



Inter-seasonal persistence and size-structuring of two minnow species within headwater streams in the Eastern Cape, South Africa

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Summary

This study examined temporal variation in population dynamics and size structuring of two cyprinid minnows, *Pseudobarbus afer* and *Barbus anoplus*, in relation to their proximate physical habitats. Population estimates were determined using three-pass depletion sampling during both summer and winter. The habitats were characterised by seasonal variation in all physico-chemical conditions and spatial variation in substrata compositions. Whereas significant differences in population size were noted between seasons for *B. anoplus*, no differences were found between seasons for density and capture probability for either species. An increase in boulders was associated with increase in population size and density for *P. afer*; for *B. anoplus*, increased percentages of bedrock and bank vegetation were associated with an increase in population size and probability of capture, respectively. According to Canonical Correspondence Analysis, size structuring in *P. afer* was explained predominantly by seasonality, with smaller length classes associated with the seasonal variable of summer, while larger length classes were associated with pH that was higher in winter. By comparison, for *B. anoplus*, the habitat variables – bank vegetation and bedrock – accounted for much of the explained variance for size structuring. Recruitment appeared to be the major driver of size structuring for the two species; refugia, especially boulders and bank vegetation, also appeared to be important. Overall, the two species were adapted to the headwater streams that were generally variable in environmental conditions. Potential invasions by non-native invasive fishes that occur within the mainstream habitats threaten these two species. Efforts should continue to protect these minnows from such invasions by constructing barriers to upstream migration of non-native fishes into these headwater habitats.

Introduction

Conservation of stream fishes requires an understanding of factors that influence population dynamics. In streams, physical habitat is often as regarded the primary factor determining fish population structure (Niaman and Latterell, 2005). The relationship between physical habitat and fish population structure has been demonstrated at various levels, including *inter alia* patterns associated with spatial and temporal changes (Jackson et al., 2001; Ostrand and Wilde, 2002), stream size (Zorn et al., 2002), microhabitat (Grossman and Ratajczak, 1998), physico-chemical environment (Casatti et al., 2006), and spatial autocorrelation (Wilkinson and Edds, 2002). Schlosser (1991) further suggested that, from a landscape ecology perspective, stream physical habitat

is a template upon which the observed patterns in fish populations are structured. Patterns in fish and habitat relationships are critical in headwater streams that, in addition to being relatively small, are subject to both high temporal and spatial variability.

Population structure of fish in headwater streams is usually considered to be non-random, and a result of a balance between net energy gain and potential risk (Capone and Kushlan, 1991; Schlosser, 1991). Net energy gain is a trade-off between food availability and swimming costs. Risk is associated with predation and unfavourable conditions especially where streams undergo periodic drying and lose connectivity with adjacent aquatic habitats (Capone and Kushlan, 1991). Predation risk in headwater streams can also be size-dependent. High predation risk in shallow habitats is for large and visible fish from terrestrial and wading predators, and in deep habitats for small fish from swimming predators (Power, 1987; Schlosser, 1988). Stream fish therefore select habitats that provide refuge and permit survival in harsh environmental conditions (Koehn et al., 1994). Population stability in these streams, based on density and relative abundance may, however, vary in response to severity of environmental conditions in habitats where populations are able to persist in both benign and harsh conditions (Keaton et al., 2005). In extreme cases, temporal variation in physical conditions can be substantial and may be the dominant factor influencing population structure (Ostrand and Wilde, 2002). The importance of local physical habitat factors in structuring populations is likely to depend on temporal variation and environmental extremes. Survival and maintenance of viable populations under environmental extremes would therefore depend on an individual species' ability to withstand, as habitat specialists, the environmental fluctuations, or to utilise, as habitat generalists, different habitats, or to recolonise when environmental conditions improve (Erős and Grossman, 2005).

The headwater streams of the Great Fish and the Sundays rivers in the Eastern Cape, South Africa provide an excellent opportunity to study fish and their relationship to their proximate habitat. Two indigenous species of cyprinid minnows, Eastern Cape redbfin, *Pseudobarbus afer*, which is listed on the IUCN Red List as endangered, and chubbyhead barb, *Barbus anoplus*, are the most widespread in the headwater streams of the Sundays and the Great Fish rivers, respectively. The streams are subject to both spatial and temporal variation in environmental conditions, with most habitats occurring as isolated pools during the dry season. The two minnows are threatened by alien invasive species, especially sharp-tooth catfish, *Clarias gariepinus*, that have established in the mainstream sections of the two rivers where these

minnows have generally disappeared. The piscivorous catfish is potamodromous and migrates upstream when rivers flood. Because of their large body size, the catfish are likely to occupy deep habitats and persist in the isolated pools that serve as refuge for the minnows during the dry season. This is likely to pose a serious threat to the populations of the indigenous minnows, as observed with the occurrence of other invasive fish in the region (Lowe et al., 2008). Previous studies already indicate the occurrence of the catfish in the headwater streams, including habitats with the indigenous minnows (Weyl et al., 2009).

The conservation priority criteria proposed by Weyl et al. (2009) indicated that the headwater streams of the Sundays River, in particular, are critical conservation priority areas for the endangered *P. afer*. Continuous monitoring of distribution and density, and an evaluation of the critical habitats for the indigenous minnows is therefore essential. A quantitative measure of the fish densities in these headwater streams is crucial in achieving this goal. Depletion sampling provides an opportunity to estimate population parameters such as absolute densities and probabilities of capture (Peterson et al., 2004) and an evaluation of population structure and their environmental correlates. Thus, the objectives of this study were to (1) evaluate the population densities and examine the influence of physical habitats and physico-chemical environment on the two widespread minnows, *P. afer* and *B. anoplus*, and (2) examine the size structuring of the populations in relation to physical habitat and temporal variation.

