The effect of rainbow trout (*Oncorhynchus mykiss*) invasions on native fish communities in the subtropical Blyde River, Mpumalanga province, South Africa

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This study investigated the effects of rainbow trout (*Oncorhynchus mykiss*) invasion on native fish communities in the upper Blyde River catchment. A fish survey was undertaken between September 2017 and October 2018 from 11 sites in the Blyde and Treur Rivers using electrofishing and fyke nets. Differences in species composition, relative abundance, and community structure among sites were tested using multivariate analysis. A total of ten fish species were captured. There were significant differences in composition and abundance between fish communities that could be attributed to *O. mykiss* invasion and variation in habitats. Populations of native species that historically occurred throughout the upper catchment, such as *Enteromius treurensis*, were greatly reduced and fragmented in the presence of *O. mykiss*. However, instream migration barriers such as waterfalls have prevented upstream migration of *O. mykiss*, and these invasion-free areas have remnant populations of native fishes that appear to be largely intact. This finding is consistent with other studies that have shown that introduced alien predatory fish can have a significant effect on fish communities and highlighted the need to prevent human-facilitated introductions in biodiversity sensitive areas, such as mountain headwater streams, that are inhabited by endemic and range-restricted minnows.

Keywords: abundance, biogeographic barriers, composition, distribution, *Enteromius treurensis*, Treur River

Introduction

The rainbow trout (*Oncorhynchus mykiss*) is a popular angling fish native to North America that has been introduced across the world, mainly for aquaculture and angling purposes (Crawford and Muir 2007). In some cases, introduced *O. mykiss* have become invasive and have caused adverse impacts to native fish and ecosystems (McDowall 2006; Young et al. 2010; Kadye et al. 2013; Stanković et al. 2015). For more than a century, *O. mykiss* has been widely introduced into lakes and rivers in South Africa to create and enhance sport-fishing opportunities (Ellender and Weyl 2014). It is a cold-water species, often introduced into cool, well-oxygenated mountain headwaters. However, these habitats are also inhabited by some threatened and endemic species (Weyl et al. 2020). These introductions of *O. mykiss* have, in some cases, caused declines in native invertebrates, frogs, and fish, extirpating some populations (Karssing et al. 2012; Rivers-Moore et al. 2013; Shelton et al. 2015a). In addition, *O. mykiss* introductions have altered food web structures (Shelton et al. 2015b), changed habitat coupling, as well as causing other overall ecosystem-level impacts (Jackson et al. 2016). These studies represent a small, but growing number of studies within South Africa, that provide evidence that trout invasions in areas of conservation concern can lead to adverse impacts on biodiversity, as demonstrated in other parts of the world (McDowall 2006; McIntosh et al. 2010).

This study investigated the impact of the *O. mykiss* on community structure of native fish species in the upper catchments of the Blyde River in Mpumalanga province, South Africa. The upper catchment of the Blyde River is located on the northern part of the Great Drakensburg Escarpment where *O. mykiss* has been introduced into several headwater streams and has established naturalised populations (Engelbrecht and Roux 1998). These upland areas form part of trout angling areas where the stocking and utilisation of trout is allowed (such as the Crocodile River from its source up to its confluence with the Santa River, the Sabie River from its source up to the waterfall in the town of Sabie, and the Elands River from its source up to its confluence with the Swartkops River) by the Mpumalanga Nature Conservation Act No. 10 of 1998.

These headwater streams are also inhabited by range-restricted and endemic river minnows, such as the Treur River barb (*Enteromius treurensis*), that are listed as critically endangered, because of alien predatory fish, such as *O. mykiss*, brown trout (*Salmo trutta*) and smallmouth bass (*Micropterus dolomieu*)

(Roux and Hoffman 2017). *Enteromius treurensis* has a small geographic range (4.5 km^2) and extant populations are fragmented and restricted to two localities within the upper Blyde River catchments (Kleynhans 1987). The species was originally described from the Treur River, a small tributary of the Blyde River (Groenewald 1958), but it became extirpated from the river in the 1960s, likely as a result of the introduction of *O. mykiss* and *M. dolomieu* (Jubb 1967; Klenyhans 1987). A small and isolated population of *E. treurensis* was then discovered in the upper Blyde River in the late 1960s (Pott 1981). The upper sections of the Blyde River are isolated by the Christmas Pool waterfalls, a natural geographic barrier that has prevented upstream migration of *O. mykiss* from the lower sections where it occurs. *Enteromius treurensis* was then re-introduced into the Treur River in 1995, where it has successfully managed to re-establish in several sections of the river that are free of alien predatory fish (Engelbrecht and Roux 1998).

The historic distribution of *E. treurensis* is thought to have encompassed the whole of the upper catchment. Its current fragmented distribution has been attributed to introduction and continued stocking of *O. mykiss*, and to gold mining activities that may have led to its decline and extirpation in river sections between the two localities with remnant populations (Kleynhans 1987). These remnant populations are largely intact, because of a series of waterfalls that prevent upstream fish migration from downstream river sections where alien fish introductions occurred.

