{OM\%}$[a]	Tol.$_{\%}$[a]	$R_{Tol\%}$[a]	P
Great Fish River							
E. mandelai	0.07	0.31	3.36	1.8	8.2	90.1	0.793
S. bainsii	0.64	0.24	1.28	29.7	11.0	59.3	0.279
L. umbratus	2.65	0.68	1.14	59.3	15.2	25.5	0.341
A. mossambica	2.28	1.39	2.19	38.9	23.6	37.4	0.112
G. callidus	5.23	1.89	1.01	64.3	23.3	12.4	0.102
O. mossambicus	10.18	0.00	0.00	100	0.0	0.0	0.140
T. sparrmanii[b]	13.64	5.09	9.07	49.1	18.3	32.6	0.012
C. gariepinus[b]	4.23	0.26	7.82	34.4	2.1	63.5	0.195
L. aeneus[b]	44.48	0.00	0.00	100.0	0.0	0.0	0.017
C. carpio[b]	10.18	0.00	0.00	100.0	0.0	0.0	0.141
Keiskamma River system							
E. mandelai	2.16	0.44	2.28	44.2	9.0	46.7	0.296
S. bainsii	1.66	3.87	2.85	19.8	46.2	34.0	0.253
A. trevelyani	3.17	0.45	1.26	64.8	9.3	25.9	0.358
G. callidus	4.03	0.00	0.00	100.0	0.0	0.0	0.765
T. sparrmanii[b]	4.03	0.00	0.00	100.0	0.0	0.0	0.768
O. mykiss[b]	8.37	0.00	0.00	100.0	0.0	0.0	0.339
Buffalo River system							
E. mandelai	1.49	1.73	1.58	31.0	36.0	33.0	0.459
S. bainsii	1.74	1.74	0.48	44.1	43.8	12.1	0.775
A. trevelyani	4.03	0.03	0.62	86.0	0.7	13.0	0.399
L. umbratus	15.15	0.00	0.00	100.0	0.0	0.0	0.123
T. sparrmanii[b]	4.52	1.30	1.56	61.2	17.6	21.2	0.221
C. gariepinus[b]	15.15	0.00	0.00	100.0	0.0	0.0	0.105

Species abbreviations used in the analysis; I_{OM}: outlying mean index (which indicates marginality); Tol: tolerance (which indicates niche breadth); R_{Tol}: residual tolerance (which indicate unexplained variance).

[a]Percentages indicate the proportion of variability from total inertia for each species.

[b]Non-native species

In the Keiskamma River, I_{OM} analysis showed that 62.2 % and 33. 6 % of the variation was explained by the first and second axes, respectively. The first axis was positively correlated with pH and negatively correlated with temperature (Figure 4.4). The second axis was positively correlated with turbidity and negatively correlated with dissolved oxygen. As in the Great Fish River, the Monte Carlo randomisation test, revealed that the fish species exhibited no significant deviation ($P > 0.05$) in their environmental niches from the average habitat conditions (Table 4.5), indicating that abiotic factors did not influence species distribution. The global test on marginality was also insignificant (Monte Carlo randomisation test, $P > 0.05$) for all species revealing that the fishes did not respond to the environmental variables within the headwater streams.

The native fish had lower I_{OM} values (1.66 – 4.03) than the non-native fish (4.03 – 8.37, Table 4.3) there was a smaller difference between the overall environmental conditions and those were the native fish are found compared to the non-native fish. With the exception of *G. callidus*, tolerance was higher for native fish (0.44 – 3.87), particularly *S. bainsii* (3.87) than for non-native fish (0.00). The niche breadth for *S. bainsii* was therefore wider and more variable than for the rest of the species. Similarly, with the exception of *G. callidus*, residual tolerance was higher for native fish (1.26 – 2.85), than for non-native fish (0.00). This indicated that there was a weaker relationship between the study's environmental conditions and the native species distribution compared to non-native species. The inertia values for the non-native species showed that 100% of the variability in the data was from marginality (I_{OM}), whereas for native species the variability of the data was spread out among marginality, tolerance and residual tolerance (Table 4.3). For E. mandelai in particular, the inertia values showed that 46.7% of the variability was from residual tolerance. This was higher than for any other species. 44.2 % of the variability of *E. mandelai* data came from marginality (I_{OM}), and only 9% of the variability came from tolerance (i.e variability in niche breadth).

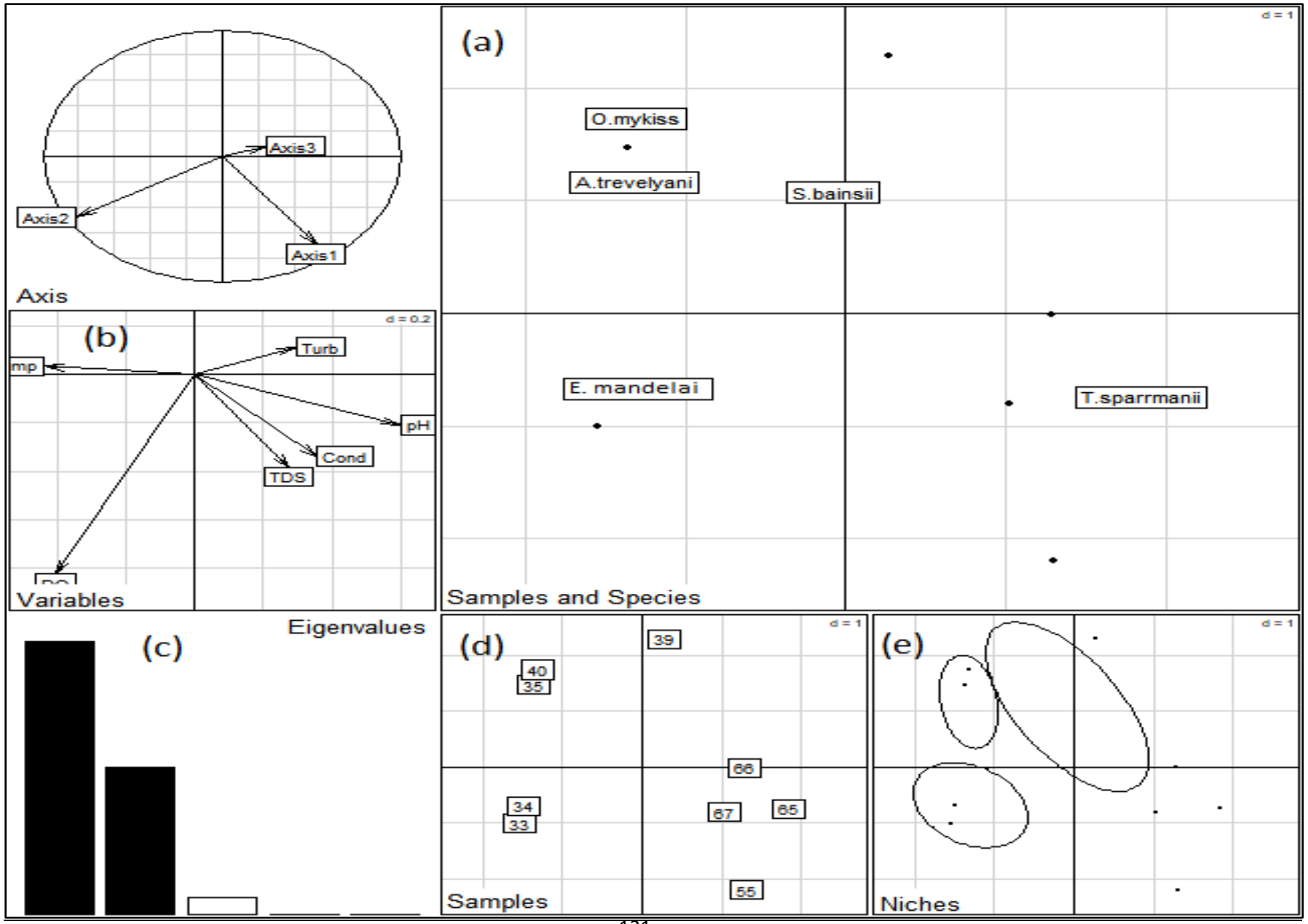

Axis3
Axis2
Axis1
Axis
(a)
d = 1
O.mykiss
A.trevelyani
S.bainsii
E. mandelai
T.sparrmanii
Samples and Species
(b)
d = 0.2
Turb
mp
pH
Cond
TDS
Variables
Eigenvalues
(c)
(d)
d = 1
40
35
39
66
34
33
67
65
55
Samples
(e)
d = 1
Niches

Figure 4.4: Outlying mean index analysis indicating the species and environmental variable vectors for the Keiskamma River. (a) Projection of the sampling sites and the mean of the distribution of the utilization weights of fishes; (b) projection of the environmental variable scores; (c) Eigenvalues showing how much variation can be explained by the different axes (d) projection of the individual sampling sites from Appendix B; (e) the individual niches for the different species.

For the Buffalo River, the first and second I_{OM} axes explained 68.2 % and 25.9 % of the data variation respectively. The first axis was positively correlated with pH and negatively correlated with temperature. The second axis was positively correlated with dissolved oxygen and negatively correlated with turbidity (Figure 4.5). There was no significant deviation of environmental niches from the average habitat conditions for any of the fish species (Monte Carlo randomisation test, $P > 0.05$) indicating that the abiotic factors did not influence species distribution (Table 4.3). The global test on marginality was also not significant (Monte Carlo randomization test, $P > 0.05$) in any of the fish species, signifying that there was no evidence provided for environmental niche segregation among the fishes along the I_{OM} axes.

The native species had the lowest marginality (I_{OM} = 1.49 – 4.03) than the non-native species (I_{OM} = 4.52 – 15.15), with *E. mandelai* having the lowest marginality (1.49). this indicated that there was no or lower difference between the overall environmental conditions and those were the native species are found compared to non-native species. Highest tolerance was found in the native species *E. mandelai* (1.73), *S. bainsii* (1.74) and the non-native *T. sparrmanii* (1.30, Table 4.3). These species are distributed along a wider variety of environmental conditions than the other three species. Residual tolerance was highest for *E. mandelai* (1.58) and *T. sparrmanii* (1.56, Table 4.3). This indicated that there was a weaker relationship between the study's environmental conditions and the two species distribution compared to the rest of the species. Inertia was dissimilar among species (Table 4.3). For *E. mandelai* in particular, the inertia values showed that 31% of the variability was from marginality (I_{OM}), 36% of the variability was from tolerance (i.e. variability in niche breadth), and 33% was from residual tolerance.

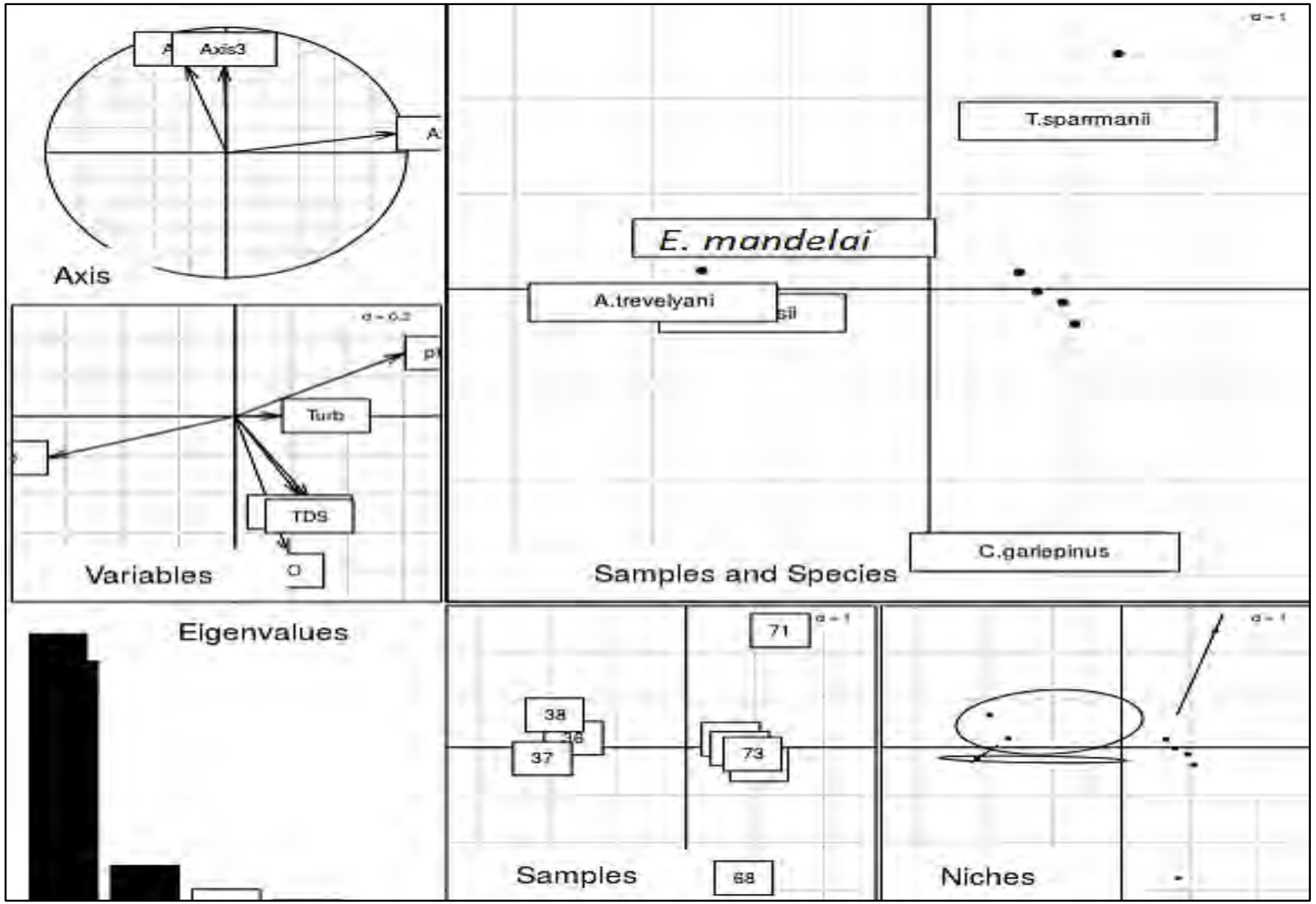

Axis3
Axis
Turb
TDS
Variables
T.sparrmanii
E. mandelai
A.trevelyani
C.gariepinus
Samples and Species
Eigenvalues
71
38
37
73
68
Samples
Niches

Figure 4.5: Outlying mean index analysis indicating the species and environmental variable vectors for the Buffalo River. (a) Projection of the sampling sites and the mean of the distribution of the utilization weights of fishes; (b) projection of the environmental variable scores; (c) Eigenvalues showing how much variation can be explained by the different axes (d) projection of the individual sampling sites; (e) the individual niches for the different species.

Discussion

This study is the first to provide detailed information on the environmental requirements of *E. mandelai* within headwater sections of three major river systems in the AWHE and STHE. These headwater streams spanned different catchment characteristics, including relatively undisturbed, agriculture-disturbed and headwater streams with riparian zones invaded by non-native vegetation. The influence of environmental factors on *E. mandelai* were thus explored to illuminate the relative influence of micro-scale (physical-chemical) then meso-scale (associated land-use patterns) and macro-scale (major river systems) variables. *Enteromius mandelai* was generally abundant and widespread in most headwater streams and in some sections of the major tributaries. In general, this species showed differences in its spatial patterns across the different river systems, but it did not exhibit any clear environmental niche patterns.

Despite occurring in a wide range of micro-habitats across different across different land-use patterns, the abundance of *E. mandelai* was affected by some environmental variables. This species had high abundances in habitats with sand substratum and high total dissolved solids and low abundance in turbid habitats. Since sand substratum was least dominant (≤ 30%) in all sites, this likely reflected the fact that this species were most abundant in large pools where sand substrate was common, together with other substrate types. This appeared to be consistent with studies elsewhere, which have shown that in headwater streams, abundances of individual species are directly correlated with the area or volume of the sampled habitats (Angermeier & Schlosser, 1989). Kadye & Booth (2014) found a significant relationship between *E. anoplus* s.s (now *E. mandelai*) abundance and coarse substratum (boulders and cobbles) in the Great Fish River, whereas fewer individuals were observed in habitats with fine substratum, such as gravel. The presence of boulders and cobbles is assumed to provide shelter, is likely to be used as spawning areas, and is associated with invertebrate prey for fish (Kadye & Booth, 2014).

The observed disparities among the different studies are due to differences in spatial scales. The spatial scale of study influences the perceived importance of abiotic factors on species distribution and abundance (Jackson et al., 2001), and small-scale studies are assumed to be limited in the range of environmental variables, whereas large-scale studies are not. Kadye & Booth's (2014) study was based in the Great Fish River. The present study encompassed two ecoregions and three river systems. Therefore, the present study may denote environmental factors important to *E. mandelai* abundance at a large scale, whereas previous

studies represent environmental factors that were important to *E. mandelai* abundance at a small scale. In other previous studies, predominantly based in the Orange River, chubbyhead barbs were generally found to be associated with cover such as fallen logs, brushwood or marginal vegetation (Cambray et al., 1978; Cambray & Bruton, 1984) which is dissimilar to associations (e.g., sand) of *E. mandelai* in headwater streams of the Eastern Cape Province. This could be due to these river systems having different environmental attributes (see Materials and Methods). Also, cryptic species have been found to have different ecological functions with respect to their morphological relatives (Schluter, 1996; Prochazka, 1998; Wellborn & Cothran, 2007b). The ecological function describes, for example, how individuals of different species interact within an ecosystem. For example, an organism that helps to degrade leaves from fallen trees occupies an ecological function of a "decomposer". Therefore, cryptic species with different ecological functions may interact differently with their environments (Bickford et al., 2007) which then requires differing management regimes for the different cryptic species.

Enteromius mandelai was found to have a ubiquitous presence within headwater sections of the three major river systems. The ubiquitous presence matches with the low genetic structuring of *E. mandelai* across river systems in the Eastern Cape Province (see Chapter 2). It, nevertheless, exhibited spatial patterns among the three rivers. Within the Great Fish River, there was an upstream to downstream gradient in fish species distribution, with *E. mandelai* occurring as the only species in most headwater streams. Within some sections of the major tributaries, this species co-occurred mostly with native species, including *S. bainsii, G. callidus* and *L. umbratus*, and was less common in habitats with non-native species. In the Keiskamma River, *E. mandelai* co-occurred mostly with *A. trevelyani* in the upper reaches of the headwater streams. Within the Buffalo River, however, *E. mandelai* occurred in lower reaches in streams with *A. trevelyani,* with the latter occurring mostly in the upper reaches of the headwater streams. This suggests the likelihood of interspecific interactions between these cyprinid minnows.

In addition to these general patterns, *E. mandelai* appeared to be less widespread in headwater sections that were invaded by riparian non-native vegetation, and in sections with non-native species. Although the impacts of non-native vegetation on these minnows are not fully known, studies elsewhere have shown that non-native riparian vegetation has had varied and often indirect effects on fish communities. For example, Roon et al. (2016) studying urban Alaskan salmon streams, found that non-native riparian vegetation reduced prey input for

headwater stream fishes. A laboratory experiment by Morrongiello et al., (2011) on southern pygmy perch *Nannoperca australis* from Broken River and Castle Creek in Australia, reported that pygmy perch exposed to *Eucalyptus* leachates were unable to reproduce, either due to stress or by habitat unsuitability.

Casatti (2005) found that fishes from river systems in south-eastern Brazil that preferred rocky substrates were impacted mostly by non-native riparian vegetation, as allochthonous matter from non-native vegetation reduced native fish habitat. In comparison, the impacts of non-native fishes, particularly piscivores on the distribution patterns of minnows in CFE and STHE region are well documented (Tweddle et al., 2009; Kadye & Booth, 2012; Ellender & Weyl, 2014). Non-native piscivores such as *Clarias gariepinus* and *Onchoryncus mykiss*, which were captured during the study, and *Micropterus salmoides*, which is known to occur in mainstem habitats, are the potential threats which are likely to influence the distribution and abundance patterns of this species in certain habitats. In addition, no native fish species that occurred in headwater streams reflected any significant responses to environmental variables. This pattern was consistent with the view that habitat factors are poor predictors of fish distribution in headwater streams that are likely subject to high environmental variability (Angermeier & Schlosser, 1989; Schlosser, 1995).

Environmental filtering is assumed to drive functionally different species to occur in different habitat conditions (Kraft et al., 2015) as observed in the Great Fish River. *Enteromius mandelai* was found dominating the most upstream habitats characterised by high dissolved oxygen values. Species with converging functional traits however, are expected to co-occur with limited interspecific inter-actions by minimising competitive interference (Schoener, 1974; McGill et al., 2006). In the Keiskamma River, *E. mandelai* was often found occurring in sympatry with another small bodied cyprinid *A. trevelyani*. These two species had similar sized environmental niche breadths and co-occurred predominantly at sites with a natural landscape pattern, high dissolved oxygen and low temperature. Studies have found that species occurring in similar habitats may coexist as a result of having different trophic habitats (Soberón & Nakamura, 2009; Chase & Myers, 2011). For *E. mandelai* and *A. trevelyani*, this assumption could be evaluated further by comparing the trophic niche patterns of the two species (Chapter 4). In the Buffalo River system, where the majority of sites had an agricultural riparian landscape, *E. mandelai* occurred at sites with both a natural and agricultural landscapes, and therefore had a wide environmental niche. *Amatolacypris trevelyani* on the other hand, was

restricted to sites with a natural landscape pattern, resulting in this species being less ubiquitous with a narrow environmental niche.

In conclusion, this study demonstrated the importance of examining environmental factors affecting species distribution and abundance in headwater streams. *Enteromius mandelai* was found to have different ecological characteristics than its similar looking relatives in the Orange River system. As a result, biological research and conservation frameworks cannot be considered complete without accounting for this new species. The three major river systems upstream sections in the headwater streams were marked by the dominance of *E. mandelai* and the occurrence of a few primary freshwater species. This species was found to be a habitat generalist with a number of environmental variables influencing its abundance patterns in the headwater streams. The findings of this study provide a critical understanding of the distribution and abundance patterns of this new species and advances our knowledge on patterns that explain minnow distribution and abundance patterns in the Amathole-Winterberg Highlands and Southern Temperate Highveld ecoregions of South Africa.