Materials and methods

Study area

Pseudobarbus afer was collected in the tributaries of the Sundays River in the Zuurberg Mountains within Addo Elephant National Park (AENP). Three other species, *Barbus pallidus*, *Glossogobius callidus* and *Anguilla mossambica* co-occurred with *P. afer* in the Zuurberg. *Barbus anoplus* was collected in the headwaters of the Koonap River, a tributary of the Great Fish River, in the Winterberg Mountains. *Anguilla mossambica* was also present in the Winterberg. The AENP has a warm temperate climate. Mean annual rainfall is relatively low (about 480 mm), falling mostly in late summer (March–May) and late winter (August). Temperatures range from 15 to 45°C in summer and 5–18°C in winter, with an annual mean of 18°C (Lombard et al., 2001). The Zuurberg Mountains, which are central to the national park, are part of the Cape Fold Mountains that are predominantly quartzite and sandstone sediments. The greater part of the AENP is dominated by unconsolidated cretaceous sediments that weather to form deep red to orange-brown fine-grained and relatively fertile soils (Macvicar, 1991). Quartzite and shale fynbos are the dominant vegetation types in the Zuurberg Mountains. Spekboom thickets, comprising leaf-succulent shrubs and evergreen sclerophylls, dominate the greater part of the national park. Other vegetation types include Karoo-bushveld, mixed-shrub and grassveld (Lombard et al., 2001). The Sundays River is the major river within the AENP. Major tributaries of the Sundays River include the Kabouga, the Uie and its tributaries, Groot and Klein, the Wit and the Krom. The Winterberg Mountains have a warm temperate climate, with an average annual rainfall of 426 mm, and summer and winter

maximum temperatures of 29 and 19°C respectively (van Zyl, 1994). Frost occurs regularly between April and September, and snow may fall in winter on the plateau. The underlying geology consists of shales and sandstones of the Beaufort series of the Karoo, while the Winterberg Plateau is comprised of resistant dolerite. Vegetation is a mosaic of several veld types including thornveld and grassveld that are mixed with dwarf *Acacia karoo*. A veld type called sourveld, which is dominated by *Thermida triandra* and other grasses, dominates the plateau slopes (Meadows and Meadows, 1988). Montane forest trees occur in deep soils in valleys and escarpment slopes. The major tributaries of the Great Fish River in the Winterberg include the Koonap River and the Kat River. Livestock farming is the major activity in this area.

Data collection

Three-pass depletion electric fishing was conducted at 21 sites for *P. afer* and 19 sites for *B. anoplus* (Fig. 1). Sites were sampled in both summer (February–May 2010) and winter (July–September 2010). Before sampling, a section of each site was blocked with 4 mm mesh nets that were secured to the streambed. At each site, temperature (°C), pH, total dissolved solids (TDS) (ppm) and conductivity ($\mu\text{S cm}^{-1}$) were measured with a HANNA HI 98129 combo meter, and turbidity (NTU) was measured using a HANNA HI 98703 turbidity meter. Fish were captured using a Samus 725GN backpack electric fisher powered by a 12 V battery. Captured fish from each pass were kept in separate buckets containing stream water. All fish were counted and measured [total length (TL) to a maximum of 50 individuals of the total catch per site] and released. To minimise bias, fish were scooped with a hand-held net from the buckets before measurement for length. After fish sampling, ten transects were set perpendicular to the direction of flow at each site, to measure physical habitat variables. The measurements made were depth, substrate type, width for each transect, and total length for the sampled section. Starting from the wetted edge, water depth was measured on three points (near the margins and at the centre of each transect) and the dominant substrate at each point was visually characterised within a radius of 25 cm. Substrate types were categorised following Gorman and Karr (1978) and Schlosser (1982) as silt (<0.05 cm), sand (0.05–2 cm), gravel (2–10 cm), pebble (10–30 cm), boulder (30–50 cm) and bedrock (>50 cm). There were therefore 30 points assessed for depth and substrate composition at each site. The proportion (%) of each substrate category was determined from these points at each site. Bank vegetation was determined from all points at the end of each transect ($n = 20$) and expressed as the proportion of points with overhanging or marginal vegetation from the total points. In addition, the total area sampled, and average depth and volume were calculated for each site.

Data analysis

Seber's (1982) maximum likelihood approach was used to estimate fish number, \hat{N} , and probability of capture, \hat{p} . Therefore, given a vector $n = (n_1, n_2, n_3)'$ of three observed removals from a population, the maximum likelihood estimates (MLEs) of \hat{N} and \hat{p} were obtained by maximising a multinomial likelihood function of the form:

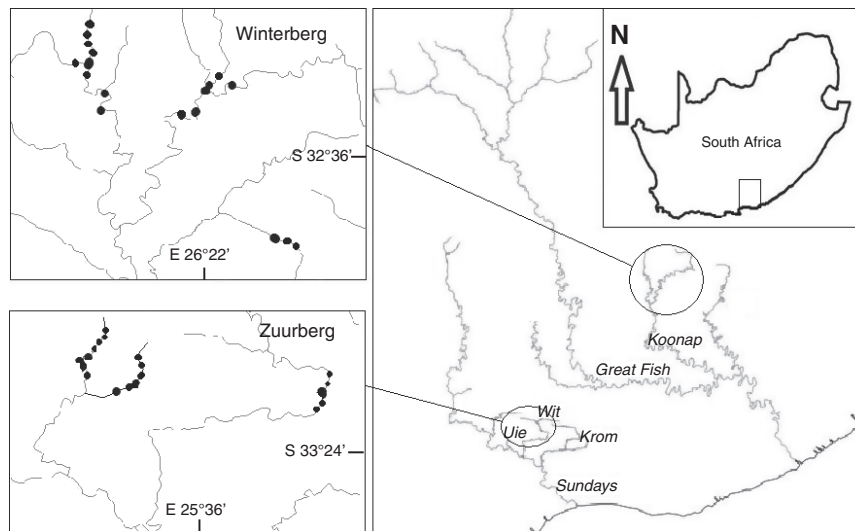


Fig. 1. Map of study area showing main rivers and sites of fish sampling in headwater streams, Zuurberg and Winterberg mountains, Eastern Cape, South Africa

$$L(\mathbf{n}|N, k) = \frac{N!}{\left(\prod_{i=1}^3 n_i!\right) (N - n_*)!} p^{n_1} (qp)^{n_2} (q^2p)^{n_3} \times (1 - p - qp - q^2p)^{N - n_*},$$

where $q = 1 - p$ and $n_* = \sum_{i=1}^3 n_i$.

The model assumes that (i) the population being sampled is closed to migration, recruitment and mortality, and that individuals have the same probability of capture for a unit of effort throughout the experiment, (ii) units of effort are independent and additive, and (c) all removals from the population are known (Seber, 1982).

The per capita seasonal change in abundance, j , was calculated as $\ln(\hat{N}_{t+1}/\hat{N}_t^{-1})$ where \hat{N}_t is the estimated abundance in season t . Potential density-dependence was examined by the relationship between j and $\ln \hat{N}_t$ (Wootton, 2007).