The present study evaluated the ecological impact of *O. mykiss* invasions on native fish communities in the upper Blyde River by comparing fish communities in river reaches invaded by *O. mykiss* and river sections free of *O. mykiss* invasion. More specifically, the study evaluated the effects of *O. mykiss* invasion on native fish species composition, relative abundance, and community structure. The study tested the hypothesis that the presence of *O. mykiss* would create specific differences in species composition and community structure, such as a decline and fragmentation of native fish species populations, in invaded river sections.

Materials and methods

Study area

The study area encompassed the headwaters of the Blyde River and the Treur River that are located on the northern part of the Great Drakensberg escarpment in Mpumalanga province, South Africa (Figure 1). The study sites were located on the upper slopes of the escarpment (1 200–1 500 m above sea level) (Table 1) that are characterised by grassland vegetation that is interspersed with Afromontane forest, cool to moderate temperatures (10–18 °C), moderate to high rainfall (800–1 200 mm), and mean annual precipitation of more than 1 000 mm (WRC 2001). To assess the impact of *O. mykiss* on fish community structure, eight sampling sites were selected from the non-invaded (sites 1 and 2) and invaded (sites 3–8) river sections of upper Blyde River (Table 1). Three additional sites (9–11) were located in the upper sections of the Treur River that are free of alien fish.

Sampling

Fish were sampled between September 2017 and October 2018 from 11 sampling sites (Figure 1; Table 1) and each site was sampled once every three months on five different occasions. Each sampling event took place over a period of 4–5 days and each site was sampled randomly depending on when landowner permission to access the site was confirmed. All the sites were sampled using a SAMUS725MP electrofisher (Samus Special Electronics, Warsaw, Poland), whereas fyke nets were used at all sites on the Blyde River, but not in the Treur River, because of the low water levels. A river stretch of approximately 50 m was blocked off to prevent fish escape and electrofishing was conducted for ~30 minutes, with the electrofisher being operated in an upstream direction with a 1 m deep × 2 m wide, 5 mm mesh mobile block net being used behind to catch stunned fish missed by the operator (Kimberg et al. 2014). Fyke nets were set in the evening and retrieved the following morning. Fish were sorted according to species, weighed (g), and measured to total length (TL, mm). A subsample of the fish was then collected and preserved in absolute ethanol, and the rest of the catch was released back into the river. At each sampling site, the following physical and chemical variables were recorded once for each sampling event using a Multi-Parameter Test 35 series water quality probe (Oakton Instruments, Vernon Hills, USA.): temperature (°C), conductivity (µS cm^{-1}), and pH. The accuracy of the multiparameter meter was 1% full scale and it was calibrated before use at each site.

Statistical analysis

To minimise biases caused by gear selectivity, catch data were transformed to presence and absence data for comparisons on differences in species composition among sites and comparisons for differences in species abundance were restricted to data obtained from electrofishing. The differences in community structure among the sites were tested using multivariate PERMANOVA based on a Bray–Curtis similarity matrix of fourth root transformed abundance data with 9 999 permutations. The PERMANOVA was based on algorithms in PAST v3. A non-metric multidimensional scaling (nMDS) was then used to assess the relationship between species abundance and two categorical factors: site and invasion status. A site was considered invaded when *O. mykiss* was present and uninvaded when it was absent, taking cognisance that it is difficult to provide absolute evidence of absence. The nMDS was based on three axis ordinations in order to achieve a good model fit (i.e. stress < 0.11). The similarity between and among sites was evaluated using an agglomerative hierarchical cluster analysis (using group-average linking), based on a Bray–Curtis similarity matrix. A Spearman rank correlation analysis was used to assess the correlation between each species and the two categorical factors, site and invasion status. The nMDS, cluster and Spearman rank analyses were all performed using Primer v6 (PRIMER-E LTD, 2007, Lutton, United Kingdom).

Canonical correspondence analysis (CCA) was then used to assess the possible effects of environmental variables on fish community structure among the sampling