CHAPTER 5

Southern temperate freshwater ecoregions revisited: a proposal to extend the Cape Fold Ecoregion to encompass the Amathole-Winterberg Highland Ecoregion

Introduction

A biogeographic province or bioregion is a complex of ecoregions that share a similar biogeographic history and thus often have strong affinities at higher taxonomic levels (Abell et al. 2000). Thieme et al., (2005) defined as a large area of land or water containing a distinct assemblage of natural communities and species, whose boundaries approximate the original extent of natural communities before major land use change. Ecoregion boundaries are determined through integration of multiple data sources, including turnover of species ranges, broad patterns of species composition, associated ecological and evolutionary histories, climatic conditions and species connectivity patterns (Matthews, 1998; Leroy et al., 2019). Freshwater ecoregions are defined as relatively large areas encompassing one or more freshwater systems with a distinct assemblage of natural freshwater species and environmental conditions that are more similar to each other than those of surrounding ecoregions (Abell et al., 2008). The freshwater ecoregions and bioregions of Africa, demarcated based on distributions of freshwater fishes, were originally delineated by Poll (1957) and Roberts (1975), and subsequently updated by Thieme et al. (2005), Abell et al. (2008), and by Snoeks & Stiassny, (2011) (Figure 5.1, 5.2). There are currently 11 bioregions and 93 freshwater ecoregions for Africa and Madagascar recognised (Thieme et al., 2005; Figure 5.1, 5.2). These regions have become integral to policy formulation and conservation prioritisation on the continent.

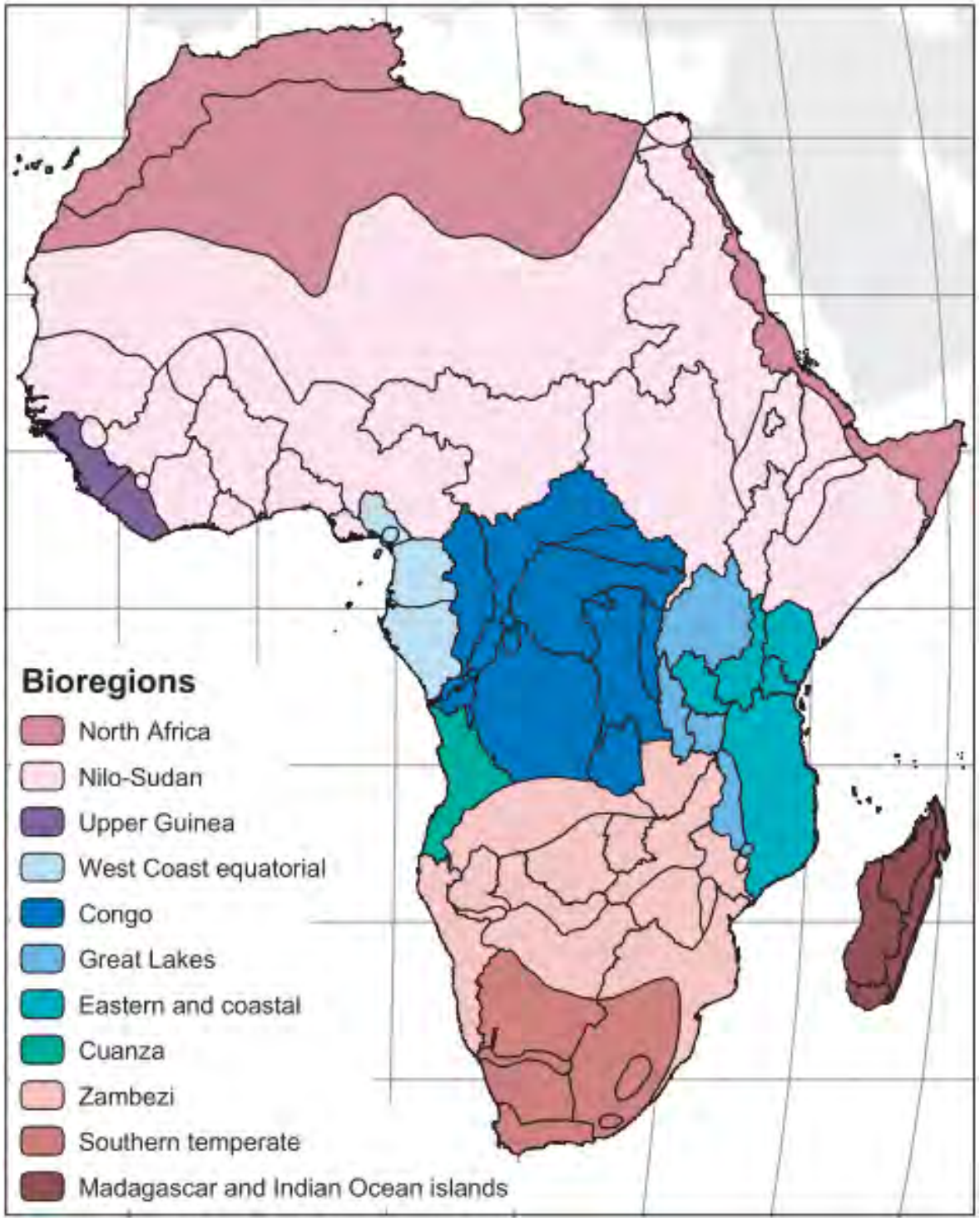
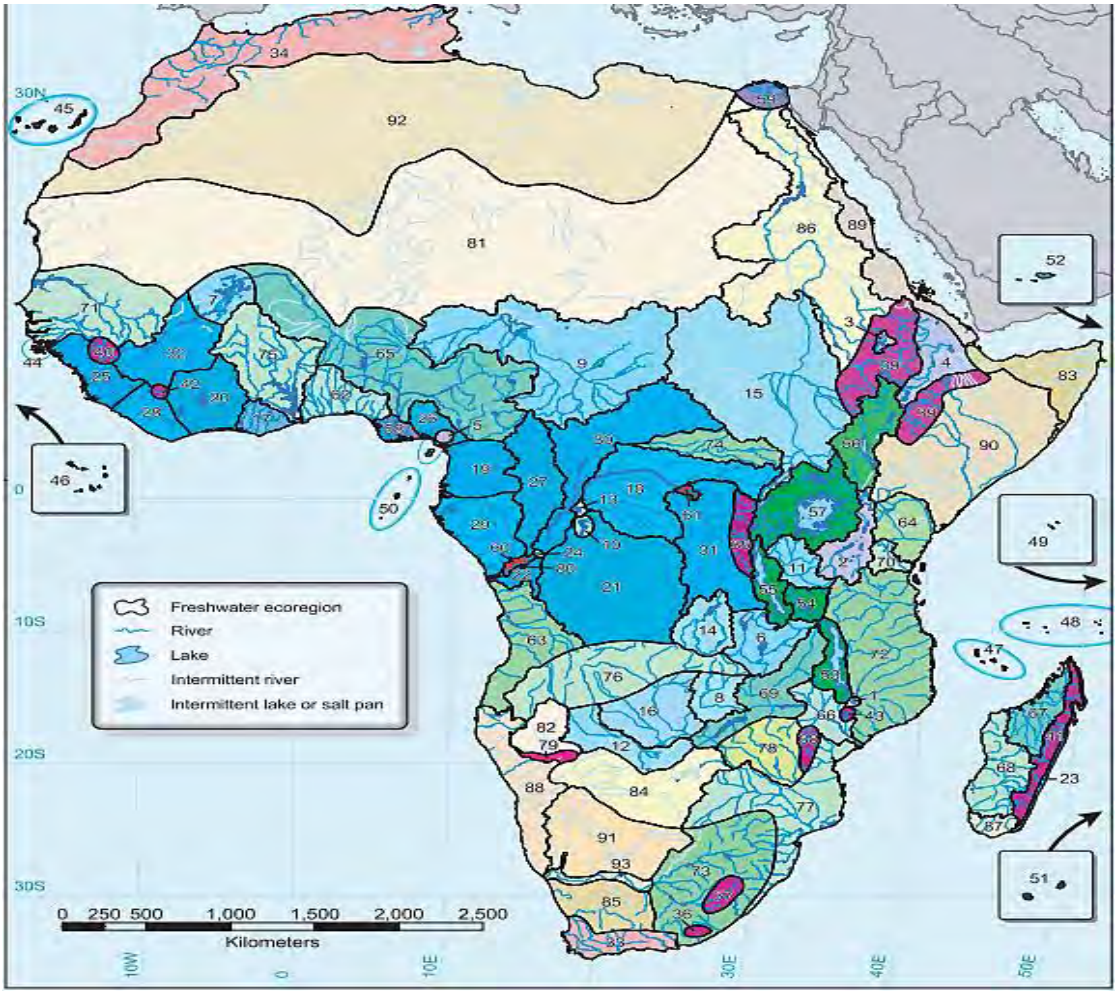


Figure 5.1: Freshwater bioregions of Africa and Madagascar (Thieme et al., 2005).



Closed Basins and Small Lakes
1 - Lakes Chilwa and Chiuta
2 - Southern Eastern Rift
3 - Lake Tana
4 - Northern Eastern Rift
5 - Western Equatorial Crater Lakes

Floodplains, Swamps, and Lakes
6 - Bangweulu-Mweru
7 - Inner Niger Delta
8 - Kafue
9 - Lake Chad Catchment
10 - Mai Ndombe
11 - Malagarasi-Moyowosi
12 - Okavango Floodplains
13 - Tumba
14 - Upper Lualaba
15 - Upper Nile
16 - Upper Zambezi Floodplains

Moist Forest Rivers
17 - Ashanti
18 - Cuvette Centrale
19 - Central West Coastal Equatorial
20 - Eburneo
21 - Kasai
22 - Lower Congo
23 - Madagascar Eastern Lowlands
24 - Malebo Pool
25 - Northern Upper Guinea
26 - Northern West Coastal Equatorial
27 - Sangha
28 - Southern Upper Guinea
29 - Southern West Coastal Equatorial
30 - Sudanic Congo (Oubangi)
31 - Upper Congo
32 - Upper Niger

Mediterranean Systems
33 - Cape Fold
34 - Permanent Maghreb

Highland and Mountain Systems
35 - Albertine Highlands
36 - Amatolo-Winterberg Highlands
37 - Drakensberg-Maloti Highlands
38 - Eastern Zimbabwe Highlands
39 - Ethiopian Highlands
40 - Fouta-Djalon
41 - Madagascar Eastern Highlands
42 - Mount Nimba
43 - Mulanje

Island Rivers and Lakes
44 - Bijagos
45 - Canary Islands
46 - Cape Verde
47 - Comoros
48 - Coralline Seychelles
49 - Granitic Seychelles
50 - São Tomé, Principe, and Annobon
51 - Mascarenes
52 - Socotra

Large Lakes
53 - Lake Malawi
54 - Lake Rukwa
55 - Lake Tanganyika
56 - Lake Turkana
57 - Lakes Kivu, Edward, George, & Victoria

Large River Deltas
58 - Niger Delta
59 - Nile Delta

Large River Rapids
60 - Lower Congo Rapids
61 - Upper Congo Rapids

Savanna–Dry Forest Rivers
62 - Bight Coastal
63 - Cuanza
64 - Kenyan Coastal Rivers
65 - Lower Niger-Benue
66 - Lower Zambezi
67 - Madagascar Northwestern Basins
68 - Madagascar Western Basins
69 - Middle Zambezi Luangwa
70 - Pangani
71 - Senegal-Gambia Catchments
72 - Eastern Coastal Basins
73 - Southern Temperate Highveld
74 - Uele
75 - Volta
76 - Zambezian Headwaters
77 - Zambezian Lowveld
78 - Zambezian (Plateau) Highveld

Subterranean and Spring Systems
79 - Karstveld Sink Holes
80 - Thysville Caves

Xeric Systems
81 - Dry Sahel
82 - Etosha
83 - Horn
84 - Kalahari
85 - Karoo
86 - Lower Nile
87 - Madagascar Southern Basins
88 - Namib Coastal
89 - Red Sea Coastal
90 - Shebelle-Juba Catchments
91 - Southern Kalahari
92 - Temporary Maghreb
93 - Western Orange

Figure 5.2: Freshwater ecoregions of Africa and Madagascar (Thieme et al., 2005).

In South Africa, freshwater systems encompass eight ecoregions; the Southern Kalahari (SKE), Western Orange (WOE), Karoo (KE), Drakensberg-Maloti Highlands (DMHE), Southern Temperate Highveld (STHE), Zambezian Lowveld (ZLE), Amathole-Winterberg Highlands (AWHE) and the Cape Fold ecoregion (CFE) (Abell et al., 2008, Figure 5.3). The Cape Fold Ecoregion incorporates the drainages that flow off the Cape Fold Mountains (de Moor & Day, 2013). These drainages are divided into the (1) Olifants, (2) Berg (3) Breede (4) Gouritz, (5) Gamtoos (6) Sundays (7) coastal drainages and (8) Baakens/Swartkops (Ellender et al., 2017, Figure 5.4). Although the CFE has a low diversity of primary freshwater fishes, the number of described and candidate species, as well as the distribution patterns of species has changed since the Skelton's (1986) analysis of distribution patterns and biogeography of the Temperate fauna (e.g. Tweddle et al., 2009; Chakona & Skelton, 2017; Ellender et al., 2017). The AWHE and DMHE contains species with close evolutionary relationships to species in the CFE such as *Sandelia bainsii*, *Amatolacypris trevelyani* and *Pseudobarbus quathlambae* (Swartz et al., 2009; Skelton et al., 2018; Chakona et al, 2020b). This pattern has been attributed to the existence of relict fauna in the Drakensberg-Maloti and Amathole-Winterberg Highlands (e.g. Skelton 1986, 1996; Swartz et al., 2009), but it could also reflect an incomplete understanding of fish diversity and distribution patterns at that time.

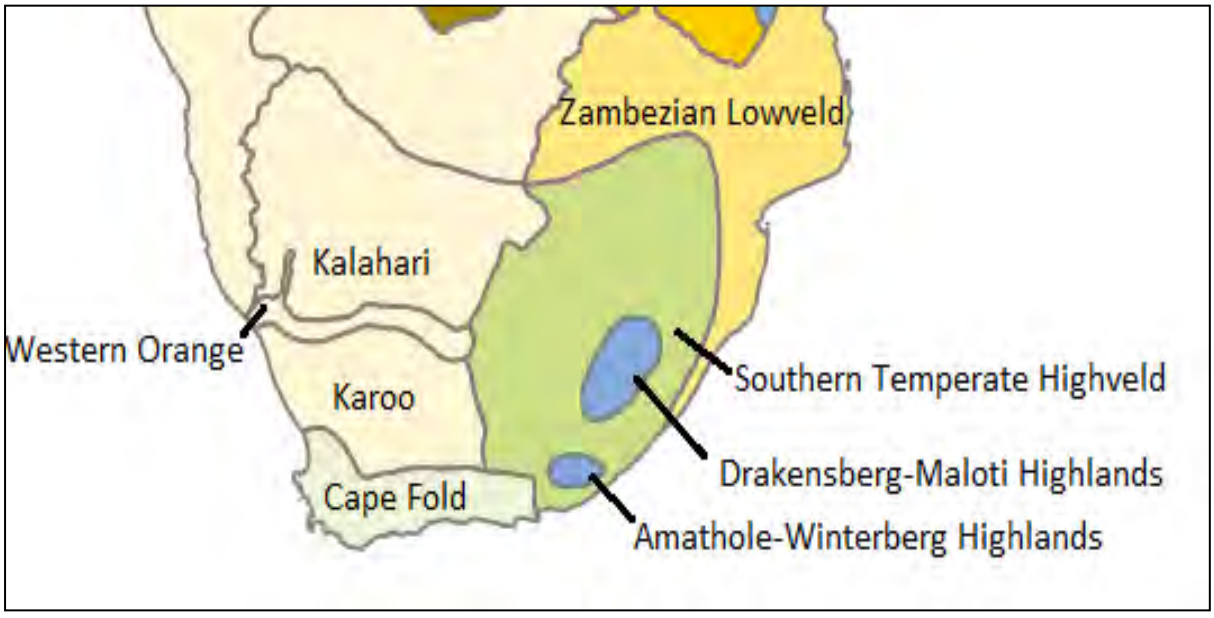


Figure 5.3: Map of freshwater ecoregions of South Africa. Adapted from Abell et al. (2008).

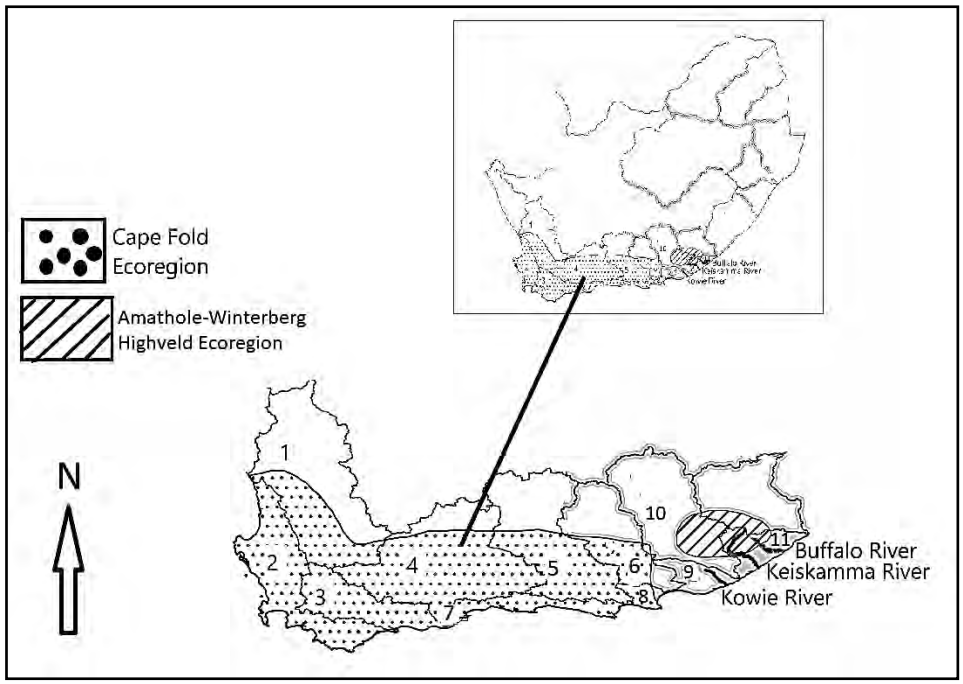


Figure 5.4: the Cape Fold Ecoregion represented by dots and the Amathole-Winterberg Highlands Ecoregion represented by slashed lines. River drainages: 1 = Olifants, 2 = Berg, 3 = Breede, 4 = Gouritz, 5 = Gamtoos, 6 = Sundays, 7 = Coastal drainages 8 = Swartkops, 9 = Bushmans, 10 = Great Fish, 11 = Keiskamma.

For example, the nesting of the AWHE within the STHE (Figure 5.3) is largely a result of two species, *E. anoplus* and *E. pallidus*, which, until only recently, were thought to have ranges that encompassed both the inland and coastal river systems in South Africa (see Skelton, 2001). Skelton (1986) suggested that the presence of a mixed fauna in these ecoregions could indicate that they are transition zones between the Cape and Karroid group of fishes. However, previous and ongoing studies are shedding more light on species diversity and distribution ranges as species which were previously considered to have wide distribution ranges encompassing more than one ecoregion have been shown to comprise multiple distinct lineages or candidate species with narrower ranges (Chakona et al., 2015b; Chapter 2). *Enteromius anoplus* in particular has been shown to be a complex of at least four distinct species and several lineages that have strong geographic affinities (Chapter 2; Engelbrecht 1996; Kambikambi et al, 2021. Similarly, *E. pallidus*, a species that was previously considered to have a disjunct distribution divided into coastal and inland populations in South Africa (Skelton, 2001), has been shown to comprise at least two distinct species, with *E. pallidus* s.s. being confined to coastal drainages that drain the CFE and the AWHE and the other lineage not closely related

to *E. pallidus* s.s. confined to inland drainages of the Orange-Vaal, Tugela, Mfolozi, Pongolo, Incomati and Limpopo river systems (Chakona et al., 2015b; Martin & Chakona, 2019). *Labeo umbratus*, also previously considered to be a widespread species encompassing the Orange River System, extending south of the Great Escarpment to the southward flowing river systems, including the Gouritz, Gamtoos, Sundays, Bushmans, Great Fish and Keiskamma Rivers (Skelton, 1986, 2001), has now been found to be composed of two distinct genetic lineages, one confined to the Orange River and the other one occurring in the southward and eastward draining rivers in the CFE and AWHE (Ramoejane et al., 2020). Studies of other stream fishes of the genera *Pseudobarbus*, *Galaxias* and *Sandelia* endemic to the CFE have uncovered similar levels of hidden diversity, and a number of new species have been described and distinct lineages identified and their distribution ranges have been revised over the past two decades (Swartz et al., 2004; 2007; 2009; Chakona & Swartz, 2013b; Chakona et al., 2013; Bronaugh et al., 2020). In light of this new information on the diversity and distribution patterns of stream fishes in the region, there is a clear need for reviewing the current freshwater ecoregion limits and areas of freshwater fish endemism in South Africa. This chapter focusses on the CFE and the AWHE to take advantage of the sampling effort that was made in these two ecoregions over the past two decades. The diversity and distribution patterns of species and genetic lineages in these regions is well known (e.g., Chapter 2; Swartz et al., 2007; 2009; Chakona & Swartz, 2013; Chakona et al., 2013b; 2014; 2015; 2020b; 2018; Ellender et al., 2017; Martin & Chakona, 2019; Bronaugh et al., 2020; Ramoejane et al., 2020), and thus provides an opportunity to address the questions about areas of endemism, and their relation to the boundaries of the freshwater ecoregions. The objectives of the present study were to (1) reanalyse spatial patterns of fish species richness and endemism, and (2) propose and revise freshwater ecoregion boundaries based on improved knowledge on species taxonomy and distribution patterns. I hypothesise that areas of endemism would coincide with drainage basin boundaries and ecoregion limits as the low vagility of freshwater fishes is expected to have promoted allopatric divergence among populations in isolated river systems.

