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EDITED BY

Eugenia López-López,
Instituto Politécnico Nacional de
México, Mexico

REVIEWED BY

Mauricio Cetra,
Federal University of São Carlos, Brazil
Fernando Mayer Pelicice,
Federal University of Tocantins, Brazil

*CORRESPONDENCE

Terence A. Bellingan,
t.bellingan@ru.ac.za

†Deceased

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Season and environment modulate aquatic invertebrates' responses to trout and indigenous fishes in three South African mountain streams

Terence A. Bellingan^{1,2,3*}, Sanet Hugo^{4,5}, Martin H. Villet¹ and Olaf L. F. Weyl^{2,4†}

¹Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa, ²Centre for Invasion Biology (CIB), South African Institute for Aquatic Biodiversity (SAIAB), Makhanda, South Africa, ³Department of Entomology and Arachnology, Albany Museum, Makhanda, South Africa, ⁴DST/NRF Research Chair in Inland Fisheries and Freshwater Ecology, South African Institute for Aquatic Biodiversity (SAIAB), Makhanda, South Africa, ⁵Department of Biological Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Thohoyandou, South Africa

Introduced organisms are seen as one of the greatest threats to resource sustainability worldwide, and aquatic macroinvertebrates are regarded as good indicators of the health of water resources. To explore these two perspectives, the responses of macroinvertebrate faunas to native and introduced fishes in three headwater tributaries of the Keiskamma River system, South Africa, were examined by comparing potential indicator communities in reaches considered to be fishless, reaches invaded by introduced salmonid species, and reaches containing native fishes. Patterns in the macroinvertebrate faunal assemblage data were driven strongly by season and flow rate, and less strongly by the presence of insectivorous fishes and biotope availability, a finding in parallel with several similar studies from the region. This affirms that aquatic macroinvertebrate faunas are responsive indicators of both environmental and biotic factors and leaves room for further studies to resolve the effects of non-native fish in the Keiskamma River system and other similar systems from South Africa.

KEYWORDS

aquatic macroinvertebrates, cyprinidae, salmonidae, river continuum hypothesis, functional feeding groups, freshwater conservation, biological monitoring

Introduction

Invasive introduced fishes have been observed globally to be the most pressing threat to native freshwater faunas (Clavero & Garcia-Berthou 2005). Their introduction can precipitate knock-on effects through trophic cascades (Brett & Goldman 1996; Tronstad et al., 2010; Ripple et al., 2016; Lane 2017) that ultimately compromise the sustainability of aquatic resources, from food and water supply to sport and tourism (Ellender & Weyl

2014). South Africa offers opportunities to study this situation because non-native fish species have been introduced into its impoundments and rivers since the late 1800s for the creation of recreational fisheries (de Moor & Bruton 1988; van Rensburg et al., 2011; McCafferty et al., 2012; Ellender & Weyl 2014), and as a result, the country is regarded as one of six global aquatic invasion hotspots where introduced fish account for more than a quarter of freshwater fish species diversity (Leprieur et al., 2008).