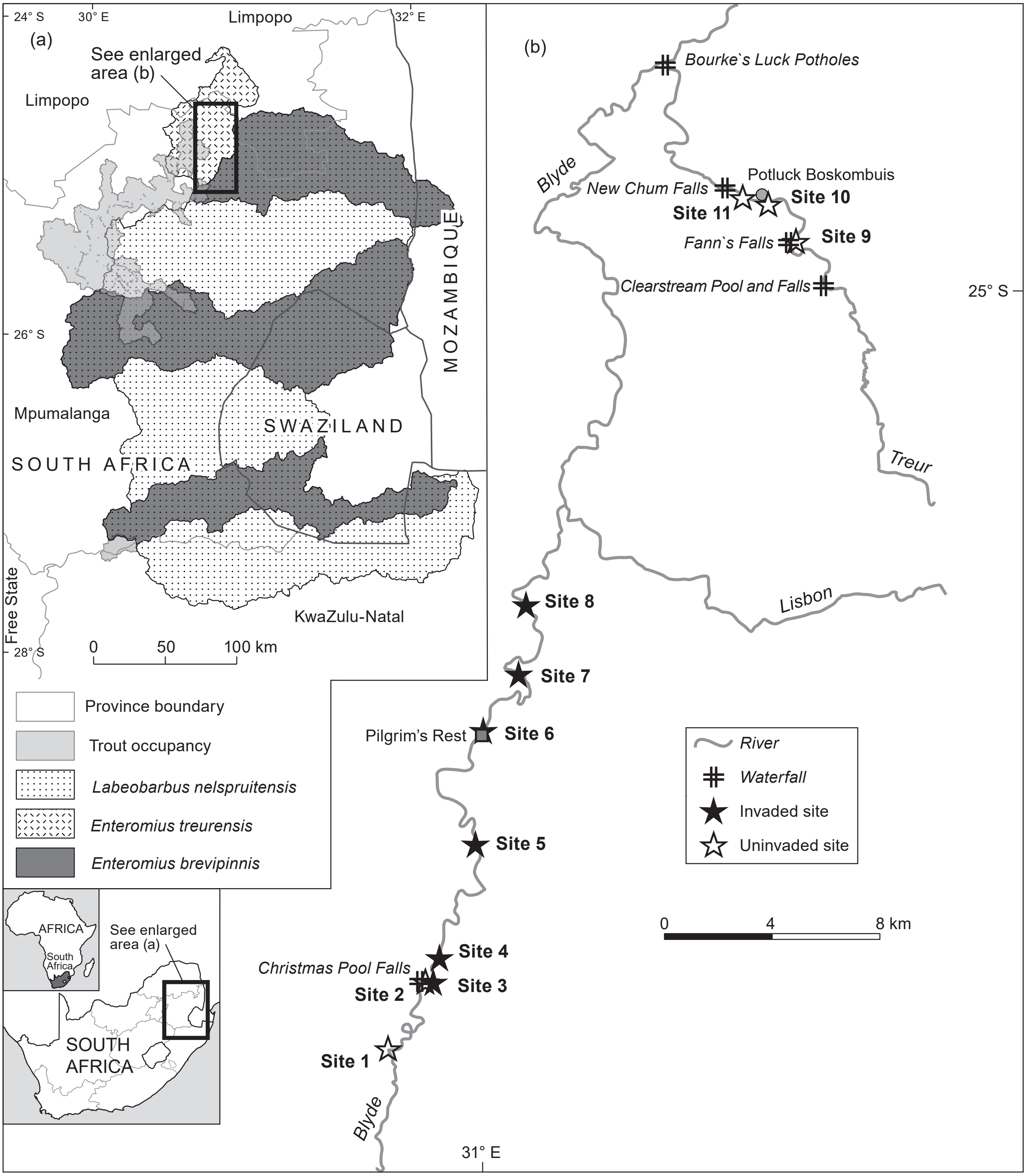

Figure 1: A map showing (a) the distribution of native and threatened fish species (*Enteromius treurensis*, *Enteromius brevipinnis* and *Labeobarbus nelspruitensis*) in relation to the presence of *Oncorhynchus mykiss*; and (b) surveyed sites with the invasive rainbow trout (*Oncorhynchus mykiss*) (★) and sites free of invasion (☆) in the upper catchment of Blyde and Treur Rivers in Mpumalanga province, South Africa. Trout occupancy refers to areas where trout is known to occur, based on a consultative stakeholder mapping process done by the Department of Forestry, Fisheries and the Environment, and the South African National Biodiversity Institute. Data sources for the distribution of native fish species were obtained from IUCN (International Union for Conservation of Nature) 2007a, 2007b, 2007c

Table 1: Sampling sites and their geographic coordinates (sites 1–11) where surveys were undertaken on the Blyde and Treur Rivers in Mpumalanga province, South Africa. Invasion status was defined as either invaded when rainbow trout (*Oncorhynchus mykiss*) was present or uninvaded when it was absent

River	Site	Location	Invasion status	Geographic coordinates	Elevation (m)	Land use
Blyde River	1	5 km above the first Christmas Pool Falls	Uninvaded	25°00′14.2″ S 30°43′06.3″ E	1 432	Forestry plantation
	2	Below the first Christmas Pool waterfall	Uninvaded	24°58′51.0″ S 30°43′50.4″ E	1 372	Forestry plantation
	3	Below the second Christmas Pools waterfall	Invaded	24°58′45.6″ S 30°44′01.9″ E	1 362	Forestry plantation
	4	3 km downstream of the second Christmas Pools waterfall	Invaded	24°58′15.9″ S 30°44′09.5″ E	1 341	Forestry plantation
	5	Third bridge from Christmas Pool Falls	Invaded	24°55′59.4″ S 30°44′52.8″ E	1 285	Forestry plantation
	6	First bridge from Pilgrim's Rest	Invaded	24°53′57.9″ S 30°44′58.9″ E	1 242	Mining, Peri urban
	7	Second bridge from Pilgrim's Rest	Invaded	24°52′40.0″ S 30°45′39.8″ E	1 215	Peri urban
	8	Third bridge from Pilgrim's Rest	Invaded	24°51′14.1″ S 30°45′46.1″ E	1 194	Agriculture
Treur River	9	Pools below Treur River waterfall, 2.5 km upstream of Potluck Boskombuis	Uninvaded	24°43′52.2″ S 30°51′18.5″ E	1 293	Protected area
	10	Pools next to Potluck Boskombuis restaurant	Uninvaded	24°43′15.0″ S 30°50′45.0″ E	1 272	Protected area
	11	Pools downstream of bridge to Potluck Boskombuis restaurant	Uninvaded	24°43′09.4″ S 30°50′14.5″ E	1 246	Protected area

