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Out of the pot and into the fire: explaining the vulnerability of an endangered

small headwater stream fish to black bass invasion

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Evolved specialisations and invasive impact

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Abstract

Introduced predatory fishes have had consistently severe impacts on native fishes in stream environments around the world, although the drivers of these impacts are often unclear. In the Swartkops River headwaters in South Africa, native Eastern Cape redfin *Pseudobarbus afer* were always absent from sites occupied by non-native black bass *Micropterus salmoides* and *Micropterus dolomieu* but generally co-occurred with the native predators *Anguilla marmorata* and *Anguilla mossambica*. A natural experiment provided by flood-mediated recolonisation of black bass-occupied sites by *P. afer* demonstrated depletion in black bass invaded sites. Field behavioural observations of *P. afer* indicated that they foraged among benthic cover during the day but suspending in open water at night. As the nocturnal *A. marmorata* and *A. mossambica* foraged actively within structural cover at night and *M. dolomieu* and *M. salmoides* are diurnal or crepuscular predators, *P .afer* is thus optimised to avoid predation by native anguillid predators and not the functionally unique predatory black bass. The integration of distributional, temporal population dynamics and behavioural data suggests that the severe impacts of *Micropterus* spp. are likely a consequence of prey naïveté and behaviour evolved to evade native predators.

Key words: behaviour; endangered fish; evolutionary response; naiveté

INTRODUCTION

Fish communities in stream headwaters are among the most highly impacted by non-native fish invasions because they often comprise unique assemblages of small bodied specialist fishes (Meyer *et al.,* 2007). This is particularly apparent in Mediterranean climate region streams, where the introduced species diversity is often higher than that of natives (Marr *et al.,* 2010), native headwater fish are typically range restricted and isolated in headwater refugia (Henriques *et al.,* 2010; Marr *et al.,* 2010; Weyl *et al.,* 2014).

Impacts by introduced black bass *Micropterus* spp. Lacepède 1802 on small native fishes appear to be particularly severe. In the Raia tributary of the Tagus River in Portugal, for example, the presence of largemouth bass *Micropterus salmoides* (Lacepède 1802) was the best biotic predictor of native fish species assemblage structure, with small cyprinids being absent from reaches invaded by *M. salmoides* (Godinho & Ferreira, 2000). In the intermittently flowing streams of the Guadiana River system, predation by non-native fish was a major contributing factor to the decline of the small cyprinid *Anaecypris hispanica* (Steindacher 1866) which is consequently at high risk of extinction (Collares-Pereira *et al.,* 1999). In an assessment of 41 tributaries of the Olifants-Doorn River system in South Africa, van der Walt *et al.,* (2016) demonstrated that *Micropterus dolomieu* Lacepède 1802 and *Micropterus punctulatus* (Rafinesque 1819) had consistently extirpated small-bodied cyprinid minnows from invaded reaches. Similarly, since the introduction of non-native species to the Cosumnes River basin California (USA), there has been a gradual disappearance of native fish. In some areas invasive redeye bass *Micropterus coosae* (Hubbs & Bailey 1940) represented >90% of fish collected (Moyle & Williams, 1990; Moyle *et al.,* 2003).

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In Mediterranean climate region streams in South Africa the endpoint of invasions by predatory fishes into headwater streams has been relatively well documented and is consistent with invasions of similar streams in other regions globally (Ellender *et al.,* 2017). Invasions of streams in South Africa typically lead to complete extirpation of native fishes in invaded zones (Ellender *et al.,* 2011; Weyl *et al.,* 2014; van der Walt *et al.,* 2016). Consequently, headwater stream fishes are increasingly being isolated in small fragmented headwater refuges due to invasion by non-native fishes (Woodford *et al.,* 2005; Clark *et al.,* 2009; van der Walt *et al.,* 2016). One such case is the Blindekloof stream, a Swartkops River system headwater tributary, where the Eastern Cape redfin *Pseudobarbus afer* (Peters 1864) were isolated in headwater refugia and extirpated from invaded reaches further downstream by *M. salmoides* and *M. dolomieu* predation (Ellender *et al.,* 2011). Although the impacts of predatory fish invasions of headwater streams have been relatively well documented, the drivers responsible for the magnitude of impacts on headwater stream fishes remain unclear. This is further complicated by the context dependent nature of impacts, with very few rules on specific effects resulting from non-native species introductions (Ricciardi & Atkinson, 2004). While there is evidence to suggest that distinctiveness (Ricciardi & Atkinson, 2004), prey naiveté (a lack of defence toward a functionally unique predator) (Cox & Lima, 2006) and behaviour (e.g., Kadye & Booth 2014) are contributory factors, the ecological impact of biological invasions remains the most poorly understood aspect of the invasion process (Parker *et al.,* 1999; Kulhanek *et al.,* 2011).