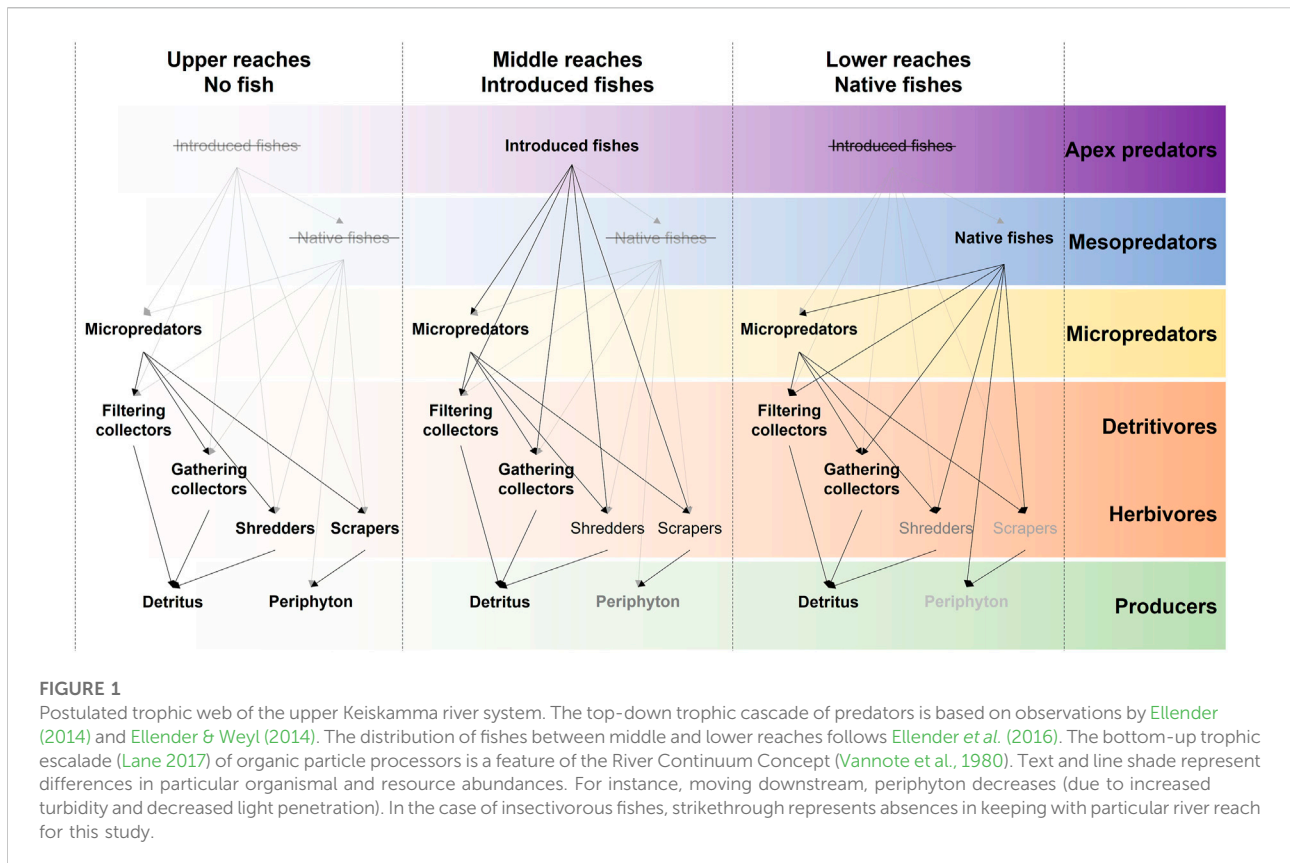
In particular, two introduced salmonid sport-fishes, rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) and brown trout (*Salmo trutta* L. 1758), are listed in the top 100 of the world's worst invasive species (Lowe et al., 2000) due to their damaging effects on recipient ecosystems (Cambray 2003). Habitat preferences and physiological tolerances limit their South African distributions to cool, clear, perennial streams and higher-altitude impoundments (Bjornn & Reiser 1991; Armstrong et al., 2003; Ellender et al., 2016), where their effects are generally deemed to exceed anthropogenic effects of pollution and habitat degradation in these rural, montane habitats (Ellender & Weyl 2014; Weyl et al., 2015). Worldwide, introduced trout are reported to affect native faunas negatively through predation and competition, which have been shown to extirpate native vertebrates and invertebrates in New Zealand (Townsend & Crowl 1991; McIntosh et al., 1992; Flecker & Townsend 1994; McIntosh & Townsend 1995; Townsend 1996) and Australia (Lintermans 2000); alter invertebrate community structure and function in Patagonia (Buria et al., 2007; Albariño & Buria 2011); alter invertebrate communities (Herbst et al., 2009; Alexiades & Kraft 2017) and disrupt reciprocal subsidies in North America (Matthews et al., 2002; Epanchin et al., 2010); and exclude native fish (Inoue et al., 2009) and disrupt trophic subsidies in Japan (Baxter et al., 2004). This makes rainbow and brown trout good candidates for studying environmental effects on indicator communities.