Materials and Methods

The study area encompasses the CFE, AWHE and STHE freshwater ecoregions (Figure 5.3). Specifically, the study focussed on freshwater fishes associated with river systems bounded by

the Olifants on the west coast and the Buffalo on the east coast of South Africa (Figure 5.4). Descriptions of the AHWE and STHE are given in Chapter 3. The CFE is located at the southernmost part of the African continent. The Table Mountain Group of sandstones is the main geological type in this region, but Malmesbury shales and alluvium areas occur as well (Silberbauer & King, 1991). Most mountain streams in this region have clear flowing and oligotrophic water as they drain over erosion-resistant quartz-rich sandstones of the Table Mountain Group (Day *et al.*, 1998). Many streams draining lowland coastal areas in this region have black waters and high nutrient content derived from phenols and tannins generated through decomposition of fynbos vegetation (Britton, 1991). The diversity of aquatic systems is linked to the different climatic regimes in the area (Thieme et al., 2005). The CFE is divided into five subregions which are based on variation in prevailing climatic conditions (Brown et al., 1996). The fynbos bioregion which covers the Olifants, Berg and Breede rivers (Figure 5.5) receives 600-2000 mm/year of winter rainfall and supports mostly oligotrophic (nutrient poor), peat stained, acidic rivers. The southern coastal bioregion encompasses the Bloukraans, Elands, Silwer, Kaaimans, Duiwe, Homtini, and Touws rivers and has more or less the same characteristics as the fynbos bioregion. The southern inland bioregion includes the Couga, Baviaanskloof and Olifants Rivers. This bioregion receives 200–600 mm per annum of rainfall and the aquatic systems have pH levels in the neutral range, with low conductivities and clear water. The alkaline interior bioregion supports alkaline seasonal or ephemeral waters of the Doring, Gamtoos and Gouritz rivers (Figure 5.5). Lastly, the drought corridor bioregion has erratic rainfall and thus supports seasonal rivers, including the Sundays, Bushmans, Kowie and Great Fish rivers (Van Nieuwenhuizen & Day, 2000).

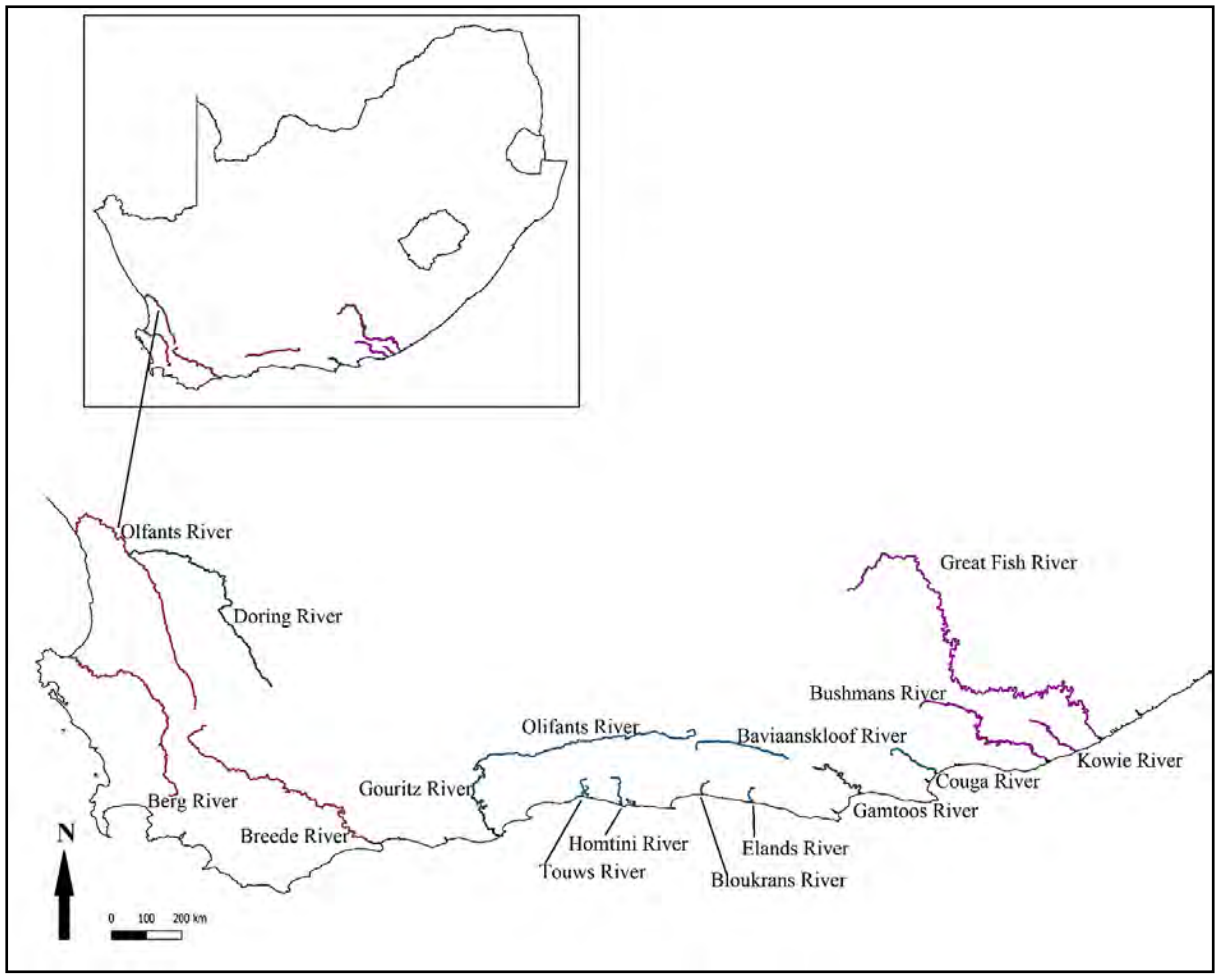


Figure 5.5: Main River systems in the Cape Fold Ecoregion

The descriptions and distribution patterns of species in this area were primarily derived from relatively recent journal articles (Chakona et al., 2020; Martin & Chakona, 2019; Sifundza et al., 2020), latest IUCN redlist assessments of fish distributions across the study region and from records in the South African Institute for Aquatic Biodiversity (NRF-SAIAB). The present study was restricted to native freshwater fish species The excluded fishes included diadromous species such as anguillid eels and marine spawned estuary-dependent species. Non-native species were also excluded. Several rivers in the temperate region of southern Africa contain complexes of species that show genetic structure that likely warrants taxonomic recognition. For example, *Galaxias zebratus, Sandelia capensis* and *Sandelia bainsii* have been found to be composed of several lineages (Chakona et al., 2013b, 2020b; Bronaugh et al., 2020). In the interest of conservation of these candidate species, they have been included in

this revision. A list of the candidate species and the references for that evidence is provided in Table 5.1.

The fauna in this region was examined for congruent distributional patterns among species. Shared species between river systems was taken to imply connectivity. Cluster analysis was used to quantify levels of ichthyofaunal similarity or dissimilarity among river systems. Cluster analysis is the task of grouping a set of objects in such a way that objects in the same group (called a cluster) are more similar (in some sense) to each other than to those in other groups (clusters). To apply cluster analyses, a resemblance matrix was constructed using the Jaccard similarity index. The Jaccard similarity index compares members for two sets to see which members are shared and which are distinct. It's a measure of similarity for the two sets of data, with a range from 0% to 100%. The higher the percentage, the more similar the two populations. Cluster analysis was determined using group average linkage. In interpreting the resulting dendrogram, initially we used the simplest possible procedure to define major basin-based regional groups: major clusters were simply "eyeballed" at the lowest dendrogram cut-offs for what appear to be discrete and substantial clusters. Analysis of similarities test (ANOSIM) (Clarke & Green, 1988) was then used to test the significance of the clusters produced by the resemblance matrix. A hypothesis of no significant differences between clusters at statistically significance level of 0.1%, under 999 permutations was used (Vavalidis et al., 2019). The similarity percentages SIMPER analysis (Clarke, 1993) was conducted in order to estimate the average similarity within and between identified clusters which also allowed a screening of the species' contribution to the cluster composition. All analysis was carried out using the statistical program Past (Hammer et al., 1999).

Table 5.1: Zoogeography of freshwater fishes in the Cape Fold Ecoregion, Amathole-Winterberg Highland Ecoregion and river systems in between.

Species	Common name	Bioregion	Distribution	Major river drainage
Amatolacypris trevelyani (Gunther 1877)	Border barb Grens-ghieliemientjie		Endemic to the Buffalo River system. Known from three locations, namely the Yellowwoods, Mgqakwabe and Cwengcwe rivers	Buffalo
1. Amatolacypris sp. "trevelyani Keiskamma"[1]			Keiskamma River system	Keiskamma
Austroglanis barnardi (Skelton 1981)	Barnard's rock-catfish	Karroid	Endemic to three tributary streams (Heks, Thee and Noordhoeks rivers) of the Olifants branch of the Olifants-Doring River system	Olifants
Austroglanis gilli (Barnard, 1943)	Clanwilliam catfish Clanwilliam-klipbaber	Karroid	Endemic to the Olifants-Doring River system where it has been recorded from 16 tributaries of the Olifants (Ratel, Oudste, Thee, Noordhoeks, Boontjies, Boskloof, Heks, Rondegat, Jan Dissels, Dwars rivers) and Doring (Biedouw, Tra-Tra, Eselbank, Matjies, Krom and Breekrans rivers) rivers	Olifants
Austroglanis sclateri Boulenger, 1901	Rock-catfish klipbaber	Karroid	Widely distributed in the Orang-Vaal River system, including the mainstem Orange and Vaal rivers. In the Orange it is found in the upper reaches in Lesotho (Senqu River) to the lower Orange at Grootpenseiland, within the Richtersveld National Park. It has been translocated through inter-basin transfer schemes to the Great Fish River system & the Olifants catchment of the Limpopo River system.	Orange-Vaal, Great Fish
Cheilobarbus capensis (Smith, 1841)	Cape whitefish	Cape	Endemic to the Berg (Atlantic drainage) and Breede (Indian Ocean drainage) river systems	Berg, Breede
Cheilobarbus serra (Peters, 1864)	Sawfin	Cape	Endemic to the Clanwilliam Olifants River (Atlantic drainage) system	Olifants
Enteromius anoplus ss (Weber 1897)[2]	Chubbyhead barb Dikkop-ghieliemientjie	Cape	Endemic to the Gouritz River system where it occurs in several tributaries including the Verkeerde Vei and Touws River; Gamka River at Latjesbosch, Kruidfontein, and Gamka Poort (Zwartberg Rnage); Bushman River, Tributary of the Gamka River, Near Letjesbosch; Buffels River, Laingsburg, and its continuation (Groote River) at Ladismith; Le Roux River, Outshoorn; Langtouw River, Herbertsdale	Gouritz
Enteromius mandelai[2]	Chubbyhead barb	Cape	Occurs in the Great Fish, Keiskamma and Buffalo River systems. As well as some coastal rivers in the Eastern Cape such as the Tyolomqa River	Great Fish, Keiskamma, Buffalo
Enteromius oraniensis[2]	Chubbyhead barb	Karroid	Found in in the Stormberg and Bossieslangspruit River of the Orange River system	Orange
Enteromius cernuus[2]	Chubbyhead barb	Cape	Endemic to the Olifants River system. Olifants River, Boontjes River, Doorn River, Troe Troe River	Olifants
Enteromius pallidus (Smith, 1841)	Goldie barb Goud-ghieliemientjie	Karroid	Occurs in at least 10 river systems in the Eastern Cape Province (the Krom, Gamtoos, Baakens, Coega, Swartkops, Sundays, Boesmans, Kariega, Kowie and Great Fish	Gamtoos, Coastal Rivers, Sundays, Swartkops, Bushmans, Kowie Great Fish

Galaxias zebratus (Castelnau, 1861)	Cape galaxias	Cape	Currently under revision	
2. *Galaxias* sp. "zebratus Breede"[3]			Hex, Bothaspruit and mainstem Breede River system	Breede
3. *Galaxias* sp. "zebratus Goukou" [3]			Goukou River system	Breede
4. *Galaxias* sp. "zebratus Heuningnes" [3]			Heuningnes and Ratel River systems	Berg
5. *Galaxias* sp. "zebratus Klein" [3]			Klein, Uilkraals and Ratel River systems	Berg
6. *Galaxias* sp. "zebratus Mollis" [3]			Onrus River system and Leeu River, Berg River	Berg
7. *Galaxias* sp. "zebratus Nebula" [3]			Widespread across the CFR from the Olifants River system in the west to the Bitou River system in the east	Olifants, Berg, Breede, Gouritz, Coastal drainages
8. *Galaxias* sp. "zebratus Rectognathus" [3]			Amandel & Du Toit Rivers, Riviersonderend sub-catchment, Breede River system	Breede
9. *Galaxias* sp. "zebratus Riviersonderend" [3]			Tributaries of the Riviersonderend River and in the Keurbooms River, Breede River system. Also occurs in the Palmiet River system	Breede
10. *Galaxias* sp. "zeebratus Slender" [3]			Uilkraals River system	Berg
Labeo seeberi (Gilchrist & Thompson, 1913)		Karroid	Endemic to the Olifants Doring River system. This species is believed to be extinct form the Olifants River system. The remaining population is confined to remnant subpopulations in the Doring River mainstem and isolated tributaries of the Doring River namely the Oorlogskloof-Koebee, Gif, Kransgat, Biedouw, Tra-Tra and Matjies rivers	Olifants
Labeo umbratus (Smith, 1841)	Moggel	Karroid	Occuring in the Orange-Vaal, Gouritz, Gamtoos, Sundays, Bushmans, Great Fish, Keiskamma, Buffalo and Nahoon rivers	Orange, Gouritz, Gamtoos, Sundays, Bushmans, Great Fish, Keiskamma
Labeobarbus seeberi (Gilchrist & Thompson, 1913)	Clanwilliam yellowfish	Karroid	Endemic to the Olifants-Doring River system. The number of locations is at least 15, consisting of the Olifants Gorge on the upper Olifants River and the Dwars, Ratels, Thee, Noorhoek, Boskloof, Rondegat, & Jan Dissles tributaries of the Olifants River, and the Oorlogskloof, Brandewyn, Biedou, Tra-Tra, Eselbank, Driehoeks-Matjies and the Groot-Leeu Tributaries of the Doring River. There are two extralimital subpopulations in the Olifants-Doring River system in the Twee River and Boontjies River, where it was stocked by the local conservation agency	Olifants
Pseudobarbus afer ss (Peters, 1864) Mandela lineage	Eastern Cape redfin Oos-Kaapse rooivlerkie	Cape	Headwater tributaries of the Baakens, Swartkops and Sundays River systems, endemic to the Eastern Cape Province	Swartkops, Baakens, Sundays

11. *Pseudobarbus* sp. "afer Forest"[4]			East coast from Klien Brak to Tsitsikamma Rivers	Coastal drainages
Pseudobarbus asper (Boulenger, 1911)	Smallscale redfin Kleinskub-rooiverkie	Cape	Restricted to the Gouritz and Gamtoos River systems	Gouritz, Gamtoos
Pseudobarbus burchelli (Smith, 1841)	Burchell's redfin Breede River redfin Breederivier-rooivlerkie	Cape	Tradouw River, Breede River system	Breede
12. Pseudobarbus sp. "burchelli Breede"[5]			Occurring across the Breede (excluding the Tradou catchment), Duiwenhoks and Goukou river systems.	Breede
13. Pseudobarbus sp. "burchelli Heuningnes" [5]			Only recorded from a single pool in the Grashoek River, a tributary of the Heuningnes River system	Berg
Pseudobarbus burgi (Boulenger, 1911)	Berg River redfin Bergrivier-rooivlerkie	Cape	Endemic to the Berg River System and occurs in the Boesmans, Goedverwacht-Platkloof, Leeu, Krom, Hugos and Wemmers (Olifants & Drakenstein Tributaries as well as in the upper reaches of the Berg River above the Berg River dam	Berg
Pseudobarbus phlegethon (Barnard, 1938)[6]	Fiery redfin Vurige rooivlerkie	Cape	Endemic to the Olifants-Doring River System. It occurs in the Oudste, Thee, Noordhoeks, Boskloof & Rondegat tributaries of the Olifants River system on the west side of the Cederberg Mountains	Olifants
14. *Psuedobarbus* sp. "phlegethon Doring" [6]		Cape	Endemic to the Olifants-Doring River system. Only been recorded from the Driehoeks-Matjies and Breekrans Rivers of the Doring River System on the Eastern side of the Cederberg Mountains.	Olifants
Pseudobarbus senticeps			Headwater tributaries of the Krom River system	coastal drainages
Pseudobarbus swartzi	Gamtoos River redfin		Headwater tributaries of the Gamtoos River system and adjacent river systems	Gamtoos
Pseudobarbus skeltoni Chakona & Swartz, 2013	Giant redfin		Endemic to the Breede River System where it is confined to three localities; the upper Riviersonderend, Krom & Tierkloof (all tributaries of the upper Breeder River	Breede
Pseudobarbus tenuis (Barnard, 1938)	Slender redfin Slank rooivlerkie	Cape	Endemic to the Gouritz River System where it occurs in many tributaries	Gouritz
15. *Pseudobarbus* sp. "tenuis Keurbooms"[5]			Headwater tributaries of the Keurbooms & Bitou River systems	Coastal drainages
Pseudobarbus verloreni Chakona, Swartz & Skelton 2014	Verlorenvlei River redfin		Endemic to the Verlorenvlei River system	Berg
Sedercypris calidus (Barnard, 1938)	Clanwilliam River redfin	Cape	Distributed in the Clanwilliam Olifants River system. Currently found in the Diepkloof, Dwars, Ratels, Oudste, Thee, Noordhoeks, Boontjies, Boskloof, Heks, Rondegat & Jan Dissles tributaries of the Olifants River & the Brandewyn, Biedouw, Tra-Tra, Eselbank, Driehoeks, Matjies, & Breekkrans & Leeu tributaries of the Doring River.	Olifants
Sedercypris erubescens (Skelton, 1974)	Twee River redfin	Cape	Endemic to the Twee River, a tributary of the Doring branch of the Clanwilliam Olifants River system. It is restricted to a single quaternary catchment & is found in the Heks, Suurvlei & Middeldeur tributaries, & the Twee River to just before its confluence with the Leeu River. There is also an introduced subpopulation in the Tuinskloof Dam.	Olifants
Sandelia capensis (Cuvier, 1829)	Cape kurper Kaapse kurper	Cape	Type locality uncertain & requires revision	Swartkops

16.	*Sandelia* sp. "capensis West Coast"[7]	The West Coast Clade is largely confined to the Langvlei, Verlorenvlei, Berg and Diep rivers	Berg
17.	*Sandelia* sp. "capensis klein" [7]	The Klein River Clade is endemic to the Klein River	Berg
18.	*Sandelia* sp. "capensis South Coast" [7]	The South Coast Clade is found everywhere else in the range of *S. capensis sensu lato.* The South Coast Clade surrounds the Klein River Clade, extends from Schusters River on the west side of the Cape Peninsula to the Coega River at Algoa bay and includes all southern coastal rivers.	Berg, Breede, Gouritz, Coastal Drainages, Gamtoos, Coastal drainages, Swartkops
19.	*Sandelia* sp. '*bainsii* Kowie'[88]	Confined to the Kowie and Great Fish River systems	Kowie, Great Fish,
20.	*Sandelia* sp. '*bainsii* Keiskamma' [8]	Confined to the Keiskamma River system	Keiskamma
21.	*Sandelia sp. 'bainsii Buffalo'*[8]	Occurs in the Buffalo and Igoda river systems	Buffalo

[1]Ellender, 2013;
[2] Kambikambi et al., unpublished;
[3] Chakona et al., 2013b:
[4] Chakona and Skelton, 2017;
[5] Swartz et al., 2009
[6] Swartz et al., 2004
[7] Bronaugh et al., 2020
[8] Chakona et al., 2020b

Results

Results of the cluster analysis revealed that the river systems did not group according to current ecoregion boundaries (Figure 5.6). Firstly, the Olifants River systems did not cluster with the other river systems that are currently assigned to the CFE. Secondly, the south-eastern clade contains river systems that are currently assigned to the CFE and AWHE ecoregions, indicating clear overlap between these ecoregions (Figure 5.6). According to the ANOSIM test, the probability of the observed value in the south-western and south-eastern clusters (R = 0.41, P = 0.0019) was less than 0.1% indicating a significant difference for the depicted clusters. The Olifants River system has 91 % average dissimilarity to the rest of the river systems and contains a distinctive fish fauna compared to the rest of the river systems in this region (Table 5.2). Species and lineages that strongly influenced clustering of the south-western river systems are *Sandelia* sp. '*capensis* South Coast', *Galaxias* sp. *'zebratus* Nebula' and *Enteromius pallidus* (Table 5.3). The clustering of the south-eastern river systems was largely driven by the sharing of *Enteromius mandelai*, *Labeo umbratus*, *Enteromius pallidus* and *Sandelia* sp. '*bainsii* Kowie' among these river systems (Table 5.3).

Interestingly, there have been some changes in the demarcation of areas of endemism in the CFE following close examination of the data. For example, the Berg and Breede River systems are now recognised as centres of diversity and endemism together with the Olifants-Doring River system in the CFE with nine lineages of *Galaxias*, four lineages of *Sandelia*, two lineages of *Pseudobarbus* and three formally described species (Table 5.1). The Olifants-Doring system has 10 formally described species and two candidate species or genetic lineages (Table 5.1). Diversity substantially declines from the Gouritz to the Kowie River, with many systems in this region containing between one and seven species or lineages (Table 5.4). While many of the species and lineages are confined to specific drainage basins, there are a few species and lineages that have wide geographic ranges, for example, *Galaxias* sp. '*zebratus* nebula', *E. mandelai*, *E. pallidus* and *Labeo umbratus* (Table 5.1).