This study integrates abundance, distributions and behavioural data to 1) document invasion at a system scale, 2) quantify impacts by documenting depletion of native fishes in invaded versus non-invaded stream reaches and, 3) uses behavioural observations to infer naiveté as a mechanism for the observed low tolerance of *P. afer*, a small, endangered, headwater stream fish, to non-native predatory fish invasions.

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MATERIALS & METHODS

STUDY SITE

The study was conducted in the headwaters of the Swartkops River system, a relatively short coastal river system that flows for 110 km from its source in the Grootwinterhoek Mountains (1000 m above sea level) before the river enters the Indian Ocean near the city of Port Elizabeth (Fig. 1). This study focussed on six first- and second-order (Strahler, 1957) headwater tributary streams (Blindekloof, Chaseskloof, Fernkloof, Nounekkloof, Vyeboomkloof and Waterkloof) of the Kwa-Zunga (third-order stream) tributary of the Swartkops River, South Africa. The headwater streams are free flowing, but the mainstem Kwa-Zunga River was dammed in 1933 to create the 96 ha Groendal Dam (Fig. 1). The stream catchments are otherwise relatively pristine and unaffected by human influence because they are located in a protected area, the Groendal Wilderness Area which forms part of the Baviaanskloof Mega-Reserve cluster.

DISTRIBUTION SURVEY (SURVEY 1)

To investigate the factors influencing distribution of native and non-native fishes at a system scale a total of 69 sites were sampled by backpack electrofishing and snorkel surveys during 2010. In order to obtain as comprehensive cover as possible, sampling sites were selected to cover the six headwater streams as well as the mainstream environment. At each sampling site, temperature, conductivity and pH were measured using a Hanna HI98129 Combo pH and electrical conductivity meter (HANNA Instruments Inc., Woonsocket, USA). Turbidity (NTU) was measured using a Hanna HI 98703 turbidimeter (HANNA Instruments Inc., Woonsocket, USA). To estimate pool volume and habitat diversity within each pool, the length of the pool was measured, followed by four to six equally spaced width measurements.

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On each width transect, three depths were measured, the outer two were each 0.2 m from the left- and right-hand stream bank and the third measurement taken midstream. At each depth measurement, the habitat type was recorded. Canopy cover was estimated as a percentage of total cover and bankside vegetation type was recorded.

At each site the choice of sampling method (snorkel survey or backpack electrofishing) depended on the dimensions of the sampling site. Snorkel surveys were conducted in streams where the depth of the sampled pools was >1 m, using a two-pass zigzag method as described in Ellender *et al.* (2011). Pass one was initiated from the tail end of the pool, with the observer swimming upstream, zigzagging to cover as much of the pool as possible. Pass two was a repeat of pass one but in a downstream direction. During each pass, all fish seen were identified to species level and counted. Sites where the average depth of the stream was <1 m were sampled by single pass electrofishing. Electrofishing was conducted using a Samus 725G backpack electrofisher (SAMUS, Poland), attached to a 12 V battery with settings standardized at the duration of 0.3 ms and a frequency of 80 Hz. The pass was conducted from the downstream side (tail) of the pool in an upstream direction, covering the entire length of each pool. Upon completion of the pass, fish were identified to species level, measured, counted, and released alive. Block nets were not used as pools were mostly isolated from each other. To ensure consistency and standardised methods of data collection, all snorkelling and electrofishing were undertaken by the same individual (BRE).