sites. First, species occurrence data were log(x + 1) transformed to reduce the effect of rare or very common species and environmental data were then Box-Cox (x + 1) transformed to dampen the influence of outliers. Second, a partial CCA was used to remove the effects of spatial autocorrelation among the eight environmental variables [temperature, pH, salinity, conductivity, total dissolved solids, altitude, and invasion status (presence or absence of *O. mykiss*)]. The partial CCA indicated collinearity among some physico-chemical variables, such as salinity, conductivity, and total dissolved solids and between temperature and altitude. As a result, only four out of the initial eight variables (altitude, pH, conductivity, and invasion status) that were non-correlated were included in the full CCA model. The significance of the CCA model, CCA axis and environmental variables were then tested using Monte Carlo permutation test with 999 unrestricted permutations. All the CCA analysis was done using individual values for each site in the VEGAN package in the R Computing Programme.

Results

Species composition and abundance

A total of 610 specimens representing ten species from five families were collected from the Blyde and Treur Rivers (Table 2). The most widespread and abundant species were *E. treurensis* (nine out of 11 sampling sites) and the line spotted barb (*E. lineomaculatus*) (seven out of 11) that collectively accounted for 62% of the catch. There were significant differences in species composition (PERMANOVA: p = 0.01) and species abundance (PERMANOVA: p < 0.001) among fish assemblages from the different sites in the two rivers (Table 2). In the Blyde River, species richness and abundance generally increased with decreasing altitude and increasing stream size (Table 2). Sampling sites (1 and 2) located in upper river sections above the Christmas Pool waterfalls had low species richness, and only two species, *E. treurensis* and the stargazer mountain catfish (*Amphilius uranoscopus*) were collected, of which *E. treurensis* was highly abundant. Sampling sites (3 and 4) located below the waterfalls were invaded by *O. mykiss* and were characterised by a lower abundance of *E. treurensis*, compared with sites 1 and 2. Sampling sites (6 to 8) located farther downstream and in lower river sections had higher species richness and were characterised by higher abundances of species, such as *E. lineomaculatus*, the largescale yellowfish (*Labeobarbus marequensis*) and the shortspine suckermouth (*Chiloglanis pretoriae*) than at other sites.

In contrast, species composition and abundance were generally similar among sites in the Treur River, except for site 11 that had markedly lower species richness than the other two sites (Table 2). Species composition was largely similar between the two rivers, the only differences were for *O. mykiss* and straightfin barb (*E. paludinosus*) that were only collected from the Blyde River and chubbyhead barb (*E. anoplus*) and banded tilapia (*Tilapia sparrmanii*) were only collected from the Treur River.

Community structure

There were significant differences in fish communities among the sampling sites, based on the location (PERMANOVA: p < 0.001) and invasion status (PERMANOVA: p = 0.01).

Table 2: Composition and abundance of fish communities in the Blyde and Treur Rivers in Mpumalanga province, South Africa. Numbers outside parentheses indicate total number of individuals with standard deviation collected using electrofishing (*n* = 436) and those in parentheses using fyke nets (*n* = 174)

Family	Species	IUCN Red List threat category	Blyde River								Treur River		
			Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11
Cyprinidae	*Enteromius lineomaculatus*	Least concern					25 ± 11.2 (33 ± 10.5)	28 ± 11.4	19 ± 6.1	31 ± 7.6 (13 ± 3.7)	13 ± 4.3	4 ± 1.1	3 ± 1.3
	Labeobarbus marequensis	Least concern						9 ± 4.0	17 ± 4.9 (5 ± 2.2)	28 ± 7.8 (16 ± 4.8)			
	Enteromius treurensis	Critical endangered	50 ± 11.9 (22 ± 9.8)	40 ± 10.7 (47± 21.0)	1 ± 0.4 (1 ± 0.4)	4 ± 1.8		13 ± 5 .8		3 ± 1.3	3 ± 1.3	6 ± 1.3	14 ± 2.9
	Labeobarbus nelspruitensis	Near threatened							1 ± 0.4	2 ± 0.9			
	Enteromius paludinosus	Least concern							(1 ± 0.4)				
	Enteromius anoplus	Least concern									15 ± 6.7	4 ± 1.8	
Mochokidae	*Chiloglanis pretoriae*	Least concern						10 ± 2.1	3 ± 0.9 (2 ± 0.9)	12 ± 1.9 (6 ± 2.7)			
Amphiliidae	*Amphilius uranoscopus*	Least concern		1 ± 0.4 (1 ± 0.4)		3 ± 1.3 (1 ± 0.4)				1 ± 0.4 (7 ± 2.2)	15 ± 3.7	9 ± 2.5	
Cichlidae	*Tilapia sparrmanii*	Least concern									4 ± 1.1	17 ± 3.5	14 ± 3.8
Salmonidae	*Oncorhynchus mykiss**	Not assessed			10 ± 2.7 (5 ± 1.8)	2 ± 0.9 (13 ± 5.7)	(1 ± 0.4)		1 ± 0.4	1 ± 0.4			

* indicates alien species and the IUCN Red List threat category indicates the conservation status of fish species caught in the upper catchment of the Blyde River (see IUCN 2021, for detailed assessments of each species).