Some of these effects have been explored in South Africa (Rivers-Moore et al., 2013; Shelton et al., 2015a, 2015b, 2016). Most work on the ecological effects of trout in southern Africa have focused on native fish and amphibians (eg. Woodford & Impson 2004; Karssing et al., 2012; Kadye et al., 2013; Avidon et al., 2018), but recent studies have examined the effects of trout on invertebrates. Large-scale field experiments (Shelton et al., 2015a; 2015b) and an *in situ* mesocosm experiment (Shelton et al., 2016) examined the effects of *O. mykiss* on the fish and invertebrate faunas of the Breede River catchment in the Cape Fold Ecoregion (*sensu* Abell et al., 2008). Native fish were found to be stronger regulators of the aquatic invertebrate community than *O. mykiss*, which preyed more heavily on terrestrial invertebrate subsidies. It was also demonstrated that *O. mykiss* depleted populations of endemic native barb species (Cyprinidae) through size-selective predation (*sensu* Rincón & Lobón-Cerviá, 1999), while alleviating predation pressure on grazing invertebrates by usurping the native fish (Shelton et al., 2015a; 2015b).

Macroinvertebrate assemblages are reliable indicators of local ecological processes because of their members' ubiquity, abundance, rapid life-cycle turnover and biological importance in the food chain

(Palmer 1996; Dickens and Graham. 2002). They therefore provide a tool for examining conservation questions. Aquatic macroinvertebrates respond predictably to water temperature, season and water quality (King, 1981; 1983; Rivers-Moore 2012; Eady et al., 2013) and have been used to monitor collateral effects of eradicating introduced fishes (Bellingan et al., 2015; 2019). Trends in invertebrate assemblage composition in response to gradients along the river, including anthropogenic influences, have been described in South Africa (King 1981; Palmer et al., 1991; 1993a; 1993b; 1994) and elsewhere (Wiberg-Larsen et al., 2000), reflecting the expectations of the river continuum concept (RCC: Vannote et al., 1980). The RCC anticipates that autochthonous and allochthonous production in canopy-covered headwaters will be physically broken up by guilds of scraping and shredding invertebrates, and that the resulting particles are washed downstream and further processed by guilds of collector invertebrates (Figure 1). This type of bottom-up trophic chain has been termed a trophic escalade (Lane 2017). The influence of upstream-downstream gradient (i.e.: the underpinning mechanism of the RCC) must be considered when using macroinvertebrates as monitoring tools. In a study of the macroinvertebrate community under the presence or absence of *O. mykiss* and *S. trutta* upstream and downstream of waterfalls along the Sterkspruit, Lotheni and Mooi Rivers in the Drakensberg-Maloti Highlands Ecoregion, South Africa, Rivers-Moore et al. (2013) could not demonstrate conclusively that introduced predatory fish negatively affected their associated macroinvertebrate assemblages, and suggested that their upstream-downstream comparisons may have been confounded by overriding river continuum variables, even though their study was conducted in high altitude, low order streams.

The Amatolo-Winterberg Highlands Ecoregion (Abell et al., 2008), where trout have been established for over a century (*O. mykiss* and *S. trutta* introduced into the Keiskamma headwaters from 1903—see de Moor & Bruton 1988), provides a unique opportunity to study the effect of native and invasive fish on macroinvertebrates. The distributions of native and introduced fish species in the upper catchment were mapped in detail (Ellender 2014; Ellender et al., 2016), and the uppermost stream reaches of the Cata, Mnyameni and Gwiligwili Rivers are naturally fishless. Brown trout have invaded sections of the Cata (0.01 fish/m⁻³) and Rainbow trout sections of the Mnyameni (0.1 fish/m⁻³) rivers, which are separated from the fishless upper reaches by waterfalls and from the lower reaches by impoundments. Below the impoundments on the Cata and Mnyameni, and lower reaches of the Gwiligwili, the rivers are populated by the insectivorous native fish species, *Amatolacypris trevelyani* (Günther, 1877) (0.2 fish/m³) and *Enteromius anoplus* (Weber, 1897) (0.004 fish/m³) (see Gaiger 1975; Cambray 1983; Ellender 2014). Macroinvertebrate communities therefore probably experience different predatory regimes and an abiotic environmental gradient, which in turn may result in trophic cascades (Brett & Goldman 1996). This system forms a “natural experiment” *sensu* DiNardo (2008), where the effects of fish predator regime on macroinvertebrate assemblages can be tested (Figures 1, 2). Indeed, river continuum effects have been reported from the Amatolo-Winterberg Ecoregion (Palmer et al., 1994).