The necessity for using a multi-method approach for overall fish distribution surveys resulted in a lack of standardised catch data between streams. Therefore, for overall distribution data only presence/absence data were analysed using CANOCO v 4.5 (ter Braak & Smilauer, 2002). For analyses, data were separated into two matrices. The first consisted of the site specific presence/absence data, and the second were the environmental data. Environmental data were standardised with z transformation by transforming the original

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distribution to a mean of 0 and standard deviation of 1. Preliminary analyses using detrended correspondence analysis (DCA), indicated gradient lengths of >2 on the first axis, therefore unimodal ordination methods were chosen for analysis of overall distribution data in the form of correspondence analysis (CA, species data only) and canonical correspondence analysis (CCA, species-environmental relation) (ter Braak, 1995). Initially eight environmental variables were used in the CCA: canopy cover, altitude, instream barriers (natural waterfalls), maximum depth, habitat diversity, surface area, temperature and turbidity. Environmental variables with variance inflation factors (VIF) >10 were then removed from further analyses due to multicollinearity with other variables (ter Braak & Smilauer, 1998). A forward stepwise procedure was then used to determine the best predictor variables. The significance of these variables' contribution to the ordination was tested using 999 Monte Carlo simulations at the $P < 0.05$ level. To test the statistical significance of the selected environmental variables on the species presence/absence model, Monte Carlo permutation tests were used.

MONTHLY MONITORING (SURVEY 2)

Baseline distributions data indicated that at a system scale, no native fishes co-occurred with black bass. A major flood followed the baseline distribution surveys (Ellender & Weyl, 2015). Post flood exploratory surveys indicated that on the Blindekloof stream, a representative Swartkops River system headwater tributary, native fishes were distributed throughout the stream and now inhabited sites that were invaded by black bass. This provided an ideal opportunity to use this incursion of native fishes into black bass invaded reaches as a natural experiment to compare their abundance, distribution and population structure in the black bass "invaded" stream reaches to those in the non-invaded "control" stream reaches. To

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do this snorkel surveys were conducted monthly (November 2011-April 2012) to assess for the distribution range, relative abundance and population structure of native fishes.

For subsequent analysis, the Blindekloof stream was divided into 15 sites that represented three invasion states according to the presence/absence of black bass [Sites 1-4 represented the 'mixed zone' (no black bass, but other non-native species present), sites 5-9 the 'invaded zone' (black bass present) and sites 10-15 the 'non-invaded zone' (only native species present)]. Despite the episodic nature of the Blindekloof stream, pools are fed by groundwater and so surface area and volume remained fairly constant throughout the study. It was therefore assumed that effort at each monitoring site was also constant and comparisons between snorkel counts would be sufficient to reflect any temporal abundance changes.

Snorkel surveys were conducted using the zigzag method described for Survey 1, except that, in addition to species-level identification and enumeration during each pass, fish were also categorised into one of four predetermined L_T classes (0-15 mm, 15-30 mm, 30-60 mm, >60 mm). To estimate fish length, the snorkeler carried a slate with a ruler attached to it, where the snout and tail of the fish are aligned with the ruler (Cunjak & Power, 1986). To avoid measuring the same individual twice, length was estimated only on the first snorkel pass.

Two native predatory eels are found in the headwaters of the Swartkops River, the giant mottled eel *Anguilla marmorata* Quoy & Gaimard 1824 and the longfin eel *Anguilla mossambica* (Peters 1852). Both eel species are nocturnal and in order to get a representative sample of the relative abundance of native predators it was necessary to undertake night snorkelling. Identical methods were used during the day and at night, except that fish lengths were not estimated at night. A 400 lumen SL4 eLED® dive torch (Underwater Kinetics, California USA) was used for night observations. For those sites sampled during the day and at night, snorkelling was undertaken during the same 24 hr period (Thurow & Schill, 1996).

Night snorkelling commenced a minimum of 45 minutes after sunset in all months except in April 2012 due to equipment failure).