As the data are dependent on one another, with the same pools sampled in both summer and winter, a linear mixed-effects model assessed the relationships between several environmental factors and population size, density and capture probability. Density, \hat{D} , was expressed as number of fish m^{-2} . Population size, density and capture probability were z -transformed prior to the analysis.

Given the i th observation from the p th pool in the s th season, the effect of one or more environmental variables, β_k , the model has the form:

$$y_{iskp} = \mu + \alpha_s + \beta_k + \delta_p + \varepsilon_{iskp}$$

where $\varepsilon_{iskp} \sim N(0, \sigma^2)$ is the residual error, and $\delta_p \sim N(0, \sigma_p^2)$ is the random effect. In the case where β_k is removed from the model, the results are equivalent to a paired t -test. The analyses were conducted using package NLME in R (R Core Development Team, 2011).

To determine size structure, fish length data for each species were categorised into the arbitrary length classes: L1 (<20 mm TL), L2 (20–29 mm TL), L3 (30–39 mm TL), L4 (40–49 mm TL), L5 (50–59 mm TL), L6 (60–69 mm TL), L7 (70–79 mm TL), L8 (80–89 mm TL), L9 (90–99 mm TL) and L10 (>100 mm TL). Each length class was expressed as

a proportion of all categories. Sexual maturity was 40 mm TL for both species (Cambray and Bruton, 1985; Cambray and Hecht, 1995). Fish lengths were therefore categorised as immature (<40 mm) and mature (>40 mm) for length frequency analysis.

A non-linear ordination method, Canonical Correspondence Analysis (CCA), was used on the standardised proportions of length categories and environmental variables to identify fish size and habitat relations. Environmental variables were physical habitat variables (H) and seasonal variable (S). Environmental variables with variance inflation factor (VIF) >10 indicate multicollinearity with other variables (ter Braak and Smilauer, 1998), and thus eliminated from the analysis. A forward selection procedure was then applied to select the best predictor variables. The significant contributions of these variables to the ordination were tested in Monte Carlo simulations at the $P < 0.05$ level. A Monte Carlo permutation test was also used to test the statistical significance of the relationship between species and environmental variables. The total variation in size composition was partitioned using partial CCA as follows: (i) variation accounted for by all environmental variables (using each of the species data and environmental variables (habitat and sampling seasons) [$H \cup S$]), (ii) variation accounted for by habitat variables after partialling out the influence of sampling seasons (pure habitat [$H|S$]), (iii) variation accounted for by sampling seasons after partialling out the effect of habitat variables (pure seasonal [$S|H$]), (iv) variation jointly explained by both groups ($H \cap S$), and (v) unexplained variation. Each component of the variation was obtained by dividing the canonical eigenvalue of the particular CCA by the total inertia. Ordinations were conducted in CANOCO v4.5. All environmental variables were standardised to mean zero and unit variance prior to the analyses.

Results

The maximum estimated population sizes (\hat{N}) were 238 and 284 fish in summer and winter, respectively, (Table 1) with no significant differences between seasons (paired $t_{17} = -0.70, P > 0.05$) for *P. afer*, and 128 and 152 fish in summer and winter, respectively, with significant differences between

Table 1

Estimated capture parameters, total number of fish captured (N_T), estimated initial population (\hat{N}), probability of capture (\hat{p}) and density of fish m^{-2} (\hat{D}) and their associated asymptotic standard errors

Species	Site	Summer				Winter			
		N_T	\hat{N}	\hat{p}	\hat{D}	N_T	\hat{N}	\hat{p}	\hat{D}
<i>Pseudobarbus afer</i>	1	137	147.6 ± 5.6	0.6 ± 0.05	0.8	77	93.2 ± 10.4	0.4 ± 0.09	0.8
	2	7	7.0 ± 0.0	0.8 ± 0.00	0.1	6	6.0 ± 0.0	0.7 ± 0.00	0.1
	3	58	58.0 ± 1.0	0.8 ± 0.06	2.5	26	26.3 ± 1.3	0.7 ± 0.10	0.6
	4	233	238.0 ± 3.0	0.7 ± 0.03	1.8	49	50.6 ± 2.1	0.7 ± 0.08	0.7
	5	57	58.0 ± 1.7	0.7 ± 0.07	1.1	102	104.4 ± 1.3	0.7 ± 0.05	1.8
	6	19	19.0 ± 2.4	0.8 ± 0.25	0.2	59	59.0 ± 1.0	0.8 ± 0.06	0.6
	7	29	29.0 ± 1.2	0.8 ± 0.10	1.5	8	8.0 ± 0.0	0.7 ± 0.00	0.2
	8	40	41.0 ± 1.8	0.7 ± 0.09	0.8	9	9.0 ± 1.6	0.6 ± 0.26	0.1
	9	45	45.1 ± 1.0	0.8 ± 0.07	3.3	29	29.7 ± 1.6	0.7 ± 0.10	0.2
	10	101	102.7 ± 1.9	0.7 ± 0.05	4.9	222	228.6 ± 1.6	0.7 ± 0.03	3.1
	11	111	111.0 ± 0.9	0.8 ± 0.04	3.8	260	262.7 ± 2.2	0.8 ± 0.03	3.5
	12	117	118.9 ± 2.0	0.7 ± 0.04	1.4	267	284.9 ± 6.9	0.6 ± 0.04	5.4
	13	22	22.0 ± 1.3	0.8 ± 0.13	1	49	54.3 ± 4.7	0.5 ± 0.10	1.4
	14	16	16.6 ± 1.8	0.6 ± 0.15	0.3	59	59.0 ± 0.9	0.8 ± 0.06	2.4
	15	81	81.8 ± 1.4	0.8 ± 0.05	1.5	62	62.2 ± 1.1	0.8 ± 0.06	2.2
	16	12	12 ± 1.3	0.7 ± 0.18	0.2	10	10.0 ± 1.3	0.6 ± 0.19	0.3
	17	23	23.0 ± 1.1	0.7 ± 0.11	0.3	22	26.9 ± 6.3	0.4 ± 0.16	0.8
	18	4	4.0 ± 0.0	0.8 ± 0.00	0.1	1	–	–	–
	19	0 ^a	–	–	–	19	19.0 ± 1.5	0.8 ± 0.16	4.7
	20	0 ^a	–	–	–	24	26.7 ± 3.6	0.5 ± 0.14	1.6
	21	0 ^a	–	–	–	13	13.0 ± 1.4	0.7 ± 0.18	0.2
<i>Barbus anoplus</i>	1	36	37.8 ± 2.4	0.6 ± 0.10	0.3	8	8.0 ± 6.3	0.7 ± 1.05	0.1
	2	79	99.6 ± 13.0	0.4 ± 0.09	1.7	28	28.4 ± 1.4	0.7 ± 0.10	0.5
	3	25	25.7 ± 1.7	0.6 ± 0.11	0.5	19	21.8 ± 4.1	0.5 ± 0.16	0.4
	4	56	56.0 ± 0.9	0.8 ± 0.06	0.7	15	15.7 ± 2.0	0.6 ± 0.16	0.2
	5	5	5.0 ± 0.0	0.8 ± 0.00	0.1	2	–	–	0.5
	6	69	71.4 ± 2.5	0.7 ± 0.07	1.1	17	17.0 ± 1.1	0.7 ± 0.13	0.1
	7	126	126.8 ± 1.4	0.8 ± 0.04	1.7	135	152.4 ± 8.6	0.5 ± 0.06	2.6
	8	11	11.0 ± 1.8	0.7 ± 0.26	0.1	1	–	–	0.6
	9	56	60.8 ± 4.1	0.6 ± 0.08	0.7	39	39.8 ± 1.6	0.7 ± 0.08	0.4
	10	74	79.8 ± 4.3	0.6 ± 0.07	0.6	30	32.4 ± 3.1	0.6 ± 0.12	0.3
	11	114	128.1 ± 7.5	0.5 ± 0.06	1.1	22	22.0 ± 1.1	0.7 ± 0.11	0.3
	12	10	10.6 ± 2.1	0.5 ± 0.21	0.4	6	6.0 ± 0.0	0.8 ± 0.00	0.2
	13	16	16.0 ± 1.4	0.7 ± 0.17	0.3	12	12.0 ± 0.0	0.85 ± 0.00	0.3
	14	18	18.0 ± 2.3	0.8 ± 0.25	0.3	16	16.0 ± 1.2	0.7 ± 0.15	0.5
	15	10	10.0 ± 1.7	0.7 ± 0.26	0.3	– ^b	–	–	–
	16	4	4.0 ± 0.0	0.8 ± 0.00	0.1	– ^b	–	–	–
	17	8	8.0 ± 1.5	0.6 ± 0.26	0.1	– ^b	–	–	–
	18	14	14.0 ± 1.2	0.7 ± 0.15	0.1	101	114.5 ± 7.6	0.5 ± 0.07	1.8
	19	5	5.0 ± 0.0	0.7 ± 0.00	0.1	– ^b	–	–	–