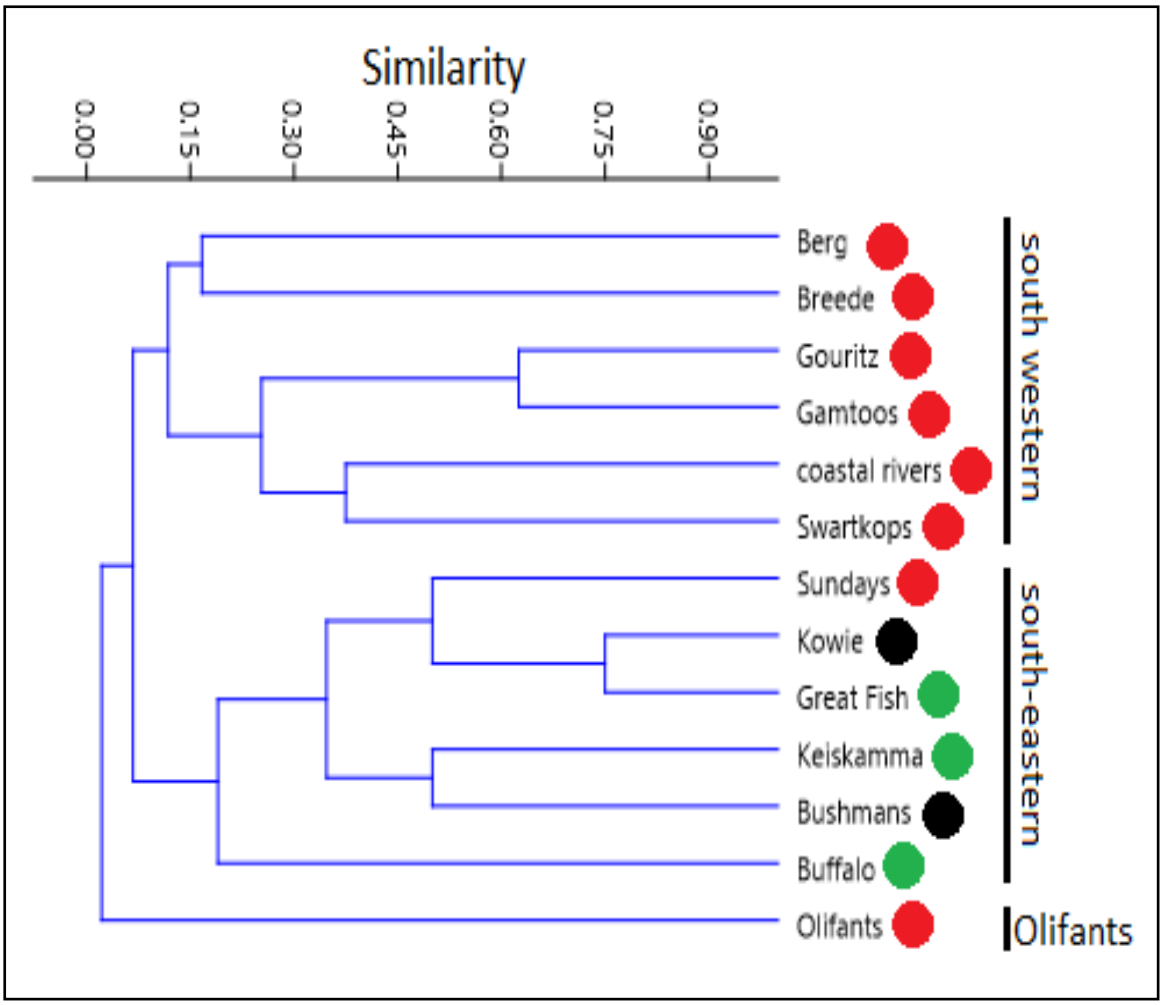


Figure 5.6: Results of cluster analysis showing grouping of river systems based on the distribution of freshwater fishes. The current ecoregion designations are shown in red (Cape Fold Ecoregion), green (Amatolo-Winterberg Highlands Ecoregion) and black (Southern Temperate Highveld Ecoregion).

Table 5.2: The depicted percentage contribution, cumulative percentage and mean contribution of fish assemblage structure for the Olifants River system and the other river systems, according to similarity percentages SIMPER analysis. The first column identifies the speices explained by that row, the second column shows the percentage dissimilarity explained by that species, the third column tallies the cumulative Bray Curtis metric for the species thus far represented in the table, and the last two columns show mean abundance in the Olifants River and mean abundance in the rest of the river systems examined.

Taxon	Contribution %	Cumulative %	Mean (Olifants)	Mean (the rest)
Pseudobarbus phlegethon	6.54	6.548	1	0
Austroglanis gilli	6.54	13.1	1	0
Enteromius cernuus	6.54	19.65	1	0
Cheliobarbus serra	6.54	26.19	1	0
Sedercypris erubescens	6.54	32.74	1	0
Sedercypris calidus	6.54	39.29	1	0
Labeobarbus seeberi	6.54	45.84	1	0
Pseudobarbus sp. '*plegethon* Doring'	6.54	52.39	1	0
Austroglanis barnardi	6.54	58.94	1	0
Labeo seeberi	6.54	65.48	1	0
Galaxias sp. '*zebratus* Nebula'	4.27	69.76	1	0.41
Enteromius mandelai	3.65	73.42	0	0.5
Labeo umbratus	3.40	76.83	0	0.5
Enteromius pallidus	3.34	80.18	0	0.5
Sandelia sp. '*capensis* South Coast'	2.89	83.07	0	0.5
Pseudobarbus afer ss	1.66	84.73	0	0.25
Enteromius anoplus ss	1.45	86.19	0	0.25
Sandelia sp. '*bainsii* Kowie'	1.20	87.39	0	0.16
Pseudobarbus asper	0.99	88.39	0	0.16
Cheilobarbus capensis	0.81	89.2	0	0.16
Amatolacypris trevelyani	0.62	89.82	0	0.08
Sandelia sp. '*bainsii* Buffalo'	0.62	90.44	0	0.08
Amatolacypris sp. '*trevelyani* Keiskamma'	0.58	91.02	0	0.08
Sandelia sp. '*bainsii* Keiskamma'	0.58	91.61	0	0.08
Pseudobarbus tenuis	0.51	92.12	0	0.08
Pseudobarbus swartzi	0.4	92.6	0	0.08
Pseudobarbus senticeps	0.45	93.06	0	0.08
Pseudobarbus sp. '*afer* Forest'	0.45	93.52	0	0.08
Pseudobarbus sp. '*tenuis* Keurbooms'	0.45	93.98	0	0.08
Pseudobarbus skeltoni	0.43	94.41	0	0.08
Galaxias sp. '*zebratus* Goukou'	0.43	94.85	0	0.08
Pseudobarbus sp. '*burchelli* Breede'	0.43	95.28	0	0.08
Galaxias sp. '*zebratus* Breede'	0.43	95.72	0	0.08
Galaxias sp. '*zebratus* Riviersonderend'	0.43	96.16	0	0.08

Galaxias sp. '*zebratus* Rectognathus'	0.43	96.59	0	0.08
Pseudobarbus burchelli	0.37	96.97	0	0.08
Pseudobarbus verlorenvlei	0.37	97.35	0	0.08
Pseudobarbus burgi	0.37	97.73	0	0.08
Sandelia sp. '*capensis* Klien'	0.37	98.11	0	0.08
Galaxias sp. '*zebratus* Mollis'	0.37	98.48	0	0.08
Sandelia sp. '*capensis* West Coast'	0.37	98.86	0	0.08
Galaxias sp. '*zebratus* Heuningnes'	0.37	99.24	0	0.08
Galaxias sp. '*zebratus* Slender'	0.37	99.62	0	0.08
Galaxias sp. '*zebratus* Klein'	0.37	100	0	0.08

Table 5.3: The depicted percentage contribution, cumulative percentage and mean contribution for the south-western and south-eastern clusters according to SIMPER analysis.

Taxon	Contribution %	Cumulative %	Mean (south-western)	Mean (south-eastern)
Enteromius mandelai	11.72	11.72	0	1
Sandelia sp. '*capensis* South Coast'	11.72	23.44	1	0
Galaxias sp. '*zebratus* Nebula'	8.69	32.14	0.83	0
Labeo umbratus	6.41	38.55	0.33	0.66
Enteromius pallidus	5.94	44.5	0.5	0.5
Pseudobarbus afer ss	5.20	49.7	0.33	0.16
Pseudobarbus asper	4.07	53.78	0.33	0
Sandelia sp. '*bainsii* Kowie'	3.81	57.6	0	0.33
Cheilobarbus capensis	2.77	60.37	0.33	0
Enteromius anoplus ss	2.03	62.41	0.16	0
Pseudobarbus swartzi	2.03	64.45	0.16	0
Pseudobarbus tenuis	2.03	66.49	0.16	0
Sandelia sp. '*bainsii* Buffalo'	2.01	68.5	0	0.16
Amatolacypris trevelyani	2.01	70.52	0	0.16
Pseudobarbus sp. '*afer* Forest'	1.83	72.36	0.16	0
Pseudobarbus sp. '*tenuis* Keurbooms'	1.83	74.2	0.16	0
Pseudobarbus senticeps	1.83	76.04	0.16	0
Amatolacypris sp. '*trevelyani* Keiskamma'	1.80	77.84	0	0.16
Sandelia sp. '*bainsii* Keiskamma'	1.80	79.65	0	0.16
Pseudobarbus skeltoni	1.53	81.19	0.16	0
Galaxias sp. '*zebratus* Goukou'	1.53	82.72	0.16	0
Pseudobarbus sp. '*burchelli* Breede'	1.53	84.26	0.16	0
Galaxias sp. '*zebratus* Breede'	1.53	85.8	0.16	0
Galaxias sp. '*zebratus* Riviersonderend'	1.53	87.34	0.16	0
Galaxias sp. '*zebratus* Rectognathus'	1.53	88.88	0.16	0
Pseudobarbus burchelli	1.23	90.11	0.16	0
Pseudobarbus verlorenvlei	1.23	91.35	0.16	0
Pseudobarbus burgi	1.23	92.59	0.16	0
Sandelia sp. '*capensis* Klien'	1.23	93.82	0.16	0

Galaxias sp. '*zebratus* Mollis'	1.23	95.06	0.16	0
Sandelia sp. '*capensis* West Coast'	1.23	96.29	0.16	0
Galaxias sp. '*zebratus* Heuningnes'	1.23	97.53	0.16	0
Galaxias sp. '*zebratus* Slender'	1.23	98.76	0.16	0
Galaxias sp. '*zebratus* Klein'	1.23	100	0.16	0

Table 5.4: Total number of fish species, number of endemic fish and percentage endemism of fish from the Olifants River system in the west coast to the Buffalo River in the east coast of South Africa.

	Olifants	Berg	Breede	Gouritz	Coastal rivers	Gamtoos	Swartkops	Sundays	Bushmans	Kowie	Great Fish	Keiskamma	Buffalo
Total number of species	11	11	10	7	6	7	3	4	3	3	6	4	3
Endemic species	10	8	7	2	3	1	0	0	0	0	0	2	2
Percentage endemism	91	73	70	29	50	14	0	0	0	0	0	50	66

Discussion

The first objective of this study was to reanalyse spatial patterns of species richness and endemism. It was hypothesized that areas of endemism would coincide with drainage basin boundaries and ecoregion limits. Many of the river systems in the study area contain endemic species or lineages. Examples are *Austroglanis barnardi*, *Austroglanis gilli*, *Sedercypris calidus*, *Sedercypris erubescens*, *Labeobarbus seeberi* and *Cheilobarbus serra* that are endemic to the Olifants-Doring River systems (see Ellender et al., 2017). Similarly, the Verlorenvlei River in the Berg River basin contains an endemic redfin, *P. verloreni* (Chakona et al., 2014; Chakona et al., in prep). *Pseudobarbus burgi* is also endemic to the Berg River system. Lineages of *Galaxias zebratus* and *Sandelia capensis* such as *Galaxias* sp. '*zebratus* Mollis', *Galaxias* sp. '*zebratus* Klein', *Galaxias* sp. '*zebratus* Slender', *Sandelia* sp. '*capensis* Klein', *Sandelia* sp. '*capensis* West Coast' are also found in the Berg River basin. The Breede has endemic *Pseudobarbus* and *Galaxiid* species and lineages such as *P. skeltoni*, *Pseudobarbus* sp. '*burchelli* Breede' lineage, *Galaxias* sp. '*zebratus* Breede', *Galaxias* sp. '*zebratus* Goukou', *Galaxias* sp. '*zebratus* Rectognathus' and *Galaxias* sp. '*zebratus*

Riviersonderend' (Swartz et al., 2009, Chakona et al., 2013b). Other examples include *E. anoplus* s.s. which is endemic to the Gouritz River system, *P. senticeps* which is endemic to the Krom River of the coastal drainages, *P. swartzi* which is endemic to the Gamtoos River system, *A. trevelyani* s.s. which is endemic to the Buffalo River system, *Sandelia* sp. '*bainsii* Buffalo' lineage from the Buffalo River system and *Sandelia* sp. '*bainsii* Keiskamma' lineage from the Keiskamma River system (Chapter 2; Chakona & Skelton, 2017; Chakona et al., 2020b). However, there are also a number of species and lineages that occur across more than one isolated river systems. These include *Galaxias* sp. '*zebratus* Nebula', *Sandelia* sp. '*capensis* South Coast', *Labeo umbratus*, *Enteromius pallidus*, *Enteromius mandelai* and *Sandelia* sp. '*bainsii* Kowie' lineage (Chakona et al., 2013b; 2020; Martin & Chakona, 2019; Bronaugh et al., 2020; Ramoejane et al., 2020).

A number of mechanisms have been invoked to explain the distribution patterns of these species, including palaeoriver systems, river captures and intermittent freshwater connections (Barnard, 1943; Skelton, 1986; Swartz et al., 2007; 2009, 2014; Chakona et al., 2013a; 2015; Bronaugh et al., 2020). Human mediated translocations are also likely to have played a role, as evidenced by the sharing of common haplotypes among a number of river systems of *E. mandelai* (Chapter 2). Also, populations of *Sandelia capensis* in the Goukou and Lourens River system are suspected to have had human-mediated translocations as shared haplotypes were found between the two despite being 220km apart and separated by a dozen coastal river systems (Bronaugh et al 2020). The uniqueness of the Olifants-Doring River system has been attributed to its previous connectivity with the Orange River system (Dingle & Hendry, 1984) which has resulted in faunal similarities between the Olifants and the Orange River systems (Skelton & Cambray, 1981; Skelton, 1986).

The high diversity and endemism on the south-coast, i.e., the area encompassing the Berg and Breede drainages has been attributed to the Miocene-Pliocene sea level transgression which are hypothesized to have acted as the engine for the diversification of stream fishes in this region (Chakona et al., 2013a). Specifically, divergence time estimates indicate that splitting of major lineages within *Galaxias, Pseudobarbus,* and *Sandelia* coincided with a period of higher sea-levels, suggesting that fragmentation of populations of these taxa in upland refugia promoted diversification. For example, the distribution limits of *Galaxias* sp. 'Riviersonderend' and *Galaxias* sp. 'Breede' suggest that these lineages could have evolved in allopatry due to vicariance caused by possible isolation of the Breede and Riviersonderend catchments during the Miocene-Pliocene marine incursion (Chakona et al., 2013a). The

attenuation of taxonomic diversity in this region eastwards could be related to the shallow continental shelf as well as climatic conditions. A special feature of the south-western CFE is the Agulhas Bank, a shallow continental shelf that would have been periodically exposed and submerged due to repeated fluctuations in sea-levels (Siesser & Dingle, 1981; Compton, 2011), resulting in the recurrent connection and isolation of adjacent river systems (Swartz, 2005; Swartz et al., 2009). Repeated expansion and contraction of freshwater habitats could have a significant impact on the distribution and diversification patterns of fish species in this region. From a climatic viewpoint, the Olifants, Berg and Breede systems are found in the fynbos subregion of the CFE which receives 600-2000 mm of rainfall per year, the highest in this Ecoregion which may allow for higher productivity. In contrast, river systems in the eastern part of the CFE are found in the drought corridor bioregion characterised by erratic rainfall (Brown et al., 1996). River systems further east in the Amathole-Winterberg highland ecoregion have non-arid climatic conditions (Thieme et al., 2005) resulting in a higher number of species than in the Sundays, Bushmans and Kowie river systems.

The second objective of this study was to propose and revise fish-based ecoregion boundaries based on improved knowledge on species taxonomy and distribution patterns. The separation of the CFE from the AWHE is supported as there is considerable overlap between these regions. Based on the findings from this study, there are two possibilities: (i) separate the region into the Olifants-Doring, the south-western and the south-eastern freshwater ecoregions as cluster analysis showed separation of these regions, or (ii) extend the CFE to encompass the AWHE. In order to maintain stability, the option to extend the CFE to encompass the AWHE seems more reasonable. This is supported by the close relationship between a number of species that are shared between these ecoregions. Examples are the occurrence of species of the genera *Sandelia* and *Enteromius*. *Amatolacypris trevelyani* also closely related to other polyploid genera in the CFE – *Pseudobarbus*, *Cheilobarbus* and *Sedercypris* (Skelton et al., 2018).

In conclusion, the reanalysis of the available data indicates that incomplete knowledge of species diversity and distribution patterns can misdirect delineation of areas of endemism, biodiversity hotspots and ecoregions. This can have serious implications on policy formulation and conservation prioritization. The present study clearly highlights the need for dedicated studies to provide a more accurate delineation of species boundaries as this is the basis for conservation and management of species.

CHAPTER 6

General discussion

In Africa, an increasing number of freshwater fishes has been found to be represented by unrealised cryptic species (e.g., Arroyave et al., 2019; Braganca et al., 2020; Jirsová et al., 2019; Kasongo et al., 2020; Mambo Baba et al., 2020). The present study builds on this research by providing information on the taxonomy, ecology and biogeography of the *E. anoplus* complex in South Africa. Chapter 1 raised questions related to some major uncertainties regarding the molecular and morphological variation among populations of *E. anoplus*. *Enteromius anoplus* was a suitable target species for molecular systematic assessment and morphological analysis for several reasons. First, *E. anoplus* had a broad distribution that spanned across a number of aquatic ecoregions and multiple, isolated river basins (Skelton, 2001; Thieme et al., 2005). Second, previous investigations of *E. anoplus* revealed evidence for genetic and morphological variation across its range (Barnard, 1943; Engelbrecht, 1996; Da Costa, unpublished). Third, previous DNA based studies for other co-occurring fish species across its distribution revealed genetic divergences among several populations (e.g. Ramoejane, 2016; van der Walt et al., 2017; Martin & Chakona, 2019; Chakona et al., 2020b).

Integrative taxonomic analysis revealed that the *E. anoplus* complex was composed of multiple species (Chapter 2). Molecular analysis confirmed the division of *Enteromius anoplus* s.l. into *Enteromius anoplus* s.s., *E. cernuus* and *E. oraniensis* previously identified by Barnard (1943) through morphological analysis as well as a new species *E. mandelai* sp. nov from the Eastern Cape Province. *Enteromius oraniensis* from the western flowing Orange River and the *Enteromius anoplus* s.s. and *E. cernuus* from the south-western Gouritz and Olifants River systems formed a clade separate from the south eastern *E. mandelai* (Chapter 2). Furthermore, the genetic diversity was highest between *E. mandelai* and the three other species (7.45–8.80%); and lowest among *E. oraniensis*, *E. cernuus* and *E. anoplus* s.s. (1.97–3.05%). Genetic divergence is related to evolutionary time (Thorpe & Sole-Cava, 1994). Therefore, the larger genetic differences between *E. mandelai* and three other species suggest that *E. mandelai* may have been isolated from *E. oraniensis*, *E. cernuus* and *E. anoplus* s.s. longer than the three species have been isolated from each other. This pattern of genetic differentiation was similar to those of other taxa in this region such as *Labeo umbratus* and *Enteromius pallidus* (Martin & Chakona, 2019; Ramoejane et al., 2020).

Although this study revealed four species present in the *E. anoplus* complex, a minimum of two more may exist. For example, populations present in the Orange River system may represent multiple species. This hypothesis is supported by the preliminary analysis carried out on DNA sequences from the various rivers in the Orange River system, as well as work carried out by Engelbrecht (1996) which suggest that Orange River chubbyhead barb represent multiple lineages. Future studies should try to establish the genetic identity of *E. anoplus* s.l. populations in rivers of the Orange River system, such as the Vaal River which were not included in the current study. Furthermore, Gilchrist and Thompson (1913) described *Barbus karkensis* from the Umgeni River system. This species was subsequently synonymized with *E. anoplus* s.l. (Lévéque and Daget, 1984). These localities are geographically separated from the populations reviewed in the present study and may represent distinct species. Future work should involve confirming whether *E. anoplus* s.l. is indeed a different species in the Umgeni River system.

In the present study, the mitochondrial cytochrome *b* (mtDNA) gene was used successfully for genetic differentiation of *E. anoplus* s.l. (Chapter 2). This gene evolves rapidly and is particularly useful for resolving relationships among recently evolved groups (Avise, 2004). However, a major disadvantage of using mtDNA is that because it is maternally inherited, it only looks through a single window of evolution. Future studies should consider using independent, genomic molecular markers such as microsatellites, single nucleotide polymorphisms and restricted fragment length polymorphism (Maqsood & Ahmad, 2017) as the phylogenies and population structures derived from mtDNA data could well differ from that overall of populations or species due to introgression or gender biased migration (Birky et al., 1989; Chow & Kishino, 1995).

In contrast to *E. anoplus* s.s., *E. cernuus* and *E. oraniensis, E. mandelai* sp. nov. in the Eastern Cape Province occurred in multiple river systems with haplotypes shared among them (Chapter 2). Specifically, there was a haplotype shared among populations in the Keiskamma, Buffalo and Tyolomnqa rivers. Another haplotype was shared between the Keiskamma River and the Koonap River, a tributary of the Great Fish River. This suggests recent or ongoing geodispersal of *E. mandelai* among these drainages. Human-mediated movement of fish may be a significant explanation for the shared haplotypes among river systems in *E. mandelai.* For example, extensive breeding and stocking of minnows as trout fodder occurred in the Pirie trout hatchery and Rooikrans hatchery located in the upper sections in the Buffalo River (Harrison, 1936). Anecdotal evidence suggests that *E. anoplus* s.s. was widely distributed in the Eastern

Cape through these hatcheries and by anglers as bait. The populations of *Labeo umbratus* that occur in the Buffalo and Nahoon rivers were also previously suspected to have introduced by anglers as bait (Jubb, 1964). Such translocations can cause intraspecific competition and genetic contamination between populations that could lead to the loss of genetic characteristics in some populations. Future investigations using additional markers would help discriminate between natural and human aided translocation of this species.

Given the species discoveries in this study (Chapter 2), the previous treatment of *E. anoplus* species as widespread and common with little conservation concern needs to be re-evaluated. *Enteromius anoplus* s.l. is currently listed as Least Concern by the International Union for conservation of Nature (IUCN) (Woodford, 2017). Because of the reduced distribution ranges of newly identified and revalidated species, coupled with the anthropogenic threats present throughout South Africa, it is expected that these newly identified species are threatened. Updated extinction list assessments are needed. Other small-bodied fish species in these systems have been found to belong to highly threatened IUCN Red List categories. For example, *Pseudobarbus phlegethon* and *Sedercypris calidus,* endemic to the Olifants River system are listed as endangered and near threatened respectively. The major decline in the distribution of these species has been caused by the establishment and spread of non-native bass species such as such as Largemouth bass *Micropterus salmoides*, Smallmouth bass *M. dolomieu* and Spotted bass *M. punctulatus* (Woodford et al., 2005; Clark et al., 2009; Weyl et al., 2014). Within the Gouritz River system, *Pseudobarbus asper*, a species which prefers mainstem and large tributary habitats, historical range has declined due to multiple impacts including loss of habitat due to invasion of the mainstem sections of rivers by non-native piscivores mainly bass species (*Micropterus* spp.) and African sharptooth catfish *Clarias gariepinus*, pollution and over abstraction of water (Ellender et al., 2017; Weyl et al., 2020). *Pseudobarbus tenuis* which prefers headwater stream habitat, is less impacted by non-native species which prefer the mainstem sections of the Gouritz River system. The exception being the rainbow trout *Oncorhynchus mykiss* which co-occurs with *P. tenuis. Pseudobarbus asper* and *P. tenuis* are listed as vulnerable and near threatened respectively. Native fish species in the AWHE and STHE in the Eastern Cape Province are threatened by the widespread invasion of river systems by non-native species such as the piscivorous *Clarias gariepinus* and *Micropterus* species, hydrological modification through inter-basin water transfer schemes, pollution from urban areas, excessive water abstraction and habitat degradation from agricultural activities (Thieme et al., 2005; Kadye & Booth, 2012) Species such as *Sandelia*

bainsii and *Amatolacypris trevelyani* with restricted distribution ranges are listed as endangered whereas *Enteromius pallidus,* with a wider distribution range is listed as least concern. An assessment on the newly described species from the present study will highlight the species and populations that are most urgently in need for conservation and management strategies. An IUCN Red List assessment is also a powerful tool for persuading the relevant stakeholders to protect threatened species and take measures to reduce their risk of extinction.