Data from the six month monitoring of the 15 monitoring sites were used to assess for temporal differences in abundance of *P. afer* irrespective of size class (Analysis 1), young of year (YOY $\leq$ 30 mm L_T;Analysis 2) and adults ($\geq$ 30 mm L_T ; Analysis 3). For size class comparisons only first-pass snorkelling data were used. First-pass data were considered representative as there was a highly significant correlation between *P. afer* abundance from the first and second passes (Spearman r = 0.908, *P* <0.001). These data were analysed using Linear Mixed Models in SPSS version 16.0 (IBM, Armonk, NY, USA). Abundance analyses were undertaken using restricted maximum likelihood models in SPSS. Fixed effects for all three analyses were specified as stream zone (mixed zone, invaded zone and non-invaded zone), sample months (1-6) were added as a covariate and site (1-15) was included as a random effect. Analysis 1 used overall *P. afer* abundance data (6 months). Analysis 2 used the number of *P. afer* young of the year (YOY). Analysis 3 used adult *P. afer* abundance. Main treatment effects were analysed using least significant difference (LSD) pairwise comparisons.

To test whether native eels were evenly distributed throughout the study reach (and therefore apply similar predation pressure throughout the study reach), the frequency of occurrence (% of sites within each stream zone where *A. mossambica* and *A. marmorata* were recorded) was tested for uniform distribution between stream zones using a χ^2 test of independence.

BEHAVIOUR (SURVEY 3)

Snorkel survey observations (BRE) of the diurnal and nocturnal behaviour of native fishes indicated that this might provide some insight into their vulnerability to introduced

predators. To investigate for diurnal differences in fish behaviour a survey of 32 sites on the Blindekloof stream was undertaken during the day and at night in November 2011 using identical methods to survey 2. To support behavioural observations using abundance data, day and night counts of *P. afer* were compared using a paired t-test (RStudio Team, 2015). All field research was reviewed by and conducted according to the ethical code for the field sampling of fishes set out by the South African Institute for Aquatic Biodiversity.

RESULTS

DISTRIBUTION

Eight fish species were recorded from the surveyed streams, four native [*P. afer,* kurper *Sandelia capensis* (Cuvier 1829), goldie barb *Enteromius pallidus* (Smith 1841), river goby *Glossogobius callidus* (Smith 1937)] and four non-natives [*M. salmoides*, *M. dolomieu*, banded tilapia *Tilapia sparrmanii* Smith, 1840 and African sharptooth catfish *Clarias gariepinus* (Burchell 1822)]. Of those sites where *P. afer* occurred (29 sites), they co-occurred with all species other than *black bass* (>60% with *S. capensis*; <11% with *G. callidus*, *E. pallidus*, *C. gariepinus* and *T. sparrmanii*). In the non-invaded stream reaches, *P. afer* were widespread, inhabiting uninvaded segments of five of the six surveyed streams (Blindekloof, Chaseskloof, Fernkloof, Nounekkloof and Waterkloof).

The CCA identified three distinct assemblages [(1) native: *P. afer* and *S. capensis*; (2) mixed: *E. pallidus*, *G. callidus, C. gariepinus* and *T. sparrmanii* and (3) black bass: *M. dolomieu* and *M. salmoides*)] explaining 98 % of the overall species-environment relation on the first two axes (Fig. 2). The CCA's explanatory variables (altitude, surface area and temperature) significantly accounted for 27.4% of the species-environmental variance (Monte Carlo permutation $P < 0.05$, of both the first axis and trace) (Table I). The "native"

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assemblage had high association with the "mixed" assemblage, but no association with the "black bass" assemblage.

CC Axis 1 suggested an association of black bass with altitude and surface area whereas *P. afer* and *S. capensis* were consistent throughout a range of altitudes and smaller surface area environments (Fig. 2). The CC Axis 2 suggested a strong positive relationship between species in the "mixed" assemblage with pool surface area, and higher temperatures and *P. afer* with lower temperatures (Fig. 2).

MONTHLY MONITORING

The overall abundance, relative abundance and distribution of fishes from the Blindekloof stream monitoring sites is summarised in Appendix S1. Nine species were recorded, six native (*P. afer, S. capensis*, *E. pallidus*, *G. callidus*, *A. marmorata, A. mossambica*) and three non-natives (*M. dolomieu*, *T. sparrmanii*, *C. gariepinus*). The 'mixed zone' was characterised by an assemblage consisting of the native species *P. afer*, *E. pallidus*, *G. callidus*, *S. capensis*, *A. mossambica* and *A. marmorata* and the non-native species *T. sparrmanii* and *C. gariepinus*. The 'invaded zone' consisted of five sampling sites, three of which were inhabited by *M. dolomieu* and two sites in between by native fishes. In the 'non-invaded zone' only *P. afer*, *S. capensis*, *G. callidus*, *A. mossambica* and *A. marmorata* were recorded.