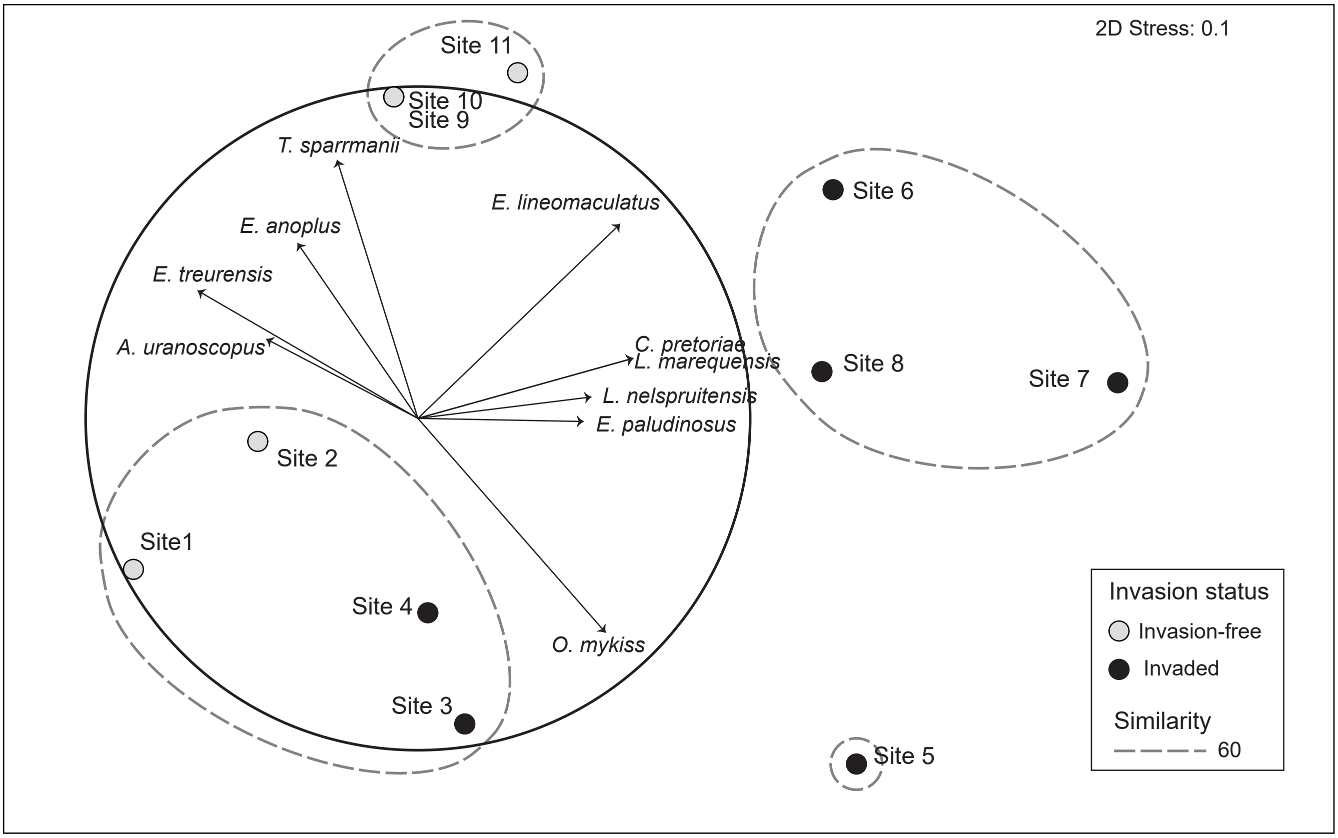

Figure 2: A non-metric multidimensional scaling (nMDS) showing sampling sites similarity, based on species composition in the invaded and uninvaded sections of Blyde River and the uninvaded Treur River in Mpumalanga province, South Africa. The dashed circles indicate similarity (60%) between and among sites evaluated using an agglomerative hierarchical cluster analysis (using group-average linking), based on a Bray–Curtis similarity matrix

The nMDS analysis (2D Stress = 0.11) indicated a complete and clear separation of sampling sites into four clusters (60% Bray–Curtis similarity) (Figure 2). The first two clusters comprised sites 1–4 from the upper section of the Blyde River. Sites 1 and 2 were characterised by the co-occurance of *E. treurensis* and *A. uranoscopus* and sites 3–4 were defined by occurrence of *O. mykiss*. The second cluster comprised sites 9–11 from the Treur River that were characterised by species, such as the *A. uranoscopus*, *E. anoplus, E. treurensis* and *T. sparrmanii* (Figure 2). The third cluster comprised of sites 6–8 was characterised by species such as *E. lineomaculatus* and *L. marequensis* (Figure 2). Site 5 formed the last cluster and was defined by the occurrence of *O. mykiss*.