Because biotic and abiotic factors influence the distribution and abundance of fish species across different spatial scales (Grossman et al., 1998; Rahel & Nibbelink, 1999) identifying these factors is a crucial step for the effective conservation and management of species. In the present study, *E. mandelai* was found to be a key component of the unique headwater stream biota that characterises river systems in the AWHE and STHE (Chapters 3 and 4). These river systems have a wide range of environmental characteristics. The Great Fish River system runs dry (average rainfall < 500 mm per annum) and the rivers are temporary, alkaline and carry very high total dissolved solids and suspended sediment loads (O'Keeffe & De Moor, 1988; Allanson et al., 1990). The Great Fish River is also affected by flow modifications such as those of the Orange-Great Fish-Sundays inter-basin water transfer scheme which altered the flow from seasonal to perennial, introduced fine sediments and non-native fauna into some portions of the Great Fish River (Cambray & Bruton, 1985; Allanson et al., 1990;). Rivers of the Keiskamma and Buffalo River systems rise in the well-watered Winterberg Mountains and have a more dependable flow with mean annual precipitation in the Keiskamma ranging from 600 mm to 1200 mm and that of Buffalo ranging from 500–2000 mm (Palmer & O'Keeffe, 1990). Furthermore, *E. mandelai* is directly threatened by non-native fishes such as *Clarias gariepinus, Oncorhynchus mykiss and Micropterus salmoides* (Kadye & Booth 2012, 2013a). It is also affected by a variety of riparian land use activities such as crop farming and silviculture (Thieme et al., 2005; Matomela, 2020). Despite the environmental and anthropogenic variability in the river systems, *E. mandelai* occurred in a wide range of habitats and was tolerant to variations in the environment (Chapters 3 and 4). The lack of specific habitat relationship is likely to be one of the reasons that has facilitated the persistence of this species throughout the Eastern Cape. From a biotic perspective, the use of stable isotope analysis revealed that *E. mandelai* had larger isotopic niches in agriculturally disturbed streams and smaller isotopic niches in streams invaded by non-native plants (Chapter 3). This can be attributed to the reports that land-use alters resource availability (García et al., 2017), habitat (Walser & Bart, 1999), and stream hydrology (Nagasaka & Nakamura, 1999), which then

affects fish distribution and abundance patterns. From an abiotic perspective, with the exception of *A. trevelyani* in some headwater streams, *E. mandelai* was found to be a higher reach species and was spatially differentiated from other native fish (Chapter 4). These spatial patterns posit the likelihood of environmental filtering which is assumed to drive functionally different species to occur in dissimilar habitat conditions.

The separation of *E. anoplus* s.l. into multiple species (Chapter 2) as well as the extensive changes in the fish fauna of this region (e.g.Chakona et al., 2014; Chakona & Skelton, 2017; Skelton et al., 2018; Martin & Chakona, 2019) also has implications for the ecoregion delimitations for southern temperate fish species (Chapter 5). The study has shown that taxonomy and the distribution patterns of fish species are the major forces weakening current ecoregion boundaries of the CFE in the east. A framework for the expansion of the CFE into AHWE and intermediate areas is established herein through cluster analysis which future larger scaled and higher resolution studies can test and build upon. This is an important step towards renewed interest in biogeographic analysis as more species are discovered and revised in this region in order to support the conservation of natural biogeographical patterns. Other important findings include the fact that species richness and endemism decreased from the west to the east, with the highest species richness and endemism being found in the Olifants, Berg and Breede River systems. This documentation and inventorying of the species richness, endemism and distribution patterns of these fishes and the habitats they occupy are useful for aquatic conservation planning.

In conclusion, this thesis contributes to the ongoing resolution of genetic diversity, ecology, distribution and biogeographic patterns of freshwater fish species in the temperate biogeographic zone of southern Africa. Using the *Enteromius anoplus* complex as a case study, this research shows that in this region, fish species with a wide distribution range encompass several river systems and considered to be common, may comprise many genetically distinct populations restricted to smaller catchments. The findings from this study also show that river systems of the CFE, AWHE and STHE contain a high number of endemic fish species that have adapted to local conditions over long periods of time. Therefore, species in these ecoregions are of scientific and conservation importance because they are integral parts of these specialised ecosystems. Additionally, even though the CFE is relatively well studied, taxonomic uncertainties and species systematics are an active research area for many fish species in the CFE and the evolving taxonomy will continue to pose ecoregion boundary questions.

REFERENCES

Da Costa, L. (n.d.). Unpublished.

Peters, W. (1853). Mittheilung über die Süsswasserfische von Mossambique. *Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlichen Preussischen Akademie der Wissenschaften zu Berlin*, 738.

Cope, E. D. (1867). Supplement on some new species of American and African fishes. *Transactions of the American Philosophical Society*, *13*, 351–410.

Gunther, A. (1867). The Record of Zoological Literature , 1866. *Annals and Magazine of Natural History*, *3*.

Gunther, A. (1868). *Catalogue of the Physostomi in the collection of the British Museum* (Vol. 7). London: British Museum. http://biodiversitylibrary.org/page/9365020.

Steindachner, F. (1894). Ichthyologische Beiträge (XVII). *Anzeiger der Kaiserlichen Akademie der Wissenschaften, Wien, Mathematisch-Naturwissenschaftliche Classe*, *31*(14), 137–140.

Weber, M. (1897). Beiträge zur Kenntniss der Fauna von Süd-Afrika. I. Zur Kenntniss der Süsswasser-Fauna von Süd-Afrika. *Zool. Jahrb*, *10*, 135–200.

Boulenger, G. A. (1903). Descriptions of four new species of *Barbus* discovered by Mr. A. Blayney Percival in East Africa. *Annals and Magazine of Natural History*, *11*, 52–54.

Boulenger, G. A. (1905). The distribution of African freshwater fishes. *Report of the British Association for the Advancement of Science*, *75*, 412–432.

Gilchrist, J. D. F., & Thompson, W. W. (1913). The freshwater fishes of South Africa. *Annals of the South African Museum*, *11*, 321–463.

Boulenger, G. A. (1916). *Catalogue of the Fresh-water Fishes of Africa in the British Museum (Natural History)*. *Trustees of the British Museum (Natural History), London* (Vol. 2). London. https://doi.org/10.5962/bhl.title.8869.

Fowler, H. (1935). Scientific results of the Vernay-Lang Kalahari expedition, March to September, 1930. Fresh-water fishes. *Annals of the Transvaal Museum*, *16*(2), 251–293.

Harrison, A. C. (1936). *Black bass in the Cape Province: second report on the progress of*

Amercian Largemouth Bass (Micropterus salmoides, Laceepeede). Pretoria.

Trewavas, E. (1936). Dr. Karl Jordan's expedition to South-West Africa and Angola: The fresh-water fishes. *Novitates Zoologicae. A Journal of Zoology in Connection with the Tring Museum*, *40*, 63–74.

Barnard, K. H. (1938). Notes on the species of *Barbus* from the Cape Province, with descriptions of new species. *Annals and Magazine of Natural History*, *2*, 80–88.

Smith, A. (1841). *Illustrations of the Zoology of South Africa, Pisces*. London: Smith, Elder and Co.

Barnard, K. H. (1943). Revision of the Indigenous Freshwater Fishes: Of the SW Cape Region. *Annals of the South African Museum*, *36*, 101–262.

Barnard, K. H., & Davies, D. H. (1947). Description of a new fish of the family Zeidae from the Cape. *Annals and Magazine of Natural History*, *13*(11).

Craig, H. (1957). Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta*, *12*, 133–149.

Hutchinson, G. E. (1957). Concludig remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, *22*, 415–427.

Poll, M. (1957). Les Genres des Poissons d'eau Douces de l'Afrique. *Ann. Mus. r. Congo belge*, *8*(54), 1–191.

Groenewald, A. A. V. J. (1958). A revision of the genera *Barbus* and *Varicorhinus* (Pisces: Cyprinidae) in Transvaal. *Annals of the Transvaal Museum*, *23*(3), 263–330.

Crass, R. (1960). Notes on the freshwater fishes of Natal with the description of four new species. *Annals of the Natal Museum*, *14*, 405–458.

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

Farquharson, F. L. (1962). The distribution of cyprinids in South Africa. *Annals of the Cape Provincal Museums*, *2*, 233–251.

Greenwood, P. (1962). A new species of *Barbus* (Pisces, Cyprinidae) from the Upper Zambezi River, Rhodesia. *Revue de Zoologie et de Botanique Africaines*, *65*, 211–216.

Jubb, R. A. (1963). A revised list of the freshwater fishes of southern Africa. *Annals of the Cape Provincial Museums*, *3*, 5–39.

Jubb, R. A. (1964). Freshwater fishes and drainage basins in Southern Africa. 1. The Orange and South Coastal drainage basisns. *South African Journal of Science*, *60*(1), 17–21.

Matthes, H. (1964). Les poissons du Lac Tumba et de la region d'Ikela. *Annales du Musée Royal d'Afrique Centrale (Sciences Zoologiques)*, *126*, 1–204.

Gabie, V. (1965). Problems asscoiated with the distribution of freshwater fishes in southern Africa. *South African Journal of Science*, *61*, 383–391.

Jubb, R. A. (1965). Freshwater fishes of the Cape Province. *Annals of the Cape Provincial Museum*, *4*, 1–72.

Jubb, R. A., & Farquharson, F. L. (1965). The freshwater fishes of the Orange River basin. *South African Journal of Science*, *61*, 118–125.

Van Valen, L. (1965). Morphological Variation and Width of Ecological Niche. *The American Naturalist*, *99*, 377–390.

Jubb, R. A. (1966). A new species of *Barbus* (Pisces, Cyprinidae) from the Sabie River, North Eastern Transvaal. *Annals of the Cape Provincal Museums*, *5*, 157–160.

Jubb, R. A. (1967). *The freshwater fishes of southern Africa*. Cape Town: Balkema.

Greenwood, P., & Jubb, R. A. (1967). The generic identity of *Labeo quathlambe* Barnard (Pisces, Cyprinidae). *Annals of the Cape Provincial Museum (Natural History)*, *6*(2), 17–37.

Colwell, R. K., & Futuyma, D. J. (1971). On the Measurement of Niche Breadth and Overlap. *Ecology*, *52*, 567–576.

Odum, E. P., & Barrett, G. W. (1971). *Fundamentals of Ecology. Third Edition. Thomson, Brooks/Cole*.

Gaigher, J. D. F., & Pott, R. M. (1973). Distribution of fishes in Southern Africa. *South African Journal of Science*, *69*, 25–27.

Jackson, P. B. N. (1973). Preliminary report on small fishes from the Hendrik Verwoerd Dam, Orange River. *South African Journal of Science*, *69*, 85–87.

Cummins, K. W. (1974). Structure and Function of Stream Ecosystems. *BioScience*, *24*(11), 631–641.

Schoener, T. W. (1974). Resource partitioning in ecological communities. *Science*, *185*(4145), 27–39.

Wedderburn, R. W. M. (1974). Quasi-likelihood functions, generalized linear models, and the gauss-newton method. *Biometrika*, *61*(3), 439–447.

Gaigher, I. G. (1975). The ecology of a minnow, *Barbus trevelyani* (Pisces, Cyprinidae) in the Tyume River, Eastern Cape. *Annals of the Cape Provincial Museums Natural History*, *11*(1), 1–19.

Hynes, H. B. N. (1975). The stream and its valley. *SIL Proceedings, 1922-2010*, *19*(1), 1–15.

Roberts, T. R. (1975). Geographical distribution of African freshwater fishes. *Zoological Journal of the Linnean Society*, *57*(4), 249–319.

Fowler, H. (1976). A catalog of World fishes. *Quarterly Journal of the Taiwan Museum (Taipei)*, *29*(XXIV), 1–110.

Skelton, P. H. (1976). Preliminary observations on the relationships of *Barbus* species from Cape coastal Rivers, South Africa (Cypriniformes: Cyprinidae). *Zoologica Africana*, *11*(2), 399–411.

Cambray, J. A., & Jubb, R. A. (1977). Dispersal of fishes via the Orange-fish tunnel, South Africa. *Journal of the Limnological Society of Southern Africa*, *3*(1), 33–35.

Southwood, T. R. E. (1977). Habitat, the templet for ecological strategies? *The Journal of Animal Ecology*, *46*(22), 337–365.

Cambray, J. A, Hahndiek, S., & Hahndiek, Q. (1978). The juvenile fish population in the marginal areas of the Hendrick Verwoerd Dam. *Journal of the Limnological Society of Southern Africa*, *4*(1), 21–30.

DeNiro, M. J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta*, *42*(5), 495–506.

Skelton, P. H. (1980). *Systematics and biogeography of the redfin Barbus species (Pisces: Cyprinidae) from southern Africa*. *Department of Ichthyology and Fisheries*. Rhodes University.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(1), 130–137.

Siesser, W. G. & Dingle, R. V. (1981). Tertiary sea-level movements around southern Africa. *The Journal of Geology 89*, 83-96.

Skelton, P. H., & Cambray, J. A. (1981). The freshwater fishes of the midlle and mower Orange River. *Koedoe*, *24*, 51–66.

Nijssen, H., van Tuijl, L., & Isbrucker, I. J. H. (1982). A catalogue of the type-specimens of Recent fishes in the Institute of Taxonomic Zoology (Zoölogisch Museum), University of Amsterdam, The Netherlands. *Verslagen en Technische Gegevens*, *33*, 1–173.

Cambray, J. A. (1983). Early development and larval behaviour of a minnow *Barbus anoplus* (Pisces: Cyprinidae). *South African Journal of Zoology*, *18*(4), 331–336.

Cambray, J. A. (1983). The feeding habits of minnows of the genus *Barbus* (Pices, Cyprinidae) in Africa, with special reference to *Barbus anoplus* Weber. *Journal of the Limnological Society of Southern Africa*, *9*(1), 12–22.

Baltz, D. M., & Moyle, P. B. (1984). The influence of riparian vegetation on stream fish communities of Carlifornia. In P. Warner & H. Hendrix (Eds.), *Carlifornia riparian systems* (pp. 183–187). Berkeley, Carlifornia: University of Carlifornia Press.

Cambray, J. A., & Bruton, M. N. (1984). The reproductive strategy of a barb, *Barbus anoplus* (Pisces: Cyprinidae), colonizing a man-made lake in South Africa. *Journal of Zoology*, *204*(2), 143–168.

Daget, J., Gosse, J. P., & Thys van den Audenaerde, D. F. E. (1984). Check list of the freshwater fishes of Africa/Catalogue des poissons d'eau douce d'Afrique. Vol I., 410. Retrieved from http://www.documentation.ird.fr/hor/fdi:36707.

Dingle, R. V., & Hendry, Q. B. (1984). Late Mesozoic and Tertiary sediment supply to the Eastern Cape Basin (SE Atlantic) and palaeo-drainage systems in Southwestern Africa. *Marine Geology*, *56*, 13–26.

Cambray, J. A., & Bruton, M. N. (1985). Age and growth of a colonizing minnow, *Barbus anoplus*, in a man-made lake in South Africa. *Environmental Biology of Fishes*, *12*(2), 131–141.

Minshall, G. W., Cummins, K. W., Petersen, R., Cushing, C. E., Bruns, D. A., Sedell, J. R., & Vannote, R. L. (1985). Developments in stream ecosystem theory. *Canadian Journal of Fisheries and Aquatic Sciences*, *43*, 1045–1055.

Laurenson, L. B. J., & Hocutt, C. H. (1986). Colonisation theory and invasive biota: The Great Fish River, a case history. *Environmental Monitoring and Assessment*, *6*(1), 71–90.

Mullahy, J. (1986). Specification and testing of some modified count data models. *Journal of Econometrics*, *33*(3), 342–365.

Skelton, P. H. (1986). Distribution patterns and biogeography of non-tropical southern African freshwater fishes. *Palaeoecology of Africa*, *17*, 211–230.

Ibarra, M., & Stewart, D. J. (1987). Catalogue of type specimens of Recent fishes in Field Museum of Natural History. *Fieldiana Zoology (New Series)*, *35*, 1–112.

Schlosser, I J. (1987). A conceptual framework for fish communities in small warmwater streams. *Community and evolutionary ecology of North American stream fishes*, *382*(1), 63–86.

Clarke, K., & Green, R. (1988). Statistical design and analysis for a "biological effects" study. *Marine Ecology Progress Series*, *46*, 213–226.

Geldenhuys, C. J., & van der Merwe, C. J. (1988). Population structure and growth of the fern *Rumohra adiantiformis* in relation to frond harvesting in the southern Cape forests. *South African Journal of Botany*, *54*(4), 351–362.

O'Keeffe, J. H., & De Moor, F. C. (1988). Changes in the physico-chemistry and benthic invertebrates of the Great Fish River, South Africa, following an interbasin transfer of water. *Regulated Rivers: Research & Management*, *2*(1), 39–55.

Skelton, P. H. (1988). The distribution of African freshwater fishes. In: Lévêque C, Bruton MN, Ssentongo GW. *Biology and Ecology of African Freshwater Fishes* (pp. 65–91). Paris: ORSTOM.

Skelton, P. H. (1988). A taxonomic revision of the redfin minnows (Pices, Cyprinidae) from southern Africa. *Annals of the Cape Provincal Museums. Natural History*, *16*, 201–307.

Southwood, T. R. E. (1988). Tactics, Strategies and Templets. *Oikos*, *52*(1), 3–18.

Angermeier, P. L., & Schlosser, I. J. (1989). Species-area relationships for stream fishes.

Ecology, *70*, 1450–1462.

Birky, C. W., Fuerst, P., & Maruyama, T. (1989). Organelle gene diversity under migration, mutation, and drift: Equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics*, *121*(3), 613–627.

Ehleringer, J. R., & Rundel, P. W. (1989). Stable Isotopes: History, Units, and Instrumentation. *Stable Isotopes in Ecological Research* (pp. 1–15).

Allanson, B. R., Hart, R. C., O'Keeffe, J. H., Robarts, R. D., O'Keeffe, J. H., & Robarts, R. D. (1990). *Inland waters of southern Africa: an ecological perspective*. *Monographiae Biologicae* (Vol. 64). Dordrecht: Springer Netherlands.

Bremner, J. M., Rogers, J., & Willis, J. P. (1990). Sedimentological aspects of the 1988 Orange River floods. *Transactions of the Royal Society of South Africa*, *47*, 247–294.

Hanchet, S. M. (1990). Effect of land use on the distribution and abundance of native fish in tributaries of the Waikato River in the Hakarimata Range, North Island, New zealand. *New Zealand Journal of Marine and Freshwater Research*, *24*(2), 159–171.

Palmer, Robert W., & O'Keeffe, J. H. (1990). Downstream effects of impoundments on the water chemistry of the Buffalo River (Eastern Cape), South Africa. *Hydrobiologia*, *202*, 71–83.

Skelton, P. H. (1990). A new *Barbus* minnow (Pisces, Cyprinidae) from the eastern Cape Province, South Africa. *South African Journal of Zoology*, *25*(3), 188–193.

Tonn, W. M., Magnuson, J. J., Rask, M., & Toivonen, J. (1990). Intercontinental comparison of small-lake fish assemblages: the balance between local and regional processes. *American Naturalist*, *136*(3), 345–375.

Britton, D. L. (1991). Fire and the chemistry of a South African mountain stream. *Hydrobiologia*, *218*, 177–192.

Fry, B. (1991). Stable Isotope Diagrams of Freshwater Food Webs. *Ecology*, *72*(6), 2293–2297.

Rahel, F. J., & Hubert, W. A. (1991). Fish Assemblages and Habitat Gradients in a Rocky Mountain–Great Plains Stream: Biotic Zonation and Additive Patterns of Community Change. *Transactions of the American Fisheries Society*, *120*(3), 319–332.

Silberbauer, M. J., & King, J. M. (1991). The distribution of wetlands in the south-western

Cape Province, South Africa. *Southern African Journal of Aquatic Sciences*, *17*, 65–81.

Skelton, P. H., Tweddle, D., & Jackson, P. B. N. (1991). Cyprinids of Africa. *Fishlit*, 211–239.

Henderson, L. (1992). Alien woody plants of the eastern Cape. *Bothalia*, *22*, 119–143.

Lambert, D. (1992). Zero-inflated poisson regression, with an application to defects in manufacturing. *Technometrics*, *34*(1), 1–14.

Hughes, R. H., & Hughes, J. S. (1992). *A directory of African wetlands*. IUCN.

Chakraborty, R. (1993). Analysis of Genetic Structure of Populations: Meaning, Methods, and Implications. In P. P. Majumder (Ed.), *Human Population Genetics: A Centennial Tribute to J. B. S. Haldane* (pp. 189–206). Boston, MA: Springer US.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*, 117–143.

Palmer, C., O'Keefe, J., Palmer, A., Dunne, T., & Radloff, S. (1993). Macroinvertebrate functional feeding groups in the middle and lower reaches of the Buffalo River, Eastern Cape, South Africa. I. Dietary variability. *Freshwater Biology*, *29*(3), 441–453.

Skelton, P. H. (1993). *A Complete Guide to the Freshwater Fishes of Southern Africa*. Southern Book Publishers, Halfway house, 338p.

Skelton, P. H. (1994). The distribution of African Freshwater fishes. *Diversity and distribution of freshwater fishes in East and Southern Africa* (pp. 64–91). ORSTOM.

Thorpe, J. P., & Sole-Cava, A. M. (1994). The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta*, *23*(1), 3–18.

van Zyl, A. J. (1994). Sex-related local movement in adult rock kestrels in the Eastern Cape Province, South Africa. *The Wilson Bulletin*, *106*(1), 145–148.