The frequency of occurrence of the native predators *A. mossambica* (n = 11 sites) and *A. marmorata* (n = 8 sites) was not significantly dependent on stream zone (*A. marmorata*; $\chi 2$ test of independence: $\chi 2 = 2.81$, d.f. = 10, $P > 0.05$; *A. mossambica*: $\chi 2 = 0.61$, d.f. = 10, $P > 0.05$). Overall *P. afer* abundances did not differ significantly between stream zones ($F_{2,23.96} = 3.13$, $P > 0.05$) but followed a downstream gradient and were highest in the non-invaded zone (mean $\pm$ se; 108.6 ± 36.8 fish 100 m^2), lower in the mixed zone (25.1 ± 8.5 fish 100 m^2)

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and lowest in the invaded zone (19.7 ± 8.5 fish 100 m^2) (Fig. 3A; Analysis 1). Overall *P. afer* abundances differed significantly by sample month ($F_{1,34.56} = 33.55$, $P < 0.05$), but no significant interactions between sample month and stream zone were observed. Abundance in all three stream zones increased throughout the monitoring period, with lowest abundance in November 2011 (non-invaded zone: 149.9 ± 50.3 fish 100 m^2; invaded zone: 20.4 ± 9.0 fish100 m^2; mixed zone: 22.9 ± 7.4 fish 100 m^2) and highest abundances in January 2012 (non-invaded zone: 212.1 ± 56.6 fish 100 m^2 invaded zone: 52.7 ± 10.2 fish 100 m^2; mixed zone: 61.7 ± 11.7 fish 100 m^2) (Fig. 3A).

Changes in YOY abundance were significantly explained by stream zone ($F_{2,16.61} = 5.08$, $P < 0.05$), and an interaction between stream zone and sample month ($F_{2,28.48} = 3.72$, $P < 0.05$). The invaded (21.0 ± 7.2 fish 100 m^2; $P < 0.05$) and mixed (25.1 ± 6.9 fish 100 m^2; $P > 0.05$) zones were significantly lower than the non-invaded zone (96.2 ± 22.9 fish 100 m^2; $P > 0.05$), but not significantly different from each other (Fig. 3B; Analysis 2). Young of the year abundances were low during November 2011 (non-invaded zone: 103.5 ± 38.9 fish 100 m^2; invaded zone: 17.3 ± 7.2 fish100 m^2; mixed zone: 12.4 ± 3.6 fish 100 m^2), increasing monthly and peaking in January 2012 (non-invaded zone: 187.4 ± 49.0 fish 100 m^2; invaded zone: 55.4 ± 10.3 fish100 m^2; mixed zone: 56.1 ± 13.1 fish 100 m^2) (Fig. 3B).

Analysis of adult *P. afer* abundances indicated that stream zone ($F_{2,40.05} = 6.51$, $P < 0.05$), and sample month and stream zone interactions ($F_{2,72} = 5.76$, $P < 0.05$) were significant factors influencing adult abundance (Fig. 3C; Analysis 3). Adult *P. afer* abundances differed significantly between zones ($P < 0.05$), with lowest abundances recorded in the invaded (1.6 ± 0.7 fish 100 m^2), higher in the mixed (10.0 ± 1.8 fish 100 m^2) and highest in the non-invaded zone (39.1 ± 8.0 fish 100 m^2). There were no differences in overall *P. afer* abundance by month in the invaded zone ($P < 0.05$). In the non-invaded zone, there was a negative relationship between *P. afer* abundance and sample month, with abundance

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decreasing from November 2011 (46.4 $\pm$ 17.7 fish 100 m^2) to April 2012 (14.4 $\pm$ 5.3 fish 100 m^2), while in the mixed zone, *P. afer* abundance was dynamic, with abundances ranging from the lowest in February 2012 (4.4 $\pm$ 2.0 fish 100 m^2) to the highest in March 2012 (14.9 $\pm$ 4.4 fish 100 m^2) (Fig. 3C).