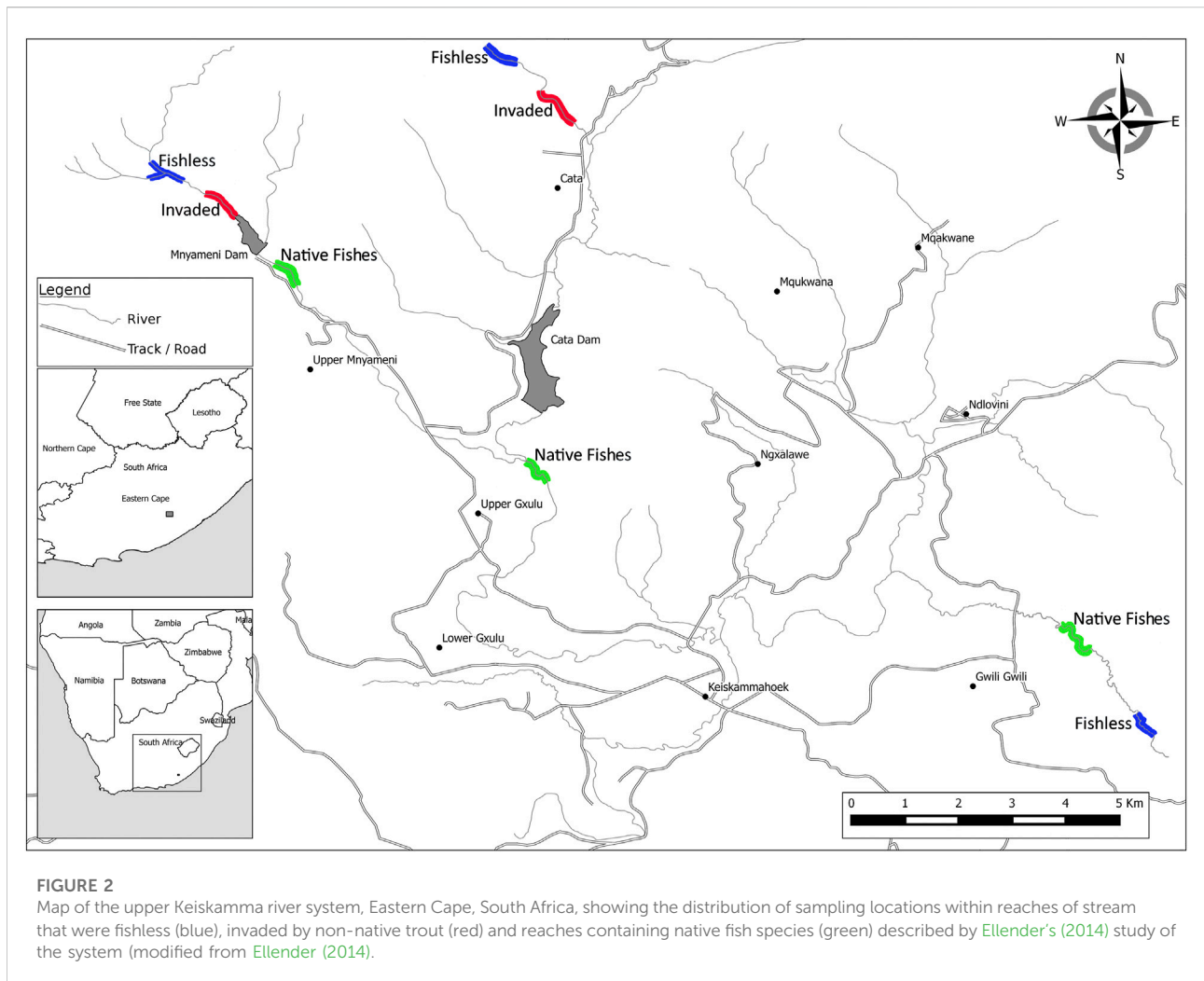


Both bottom-up environmental gradients and top-down fish predation are likely to drive aquatic macroinvertebrate community structure. The RCC predicts that the dominant functional feeding groups (FFGs) will change from scrapers, shredders and gathering collectors in the upper reaches to filter feeders in the lower reaches as light penetration and the particle sizes of particulate organic matter decrease (cf. Vannote et al., 1980). Macroinvertebrate species richness and diversity should increase toward the lower reaches as food resources diversify (Clarke et al., 2008). Conversely, the effects of predatory fish may decrease macroinvertebrate diversity through a trophic cascade (cf. Ripple et al., 2016). Certain functional feeding groups like gathering collectors and micropredators (i.e. larger macroinvertebrates) will be disproportionately affected by predation by fish (Flecker & Allan 1984; Meissner & Muotka 2006). Moreover, the invasive trout and the native fish communities may have different effects on macroinvertebrates. On the one hand, trout may have a greater impact on native macroinvertebrates if they have a higher functional response compared to native fish species (Alexander et al., 2014). On the other hand, as found previously, trout may be more dependent on terrestrial food sources (Li et al., 2016) and native fish may have a greater impact on aquatic macroinvertebrates (Ripple et al., 2016).

We examined the response of native aquatic macroinvertebrates to predation pressure by native and invasive insectivorous fish species in three headwater tributaries of the Keiskamma River system in the Eastern Cape, by examining variation in macroinvertebrate species richness, diversity, and community composition. We hypothesise that the macroinvertebrate community is predominantly structured by a longitudinal environmental gradient along the river (bottom-up hypothesis), taking seasonal effects into account. Specifically, the following patterns were expected from bottom-up drivers, following predictions from the RCC:

- 1) Species from the functional feeding groups scrapers, shredders and gathering collectors should be more abundant where forest canopy covers the stream and input of leaf litter is greater (Vannote et al., 1980).
- 2) Filtering collectors should increase in abundance toward the lower reaches as food particle size decreases (Vannote et al., 1980).
- 3) Species richness and diversity should increase towards the lower reaches, due to greater quantities and variety of food resources (Clarke et al., 2008; Ellender et al., 2016).