^aWater present but no fish sampled within the pools.

^bPools had dried up, no fish sampled.

seasons (paired $t_{14} = 2.64$, $P < 0.05$) for *B. anoplus*. Adults were not significantly different in number for *P. afer* but were significantly different (paired $t_{14} = 2.30$, $P < 0.05$) for *B. anoplus* between seasons. *P. afer* densities ranged from 0.1–5.0 to 0.1–5.7 fish m^{-2} in summer and winter, respectively (Table 1), with no significant differences (paired $t_{17} = 0.11$, $P > 0.05$) between sampling seasons. By comparison, *B. anoplus* densities were lower and ranged between 0.1–1.7 fish m^{-2} in summer and 0.1–2.6 fish m^{-2} in winter (Table 1), with no significant differences between seasons. Adult densities were not significantly different between seasons for either species. Capture probability ranged between 0.4 and 0.8 for both *P. afer* and *B. anoplus* (Table 1), with no significant differences between seasons. There was no significant relationship between per capita seasonal change in abundance and summer abundance for either species.

Water temperatures were higher in summer, with averages of 23.6 and 23.1°C, than in winter, with averages of 14.1 and 10.3°C at the Zuurberg and Winterberg sites, respectively (Table 2). The pH was neutral in summer (7.1) and alkaline

in winter (8.0) in the Zuurberg, and alkaline in both summer and winter (8.1 and 8.4 respectively) in the Winterberg. Total dissolved solids (TDS) and turbidity were all higher in summer than winter in the Zuurberg. By comparison, the Winterberg had higher TDS in winter than summer, while turbidity was higher in summer than winter (Table 2). Conductivity was higher in winter than summer in both the Zuurberg and the Winterberg. Except for three pools that had no fish in summer in the Zuurberg, and four pools that were dry in winter in the Winterberg, all pools sampled were persistent in both summer and winter. Substrate type was generally variable among all sites in both the Zuurberg and the Winterberg (Table 2). Bank vegetation was present at most sites in both localities.

The estimated population of *Pseudobarbus afer* significantly increased with increasing proportion of boulders and volume (Fig. 2, Table 3); boulders were also the best predictors for increasing density. Bedrock was the best predictor for increasing estimated population while silt was associated with decreasing density for *B. anoplus* (Fig. 2, Table 3).

Table 2

Physico-chemical and habitat variables (mean \pm standard deviation) sampled at *Pseudobarbus afer* and *Barbus anoplus* sites in Zuurburg Mountains and Winterberg Mountains, respectively, during summer and winter. Number of sites (n) sampled during each period are indicated

	Zuurburg		Winterberg	
	Summer (n = 18)	Winter (n = 21)	Summer (n = 19)	Winter (n = 15)
Temperature ($^{\circ}\text{C}$)	23.6 \pm 0.5	14.1 \pm 0.3	23.1 \pm 1.0	10.3 \pm 0.6
pH	7.1 \pm 0.1	8.0 \pm 0.2	8.1 \pm 0.1	8.4 \pm 0.0
TDS (ppm)	246.2 \pm 29.5	243.8 \pm 36.2	135.7 \pm 20.0	293.7 \pm 16.3
Conductivity ($\mu\text{S cm}^{-1}$)	379.0 \pm 30.4	413.2 \pm 61.1	239.6 \pm 36.8	508.8 \pm 28.6
Turbidity (NTU)	7.2 \pm 1.5	2.6 \pm 0.5	323.0 \pm 72.1	13.2 \pm 2.3
Boulder (%)	12.4 \pm 2.9	36.9 \pm 4.7	15.6 \pm 2.9	15.9 \pm 4.4
Gravel (%)	14.6 \pm 2.3	2.8 \pm 1.3	12.5 \pm 3.0	10.9 \pm 5.2
Pebble (%)	43.1 \pm 4.3	38.3 \pm 4.6	31.1 \pm 4.7	28.8 \pm 5.9
Bedrock (%)	18.3 \pm 5.2	16.3 \pm 6.0	14.9 \pm 5.5	27.9 \pm 7.3
Silt (%)	8.1 \pm 3.3	5.7 \pm 3.8	18.8 \pm 7.6	12.6 \pm 4.4
Sand (%)	3.3 \pm 1.2	0.0 \pm 0.0	7.4 \pm 1.5	3.9 \pm 1.7
Bank vegetation (%)	20.9 \pm 3.7	20.6 \pm 3.8	13.3 \pm 4.2	17.4 \pm 4.1
Average depth (cm)	37.6 \pm 3.1	34.8 \pm 2.8	31.9 \pm 2.7	28.8 \pm 2.0
Maximum depth (cm)	64.1 \pm 5.7	57.5 \pm 4.5	54.9 \pm 5.6	45.5 \pm 2.7
Area (m^2)	58.9 \pm 9.5	60.2 \pm 7.4	77.8 \pm 10.3	71.6 \pm 8.5
Volume (m^3)	22.7 \pm 4.4	20.2 \pm 2.8	26.1 \pm 5.7	20.9 \pm 2.9