At sites within the invaded zone where *M. dolomieu* was consistently recorded, *P. afer* adult abundance decreased over time (Fig. 4A) compared to those pools where *M. dolomieu* was not recorded (Fig. 4B). During months of decreasing rainfall (November 2011-January 2012) *P. afer* abundance decreased to non-detectable levels in *M. dolomieu*-inhabited sites (November 2011 = 0.9 $\pm$ 0.9 fish 100 m^2 - February = 0 fish). Thereafter, flows following high rainfall in February and March resulted in *P. afer* again dispersing into these sites at very low abundances (0.2 $\pm$ 0.1 fish 100 m^2). In sites where *M. dolomieu* was absent, *P. afer* adult abundance was initially high during November 2011 (10.5 $\pm$ 1.0 fish 100 m^2), dropped to zero in December 2011 and thereafter fluctuated between January 2012 (2.5 $\pm$ 1.7 fish 100 m^2) and April 2012 (4.8 $\pm$ 1.5 fish 100 m^2). There was also a lag effect in the dispersal of adults into the invaded zone, with adults appearing two months after high rainfall during February and March 2012.

BEHAVIOUR

During the day *P. afer* were observed swimming in the water column and hiding in cracks and under rocks while at night they were predominantly suspended in the water column, well off the bottom of the stream. At night *A. mossambica* and *A. marmorata* were hunting on the bottom of the stream, examining cracks and crevices while they systematically covered a pool and neither species were observed during the day. The majority of surveyed sites (68.8%) had higher *P. afer* night compared to day counts. Day and night counts differed significantly (day: 18.0 $\pm$ 4.2 fish 100 m^2; night: 27.0 $\pm$ 5.6 fish 100 m^2; $P < 0.05$).

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DISCUSSION

Surveys under base flow conditions demonstrated that reaches invaded by black bass (then *M. salmoides* and *M. dolomieu*) were devoid of *P. afer* and other small native fishes, a finding consistent with their impacts on other headwater streams (Moyle *et al.,* 2003; Kimberg *et al.,* 2014; van der Walt *et al.,* 2017). By integrating approaches to investigate distribution, temporal population dynamics and behavioural data, the drivers of this observed impact could be further elucidated.

In the headwaters of the Swartkops River system, the three distinct assemblages identified under base flow conditions (native; mixed and black bass) were expected as biotic (predation, competition) and abiotic features of streams over any spatial scale (stream reach or stream order) typically result in variable species composition of fish assemblages (Angermeier & Winston, 1999; Jackson *et al.,* 2001; Smith & Kraft, 2005). There was considerable overlap between the mixed (*B. pallidus*, *G. callidus*, *T. sparrmanii*, *C. gariepinus*)and native (*P. afer*, *S. capensis*) assemblages, but species from neither assemblage co-occurred with the black bass assemblage. Associations between the three assemblages and both physical (surface area and altitude) and physico-chemical (temperature) factors were weak, therefore, separation on an environmental gradient did not adequately explain the absence of both the mixed and native assemblage in black bass invaded zones. The three distinct assemblages are postulated to be controlled by predation, as has been found in other headwater stream environments invaded by black bass (Schlosser, 1987; Godinho & Ferreira, 2000; MacRae & Jackson, 2001).

Piscivory can be a major contributor to the variation and structure of stream fish assemblages (Jackson *et al.,* 2001; Moyle *et al.,* 2003) as is demonstrated by this study where the presence of black bass resulted in the absence of all other fishes from invaded zones.