Conversely, we hypothesise the macroinvertebrate community is structured by insectivorous fishes (top-down hypothesis). The following patterns were expected from top-down drivers:



- 4) Invertebrate taxa, especially large-bodied species from the predator and gathering collector groups, should be less abundant in the presence of fish (Flecker & Allan 1984; Meissner & Muotka 2006).
- 5) Species richness and diversity should decrease in river reaches containing fish (e.g., Flecker & Townsend 1994).
- 6) The degree of predation impact differs between introduced and native fish species (Alexander et al., 2014; Ripple et al., 2016).

Methods

Study area

The riverine habitat consisted of three tributaries of the Keiskamma River, in the Eastern Cape, South Africa: the Cata, Gwili Gwili and Mnyameni Rivers (Figure 2), which are swift-flowing streams of 3rd-, 2nd- and 2nd-order, respectively. Reaches selected for sampling macroinvertebrate assemblages were based on the distribution of fishes along these streams,

following Ellender (2014). The riverbanks of the fishless reaches of all three rivers, and the invaded reaches of the Mnyameni and Cata Rivers, were completely shaded by a canopy of indigenous mist-belt forest (Mucina et al., 2006), a source of allochthonous primary production in the rivers. The riparian vegetation along the native fish reaches of the Gwili Gwili, Cata and Mnyameni rivers was Savannah Thornveld with sparsely distributed indigenous *Podocarpus* (Podocarpaceae) trees.

The fishless reach of the Mnyameni River was characterized by large, fairly deep pools broken up by short riffles over cobbled beds; this reach thus has the greatest average depth of 0.35 ± 0.16 m, and the highest pool-to-riffle ratio (Table 1). The Gwili Gwili River was shallowest, ranging from 0.06 ± 0.03 to 0.07 ± 0.04 m deep along the fishless and native fish reaches, respectively. The fishless and invaded reaches of the Cata River were similar in substrate proportions, while the native fish reach contained the highest proportion of riffle compared to the remaining sites (Table 1). The mean stream width ranged from 7.80 ± 3.30 m at the fishless Mnyameni reach to 1.2 ± 0.4 m at the fishless Gwili Gwili reach. The Gwili Gwili River, being

TABLE 1 Summary of the location, habitat and physicochemical characteristics (mean \pm SD) for eight reaches sampled quarterly on three headwater tributaries of the Keiskamma River from July 2012 - April 2013. Values marked with an asterisk (*) indicate qualitative estimations.