Capture probability for *B. anoplus* increased in relation to the proportion of bank vegetation and average depth (Fig. 2, Table 3).

Length frequencies showed distinct modal peaks for the L5 length class for both species in summer, and the L3 length class for *B. anoplus* and L5 length class for *P. afer* in winter (Fig. 3). There was also a higher frequency of the small length class, L2, in summer than winter for both species. Mature *P. afer* were dominant in both summer and winter. By comparison, the *B. anoplus* population was dominated by mature fish in summer and immature fish in winter.

The best predictor habitat variables, pH and temperature, and the seasonal variable of summer (Monte Carlo permutations of both first axis and trace, $P < 0.01$) accounted for a significant 13.2% of variation in size structure for *P. afer*. The explained variation was partitioned as pure habitat ($H|S$), 2.4%, pure seasonal ($S|H$), 2.4%, shared/redundant variation ($H \cap S$), 8.3%, and unexplained, 73.6% (Table 4). CCA Axis 1 suggested an association of smaller length classes (L1, L2 and L3) with seasonal variable summer, and length class L7 with pH, while length classes L4, L5, L6, L8 and L9 appeared to be consistent throughout the sampling seasons (Fig. 4). Habitat variables, bedrock and bank vegetation, and the seasonal variables of summer (Monte Carlo permutations, $P < 0.05$) accounted for a significant 21.8% of variation in species size structure for *B. anoplus* (Table 4). The explained variation was mostly pure habitat ($H|S$), which (Monte Carlo permutations, $P < 0.05$) explained a significant 17.8% variation. Pure seasonal variation ($S|H$) was insignificant (Monte Carlo permutation, $P > 0.05$), explaining only 4.4% of the variation, while no variation was shared between habitat and season ($H \cap S$). A total of 56.4% of the variation was unexplained (Table 4). CCA axis 1 showed the association of the length classes L3 and L4 with bank vegetation, and length classes L7 and L8 with bedrock (Fig. 4). CCA axis 2 was a seasonal gradient that indicated an association of the smallest length classes (L1 and L2) and the biggest length classes (L9 and L10) with the seasonal variable, summer (Fig. 4).

Discussion

The habitats for both *P. afer* and *B. anoplus* were characterised by seasonal variability in all physico-chemical variables, especially temperature, conductivity and turbidity. Spatially, the habitats were heterogeneous with varying proportions of the different substrate types. While the estimated populations were variable between seasons, especially for *B. anoplus*, the populations of the two species were relatively stable in their habitats with no differences in population parameter estimates for densities and capture probability between seasons. There was no evidence that per capita loss was dependent on summer abundance. This suggests that population dynamics were density-independent and largely driven by extrinsic factors. These factors could be inferred from the substantial seasonal differences in environmental variables, notably substrate in the Zuurburg and physicochemical variables in the Winterberg. During winter, boulder percentage increased in the Zuurburg whereas turbidity decreased substantially as the habitats receded into isolated pools compared to summer when there was high water flow. The two minnow species were nonetheless able to persist and maintain stable populations between seasons in the headwater streams where they occur.

Pseudobarbus afer generally occur in relatively stable, clear mountain streams. The genus is, nevertheless, known to persist within unstable and changing environmental conditions owing to a combination of flexible life history patterns, physiological tolerances and both morphological and dietary plasticity (Skelton, 1988; Cambray, 1994). Similarly, *Barbus anoplus* is known to adapt and persist in unstable environments, such as headwater streams, where it usually occur as the only fish species (Cambray and Bruton, 1985). The patterns in densities indicate stable populations within the headwater streams for the two species. Maintaining stable populations within unstable environments by both species suggests adaptations that are typical of opportunist or habitat generalists species (Erős and Grossman, 2005; Keaton et al., 2005). This supports observations from other studies