Chow, S., & Kishino, H. (1995). Phylogenetic relationships between tuna species of the genus *Thunnus* (Scombriidae: Teleosrei): inconsistent implications from morphology, nuclear and mitochondrial genomes. *Journal of Molecular Evolution*, *41*, 741–748.

Schlosser, Isaac J. (1995). Critical landscape attributes that influence fish population dynamics in headwater streams. *Hydrobiologia*, *303*(1–3), 71–81.

Brown, C. A., Eekhout, S., & King, J. M. (1996). *National Biomonitoring Programme for*

Riverine Ecosystems: Proceedings of spatial frame workshop. Pretoria, South Africa.

Engelbrecht, J. (1996). *Phylogenetic relationships between morphologically similar Barbus species, with reference to their taxonomy, distribution and conservation*. Rand Afrikaans University.

Palmer, C. G., Maart, B., Palmer, A. R., & O'Keeffe, J. H. (1996). An assessment of macroinvertebrate functional feeding groups as water quality indicators in the Buffalo River, Eastern Cape Province, South Africa. *Hydrobiologia*, *318*, 153–164.

Palmer, R W. (1996). Invertebrates in the Orange River, with emphasis on conservation and management. *Southern African Journal of Aquatic Sciences*, *22*, 3–51.

Schluter, D. (1996). Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *351*(1341), 807–814.

Skelton, P. H. (1996). A historical review of the taxonomy and biogeography of freshwater fishes in South Africa - the past 50 years. *Transactions of the Royal Society of South Africa*, *51*(1), 91–114.

Sunnucks, P., & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, *13*(3), 510–524.

Brett, M. T., & Goldman, C. R. (1997). Consumer versus resource control in freshwater pelagic food webs. *Science*, *272*(5298), 384–386.

Gannes, L. Z., O´Brien, D. M., & Martinez del Rio, C. (1997). Stable isotopes in animal ecology: assumtions, caveats, and a call for more laboratory experiments. *Ecology*, *78*(4), 1271–1276.

Hicks, B. J. (1997). Food webs in forest and pasture streams in the Waikato region, New Zealand: A study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. *New Zealand Journal of Marine and Freshwater Research*, *31*(5), 651–664.

Poff, N. L. R. (1997). Landscape filters and species traits: Towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society*, *16*(2), 391–409.

Whitledge, G. W., & Rabeni, C. F. (1997). Energy sources and ecological role of crayfishes in

an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(11), 2555–2563.

Day, J. A., Dallas, H. F., & Wackernagel, A. (1998). Delineation of management regions for South African rivers based on water chemistry. *Aquatic Ecosystem Health and Management*, *1*, 183–197.

Grossman, G. D., Ratajczak, R. E., Crawford, M., & Freeman, M. C. (1998). Assemblage organization in stream fishes: Effects of environmental variation and interspecific interactions. *Ecological Monographs*, *68*(3), 395–420.

Matthews, W. J. (1998). *Patterns in Freshwater Fish Ecology*. *Patterns in Freshwater Fish Ecology*. New York: Chapman & Hall.

Prochazka, K. (1998). Spatial and trophic partitioning in cryptic fish communities of shallow subtidal reefs in False Bay, South Africa. *Environmental Biology of Fishes*, *51*(2), 201–220.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (1999). PAST: Paleontological statistics software package. *Palaeontologia Electronica*, *4*(1), 1–9.

Hoare, D B, & Bredenkamp, G. J. (1999). Grassland communities of the Amatola/Winterberg mountain region of the Eastern Cape, South Africa. *South African Journal Of Botany*, *65*(1), 75–82.

Nagasaka, A., & Nakamura, F. (1999). The influences of land-use changes on hydrology and riparian environment in a northern Japanese landscape. *Landscape Ecology*, *14*, 543–556.

Rahel, F. J., & Nibbelink, N. P. (1999). Spatial patterns in relations among brown trout (*Salmo trutta*) distribution, summer air temperature, and stream size in Rocky Mountain streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*, 43–51.

Rall, J. L. (1999). *Development of a conservation program on Pseudobarbus quathlambae in the catchment area of phase 1B of the Lesotho Highlands Water Project*. Rand Afrikaans University, South Africa.

Walser, C. A., & Bart, H. L. (1999). Influence of agriculture on in-stream habitat and fish community structure in Piedmont watersheds of the Chattahoochee River System. *Ecology of Freshwater Fish*, *8*(4), 237–246.

Aparicio, E., Vargas, M. J., Olmo, J. M., & de Sostoa, A. (2000). Decline of native freshwater fishes in a Mediterranean watershed on the Iberian Peninsula: A quantitative assessment. *Environmental Biology of Fishes*, *59*(1), 11–19.

Dolédec, S., Chessel, D., & Gimaret-Carpentier, C. (2000). Niche separation in community analysis: A new method. *Ecology*, *81*(10), 2914–2927.

Dynesius, M., & Jansson, R. (2000). Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proceedings of the National Academy of Sciences of the United States of America*, *19*(16), 9115–9920.

Farm, B. P. (2000). New "*Barbus*" (Teleostei: Cyprinidae) from Serengeti National Park, Tanzania. *Copeia*, *4*, 973–982.

Le Maitre, D. C., Versfeld, D. B., & Chapman, R. A. (2000). The impact of invading alien plants on surface water resources in South Africa: A preliminary assessment. *Water SA*, *26*(3), 397–408.

Van Nieuwenhuizen, G. D. P., & Day, J. A. (2000). *Cape action plan for the environment: the conservation of freshwater ecosystems in the Cape Floral Kingdom*. Cape Town, South Africa.

Gilliam, J. F., & Fraser, D. F. (2001). Movement in corridors: Enhancement by predation threat, disturbance, and habitat structure. *Ecology*, *82*(1), 258–273.

Jackson, D. A., Peres-Neto, P. R., & Olden, J. D. (2001). What controls who is where in freshwater fish communities – the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Sciences*, *58*(1), 157–170.

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., D'Amico, J. A., et al. (2001). Terrestrial ecoregions of the world: A new map of life on Earth. *BioScience*, *51*(11), 933–938.

Skelton, P. H. (2001). *A complete guide to the Freshwater Fishes of Southern Africa* (2nd edn.). Cape Town, South Africa: Struik.

Unmack, P. J. (2001). Biogeography of Australian freshwater fishes. *Journal of Biogeography*, *28*(9), 1053–1089.

Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic

fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*(8), 2061–2066.

Burnham, K.P., & Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* (Second edi.). Springer.

Day, J. A, & de Moor, I. J. (2002). Guides to the Freshwater Invertebrates of Southern Africa - Volume 5: Non-Arthropods. *Africa*, *5*, 304.

Fagan, W. F. (2002). Connectivity, fragmentation, and extinction risk in dendritic metapopulations. *Ecology*, *83*(12), 3243–3249.

Gomi, T., Sidle, R., & Richardson, J. S. (2002). Headwater and channel network - understanding processes and downstream linkages of headwater systems. *BioScience*, *52*, 905–916.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, *83*(3), 703–718.

Quinn, G. P., & Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge University Press.

Swofford, D. L. (2002). *PAUP. Phylogenetic Analysis Using Parsimony (and Other Methods).Version 4.0b10*. *Options*. Sunderland, Massachusettes: Sinauer Associates.

Cambray, J. A. (2003). Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. *Hydrobiologia*, *500*, 217–230.

Vizzini, S., & Mazzola, A. (2003). Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of primary producers and consumers in a western Mediterranean coastal lagoon. *Marine Biology*, *142*(5), 1009–1018.

Yau, K. K. W., Wang, K., & Lee, A. H. (2003). Zero-inflated negative binomial mixed regression modeling of over-dispersed count data with extra zeros. *Biometrical Journal*, *136*(3), 345–375.

Allan, J. D. (2004). Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 257–284.

Avise, J. C. (2004). *Molecular Markers, Natural History, and Evolution*. Sunderland, Massachusetts: Sinauer Associates, Inc. Publishers.

Burnham, Kenneth P., & Anderson, D. R. (2004). Multimodel inference: Understanding AIC and BIC in model selection. *Sociological Methods and Research*, *33*(2), 261–304.

Hill, M O, Preston, C. D., & Roy, D. B. (2004). Plantatt Atributes of British and Irish Plants: Status, Size, Life history, Geography and Habitats. *Centre for Ecology and Hydrology*, Huntingdon, 72pp.

South Africa. Department of Water Affairs and Forestry. (2004). *State-of-rivers report*: *Buffalo River system*. Dept. of Water Affairs and Forestry.

Tooth, S., & McCarthy, T. S. (2004). Anabranching in mixed bedrock-alluvial rivers: The example of the Orange River above Augrabies Falls, Northern Cape Province, South Africa. *Geomorphology*, *57*, 235–262.

Cummins, K. W., Merritt, R. W., & Andrade, P. C. N. (2005). The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. *Studies on Neotropical Fauna and Environment*, *40*(1), 69–89.

Greene, W. (2005). The bivariate Poisson model. *Foundations and trends in econometrics*, *1*(2), 161+.

Lowe, W. H., & Likens, G. E. (2005). Moving headwater streams to the head of the class. *BioScience*, *55*(3), 196–197.

Thieme, M. L., Abell, R., Lehner, B., Dinerstein, E., Stiassny, M. L. J., Skelton, P. H., Teugels, G. G., et al. (2005). *Freshwater Ecoregions of Africa and Madagascar: A Conservation Assessment*. *Island Press* (Vol. 43). Washington, D.C.: Island Press.

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*(2), 167–173.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., Naiman, R. J., et al. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, *81*(2), 163–82.

Hoare, David B, Mucina, L., Rutherford, M. ., Vlok, J. H. ., Euston-Brown, D., Palmer, A., Powrie, L., et al. (2006). Albany Thicket Biome. *Strelitzia*, *19*, 540–567.

McGill, B. J., Maurer, B. A., & Weiser, M. D. (2006). Empirical evaluation of neutral theory. *Ecology*, *87*(6), 1411–1423.

Smith, K. G., & Darwall, W. R. T. (2006). *The Status and Distribution of Freshwater Fish Endemic to the Mediterranean Basin*. *Assessment*.

Wang, L., Seelbach, P. W., & Hughes, R. M. (2006). Introduction to landscape influences on stream habitats and biological assemblages. *American Fisheries Society Symposium*, *2006*(48), 1–23.

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.

Abell, R., Allan, J. D., & Lehner, B. (2007). Unlocking the potential of protected areas for freshwaters. *Biological Conservation*, *134*(1), 48–63.

Beheregaray, L. B., & Caccone, A. (2007). Cryptic biodiversity in a changing world. *Journal of Biology*, *6*(4), 9.1-9.5.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L. L., Meier, R., Winker, K., Ingram, K. K., et al. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, *22*(3), 148–155.

Hill, Mark O., Preston, C. D., Bosanquet, S. D. S., & Roy, D. B. (2007). *BRYOATT - Attributes of British and Irish Mosses, Liverworts and Hornworts*. *Centre for Ecology and Hydrology*.

Larkin, M., Blackshields, G., Brown, N., Chenna, R., McGettigan, P., McWilliam, H., Valentin, F., et al. (2007). ClustalW and ClustalX version 2. *Bioinformatics*, *23*(21), 2947–2948.

Meyer, J. L., Strayer, D. L., Wallace, J. B., Eggert, S. L., Helfman, G. S., & Leonard, N. E. (2007). The contribution of headwater streams to biodiversity in river networks. *Journal of the American Water Resources Association*, *43*(1), 86–103.

Nel, J. L., Roux, D. J., Maree, G., Kleynhans, C. J., Moolman, J., Reyers, B., Rouget, M., et al. (2007). Rivers in peril inside and outside protected areas: a systematic approach to conservation assessment of river ecosystems. *Diversity and Distributions*, *13*, 341–352.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi & C. G. Montan˜a. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia, 152,* 179–189.

Program, M. S., Miami, N., District, L. R., Biology, E., Haven, N., Layman, C. A., Arrington, D. A., et al. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure ? *Ecology*, *88*(1), 42–48.

Richardson, J. S., & Danehy, R. J. (2007). A synthesis of the ecology of headwater streams and their riparian zones in temperate forests. *Forest Science*, *53*(2), 131–147.

Stiassny, M. L. J., Tuegels, G. G., & Hopkins, C. D. (2007). *The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa*. Paris: Muséum national d'Histoire naturelle, *42*(2), 1456 pp.

Súarez, Y. R., Valério, S. B., Tondado, K. K., Florentino, A. C., Felipe, T. R. A., Ximenes, L. Q. L., & Da Silva Lourenço, L. (2007). Fish species diversity in headwaters streams of Paraguay and Paraná basins. *Brazilian Archives of Biology and Technology*, *50*(6), 1033–1042.

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*(12), 2086–2099.

Wellborn, G. A., & Cothran, R. D. (2007). Niche diversity in crustacean cryptic species: Complementarity in spatial distribution and predation risk. *Oecologia*, *154*(1), 175–183.

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., et al. (2008). Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *BioScience*, *58*(5), 403–413.

Kadye, Wilbert T., Magadza, C. H. D., Moyo, N. A. G., & Kativu, S. (2008). Stream fish assemblages in relation to environmental factors on a Montane Plateau (Nyika Plateau, Malawi). *Environmental Biology of Fishes*, *83*, 417–428.

Kadye, Wilbert Takawira, & Magadza, C. H. D. (2008). Trout induces a shift from preferred habitat types for indigenous species: The example of the indigenous catfish, *Amphilius uranoscopus* (Pfeffer, 1889), on an African Montane Plateau. *Hydrobiologia*, *614*, 329–

337.

Moore, J. W., & Semmens, B. X. (2008). Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters*, *11*(5), 470–480.

Rall, J. L., & Sephaka, T. (2008). *Re-evaluation of the relevance to construct barriers as in-situ conservation measures for the protection of the Maltoi Minnow in the Senqunyane catchment*. *Environmental consultants for the sustainable utilisation of nature (ESOSUN)*. South Africa.

Rybczynski, S. M., Walters, D. M., Fritz, K. M., & Johnson, B. R. (2008). Comparing trophic position of stream fishes using stable isotope and gut contents analyses. *Ecology of Freshwater Fish*, *17*(2), 199–206.

Allen, A. R., Peterson, B. J., Huryn, A. D., Flinn, M. B., & Bowden, W. B. (2009). *Foodweb characteristics based on stable isotopic analyses of carbon and nitrogen from streams in the central and upper Noatak River basin: Gates of the Arctic Park and Preserve and the Noatak National Preserve, Alaska*. *Final Report to the National Park Service, Arctic Network Fiarbanks, Alaska*. http://www.uvm.edu/bwrl/arcn/docs/2009_Allen_etal_foodweb.pdf.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, *24*(3), 127–135.

Clark, B. M., Impson, D., & Rall, J. (2009). Present status and historical changes in the fish fauna of the Berg River, South Africa. *Transactions of the Royal Society of South Africa*, *64*(2), 142–163.

Krebs, C. J. (2009). *Ecology* (Sixth Edit.). San Fransisco CA: Pearson Benjamin Cummings.

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*(11), 1451–1452.

Van Oosterhout, M. P., Van Der Velde, G., & Gaigher, I. G. (2009). High altitude mountain streams as a possible refuge habitat for the catfish *Amphilius uranoscopus*. *Environmental Biology of Fishes*, *84*, 109–120.

Smith, T. M., & Smith, R. L. (2009). *Elements of ecology* (Seventh Ed.). San Fransisco: Pearson Benjamin Cummings.

Soberón, J., & Nakamura, M. (2009). Niches and distributional areas: Concepts, methods, and assumptions. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 19644–19650.

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*(1), 75–84.

Tweddle, D., Darwall, W. R. T., Smith, K. G., & Skelton, P. H. (2009). *The status and distribution of freshwater biodiversity in southern Africa*. *The status and distribution of freshwater biodiversity in southern Africa*. Gland, Switzerland: International Union for Conservation of Nature and South African Institute for Aquatic Biodiversity.

Vitule, J. R. S., Freire, C. A., & Simberloff, D. (2009). Introduction of non-native freshwater fish can certainly be bad. *Fish and Fisheries*, *10*(1), 98–108.

Ellender, B. R., Weyl, O. L. F., Winker, H., & Booth, A. J. (2010). Quantifying the annual fish harvest from South Africa's largest freshwater reservoir. *Water SA*, *36*(1), 45–52.

Herbers, J. M. (2010). Evolution: Fundamentals. In M. D. Breed & J. Moore (Eds.), *Encyclopedia of animal behaviour* (First Edit., pp. 670–678). London: Elsevier.

Linder, H. P., Johnson, S. D., Kuhlmann, M., Matthee, C. A., Nyffeler, R., & Swartz, E. R. (2010). Biotic diversity in the Southern African winter-rainfall region. *Current Opinion in Environmental Sustainability*, *2*, 109–116.

O'Hara, R. B., & Kotze, D. J. (2010). Do not log-transform count data. *Methods in Ecology and Evolution*, *1*(2), 118–122.

Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution and Systematics*, *42*(1), 411–440.

Bouillon, S., Connolly, R. M., & Gillikin, D. P. (2011). Use of stable isotopes to understand food webs and ecosystem functioning in estuaries. In E. Wolasnsky & D. McLusky (Eds.), *Treatise on Estuarine and Coastal Science* (Vol. 7, pp. 143–173). Waltham, Massachusetts, USA: Academic Press.

Chase, J. M., & Myers, J. A. (2011). Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society B:*

Biological Sciences, *366*(1576), 2351–2363.

Clark, V. R., Barker, N. P., & Mucina, L. (2011). The Great Escarpment of southern Africa: A new frontier for biodiversity exploration. *Biodiversity and Conservation*, *20*(20), 2543–2561.

Clulow, A. D., Everson, C. S., & Gush, M. B. (2011). The long-term impact of *Acacia Mearnsii* trees on evaporation, streamflow and groundwater resources. *Report to the Water Research Commission, Report No TT 505/11*.

Compton, J. S. (2011). Pleistocene sea-level fluctuations and human evolution on the southern coastal plain of South Africa. *Quaternary Science Reviews, 30*, 506-527.

Cucherousset, J., & Olden, J. D. (2011). Ecological Impacts of non-native freshwater fishes. *Fisheries*, *36*(5), 215–230.

Ellender, Bruce R, Weyl, O. F., & Swartz, E. R. (2011). Invasion of a headwater stream by non-native fishes in the Swartkops River system, South Africa. *African Zoology*, *46*(1), 39–46.

Finn, D. S., & Poff, N. L. (2011). Examining spatial concordance of genetic and species diversity patterns to evaluate the role of dispersal limitation in structuring headwater metacommunities. *Journal of the North American Benthological Society*, *30*(1), 273–283.

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*(3), 595–602.

Morrongiello, J. R., Bond, N. R., Crook, D. A., & Wong, B. B. M. (2011). Eucalyptus leachate inhibits reproduction in a freshwater fish. *Freshwater Biology*, *56*(9), 1736–1745.

van Rensburg, B., Weyl, O. F., Davies, S., van Wilgen, N., Spear, D., Chimimba, C., & Peacock, F. (2011). Invasive vertebrates of South Africa. *Biological Invasions: Economic and environmetal costs of alien plant, animal, and microbe species.* (pp. 326–378). Boca Raton, Florida: CRC Press.

Saffari, S. E., & Robiah, A. (2011). Zero-Inflated Negative Binomial Regression Model with Right Censoring Count Data. *Journal of Materials Science and Engineering B*, *1*(9), 551–554.

Snoeks, J. H., & Stiassny, M. L. J. (2011). The status and distribution of freshwater fishes. In D. WRT, K. G. Smith, D. J. Allen, R. A. Holland, I. J. Harrison, & B. EGE (Eds.), *The diversity of life in African freshwaters: Under water, under threat. An analysis of the status and distribution of freshwater species throughout mainland Africa* (p. 429). Cambridge, UK: IUCN.

Butler, G. L., & Wooden, I. J. (2012). Dietary habits of a large, long-lived endangered Australian percichthyid, the eastern freshwater cod *Maccullochella ikei*. *Endangered Species Research*, *16*, 199–209.

Chakona, A., & Swartz, E. R. (2012). Contrasting habitat associations of imperilled endemic stream fishes from a global biodiversity hot spot. *BMC Ecology*, *12*(1), 19.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). JModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*(8), 772.

Jiang, W., Ng, H. H., Yang, J., & Chen, X. (2012). A taxonomic review of the catfish identified as *Glyptothorax zanaensis* (Teleostei: Siluriformes: Sisoridae), with the descriptions of two new species. *Zoological Journal of the Linnean Society*, *165*(2), 363–389.

Kadye, W. T., & Booth, A. J. (2012). Inter-seasonal persistence and size-structuring of two minnow species within headwater streams in the Eastern Cape, South Africa. *Journal of Applied Ichthyology*, *28*(5), 791–799.

Kadye, Wilbert T., & Booth, A. J. (2012). An invader within an altered landscape: one catfish, two rivers and an inter-basin water transfer scheme. *River Research and Applications*, *29*, 1131–1146.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., et al. (2012). Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539–542.

Sterling, K. A., Reed, D. H., Noonan, B. P., & Warren, M. L. (2012). Genetic effects of habitat fragmentation and population isolation on *Etheostoma raneyi* (Percidae). *Conservation Genetics, 13*, 859–872.

Chakona, A., & Swartz, E. R. (2013). A new redfin species, *Pseudobarbus skeltoni* (Cyprinidae, Teleostei), from the Cape Floristic Region, South Africa. *Zootaxa*, *3686*(5), 565–577.

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*(8).

Chakona, A., Swartz, E. R., Gouws, G., & Bloomer, P. (2013). A freshwater fish defies ancient mountain ranges and drainage divides: Extrinsic and intrinsic influences on the evolutionary history of a recently identified galaxiid. *Journal of Biogeography*, *40*(7), 1399–1412.

Ellender, Bruce R. (2013). *Ecological consequences of non-native fish invasions in Eastern Cape headwater streams*. Rhodes University.

Griffiths, N. A., Tank, J. L., Royer, T. V., Roley, S. S., Rosi-Marshall, E. J., Whiles, M. R., Beaulieu, J. J., et al. (2013). Agricultural land use alters the seasonality and magnitude of stream metabolism. *Limnology and Oceanography*, *58*(4), 1513–1529.

Kadye, W. T., & Booth, A. J. (2013a). Movement patterns and habitat selection of invasive African sharptooth catfish. *Journal of Zoology*, *289*(1), 41–51.

Kadye, W. T. & A. J. Booth. (2013b). An invader within an altered landscape: one catfish, two rivers and an interbasin water transfer scheme. *River Research and Applications 29*, 1131–1146.