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Similar results have been reported from other studies on invaded river systems where black bass introduction has resulted in two distinct assemblages, one dominated by small native fishes and the other by large-bodied *Micropterus* spp. (Godinho & Ferreira, 2000; MacRae & Jackson, 2001; van der Walt *et al.,* 2017). In *Micropterus*-dominated assemblages, the number of other species present has also been shown to be directly related to habitat complexity and heterogeneity, providing refuge from predation (Tonn & Magnuson, 1982; Schlosser, 1987; MacRae & Jackson, 2001; Moyle *et al.,* 2003). Swartkops headwater tributaries are episodic and pools are predominantly fed by interstitial flows and isolated on the surface. During dry periods (> 95% of the time) native species are therefore confined with predatory black bass in invaded pools. For fishes inhabiting episodic environments, their persistence relies on refugia not prone to desiccation (Magoulick & Kobza, 2003), but if these are occupied by invasive predatory fishes (as was the case on the Blindekloof stream), the persistence of the species is severely compromised. This has been demonstrated in systems with seasonal rainfall fluctuations, such as the Cosumnes River, California, USA (Moyle *et al.,* 2003) and the Guadiana River Basin in Portugal (Collares-Pereira *et al.,* 1998), where seasonal droughts results in the extirpation of native fishes from isolated pools inhabited by black bass.

In situations where black bass invasion results in the total extirpation or exclusion of native fishes from invaded zones, impacts beyond loss of habitat become difficult to quantify. An above average rainfall year, however, provided an opportunity to corroborate stable state findings after *P. afer* recolonised black bass-invaded stream reaches, that were previously devoid of small native fishes. Over the monitoring period in the Blindekloof stream, overall abundances of *P. afer* in pools inhabited by *M. dolomieu* were not significantly lower than in the non-invaded zone in the upper stream reaches, as well as in the mixed zone in the lower reaches. There were, however, size specific differences in temporal abundance between

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stream zones. The maintenance of *P. afer* in the invaded zone was most probably a result of dispersal into invaded stream reaches from non-invaded source populations. Similar meta-population dynamics, where the maintenance of native fishes in invaded zones is due to immigration from non-invaded source populations, have been observed for Canterbury galaxias *Galaxias vulgaris* Stokell 1949 in salmonid invaded streams in New Zealand (Woodford & McIntosh, 2010, 2011). Specifically, *G. vulgaris* only persisted in reaches invaded by *Oncorhynchus mykiss* (Walbaum 1792) and *Salmo trutta* Linnaeus 1758 when invader-free source populations were present (Woodford & McIntosh, 2011).

In the Blindekloof stream, YOY *P. afer* increased in abundance over the sampling period, as this corresponded to the summer spawning season for this species (Cambray, 1994). Despite the extremely low abundance of adult *P. afer* in the invaded zone, YOY *P. afer* abundances did not differ significantly between invaded and non-invaded zones. This is contrary to findings by Godinho & Ferreira (2000), who found that in the Raia stream in Portugal, juvenile abundance of native fishes was significantly lower in stream reaches invaded by non-native *M. salmoides* and pumpkinseed *Lepomis gibbosus* (Linnaeus 1758).

In the case of the Blindekloof, the low abundance of *M. dolomieu* in invaded zones and the massive recruitment of YOY *P. afer* into the invaded zone probably resulted in a lag period between the influx and any noticeable changes in abundance due to predation over the relatively short duration of the study. In the mixed zone similar increases in YOY *P. afer* abundances were observed, but they were significantly lower than in the invaded and non-invaded zones. Two possible causes for this are: a decreased adult abundance in the mixed zone, resulting in poor recruitment (and thus lower downstream dispersal) , or variable reproductive potential in stream habitats due to physical or environmental factors making them less favourable in these respects than other stream reaches (Labbe & Fausch, 2000).

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Adult *P. afer* abundance differed significantly between stream zones, with the low abundance in the invaded zone, intermediate abundance in the mixed zone and high abundance in the non-invaded zone. *Pseudobarbus afer* adult abundance also displayed temporal changes between zones. In the Blindekloof stream, the invaded zone may therefore be acting as a filter/sink, resulting in reduced abundances of *P. afer* adults downstream, as this longitudinal decrease in abundance was not observed from the adjacent non-invaded Fernkloof and Waterkloof tributaries of the Swartkops River system (Ellender & Weyl, 2015). The impacts of other non-native fishes (e.g., *T. sparrmanii* and *C. gariepinus*) on *P. afer* recruitment in the mixed zone can also not be discounted. There were, however, indications of adult dispersal from the upper to lower stream reaches.