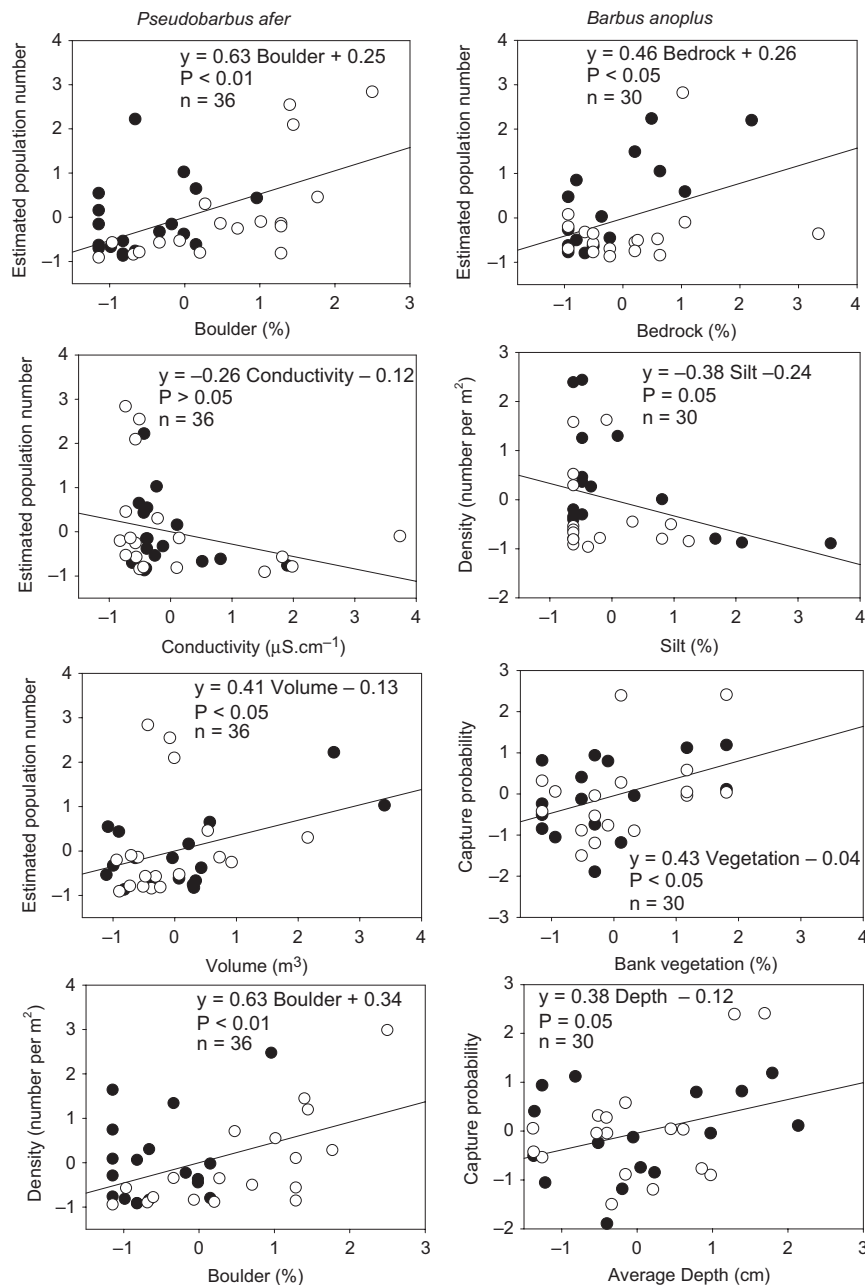


Fig. 2. *Pseudobarbus afer* and *Barbus anoplus* population number, density and capture probability in relation to important habitat variables. Points = summer (●) and winter (○) samples

suggesting that headwater streams characterised by high spatial and temporal variation in environmental conditions are inhabited by small cyprinids and other minnows that are persistent and tolerant to such changes (Butler and Fairchild, 2005).

Substrate type appeared to be the major determinant for persistence in this study. The influence of boulders and bedrock on population number and density suggests the importance of large substrate types for the two species. Large substrates are known to provide refuge and result in greater habitat stability, which also produces abundant macroinvertebrates on which fish feed (Guenther and Spacie, 2006; Mueller and Pyron, 2010). It is likely that persistence of *P. afer* during environmental extremes would be mediated by boulder substrate as the most important refugia. By comparison, persistence of *B. anoplus* that was abundant in habitats

with bedrock substrate would be mediated by bank vegetation. Bank vegetation provides a refuge that not only reduces predation risk, especially from terrestrial predators such as birds, but also from interference among individuals, and provides food in the form of aquatic invertebrates that are associated with vegetation (Edo and Suzuki, 2003).

Seasonality was the major influence for *P. afer* size structure, shown especially by a high association of smaller length classes with the seasonal variable, summer. *Pseudobarbus afer* spawn in response to an increasing water flow in summer, usually between October and November (Cambray, 1994). This suggests that size structuring observed in this study for *P. afer* was mainly recruitment driven. This pattern was reflected as a seasonal gradient of CCA, an observation that was also supported by length frequency analysis where there was a high frequency of the small length class L2 in summer

Table 3
Linear mixed-effects model coefficients for *Pseudobarbus afer* and *Barbus anoplus* relating to estimated number, density and capture probability in relation to several habitat variables

	Estimate	SE	d.f.	t	P
<i>Pseudobarbus afer</i>					
Number					
Boulder	0.63	0.17	16	3.69	0.00
Volume	0.41	0.17	16	2.42	0.03
Conductivity	-0.26	0.17	16	-1.52	0.15
Density					
Boulder	0.63	0.25	16	3.63	0.00
<i>Barbus anoplus</i>					
Number					
Bedrock	0.46	0.16	13	2.83	0.01
Density					
Silt	-0.38	0.17	13	-2.21	0.05
Capture probability					
Vegetation	0.43	0.17	13	2.47	0.03
Average depth	0.38	0.17	13	2.19	0.05

and a gradual shift in length with season. Large length classes, on the other hand, showed high association with pH, which was relatively high in winter suggesting that these were the over-wintering length classes. By comparison, seasonality appeared to have less influence on size structuring for *B. anoplus*. Instead, habitat variables, bedrock and bank vegetation, were the major influence for *B. anoplus* size structure, suggesting the importance of physical habitat and possibly refugia. Refugia, in the form of bank vegetation, appeared to influence the over-wintering size classes, L3 and L4, for this species. The differences in the importance of seasonality for the two minnow species could be explained by their different spawning strategies. Unlike *P. afer*, *B. anoplus* is known to be iteroparous, with a primary spawning period between November and January and a secondary spawning period between February and March (Cambray and Bruton, 1985). The significance of this strategy, which may have

Table 4
Summary statistics for Canonical Correspondence Analysis, *Pseudobarbus afer* and *Barbus anoplus* ordinations

	<i>Pseudobarbus afer</i>		<i>Barbus anoplus</i>	
	Axis 1	Axis 2	Axis 1	Axis 2
Eigenvalues	0.13	0.02	0.14	0.06
Species-environment correlations	0.62	0.28	0.59	0.53
Cumulative percentage variance				
Species data	10.6	12.0	13.8	19.6
Species-environment relationship	80.8	91.3	62.5	88.5
Correlations with axes				
pH	0.75	0.62		
Temperature	-0.94	-0.11		
Summer	-0.99	0.10	0.14	-0.37
Bank vegetation			-0.39	0.27
Bedrock			0.45	0.34
Total inertia			1.21	1.01
Sum of all canonical eigenvalues			0.16	0.22
Canonical eigenvalue $H S$			0.03	0.18
Canonical eigenvalue $S H$			0.03	0.04
Canonical eigenvalue $H \cap S$			0.10	0.00

evolved as an adaptation to unstable environments, is to stagger the breeding period to increase net survival probability (Cambray and Bruton, 1985). The first spawned fish allocate resources towards growth and survival and participate in reproduction in their second summer, whereas the second spawned fish invest in growth and survival throughout the first spawning season and participate in reproduction in the second spawning season (Cambray and Bruton, 1985). This would suggest that this species was less likely to exhibit