Effect of environmental variables

The ordination results from the CCA indicated that the first two axes explained 38.2% variation in the fish community structure. The first CCA axis accounted for 26.6% of the total species variance and was positively correlated with pH, conductivity, and invasion status, but negatively correlated with elevation (Figure 3). The second CCA axis accounted for 11.6% of the total variance and was positively correlated with pH and conductivity, but negatively correlated with altitude and invasion status. The permutation tests indicated that the CCA model, first CCA axis, and two environmental variables (altitude and invasion status) were significant predictors of variation in the fish community structure (p < 0.01). Sampling sites formed four clusters, based on invasion status and water quality variables (Figure 3). Sites 1–2 and sites 9–11 represented sites that were free of *O. mykiss*, whereas sites 3–4 and 5–8 represented trout-invaded areas.

In addition, sites from areas free of *O. mykiss* invasion could be further separated into two groups: sites 1–2 on the Blyde River that were at a higher elevation and had lower water temperatures versus sites 9–11 on the Treur River that were at a lower elevation with relatively higher temperature and pH (Table 3). Similarly, sites from invaded areas could be split into two groups based on physico-chemical variables: sites 5–8 with higher conductivity versus sites 3–4 with relatively lower conductivity values.

Discussion

Effect of O. mykiss invasion

The establishment of invasive predatory species within recipient systems is known to reduce native populations through various mechanisms, such as predation and competitive exclusion for resources such as food and