Characteristic	Cata river			Mnyameni river			Gwiligwili river	
	Fishless	Invaded	Native fish	Fishless	Invaded	Native fish	Fishless	Native fish
Geo-coordinates	32.588 S 27.050 E	32.593 S 27.059 E	32.612 S 27.074 E	32.571 S 27.116 E	32.577 S 27.124 E	32.641 S 27.112 E	32.688 S 27.228 E	32.674 S 27.218 E
Altitude (masl)	1,013	944	836	980	889	717	776	700
Length (m)	27	30	30	22	26	30	28	30
Mean width (m)	2.83 \pm 0.45	3.83 \pm 1.55	5.20 \pm 1.83	7.80 \pm 3.30	4.03 \pm 0.68	7.30 \pm 1.47	1.2 \pm 0.41	1.76 \pm 0.49
Mean depth (m)	0.20 \pm 0.12	0.20 \pm 0.10	0.23 \pm 0.10	0.35 \pm 0.16	0.25 \pm 0.10	0.24 \pm 0.15	0.06 \pm 0.03	0.07 \pm 0.04
Surface Area (m ²)	76.5	115	156	171.6	104.8	219	53	34.5
Canopy cover (%)	90*	70*	0	87*	80*	0	70*	76*
Stone (%)	54	66	72	42	73	29	17	14
Litter (%)	46	34	8	51	18	62	80	23
Sand/Gravel (%)	0	0	20	7	19	9	3	63
Pool (%)	62	54	28	68	38	62	55	59
Riffle/Run (%)	38	46	72	32	62	38	45	41
Temperature	11.35 \pm 3.54	11.24 \pm 2.80	16.30 \pm 4.10	11.20 \pm 3.92	11.43 \pm 3.56	14.30 \pm 3.19	12.90 \pm 2.92	13.05 \pm 5.23
pH	7.19 \pm 0.68	6.85 \pm 0.26	7.88 \pm 0.29	6.98 \pm 0.20	7.15 \pm 0.25	7.46 \pm 0.24	7.09 \pm 0.30	6.99 \pm 0.14
Conductivity	17.30	24.95	106.83	34.27	37.70	71.83	75.40	69.40

a narrower stream with a smaller catchment, flowed less strongly than the Cata and Mnyameni Rivers, with flow increasing downstream as the stream profile widened.

Sampling, field measurements and taxon classification

Sampling was carried out quarterly, in July and October of 2012, and January and April of 2013, corresponding to winter, spring, summer and autumn seasons. For each season, we collected ten samples in each reach in each river from sites roughly 30 m long (Table 1; Figure 2). Samples were collected with a modified Surber sampling technique (Hauer & Resh 1996). Two frames of 300 mm \times 300 mm were constructed from polyvinyl chloride (PVC) tubing and supported 120 mm apart by clear Perspex panes, to allow a channel of water to flow from upstream to downstream through the Surber box and into the net. A long-handled water net with frame dimensions of 300 mm \times 300 mm and mesh size 500 μ m, known commonly as a standard 'SASS' net (Dickens & Graham 2002), was held flush behind the box and the substrate within the box area was disturbed vigorously by hand for 1 minute to dislodge invertebrates. The water flowing through the box carried the invertebrates into the net. Where stream flow was low or negligible, water was flushed manually through the box sampler. At each sample, the flow from the area of stream was recorded using a Marsh McBirney, Inc. Flo-Mate (Model 2000) portable electromagnetic flow meter and flow was categorised, based on current speed, as no flow (0 m/s), slow flow (0.05–0.3 m/s), medium flow (0.3–0.5 m/s) and fast flow

(>0.5 m/s), as flow is a key differentiator between biotope type; visually estimated the percentage canopy cover; and categorised biotope from which the sample was collected as stones, litter or gravel, broadly following Wadson & Rowntree (1998) and Dickens & Graham (2002) (Table 1). Conductivity, pH and temperature were recorded at each site and each sampling event (Hanna HI98219 Combo pH and electrical conductivity meter, HANNA Instruments Inc., Woonsocket, United States) (Table 1).

Macroinvertebrates were identified to species or morphospecies level in the laboratory using published keys for the southern African region (for example, see Barber-James & Lugo-Ortiz 2003; de Moor & Scott 2003), except for earthworms (Oligochaeta) and roundworms (Nematoda) that were identified to subclass level. Further, we allocated the insect species to functional feeding groups (filtering collectors, gathering collectors, predators, scrapers and shredders) following Cummins & Klug (1979) and Merritt & Cummins (1996), with additional input from, e.g., Agnew (1962) and Palmer *et al.* (1993a).