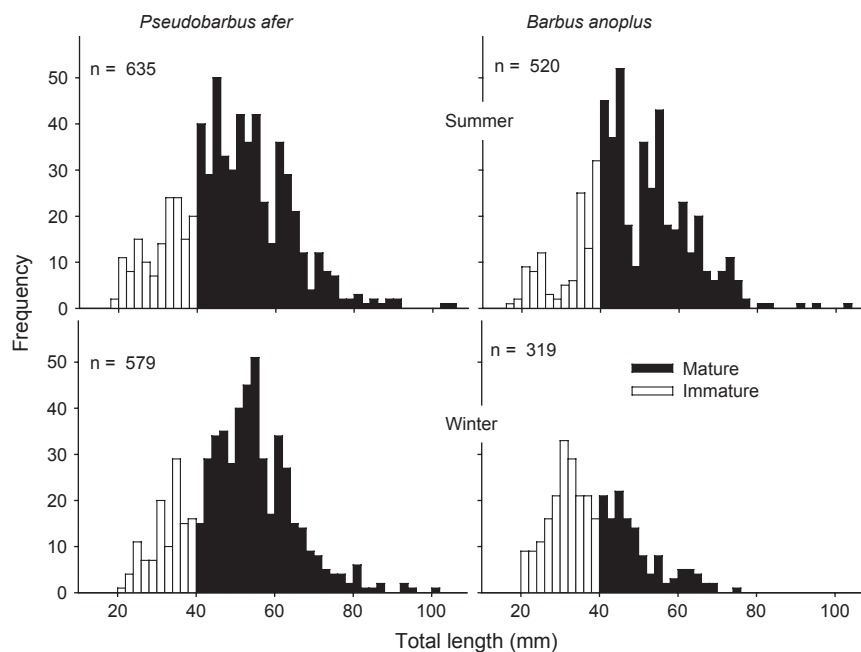


Fig. 3. Length frequencies of immature and mature *Pseudobarbus afer* and *Barbus anoplus* sampled during summer (February–May 2010) and winter (July–September 2010)

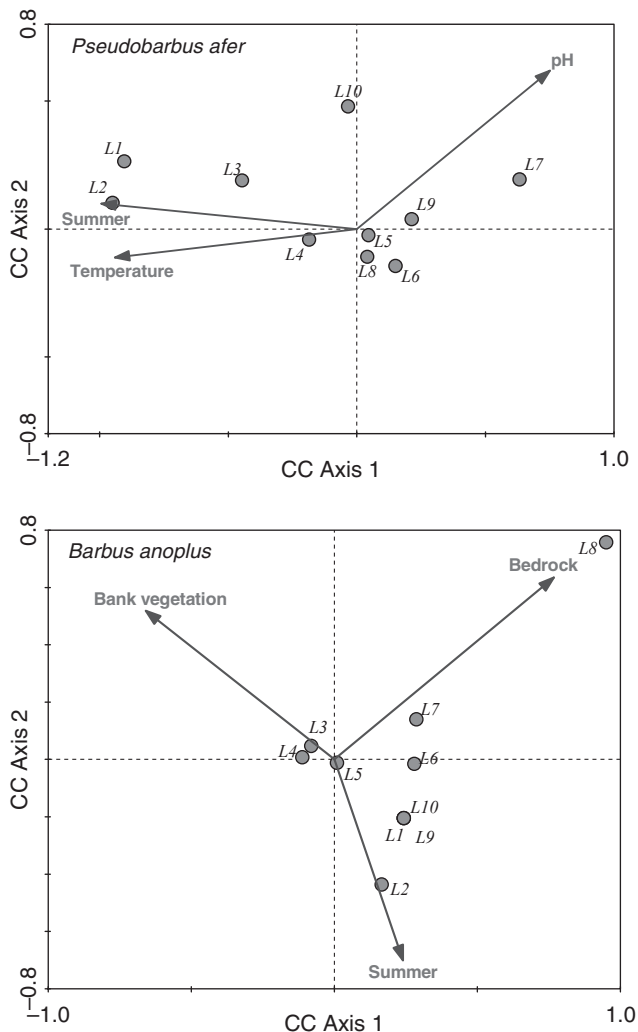


Fig. 4. Canonical Correspondence Analysis ordination plots for *Pseudobarbus afer* and *Barbus anoplus* size classes. Size classes: L1 (<20 mm TL), L2 (20–29 mm TL), L3 (30–39 mm TL), L4 (40–49 mm TL), L5 (50–59 mm TL), L6 (60–69 mm TL), L7 (70–79 mm TL), L8 (80–89 mm TL), L9 (90–99 mm TL) and L10 (>100 mm TL)

seasonal patterns in size structuring as reflected by the occurrence of small length classes throughout the sampling period.

The lack of a relationship between dominant substrates and size structuring was a possible indication of lack of habitat specialisation for the different size classes for both species. This, as hypothesised by Angermeier and Schlosser (1989), may be a consequence of an imbalance between fish and their habitats due to high environmental variation, as they are habitat generalists. Such a strategy would enable species to maintain stable populations as habitat generalists in response to an unstable environment, with refuge availability as the critical factor. Recruitment appeared to be the major driver in size structuring, with the two species, however, having different well-studied spawning strategies (e.g. Cambray and Bruton, 1985; Cambray, 1994), and reflected as seasonal gradients in this study. Refugia, especially in the form of boulders, appeared to be important for *P. afer*, whereas bank vegetation was important for *B. anoplus* overwintering size classes.

To conclude, both *P. afer* and *B. anoplus* are well adapted to the headwater streams that were generally variable in

environmental conditions. Additional stressors, especially in the form of alien invasive fishes, may have more confounding effects that would affect the minnows in these habitats. Efforts should be made to protect these minnows, especially the endangered *P. afer*, by enforcing the recommendations from previous studies (e.g. Weyl et al., 2009), such as constructing barriers to the upstream migration of alien invasive species such as sharp-tooth catfish *Clarias gariepinus* and largemouth bass *Micropterus salmoides*.