In the non-invaded zone in the upper stream reaches there was a negative relationship between abundance and sample month, and concomitantly the opposite was true for the mixed zone in the lower stream reaches, where there was an accumulation of *P. afer* adults over time. Despite the net movement of *P. afer* adults downstream, which continually flushed individuals through the invaded zone, by the end of the study adult *P. afer* were once again rare in the invaded zone, and absent from the pool with the highest abundance of *M. dolomieu.* In pools where *M. dolomieu* were consistently recorded, *P. afer* abundance decreased rapidly to undetectable levels within 3 months. As a result of flows associated with a high rainfall month resulted in adult *P. afer* being observed again in the invaded zone. Within the invaded zone, but at sites where *M. dolomieu* was not recorded, the opposite was true, and *P. afer* abundance increased over time to >15 times that recorded at invaded sites.

Whether *P. afer* naturally displays source-sink population dynamics [where the upstream reaches act as a source and downstream reaches a demographic sink; (Pulliam & Danielson, 1991; Dias, 1996)] is not known. Alternatively, as has been documented for the Arkansas darter *Etheostoma cragini* Gilbert, 1885 in intermittent Colorado plains streams;

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(Labbe & Fausch 2000)], *P. afer* may migrate downstream to use inundated habitats and migrate back upstream into refuge habitats as the stream dries up before the onset of the dry season [. In the case of *E. cragini* the ability to disperse between refuge pools and habitats prone to seasonal drying was shown to be vital for rearing young of the year, which highlights the importance of connectivity at much finer spatial scales than previously considered (Schlosser, 1991; Labbe & Fausch, 2000; Fausch *et al.,* 2002). If *P. afer* display similar behaviour, the biological barrier created by the presence of black bass in certain stream segments may inhibit both dispersal and migration between important habitats. This seems most likely to impact adult *P. afer*, which exhibited the highest vulnerability to black bass invasion.

A question arising is why native predators appear to have lower impacts than non-native predators. Separating the impact of non-native black bass and native predators (*A. mossambica*, *A. marmorata* and *S. capensis*) on the *P. afer* population is complex, and some sort of natural predation pressure on *P. afer* has to be realistically assumed as all three species are reported to eat fish (Bruton *et al.,* 1987). *Anguilla marmorata* and *A. mossambica* were ubiquitous in headwater streams inhabited by *P. afer*. Having co-evolved in these headwater streams could explain why *P. afer* may possibly also be less impacted less by these native predators, than they are by non-native predators.

Naïveté by native fishes to introduced predators has been shown to result in large impacts on native fishes (Whitlow *et al.,* 2003; Cox & Lima, 2006). From laboratory experiments Kadye & Booth (2014) eluded to inactivity and use of structure at night as evolved behaviour by *P. afer* to avoid native eel predation. During this study nocturnal field observations of *P. afer* position in the water column provide additional insight into this. While observing *P. afer* behaviour during day and night snorkelling surveys conducted on the Blindekloof stream it was apparent that at night, *P. afer* did not use structure but was

predominantly suspended in the water column, well off the bottom of the stream while during the day, individuals were observed swimming in the water column and hiding in cracks and under rocks. At night, eels were observed hunting on the bottom of the stream, examining cracks and crevices while they systematically covered a pool. This suggests that the suspension in the water column is most likely an evolved predator-avoidance behaviour that might reduce predatory impacts by native eels. As predatory success of black bass has been shown to be negatively affected by habitat complexity (e.g., Savino & Stein, 1982; Alexander *et al.,* 2015) such behaviour increase the vulnerability of *P. afer* to predation.

In conclusion, evidence suggests that the invasion of *M. dolomieu* in the Blindekloof stream may not disrupt YOY dispersal and recruitment, but rather acted as an effective filter reducing adult abundance, and hence also recruitment downstream. It remains to be seen whether *P. afer* can indefinitely maintain the current isolated metapopulations that characterise its distribution due to black bass invasions. The only way to accurately determine long term impact would be to establish whether these populations can maintain themselves, or whether they rely on inputs from others within the stream network. To effectively conserve small native stream fishes, a multilevel understanding of population processes at multiple scales (from reach to system) is needed to prioritise efforts for remediation and rehabilitation (Schlosser & Angermeier, 1995; Labbe & Fausch, 2000).

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