Marr, S. M., Olden, J. D., Leprieur, F., Arismendi, I., Ćaleta, M., Morgan, D. L., Nocita, A., et al. (2013). A global assessment of freshwater fish introductions in Mediterranean-climate regions. *Hydrobiologia*, *719*(1), 317–329.

de Moor, F. C., & Day, J. A. (2013). Aquatic biodiversity in the mediterranean region of South Africa. *Hydrobiologia*, *719*, 237–268

Michaud, W. K., J. B. Dempson, J. D. Reist & M. Power. (2013). Ecological influences on the difference in $\delta^{15}N$ and $\delta^{13}C$ values between fish tissues: implications for studies of temporal diet variation. *Ecology of Freshwater Fish, 22*, 520–529.

Unmack, P. J., Hammer, M. P., Adams, M., Johnson, J. B., & Dowling, T. E. (2013). The role of continental shelf width in determining freshwater phylogeographic patterns in south-eastern Australian pygmy perches (Teleostei: Percichthyidae). *Molecular Ecology*, *22*(6), 1683–1699.

Bartón, K. (2014). MuMIn: Multi-model inference. *R package version 1.10.0*.

Chakona, A., Swartz, E. R., & Skelton, P. H. (2014). A new species of redfin (Teleostei, Cyprinidae, *Pseudobarbus*) from the Verlorenvlei River system, South Africa. *ZooKeys*, *137*(453), 121–137.

Clarke, K. R., Gorley, R., Somerfield, P., & Warwick, R. (2014). Change in marine communities: an approach to statistical analysis and interpretation, 3rd edition. *PRIMER-E, Plymouth UK*.

Cook, B. D., Unmack, P. J., Huey, J. A., & Hughes, J. M. (2014). Did common disjunct populations of freshwater fishes in Northern Australia form from the same biogeographic events? *Freshwater Science*, *33*(1), 263–272.

Ellender, Bruce 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*(2), 117–132.

Junker, J. R., & Cross, W. F. (2014). Seasonality in the trophic basis of a temperate stream invertebrate assemblage: Importance of temperature and food quality. *Limnology and Oceanography*, *59*(2), 507–518.

Kadye, Wilbert T., & Booth, A. J. (2014). Alternative responses to predation in two headwater stream minnows is reflected in their contrasting diel activity patterns. *PLoS ONE*, *9*(4).

Milanovich, J. R., Berland, A., & Hopton, M. E. (2014). Influence of catchment land cover on stoichiometry and stable isotope compositions of basal resources and macroinvertebrate consumers in headwater streams. *Journal of Freshwater Ecology*, *29*(4), 565–578.

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., Semmens, B. X., et al. (2014). Best practices for use of stable isotope mixing models in food-web studies. *NRC Research Press*, *92*, 823–835.

Skaug, H., Fournier, D., Bolker, B., Magnusson, A., & Nielsen, A. (2014). Generalized Linear Mixed Models using AD Model Builder. *R Statistical Program*.

Swartz, E. R., Chakona, A., Skelton, P. H., & Bloomer, P. (2014). The genetic legacy of lower sea levels: Does the confluence of rivers during the last glacial maximum explain the contemporary distribution of a primary freshwater fish (*Pseudobarbus burchelli*, Cyprinidae) across isolated river systems? *Hydrobiologia*, *726*(1), 109–121.

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*(6), 270–279.

Carvalho, Rodrigo Assis, & Tejerina-Garro, F. L. (2015). The influence of environmental variables on the functional structure of headwater stream fish assemblages: A study of two tropical basins in Central Brazil. *Neotropical Ichthyology*, *13*(2), 349–360.

Chakona, A., Malherbe, W. S., Gouws, G., & Swartz, E. R. (2015). Deep genetic divergence between geographically isolated populations of the goldie barb (*Barbus pallidus*) in South Africa: potential taxonomic and conservation implications. *African Zoology*, *50*(1), 5–10.

Chakona, G., Swartz, E. R., & Chakona, A. (2015). Historical abiotic events or human-aided dispersal: Inferring the evolutionary history of a newly discovered galaxiid fish. *Ecology and Evolution*, *5*(7), 1369–1380.

Cristescu, M. E. (2015). Genetic reconstructions of invasion history. *Molecular Ecology*, *24*(9), 2212–2225.

Ellender, Bruce R., & Weyl, O. L. F. (2015). Resilience of imperilled headwater stream fish to an unpredictable high-magnitude flood. *Koedoe*, *57*(1), 1–8.

González-Orozco, C. E., Mishler, B. D., Miller, J. T., Laffan, S. W., Knerr, N., Unmack, P., Georges, A., et al. (2015). Assessing biodiversity and endemism using phylogenetic methods across multiple taxonomic groups. *Ecology and Evolution*, *5*(22), 5177–5192.

Kraft, N. J. B., 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*(5), 592–599.

Ringle, Christian M., Wende, Sven, & Becker, Jan-Michael. (2015). SmartPLS 3. Bönningstedt: SmartPLS. Retrieved from http://www.smartpls.com

Yang, L., Sado, T., Vincent Hirt, M., Pasco-Viel, E., Arunachalam, M., Li, J., Wang, X., et al. (2015). Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution*, *85*, 97–116.

Arthington, A. H., Dulvy, N. K., Gladstone, W., & Winfield, I. J. (2016). Fish conservation in freshwater and marine realms: status, threats and management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*(5), 838–857.

De Castro, D. M. P., De Carvalho, D. R., Pompeu, P. D. S., Moreira, M. Z., Nardoto, G. B., & Callisto, M. (2016). Land use influences niche size and the assimilation of resources by benthic macroinvertebrates in tropical headwater streams. *PLoS ONE*, *11*(3).

Dodds, W. K., & Smith, V. H. (2016). Nitrogen, phosphorus, and eutrophication in streams. *Inland Waters*, *6*(2), 155–164.

Kadye, Wilbert 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*(19), 7141–7155.

Ramoejane, Mpho. (2016). *Genetic diversity , evolutionary relationships and conservation of southern African Labeo fishes in relation to water management*. Rhodes University.

Roon, D. A., Wipfli, M. S., Wurtz, T. L., & Blanchard, A. L. (2016). Invasive European bird cherry (*Prunus padus*) reduces terrestrial prey subsidies to urban Alaskan salmon streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*, 1679–1690.

Shen, Y., Guan, L., Wang, D., & Gan, X. (2016). DNA barcoding and evaluation of genetic diversity in Cyprinidae fish in the midstream of the Yangtze River. *Ecology and Evolution*, *6*(9), 2702–2713.

Skelton, P. H. (2016). Name changes and additions to the southern African freshwater fish fauna. *African Journal of Aquatic Science*, *41*(3), 345–351.

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.

Ellender, Bruce 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*(4), 867–879.

García, L., Cross, W. F., Pardo, I., & Richardson, J. S. (2017). Effects of landuse intensification on stream basal resources and invertebrate communities. *Freshwater Science*, *36*(3), 609–625.

Van Ginneken, M., Decru, E., Verheyen, E., & Snoeks, J. (2017). Morphometry and DNA barcoding reveal cryptic diversity in the genus *Enteromius* (Cypriniformes: Cyprinidae) from the Congo basin, Africa. *European Journal of Taxonomy*, *314*(310), 1–8.

Hargrove, J. S., Weyl, O. L. F., & Austin, J. D. (2017). Reconstructing the introduction history of an invasive fish predator in South Africa. *Biological Invasions*, *19*, 2261–2276.

Hayes, M. M., & Armbruster, J. W. (2017). The taxonomy and relationships of the African small barbs (Cypriniformes: Cyprinidae). *Copeia*, *105*(2), 348–362.

Khaefi, R., Esmaeili, H. R., Geiger, M. F., & Eagderi, S. (2017). Taxonomic review of the cryptic *Barbus lacerta* species group with description of a new species (Teleostei: Cyprinidae). *FishTaxa*, *2*(2), 90–115.

Kubheka, P. S., Chakona, A., & Mazungula, D. N. (2017). The Maloti minnow *Pseudobarbus quathlambae* (Barnard, 1938) is not extinct in South Africa. *African Journal of Aquatic Science*, *42*(3), 303–306.

Maqsood, H. M., & Ahmad, S. M. (2017). Advances in molecular markers and their applications in aquaculture and fisheries. *Genetics of Aquatic Organisms*, *1*(1), 27–41.

Merciai, R., Molons-Sierra, C., Sabater, S., & García-Berthou, E. (2017). Water abstraction affects abundance, sizestructure and growth of two threatened cyprinid fishes. *PLoS ONE*, *12*(4).

Schanke, K. L., Yamashita, T., & Gagen, C. J. (2017). Reduced gene flow in two common headwater fishes in the Ouachita mountains: A response to stream drying and in-stream barriers. *Copeia*, *105*(1), 33–42.

van der Walt, K. A., Swartz, E. R., Woodford, D., & Weyl, O. (2017). Using genetics to prioritise headwater stream fish populations of the Marico barb, *Enteromius motebensis* Steindachner 1894, for conservation action. *Koedoe*, *59*(1), 1–7.

Woodford, D. (2017). Enteromius anoplus. *The IUCN Red List of Threatened Species*. http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T63249A100115110.en

Bartáková, V., Bryja, J., & Reichard, M. (2018). Fine-scale genetic structure of the European bitterling at the intersection of three major European watersheds. *BMC Evolutionary Biology*, *18*, 105.

Chakona, A., Kadye, W. T., Bere, T., Mazungula, D. N., & Vreven, E. (2018). Evidence of hidden diversity and taxonomic conflicts in five stream fishes from the Eastern Zimbabwe Highlands freshwater ecoregion. *ZooKeys*, *2018*(768), 69–95.

Chakona, G., Swartz, E. R., & Chakona, A. (2018). The status and distribution of a newly identified endemic galaxiid in the eastern Cape Fold Ecoregion, of South Africa. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*(1), 55–67.

Lee, K. Y., Graham, L., Spooner, D. E., & Xenopoulos, M. A. (2018). Tracing anthropogenic inputs in stream foods webs with stable carbon and nitrogen isotope systematics along an agricultural gradient. *PLoS ONE*, *13*(7), e0200312.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904.

Schmidt, R. C., Bart, H. L., & Nyingi, W. D. (2018). Integrative taxonomy of the red-finned barb, *Enteromius apleurogramma* (Cyprininae: Smiliogastrini) from Kenya, supports recognition of *E. amboseli* as a valid species. *Zootaxa*, *4482*(3), 566–578.

Shelton, J. M., Weyl, O. L. F., Chakona, A., Ellender, B. R., Esler, K. J., Impson, N. D., Jordaan, M. S., et al. (2018). Vulnerability of Cape Fold Ecoregion freshwater fishes to climate change and other human impacts. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*(1), 68–77.

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.

Thiébaut, G., Thouvenot, L., & Rodríguez-Pérez, H. (2018). Allelopathic effect of the invasive *Ludwigia hexapetala* on growth of three macrophyte species. *Frontiers in Plant Science*, *9*, 1835.

Tronquart, N. H., Oberdorff, T., Tales, E., Amandine, Z., & Belliard, J. (2018). Biological impacts of local vs. regional land use on a small tributary of the Seine River (France): insights from a food web approach based on stable isotopes. *Environmental Science and Pollution Research*, *25*, 23583–23594.

Wanyama, I., Rufino, M. C., Pelster, D. E., Wanyama, G., Atzberger, C., van Asten, P., Verchot, L. V., et al. (2018). Land Use, Land Use History, and Soil Type Affect Soil Greenhouse Gas Fluxes From Agricultural Landscapes of the East African Highlands. *Journal of Geophysical Research: Biogeosciences*, *123*(3), 976–990.

Arroyave, J., Martinez, C. M., & Stiassny, M. L. J. (2019). DNA barcoding uncovers extensive cryptic diversity in the African long-fin tetra *Bryconalestes longipinnis* (Alestidae: Characiformes). *Journal of Fish Biology*, *95*(2), 379–392.

Barbosa, H. de O., Borges, P. P., Dala-Corte, R. B., Martins, P. T. de A., & Teresa, F. B. (2019). Relative importance of local and landscape variables on fish assemblages in streams of Brazilian savanna. *Fisheries Management and Ecology*, *26*(2), 119–130.

Chakona, A., Jordaan, M. S., & Kadye, W. T. (2019). Distribution and summer habitat associations of three narrow-range endemic fishes in an intermittent southern temperate Mediterranean river system. *Fundamental and Applied Limnology*, *193*(1), 65–77.

Fricke, R., Eschmeyer, W. N. & Van der Laan, R. (2019). Eschmeyer's catalog of fishes: genera, species, references. *California Academy of Sciences (Electronic version)*.

Froese, R., & Pauly, D. (2019). www.fishbase.org. *World Wide Web electronic publication*.

Hammer, M. P., Adams, M., Thacker, C. E., Johnson, J. B., & Unmack, P. J. (2019). Comparison of genetic structure in co-occurring freshwater eleotrids (Actinopterygii: Philypnodon) reveals cryptic species, likely translocation and regional conservation hotspots. *Molecular Phylogenetics and Evolution*, *139*(106556).

Hammerschlag, N., Schmitz, O. J., Flecker, A. S., Lafferty, K. D., Sih, A., Atwood, T. B., Gallagher, A. J., et al. (2019). Ecosystem function and services of aquatic predators in the anthropocene. *Trends in Ecology and Evolution*, *34*(4), 369–383.

Jirsová, D., Štefka, J., Blažek, R., Malala, J. O., Lotuliakou, D. E., Mahmoud, Z. N., & Jirků, M. (2019). From taxonomic deflation to newly detected cryptic species: Hidden diversity in a widespread African squeaker catfish. *Scientific Reports*, *9*(1), 15748.

Kambikambi, M. J., Chakona, A., & Kadye, W. T. (2019). Tracking seasonal food web dynamics and isotopic niche shifts in wild chubbyhead barb *Enteromius anoplus* within a southern temperate headwater stream. *Hydrobiologia*, *837*(1), 87–107.

Katemo Manda, B., Snoeks, J., Decru, E., Bills, R., & Vreven, E. (2019). *Enteromius thespesios* (Teleostei: Cyprinidae): a new minnow species with a remarkable sexual dimorphism from the south-eastern part of the Upper Congo River. *Journal of Fish Biology*, *96*, 1160–1175.

Koel, T. M., Tronstad, L. M., Arnold, J. L., Gunther, K. A., Smith, D. W., Syslo, J. M., &

White, P. J. (2019). Predatory fish invasion induces within and across ecosystem effects in Yellowstone National Park. *Science Advances*, *5*(3), 1–12.

Leroy, B., Dias, M. S., Giraud, E., Hugueny, B., Jézéquel, C., Leprieur, F., Oberdorff, T., et al. (2019). Global biogeographical regions of freshwater fish species. *Journal of Biogeography*, *46*(11), 2407–2419.

Mamonekene, V., Zamba, A. I., & Stiassny, M. L. J. J. (2019). A new small barb (Cyprininae, Smiliogastrini) from the Louesse, Lekoumou (upper Niari Basin), and Djoulou (upper Ogowe Basin) rivers in the Republic of Congo, west-central Africa. *American Museum of Natural History*, *2018*(3917), 1–16.

Martin, M. B., & Chakona, A. (2019). Designation of a neotype for *Enteromius pallidus* (Smith, 1841), an endemic cyprinid minnow from the Cape Fold Ecoregion, South Africa. *ZooKeys*, 848, 103–118.

Mipounga, H. K., Cutler, J., Mve Beh, J. H., Adam, B., & Sidlauskas, B. L. (2019). *Enteromius pinnimaculatus* sp. nov. (Cypriniformes: Cyprinidae) from southern Gabon. *Journal of Fish Biology*, *96*(5), 1218–1233.

Oficialdegui, F. J., Clavero, M., Sánchez, M. I., Green, A. J., Boyero, L., Michot, T. C., Klose, K., et al. (2019). Unravelling the global invasion routes of a worldwide invader, the red swamp crayfish (*Procambarus clarkii*). *Freshwater Biology*, *64*(8), 1382–1400.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., et al. (2019). vegan: Community Ecology Package. R package version 2.5-2. *Community ecology package*, 1–296.

Richardson, J. S. (2019). Biological diversity in headwater streams. *Water*, *11*(2), 1–19.

Rivers-Moore, N. A., Ellender, B. R., & Weyl, O. L. F. (2019). Modelling expected trout ranges under current and future water temperature regimes in the Eastern Cape, South Africa. *African Journal of Aquatic Science*, *44*(1), 35–42.

Schmidt, R. C., Dillon, M. N., Kuhn, N. M., Bart, H. L., & Pezold, F. (2019). Unrecognized and imperilled diversity in an endemic barb (Smiliogastrini, *Enteromius*) from the Fouta Djallon highlands. *Zoologica Scripta*, *48*(5), 605–613.

Shelley, J. J., Dempster, T., Le Feuvre, M. C., Unmack, P. J., Laffan, S. W., & Swearer, S. E. (2019). A revision of the bioregionalisation of freshwater fish communities in the

Australian Monsoonal Tropics. *Ecology and Evolution*, *9*(8), 4568–4588.

Stegmann, L. F., Leitão, R. P., Zuanon, J., & Magnusson, W. E. (2019). Distance to large rivers affects fish diversity patterns in highly dynamic streams of Central Amazonia. *PLoS ONE*, *14*(10), 1–17.

Braganca, P. H. N., Smith, T. G., Vreven, E. J. W. M. N., & Chakona, A. (2020). Integrative taxonomy reveals hidden diversity in the southern African darters genus *Nannocharax* Günther 1867 (Characiformes: Distichodontidae). *Journal of Fish Biology*, *97*(6), 1713–1723.

Bronaugh, W. M., Swartz, E. R., & Sidlauskas, B. L. (2020). Between an ocean and a high place: coastal drainage isolation generates endemic cryptic species in the Cape kurper *Sandelia capensis* (Anabantiformes: Anabantidae), Cape Region, South Africa. *Journal of Fish Biology*, *96*(5), 1087–1099.

Carvalho, Rodrigo A., Santana, H. T., & Tejerina-Garro, F. L. (2020). Environmental influence on higher fish taxonomic levels: relationships in tropical headwater streams. *Studies on Neotropical Fauna and Environment*, *55*, 193–202.

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*(1), 144–158.

Chakona, A., Gouws, G., Kadye, W. T., & Mpopetsi, P. P. (2020). Probing hidden diversity to enhance conservation of Sandelia bainsii (Castelnau 1861), *62*(1), 1–6.

Englmaier, G. K., Tesfaye, G., & Bogutskaya, N. G. (2020). A new species of *Enteromius* (Actinopterygii, cyprinidae, smiliogastrinae) from the Awash river, Ethiopia, and the re-establishment of *E. kakianus*. *ZooKeys*, *902*, 107–150.

Jordaan, M. S., Chakona, A., Colff, D. Van Der, Tharme, R. E., Beatty, S. J., & Brien, G. O. (2020). Protected areas and endemic freshwater fishes of the Cape Fold Ecoregion: Missing the Boat for Fish Conservation? *Frontiers in Environmental Science*, *8*, 1–13.

Kadye, W. T., & Booth, A. J. (2020). Environmental niche patterns of native and non-native fishes within an invaded African river system. *Journal of Fish Biology*, *96*(5), 1269–1277.

Kadye, W. T., Redelinghuys, S., Parnell, A. C., & Booth, A. J. (2020). Exploring source

differences on diet-tissue discrimination factors in the analysis of stable isotope mixing models. *Scientific Reports*, *10*(1), 15816.

Kadye, W. T, Leigh, S., & Booth, A. J. (2020). Predator naïve minnows respond to their conspecific alarm substance but not the odour from a non-native predator. *African Journal of Ecology*, *58*(4), 757–765.

Kasongo, I., Abwe, E., Decru, E., Chocha Manda, A., & Vreven, E. (2020). Description of a new small-sized Synodontis species (Siluriformes: Mochokidae) that is important for local subsistence fisheries in the middle Lufira (upper Congo River, DR Congo). *Journal of Fish Biology*, *96*, 1142–1159.

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*(7), 2223–2233.

Mambo Baba, T., Kisekelwa, T., Danadu MMizani, C., Decru, E., & Vreven, E. (2020). Hidden species diversity in *Marcusenius mooorii* (Teleostie: mormyridae) from the Congo Basin. *Journal of Fish Biology*, *96*, 1100–1122.

Matomela, N. (2020). *Assessing community structure and trophic interrelationships in three differently impacted headwater streams in the Amathole-Winterberg freshwater ecoregion, South Africa*. Rhodes University.

Weyl, Olaf L. F., Ellender, B. R., Wassermann, R. J., Truter, M., Dalu, T., Zengeya, T. A., & Smit, N. J. (2020). Alien Freshwater Fauna in South Africa. *Biological Invasions in South Africa* (pp. 153–183).

Kambikambi, M.J., Kadye, W.T., & Chakona, A. (2021) Allopatric differentiation in the *Enteromius anoplus* complex in South Africa, with the revalidation of *E. cernuus* and *E. oraniensis*, and description of a new species, *E. mandelai* (Teleostei: Cyprinidae). *Journal of Fish Biology:* N/A. https://doi.org/10.1111/jfb.14780.

Ramoejane, M., Weyl, O. L. F., Swartz, E. R., & Gouws, G. (2021). Identifying multiple geographically restricted phylogeographic lineages of Moggel (Cyprinidae: *Labeo umbratus*) in South Africa. *African Journal of Aquatic Science*, *42*(2), 225–235.

Sifundza, D. S., Chakona, A., & Kadye, W. T. (2021). Distribution patterns and habitat associations of Sandelia bainsii (Teleostei: Anabantidae), a highly threatened narrow-

range endemic freshwater fish. *Journal of Fish Biology*, *98*, 292–303.

APPENDICES

Appendix A: Physical-chemical, substrate types and land-use patterns of all sampled sites where fishes were captured in the Great Fish, Keiskamma and Buffalo River systems.