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References

- Angermeier, P. L.; Schlosser, I. J., 1989: Species-area relationships for stream fishes. *Ecology* **70**, 1450–1462.
- ter Braak, C. J. F.; Smilauer, P., 1998: CANOCO reference manual and user's guide to CANOCO for Windows: software for canonical community ordination (version 4). Microcomputer Power, Ithaca, NY.
- Butler, L. H.; Fairchild, W. G., 2005: Response of fish assemblages to winter in two adjacent warmwater streams. *Am. Midl. Nat.* **154**, 152–165.
- Cambray, J. A., 1994: The comparative reproductive styles of two closely related African minnows (*Pseudobarbus afer* and *P. asper*) inhabiting two different sections of the Gamtoos River system. *Environ. Biol. Fishes* **41**, 247–268.
- Cambray, J. A.; Bruton, M. N., 1985: Age and growth of a colonizing minnow, *Barbus anoplus*, in a man-made lake in South Africa. *Environ. Biol. Fishes* **12**, 131–141.
- Cambray, J. A.; Hecht, T., 1995: Comparison of the growth of two closely related redfin minnows, *Pseudobarbus afer* (Peters, 1864) and *P. asper* (Boulenger, 1911) (Pisces, Cyprinidae), in the Gamtoos River System, South Africa. *J. Afr. Zool.* **109**, 350–376.
- Capone, T. A.; Kushlan, J.A., 1991: Fish community structure in dry-season stream pools. *Ecology* **72**, 983–992.
- Casatti, L.; Langeani, F.; Silva, A. M.; Castro, R. M. C., 2006: Stream fish, water and habitat quality in a pasture dominated basin, southeastern Brazil. *Braz. J. Biol.* **66**, 681–696.
- Edo, K.; Suzuki, K., 2003: Preferable summering habitat of returning adult masu salmon in the natal stream. *Ecol. Res.* **18**, 783–791.
- Erős, T.; Grossman, G. D., 2005: Effects of within-patch habitat structure and variation on fish assemblage characteristics in the Bernecei stream, Hungary. *Ecol. Freshw. Fish* **14**, 256–266.
- Gorman, O. T.; Karr, J. R., 1978: Habitat structure and stream fish communities. *Ecology* **74**, 1856–1870.
- Grossman, G. D.; Ratajczak, R. E., 1998: Long-term patterns of microhabitat use by fish in a southern Appalachian stream from 1983 to 1992: effects of hydrologic period, season and fish length. *Ecol. Freshw. Fish* **7**, 108–131.
- Guenther, C. B.; Spacie, A., 2006: Changes in fish assemblage structure upstream of impoundments within the Upper Wabash River basin, Indiana. *Trans. Am. Fish. Soc.* **135**, 570–583.
- 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. *Can. J. Fish. Aquat. Sci.* **58**, 157–170.
- Keaton, M.; Haney, D.; Andersen, B. C., 2005: Impact of drought upon fish assemblage structure in two South Carolina Piedmont streams. *Hydrobiologia* **545**, 209–223.
- Koehn, J. D.; O'Connor, N. A.; Jackson, P. D., 1994: Seasonal and size-related variation in microhabitat use by a Southern Victorian stream fish assemblage. *Aust. J. Mar. Freshw. Res.* **45**, 1353–1366.
- Lombard, A. T.; Johnson, C. F.; Cowling, R. M.; Pressey, R. L., 2001: Protecting plants from elephants: botanical reserve scenarios within the Addo Elephant National Park, South Africa. *Biol. Conserv.* **102**, 191–203.
- Lowe, S. R.; Woodford, D. J.; Impson, D. N.; Day, J. A., 2008: The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. *Afr. J. Aquat. Sci.* **33**, 51–62.
- Macvicar, C. N., 1991: . Soil classification: a taxonomic system for South Africa. *Memoirs of the Agricultural Natural Resources of South Africa*, 15. Department of Agricultural Development, Pretoria, South Africa.
- Meadows, M. E.; Meadows, K. F., 1988: Late quaternary vegetation history of the Winterberg Mountains, eastern Cape, South Africa. *S. Afr. J. Sci.* **84**, 253–259.
- Mueller, R.; Pyron, M., 2010: Fish assemblages and substrates in the Middle Wabash River, USA. *Copeia* **1**, 47–53.
- Nieman, R. J.; Latterell, J. J., 2005: Principles for linking fish habitat to fisheries management and conservation. *J. Fish Biol.* **67**, 166–185.
- Ostrand, K. G.; Wilde, G. R., 2002: Seasonal and spatial variation in prairie stream-fish assemblage. *Ecol. Freshw. Fish* **11**, 137–149.
- Peterson, J. T.; Thurow, R. F.; Guzevich, J. W., 2004: An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. *Trans. Am. Fish. Soc.* **133**, 462–475.
- Power, M. E., 1987: Predator avoidance by grazing fishes in temperate and tropical streams: importance of stream depth and prey size. In: Predation: direct and indirect impacts on aquatic communities. W. C. Kerfoot, A. Sih (Eds). University Press of New England, Hanover, NH. pp. 333–353.
- R Development Core Team, 2011: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Schlosser, I. J., 1982: Fish community structure and function along two habitat gradients in headwater stream. *Ecol. Monogr.* **52**, 395–414.
- Schlosser, I. J., 1988: Predation risk and habitat selection by two size classes of a stream cyprinid: experimental test of a hypothesis. *Oikos* **52**, 36–40.
- Schlosser, I. J., 1991: Stream fish ecology: a landscape perspective. *Bioscience* **41**, 704–712.
- Seber, G. A. F., 1982: The estimation of animal abundance, 2nd edn. Charles Griffin & Co., London. 654 pp.
- Skelton, P. H., 1988: A taxonomic revision of the redfin minnows (Pisces, Cyprinidae) from southern Africa. *Ann. Cape Town Mus. (Natural History)* **16**, 201–307.
- Weyl, O. L. F.; Booth, A. J.; Traas, G. R. L.; Swartz, E., 2009: Determination of critical conservation areas in the Sundays River system for indigenous fish species. *Sundays River Freshwater Fish Research Project ID: PO64438*.
- Wilkinson, C. D.; Edds, D. R., 2002: Spatial pattern and environmental correlates of a midwestern stream fish community: including spatial autocorrelation as a factor in community analyses. *Am. Midl. Nat.* **146**, 271–289.
- Wootton, R. J., 2007: Over-wintering growth and losses in a small population of the threespine stickleback, *Gasterosteus aculeatus* (L.), in mid-Wales. *Ecol. Freshw. Fish* **16**, 476–481.
- Zorn, T. G.; Seelbach, P. W.; Wiley, M. J., 2002: Distribution of stream fish and their relationship to stream size and hydrology in Michigan's lower peninsula. *Trans. Am. Fish. Soc.* **131**, 70–85.
- van Zyl, A. J., 1994: Sex-related local movement in adult rock kestrels in the Eastern Cape Province, South Africa. *Wilson. Bull.* **106**, 145–148.
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