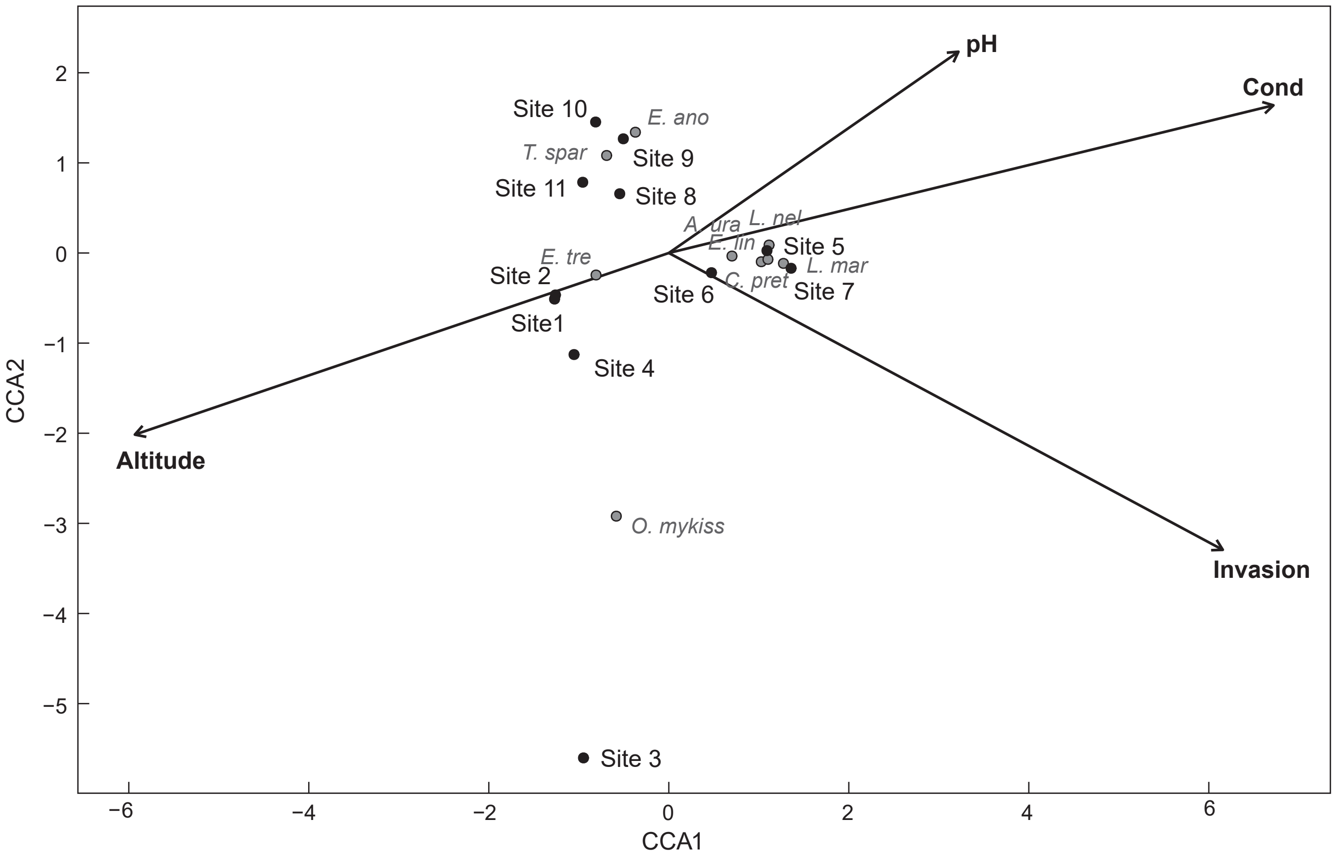

Figure 3: Canonical correspondence analysis showing the relationship between species composition and environmental variables among the invaded and uninvaded sections of Blyde River and the uninvaded Treur River in Mpumalanga province, South Africa. Abbreviations: *A. ura = Amphilius uranoscopus, C. pre = Chiloglanis pretoriae, E. ano = Enteromius anoplus, E. lin = Enteromius lineomaculatus, E. tre = Enteromius treurensis, L. mar = Labeobarbus marequensis, L. nel = Labeobarbus nelspruitensis, O. myk = Oncorhynchus mykiss, T. spa = Tilapia sparrmanii*, Cond = conductivity. Invasion refers to the presence or absence of *O. mykiss*

habitat (Eby et al. 2006). Salmonid fishes, in particular *O. mykiss* and *S. trutta* have caused major declines in native fish populations in areas of introduction around the world (e.g. McDowall 2006). In the current study, there was low abundance of native fish species, specifically *E. treurensis* (96% lower), in areas where *O. mykiss* occurred. This is likely because of predation. This assertion is supported by observations from elsewhere that have shown that *O. mykiss* selectively consume small river minnows (McIntosh et al. 2010; Shelton et al. 2015a). In addition, other recent assessments have demonstrated that predation by *O. mykiss* can lead to the decline, and in some cases, the local extirpation of fish in invaded areas (Weyl et al. 2020). For example, *O. mykiss* has been implicated in the extirpation of the endangered boarder barb (*Amatolacypris trevelyani*) in the headwaters of the Keiskamma River system (Ellender 2013) and causing a decline in the abundance of native fish, such as the Breede River redfin (*Pseudobarbus burchelli*), the Cape kurper (*Sandelia capensis*), and the Cape galaxias (*Galaxias zebratus*) in the Breede River system (Shelton et al. 2015a).

Although *O. mykiss* had a demonstrable impact on native species at sampling sites below the Christmas Pool waterfalls where it was abundant, its effect was less pronounced at sites located farther downstream where it was less abundant. This is a well-known and characterised phenomenon in trout invasions globally, where impacts are more severe in river segments with high trout

Table 3: Mean values (± standard deviation) of water quality parameters recorded at sites where fish surveys were undertaken in the Blyde and Treur Rivers in Mpumalanga province, South Africa. pH values are given as a range (min–max)

River	Site	Temperature (°C)	pH	Conductivity (µS cm^{-1})
Blyde River	1	15.5 ± 2.3	7.8–9.0	21.9 ± 4.5
	2	15.5 ± 3.6	8.0–9.0	22.9 ± 8.3
	3	15.1 ± 5.0	8.0–8.4	23.2 ± 1.9
	4	16.3 ± 6.2	7.9–8.3	24.5 ± 1.4
	5	17.4 ± 2.8	8.1–8.5	71.9 ± 21.9
	6	17.4 ± 2.8	8.6–9.0	105.5 ± 28.3
	7	17.6 ± 2.9	8.5–9.0	140.3 ± 35.9
	8	17.2 ± 3.5	8.6–9.0	142.5 ± 35.0
Treur River	9	19.9 ± 7.1	8.2–8.8	52.4 ± 11.0
	10	19.6 ± 3.2	8.1–9.0	53.8 ± 6.7
	11	19.6 ± 3.3	8.5	49.5 ± 7.9

density than in segments with low densities (Woodford and Impson 2004; McIntosh et al. 2010; Shelton et al. 2015a). *Oncorhynchus mykiss* is a cold-water species that inhabits cool, well-oxygenated headwater streams and its abundance declines naturally with decreasing altitude (Rivers-Moore et al. 2019). This habitat preference may limit downstream migration to lower river sections that are characterised by higher water temperatures. In addition, the higher species richness observed in lower river sections, including native omnivores, such as *L. marequensis*, that are capable of piscivory (Lombard et al. 2018), may also explain the diminished vulnerability to *O. mykiss* predation observed in small-bodied fish, such as *E. lineomaculatus*. Furthermore, *E. lineomaculatus* is a widespread species occurring across the Limpopo basin, and it has coevolved with facultative piscivores like the African sharptooth catfish (*Clarias gariepinus*) and the canary kurper (*Chetia flaviventris*). This is in sharp contrast to *E. treurensis*, which being a headwater stream endemic is likely to be far more vulnerable to novel invasive predators. This contrast has been observed before with different responses to the largemouth bass (*Micropterus salmoides*) invasions by the Marico barb (*E. motebensis*), a headwater specialist, and the straight fin barb (*E. paludinosus*), a lowland specialist, in the Groot Marico River (Kimberg et al. 2014). Species rich river systems with multiple native top predators are therefore likely to be more robust to the addition of introduced predators (Finke and Denno 2004).