System	River	Land-use	Temp (°C)	Cond ($\mu S.m^{-1}$)	pH	TDS (ppm)	Turb (NTU)	DO (mg/L)	A.depth (cm)	A.width (m)	Area (m^2)	% Bd	% Bl	% Sn	% Sl	% Cl	% Gr	% V
Great Fish	Kat	Agriculture	24.2	161	8.66	94	40.4	7.8	41.9	6.7	293.5	0.0	0.4	0.1	0.4	0.2	0.0	0.2
Great Fish	Kat	Agriculture	28.5	216	8.42	127	28.9	5.5	33.2	6.8	245.1	0.0	0.1	0.1	0.4	0.2	0.2	0.1
Great Fish	Kat	Agriculture	26.3	202	9.24	123	22.9	10.5	29.2	3.3	66.8	0.1	0.2	0.0	0.0	0.6	0.2	0.1
Great Fish	Kat	Agriculture	25.1	132	8.68	77	64.1	8.3	32.6	8.7	257.5	0.0	0.8	0.0	0.1	0.0	0.3	0.4
Great Fish	Kat	Agriculture	26.3	97	8.92	57	18.7	7.8	28.6	7.6	336.5	0.6	0.1	0.0	0.3	0.0	0.1	0.2
Great Fish	Kat	Agriculture	23.4	118	9.22	69	275.0	7.2	83.2	21.5	505.3	0.0	0.0	0.3	0.3	0.0	0.4	0.2
Great Fish	Kat	Agriculture	25.6	121	9.10	71	31.8	8.1	86.5	12.3	271.3	0.6	0.1	0.0	0.0	0.3	0.1	0.0
Great Fish	Kat	Agriculture	27.3	130	8.12	74	518.0	7.2	44.6	3.9	89.3	0.1	0.6	0.0	0.0	0.0	0.4	0.2
Great Fish	Kat	Agriculture	30.7	1678	9.84	990	8.7	14.6	35.2	4.0	115.2	1.6	0.0	0.0	0.0	0.0	0.0	1.6
Great Fish	Kat	Agriculture	24.5	383	9.40	227	51.2	3.2	36.2	4.2	306.6	0.9	0.0	0.0	0.0	0.1	0.0	0.6
Great Fish	Kat	Agriculture	29.0	185	9.32	109	699.0	7.0	36.4	10.3	715.2	0.0	0.3	0.0	0.7	0.0	0.1	0.6
Great Fish	Kat	Agriculture	28.3	98	9.72	57	58.5	8.0	24.9	4.6	247.3	0.6	0.0	0.0	0.1	0.1	0.2	0.6
Great Fish	Kat	Agriculture	23.7	156	8.44	90	118.0	8.1	15.3	3.1	77.4	0.1	0.6	0.0	0.2	0.0	0.2	0.3
Great Fish	Kat	Agriculture	26.3	106	8.50	62	29.3	7.4	16.4	3.3	148.1	0.1	0.5	0.0	0.3	0.1	0.1	0.3
Great Fish	Kat	Agriculture	26.2	94	8.57	55	146.0	7.8	27.2	5.3	212.3	0.0	0.0	0.3	0.3	0.0	0.4	0.8
Great Fish	Kat	Agriculture	23.2	185	9.37	109	720.0	7.5	31.4	8.7	574.5	0.0	0.2	0.0	0.2	0.6	0.0	0.2

System	River	Landscape	Temp (°C)	Cond (µS)	pH	TDS (ppm)	Turb (NTU)	DO (mg/L)	A.depth (cm)	A.width (m)	Area (m^2)	% Bd	% Bl	% Sn	% Sl	% Cl	% Gr	% V
Great Fish	Kat	Agriculture	17.6	129	8.21	77	98.6	8.8	44.4	8.6	305.3	0.0	0.5	0.1	0.0	0.3	0.2	0.0
Great Fish	Kat	Agriculture	17.9	87	8.01	51	61.5	10.0	55.4	4.4	57.2	0.0	0.3	0.1	0.4	0.2	0.0	0.0
Great Fish	Kat	Agriculture	16.1	101	8.10	60	22.0	9.3	37.5	4.5	131.6	0.2	0.4	0.3	0.0	0.1	0.0	0.0
Great Fish	Kat	Agriculture	18.3	105	8.27	63	23.6	14.2	52.9	5.7	143.6	0.2	0.1	0.1	0.3	0.3	0.0	0.0
Great Fish	Kat	Invaded(wattle)	30.2	86	8.06	41	55.8	7.0	24.1	2.9	139.2	0.4	0.5	0.0	0.1	0.1	0.2	0.3
Great Fish	Kat	Invaded(wattle)	24.1	33	7.83	19	19.2	6.4	22.3	2.5	67.1	0.4	0.2	0.0	0.5	0.0	0.0	0.5
Great Fish	Kat	Invaded(wattle)	24.1	65	8.08	39	25.9	6.8	27.8	4.2	149.3	0.1	0.9	0.0	0.0	0.0	0.1	1.2
Great Fish	Kat	Invaded(wattle)	19.0	103	8.40	59	59.0	9.2	31.3	5.8	95.7	0.8	0.3	0.0	0.0	0.0	0.0	0.0
Great Fish	Kat	Invaded(wattle)	9.8	39	7.63	24	24.2	13.4	31.0	2.7	63.2	0.9	0.2	0.0	0.0	0.0	0.0	0.0
Great Fish	Kat	Invaded(wattle)	12.1	56	7.68	33	42.5	12.4	35.1	5.3	199.8	0.5	0.1	0.2	0.0	0.2	0.0	0.0
Great Fish	Kat	Invaded(wattle)	13.8	54	8.08	32	46.1	13.9	65.2	6.1	206.9	0.1	0.7	0.3	0.0	0.0	0.0	0.0
Great Fish	Kat	Near natural	26.0	186	9.19	109	43.3	7.6	18.4	2.9	148.5	0.0	0.6	0.1	0.3	0.0	0.1	0.1
Great Fish	Kat	Near natural	23.5	150	8.19	86	26.9	7.4	29.8	6.9	205.0	0.4	0.1	0.0	0.5	0.0	0.0	0.4
Great Fish	Kat	Near natural	27.4	380	8.38	223	8.4	6.5	37.0	2.7	27.8	0.1	0.0	0.0	0.0	0.0	1.1	0.9
Great Fish	Kat	Near natural	22.9	129	9.67	76	70.3	8.0	30.3	8.1	97.5	1.5	0.0	0.0	0.0	0.0	0.0	0.4
Great Fish	Kat	Near natural	24.2	130	8.70	76	69.9	8.0	41.7	5.7	220.4	1.2	0.1	0.0	0.0	0.0	0.0	0.2
Great Fish	Kat	Near natural	25.0	110	8.35	64	272.0	8.2	24.2	6.8	280.1	0.0	0.8	0.0	0.1	0.0	0.2	0.6
Great Fish	Kat	Near natural	24.3	82	9.13	48	33.2	7.5	19.2	3.7	55.2	0.3	0.4	0.0	0.3	0.1	0.0	0.5
Great Fish	Kat	Near natural	25.0	523	9.20	337	337.0	10.6	22.6	2.2	26.5	0.1	0.2	0.0	0.2	0.2	0.0	0.2
Great Fish	Kat	Near natural	23.4	60	9.74	34	29.1	7.8	15.9	5.7	246.9	0.0	1.3	0.0	0.0	0.0	0.0	0.2
Great Fish	Kat	Near natural	22.3	43	9.31	24	17.2	8.2	13.7	2.9	131.3	0.0	0.1	0.0	0.0	1.2	0.0	0.4

System	River	Landscape	Temp (°C)	Cond (µS)	pH	TDS (ppm)	Turb (NTU)	DO (mg/L)	A.depth (cm)	A.width (m)	Area (m^2)	% Bd	% Bl	% Sn	% Sl	% Cl	% Gr	% V
Great Fish	Kat	Near natural	16.8	120	8.16	71	37.2	9.0	49.8	8.4	363.1	0.0	0.3	0.0	0.0	0.4	0.2	0.0
Great Fish	Kat	Near natural	12.8	64	7.33	36	77.3	15.9	49.7	3.9	102.9	0.1	0.3	0.2	0.0	0.4	0.0	0.0
Great Fish	Kat	Near natural	15.5	63	7.75	36	80.0	11.5	31.3	4.1	85.8	0.2	0.4	0.2	0.0	0.2	0.0	0.0
Great Fish	Kat	Near natural	10.7	62	7.56	36	82.4	11.4	46.4	2.2	0.0	0.3	0.6	0.1	0.0	0.0	0.0	0.0
Great Fish	Kat	Near natural	20.5	3999	8.44	200	14.7	4.6	20.4	5.7	174.0	0.0	0.0	0.0	0.2	0.0	0.2	0.7
Great Fish	Kat	Near natural	19.9	117	8.22	68	18.7	7.8	43.5	3.9	150.1	0.7	0.1	0.0	0.0	0.3	0.0	0.3
Great Fish	Kat	Near natural	26.6	428	7.21	192	82.3	4.4	23.5	2.3	29.9	0.0	0.7	0.1	0.2	0.0	0.0	0.4
Great Fish	Kat	Near natural	19.3	137	7.93	78	35.7	6.7	34.9	5.4	110.2	0.0	0.0	0.2	0.9	0.0	0.0	0.8
Great Fish	Kat	Near natural	24.0	106	8.67	62	13.5	7.1	33.1	3.5	105.7	0.0	0.8	0.1	0.0	0.2	0.0	0.1
Great Fish	Kat	Near natural	25.8	109	8.54	63	19.3	7.8	30.9	5.1	71.9	0.7	0.4	0.0	0.0	0.0	0.0	0.0
Great Fish	Kat	Near natural	27.6	118	8.34	70	30.0	5.7	51.7	5.9	266.8	0.3	0.5	0.0	0.0	0.0	0.1	0.1
Great Fish	Koonap	Agriculture	20.3	225	9.92	132	1000.0	5.5	48.2	6.4	193.6	0.0	0.1	0.1	0.4	0.1	0.3	0.2
Great Fish	Koonap	Agriculture	25.5	478	9.72	283	54.5	10.4	28.9	8.7	203.8	0.2	0.2	0.0	0.6	0.1	0.0	0.5
Great Fish	Koonap	Agriculture	27.5	335	9.02	196	4.6	9.3	23.6	3.3	92.4	0.0	1.1	0.0	0.0	0.1	0.0	0.1
Great Fish	Koonap	Agriculture	25.9	633	8.81	371	10.6	8.0	54.0	3.6	150.7	0.3	0.8	0.0	0.0	0.0	0.0	0.0
Great Fish	Koonap	Agriculture	20.1	134	10.71	77	10.6	9.9	29.7	7.4	612.7	1.1	0.0	0.0	0.0	0.1	0.0	0.2
Great Fish	Koonap	Near natural	28.0	525	9.48	310	31.7	8.0	49.5	8.5	435.5	0.0	0.1	0.3	0.0	0.5	0.1	0.3
Great Fish	Koonap	Near natural	29.5	285	8.59	166	384.0	3.7	59.4	5.3	70.7	0.1	0.1	0.0	0.8	0.0	0.1	0.0
Great Fish	Koonap	Near natural	21.0	310	7.95	182	92.2	7.1	54.7	9.8	186.8	1.1	0.1	0.0	0.0	0.0	0.0	0.0
Great Fish	Koonap	Near natural	14.9	329	7.74	192	17.2	4.8	44.4	5.6	58.8	0.1	0.1	0.1	0.4	0.2	0.1	0.0
Great Fish	Koonap	Near natural	16.5	97	8.53	54	4.3	7.6	40.3	5.5	65.6	0.4	0.3	0.0	0.0	0.2	0.0	0.0

System	River	Landscape	Temp (°C)	Cond (µS)	pH	TDS (ppm)	Turb (NTU)	DO (mg/L)	A.depth (cm)	A.width (m)	Area (m^2)	% Bd	% Bl	% Sn	% Sl	% Cl	% Gr	% V
Great Fish	Koonap	Near natural	18.9	205	8.31	120	719.0	5.9	60.7	12.1	239.6	0.6	0.0	0.0	0.0	0.1	0.2	0.0
Keiskamma	Keiskamma	Near natural	17.6	201	7.79	118	36.9	7.1	45.7	4.4	52.4	0.0	0.4	0.0	0.3	0.3	0.0	0.0
Keiskamma	Keiskamma	Near natural	12.2	498	8.10	249	3.0	11.4	28.2	5.6	119.8	0.3	0.2	0.0	0.0	0.0	0.0	0.5
Keiskamma	Keiskamma	Near natural	16.0	215	7.76	126	16.0	13.3	30.8	2.9	67.0	0.7	0.3	0.0	0.0	0.1	0.0	0.0
Keiskamma	Keiskamma	Near natural	20.9	237	8.04	138	6.1	11.8	27.5	2.9	136.9	0.4	0.4	0.0	0.0	0.2	0.0	0.0
Keiskamma	Keiskamma	Near natural	17.5	167	7.69	102	8.3	8.7	29.5	6.3	119.4	0.6	0.4	0.0	0.0	0.0	0.0	0.0
Keiskamma	Keiskamma	Near natural	20.5	70	8.12	41	15.7	9.1	58.3	11.0	132.0	0.1	0.7	0.1	0.0	0.2	0.0	0.0
Buffalo	Buffalo	Near natural	17.2	145	7.64	84	6.7	8.4	42.1	7.1	112.8	0.3	0.5	0.0	0.0	0.2	0.0	0.0
Buffalo	Buffalo	Near natural	23.7	487	7.87	289	45.1	7.9	60.7	8.3	116.2	0.3	0.2	0.2	0.0	0.3	0.0	0.0
Buffalo	Buffalo	Near natural	22.0	460	8.02	268	4.6	6.6	48.5	7.2	122.9	0.0	0.8	0.0	0.0	0.2	0.0	0.0

Physical-chemical and habitat variables are abbreviated as: Temp = temperature, Cond = conductivity, TDS = total dissolved solids, Turb = Turbidity, D0 = dissolved oxygen, A.depth = Average depth. A.width = average width, Bd = Bedrock, Bl = Boulder, Cl = Cobble, Gr = Gravel, Sn = Sand, Sl = Silt, V = Vegetation.

Appendix B: Fish species collected during the current survey at sites in the Great Fish, Keiskamma and Buffalo River systems. The ✓ symbol represents present and ✗ symbol represents absent. * represents non-native fish species.

	System	Landscape	*Enteromius mandelai*	*Sandelia bainsii*	*Amatolacypris trevelyani*	*Labeo umbratus*	*Anguilla mossambica*	*Glossogobius callidus*	*Oreochromis mossambicus*	* *Tilapia sparrmanii*	* *Clarias gariepinus*	* *Labeobarbus aeneus*	* *Oncorhynchus mykiss*
1	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
2	Great Fish	Agriculture	✓	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗
3	Great Fish	Agriculture	✓	✓	✗	✗	✗	✓	✗	✗	✗	✗	✗
4	Great Fish	Agriculture	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗
5	Great Fish	Agriculture	✓	✓	✗	✓	✗	✓	✗	✗	✗	✗	✗
6	Great Fish	Agriculture	✗	✓	✗	✓	✗	✓	✗	✗	✗	✗	✗
7	Great Fish	Agriculture	✓	✓	✗	✗	✗	✓	✗	✗	✗	✗	✗
8	Great Fish	Agriculture	✗	✗	✗	✗	✗	✓	✗	✓	✓	✓	✗
9	Great Fish	Agriculture	✗	✗	✗	✗	✗	✗	✗	✗	✓	✗	✗
10	Great Fish	Agriculture	✓	✗	✗	✓	✓	✓	✗	✓	✓	✗	✗
11	Great Fish	Agriculture	✓	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗
12	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
13	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
14	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
15	Great Fish	Agriculture	✗	✗	✗	✓	✗	✓	✗	✗	✗	✗	✗
16	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
17	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
18	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
19	Great Fish	Agriculture	✓	✗	✗	✓	✓	✓	✗	✗	✗	✗	✗
20	Great Fish	Agriculture	✓	✓	✗	✗	✗	✓	✗	✗	✗	✗	✗
21	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
22	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
23	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
24	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
25	Great Fish	Agriculture	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗

	System	Landscape	*Enteromius mandelai*	*Sandelia bainsii*	*Amatolacypris trevelyani*	*Labeo umbratus*	*Anguilla mossambica*	*Glossogobius callidus*	*Oreochromis mossambicus*	* *Tilapia sparrmanii*	* *Clarias gariepinus*	* *Labeobarbus aeneus*	* *Oncorhynchus mykiss*
26	Great Fish	Agriculture	✓	×	×	×	×	×	×	×	×	×	×
27	Great Fish	Invaded (wattle)	✓	✓	×	×	×	×	×	×	×	×	×
28	Great Fish	Invaded (wattle)	✓	✓	×	×	×	×	×	×	×	×	×
29	Great Fish	Invaded (wattle)	×	✓	×	×	×	×	×	×	×	×	×
30	Great Fish	Invaded (wattle)	✓	✓	×	×	✓	✓	×	×	×	×	×
31	Great Fish	Invaded (wattle)	×	✓	×	×	×	×	×	×	×	×	×
32	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
33	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
34	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
35	Great Fish	Near natural	✓	✓	×	✓	×	×	×	×	×	×	×
36	Great Fish	Near natural	✓	✓	×	✓	×	✓	×	×	×	×	×
37	Great Fish	Near natural	✓	✓	×	×	✓	✓	×	×	×	×	×
38	Great Fish	Near natural	×	×	×	×	✓	×	×	×	×	×	×
39	Great Fish	Near natural	×	✓	×	×	×	×	×	×	×	×	×
40	Great Fish	Near natural	×	✓	×	×	×	×	×	×	×	×	×
41	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
42	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
43	Great Fish	Near natural	✓	✓	×	×	×	✓	×	×	×	×	×
44	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
45	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
46	Great Fish	Near natural	×	×	×	✓	×	×	✓	×	✓	×	×
47	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
48	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×

	System	Landscape	*Enteromius mandelai*	*Sandelia bainsii*	*Amatolacypris trevelyani*	*Labeo umbratus*	*Anguilla mossambica*	*Glossogobius callidus*	*Oreochromis mossambicus*	* *Tilapia sparrmanii*	* *Clarias gariepinus*	* *Labeobarbus aeneus*	* *Oncorhynchus mykiss*
49	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
50	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
51	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
52	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
53	Great Fish	Near natural	✓	×	×	×	×	×	×	×	×	×	×
54	Great Fish	Near natural	✓	✓	×	×	×	×	×	×	×	×	×
55	Great Fish	Near natural	×	×	×	×	✓	×	×	×	×	×	×
i	Keiskamma	Agriculture	×	×	×	×	×	✓	×	✓	×	×	×
ii	Keiskamma	Agriculture	×	✓	×	×	×	×	×	×	×	×	×
ii	Keiskamma	Agriculture	✓	✓	✓	×	×	×	×	×	×	×	×
iv	Keiskamma	Invaded	×	✓	×	×	×	×	×	×	×	×	×
v	Keiskamma	Near natural	✓	×	✓	×	×	×	×	×	×	×	×
vi	Keiskamma	Near natural	✓	×	✓	×	×	×	×	×	×	×	×
vii	Keiskamma	Near natural	✓	×	✓	×	×	×	×	×	×	×	×
viii	Keiskamma	Near natural	×	✓	✓	×	×	×	×	×	×	×	✓
ix	Keiskamma	Near natural	✓	✓	×	×	×	×	×	×	×	×	×
A	Buffalo	Agriculture	×	×	×	✓	×	×	×	×	✓	×	×
B	Buffalo	Agriculture	✓	×	×	×	×	×	×	✓	×	×	×
C	Buffalo	Agriculture	✓	✓	×	×	×	×	×	×	×	×	×
D	Buffalo	Agriculture	✓	×	×	×	×	×	×	✓	×	×	×
E	Buffalo	Agriculture	✓	✓	×	×	×	×	×	×	×	×	×
F	Buffalo	Agriculture	✓	×	×	×	×	×	×	×	×	×	×
G	Buffalo	Near natural	×	×	✓	×	×	×	×	×	×	×	×
H	Buffalo	Near natural	✓	✓	✓	×	×	×	×	×	×	×	×

I	Buffalo	Near natural	✓	×	×	×	×	×	×	×	×	×	×	

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

Scientific name	Common name
Amatolacypris trevelyani	Border barb, Grens-ghieliemientjie
Anguilla mossambica	African longfin eel
Austroglanis barnardi	Barnard's rock-catfish
Austroglanis gilli	Clanwilliam catfish, Clanwilliam-klipbaber
Austroglanis sclateri	Rock-catfish, klipbaber
Cheilobarbus capensis	Cape whitefish
Cheilobarbus serra	Sawfin
Clarias gariepinus	sharptooth catfish
Cyprinus carpio	common carp
Enteromius amatolicus	Amatola barb
Enteromius annectens	Broadstriped barb
Enteromius anoplus	Chubbyhead barb
Enteromius barotseensis	Barotse barb
Enteromius bifrenatus	Hyphen barb
Enteromius breviceps	Shorthead barb
Enteromius brevipinnis	Shortfin barb
Enteromius cernuus	Chubbyhead barb
Enteromius fasciolatus	African banded barb
Enteromius gurneyi	Redtail barb
Enteromius karkensis	Chubbyhead barb
Enteromius lineomaculatus	Line-spotted barb
Enteromius mandelai	Chubbyhead barb
Enteromius motebensis	Marico barb
Enteromius neefi	Sidespot barb
Enteromius oraniensis	Chubbyhead barb
Enteromius pallidus	Goldie barb, Goud-ghieliemientjie
Enteromius radiatus	Redeye barb
Enteromius treurensis	Treur barb
Enteromius unitaeniatus	Slender barb
Enteromius viviparus	Bowstripe barb
Galaxias zebratus	Cape galaxias
Glossogobius callidus	River goby
Labeo umbratus	Moggel
Labeobarbus aeneus	Smallmouth yellowfish
Labeobarbus capensis	Orange River mudfish
Labeobarbus kimberlyensis	Largemouth yellowfish
Labeobarbus seeberi	Clanwilliam yellowfish
Micropterus dolomieu	Smallmouth bass
Micropterus salmoides	Largemouth bass
Nannoperca australis	Southern pygmy perch
Onchorhynchus mykiss	rainbow trout
Oreochromis mossambicus	Mozambique tilapia
Pseudobarbus afer	Eastern Cape redfin, Oos-Kaapse rooivlerkie
Pseudobarbus asper	Smallscale redfin, Kleinskub-rooiverkie
Pseudobarbus burchelli	Burchell's redfin, Breede River redfin, Breederivier-rooivlerkie
Pseudobarbus burgi	Berg River redfin, Bergrivier-rooivlerkie

Pseudobarbus phlegethon	Fiery redfin, Vurige rooivlerkie
Pseudobarbus quathlambae	Maloti minnow
Pseudobarbus senticeps	N/A
Pseudobarbus skeltoni	Giant redfin
Pseudobarbus swartzi	Gamtoos River redfin
Pseudobarbus tenuis	Slender redfin
Pseudobarbus verloreni	Verlorenvlei River redfin
Salmo trutta	Brown trout
Sandelia bainsii	Cape rocky
Sandelia bainsii	Eastern Cape rocky
Sandelia capensis	Cape kurper
Sedercypris calidus	Clanwilliam River redfin
Sedercypris erubescens	Twee River redfin
Tilapia sparrmanii	Banded tilapia, Vlei kurper