There are other river minnows that are known to occur in the upper Blyde River catchment that were not recorded in this study. These include the shortfin barb (*E. brevipinnis*) and the side spot barb (*E. neefi*), whose known distribution partly overlaps with trout angling areas in the catchment (Figure 1). This is of concern, especially for *E. brevipinnis* that is listed as near threatened, because of habitat loss and deterioration, and predation by alien fish, such as trout and bass (*Micropterus* spp.) (Engelbrecht et al. 2017). In addition, evidence from previous and ongoing taxonomic revisions of freshwater fishes in southern Africa is showing that some of the species that were considered to have wide distribution ranges comprise several narrow range endemic species (e.g. Chakona and Skelton 2017; Mazungula and Chakona 2021). Many of these are highly threatened. This could be the case for some of the species in the study area, such as *E. neefi* and the goldie barb (*E. pallidus*) (Engelbrecht et al. 2002; Chakona et al. 2015). This raises additional concerns on the potential impacts of trout invasions on the revised native species that are endemic, and range restricted to the river system.

Effect of environmental variables

Some of the differences in the fish assemblages among sampling sites were attributed to habitat differences related to altitudinal changes in river profiles, natural biogeographic barriers, such as waterfalls, that prevent upstream fish migration, and water quality variables. In the Blyde River, sampling sites located in the headwater river sections had low species richness relative to sampling sites located farther downstream and in the lower river sections. It has been suggested that the direct relationship between fish species richness and stream size can be attributed to the fact that larger streams tend to provide a wide variety of habitats that are able to support a greater diversity of species than smaller streams (Kadye and Marshall 2007; Weyl et al. 2013). For example, headwater sections of mountain streams, such as the Blyde River, have limited habitat diversity and are inhabited by a few habitat-specific species, such as *E. treurensis* and *A. uranoscopus*, that are known to prefer rocky pools, riffles of clear, and cool waters (Kleynhans 1987), whereas lower sections had relatively higher number of species and were characterised by species, such as *E. lineomaculatus* and *L. marequensis*, that tend to favour vegetated habitats and slow-moving water. In addition, the low number of fish species in the headwater sections of the Blyde River can also be attributed to the presence of the Christmas Pool waterfalls that forms a geographic barrier for upstream fish migration. Habitat connectivity is a key factor that determines the distribution of species in aquatic ecosystems (Rahel 2007). Physical barriers, such as waterfalls, rapids and steep gradients, impede the upstream movement of species and this often leads to different fish assemblages among isolated river reaches in freshwater systems (Dias et al. 2013). Well-known examples of waterfall barriers within the region that have promoted distinct ichthyofauna among isolated river reaches include the Augrabies Falls on the Orange River and the Victoria Falls on the Zambezi River (Skelton and Cambray 1981; Zengeya and Marshall 2008; Tweddle 2010). In addition, the instream barriers also protect biodiversity by preventing natural upstream dispersal of introduced alien fish. For example, waterfalls have prevented the upstream invasion of headwater streams by largemouth bass (*M. salmoides*) in the Groot Marico River system (Kimberg et al. 2014) and trout (*O. mykiss* and *S. trutta*) in the uKhahlamba Drakensberg Park (Karssing et al. 2012; Rivers-Moore et al. 2013).

Differences in the fish community structure may also reflect the effects of anthropogenic pollution in some sections of the upper catchment, as suggested by Kleynhans (1987). Fish abundance was markedly lower at sites (6–7) that were adjacent to point sources of pollution such as gold mining and sewerage discharge, that were characterised by higher conductivity values likely as a result of elevated nutrients and inorganic chemicals than at sites farther downstream of the pollution sources and sites located on the Treur River, which is considered to be in a near pristine state (Nel et al. 2011). Although species composition was broadly similar between the Blyde and Treur Rivers, the latter was characterised by species such as *A. uranoscopus* and *T. sparrmanii*, which have a wider altitudinal range and are known to tolerate a wide range of temperatures (Skelton 2001). Sampling sites located on the Treur River had a higher mean water temperature relative to sampling sites in the Blyde River. These differences in water temperatures can be related to differences in stream hydrology. Sites on the Treur River are relatively shallow and have a rocky riverbed and the elevated temperatures likely result from greater solar heating of the rocky riverbed surface.

Conclusions

This study demonstrated that the populations of native fish species, such as *E. treurensis*, that historically occurred throughout the upper catchment were greatly reduced and fragmented in the presence of *O. mykiss*. However, instream migration barriers, such as waterfalls, have prevented upstream migration of *O. mykiss*, and these invasion free areas have remnant populations of native fishes that appear to be largely intact. Alien fish species have often-circumscribed natural migration barriers through intentional stocking of upstream reaches by humans. This is of concern as stocking of trout in the upper Blyde River is still on going, as parts of the catchment are designated as trout angling areas. However, areas with remnant populations of *E. treurensis* have remained free of human facilitated alien fish introductions, because they were designated as natural heritage sites. This designation of areas where trout utilisation is allowed and prohibited appears to have diffused potential conflicts between utilisation of trout and conservation concerns, unlike other areas in the country (see Woodford et al. 2017 and Zengeya et al. 2017). It is therefore crucial that the conservation status of these alien-free areas is maintained and that they are regularly monitored and kept free of alien fish species for the conservation of endemic and range-restricted minnows that occur in the system.

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