Analysis of species composition

We used generalised linear models for multivariate abundance data ('*manyglm*' from R package '*mvabund*', Wang *et al.*, 2020), with a negative binomial distribution family, to study the relationship between macroinvertebrate community composition and river reaches with different fish predation regimes (hereafter referred to as "river reach"), flow rate,

TABLE 2 General results from a multivariate abundance generalised linear model with the combination of predictor variables that best explain variation in macroinvertebrate species composition. Results are expressed as a marginal analysis of variance, with model degrees of freedom (d.f.), Rao's score test statistic, and *p*-value for each predictor. Residual d.f. = 302.

Predictor	d.f	Rao's score	<i>p</i>
Season	3	403.1	<0.0001
Flow	3	355.4	<0.0001
River reach	2	310.3	<0.0001
Biotope	2	200.1	<0.0001
Canopy cover	1	180.5	<0.0001
Conductivity	1	157.6	<0.0001
pH	1	114.1	<0.0001

canopy cover, temperature, season, biotope, pH, depth and electroconductivity. Preliminary analyses using *manyglm* and Bayesian ordination (see below) indicated negligible differences in species composition between rivers, and we therefore focused on the longitudinal gradient, disregarding river identity. Compared to distance-based methods, model-based multivariate methods do not require data transformation, are not affected by differences in β -diversity among groups, and are not disproportionately influenced by a few relatively highly abundant species (Warton et al., 2012). Nevertheless, only species present in 5% or more of all samples were included in the species matrix.

Collinearity between predictor variables was examined to identify redundant variables before constructing the model (function *vif*, R package *car*, Fox & Weisberg 2019), and the model was further simplified using a backwards stepwise model selection procedure and Akaike's information criterion values to identify the combination of predictor variables that best explained variation in species composition. A Dunn-Smyth residuals-fitted values plot and a quantile-quantile plot was used to evaluate whether the model was appropriate for the data (Dunn & Smyth 1996; Wang et al., 2012). Results were expressed as a marginal analysis of variance (each predictor is tested after all other predictors have been taken into account), with Rao's score test statistics and *p*-values that have been adjusted to account for correlation among species.

Manyglm also supplied univariate regression output for each species with *p*-values adjusted for multiple testing, which we used for a more detailed analysis of those species most affected by the presence of fish or the environmental gradient, noting each species' functional group. This allowed us to probe the potential mechanisms underlying any significant community ensemble response (see predictions 1, 2 and 4).

To illustrate the results, we used a Bayesian ordination technique (function *boral* from R package *boral*, Hui 2020) that complements *manyglm*, where no predictor variables are specified except for a fixed row-effect and two latent variables that represent the scores for an ordination plot with two axes

(Hui 2016). The predictor variables found to best explain variation in species composition were then superimposed on the ordination plot with functions *'ordispider'* to indicate groups, and *'envfit'* to indicate vectors for continuous variables (R package *'vegan'*, Oksanen et al., 2019).

Analysis of species richness and diversity

We examined variation in macroinvertebrate species richness and diversity among the three predation regimes by constructing species accumulation curves (Mao Tau estimate: Colwell et al., 2012) and Tsallis's diversity accumulation curves (Tsallis 1988) equivalent to the Shannon diversity index (R *vegan*'s *'specaccum'* and *'tsallisaccum'* respectively) based on all recorded species (see predictions 3 and 5). We were primarily interested in the effect of invasive or native insectivorous fish presence (i.e. differences between river reaches), but we used the findings from the species composition analyses to verify whether it is reasonable to pool samples from different seasons. Accumulation curves were more useful than single index values because Gwiligwili lacks trout and consequently the trout-invaded reaches are represented by fewer samples than the fishless or native fish reaches. Accumulation curves were fitted to the asymptotic Lomolino model (Dengler 2009) to compare estimated richness and diversity values at equal sample sizes, and to estimate total species richness or diversity (curve asymptotes) under the hypothetical condition of complete sampling.

Finally, the presence or absence of rare species and singletons (i.e., those species that were not included in the species matrix for *manyglm*) may contribute to the differences in species richness among river reaches (Spiller & Schoener 1998; Thompson & Withers 2003; Lopez et al., 2012). Such species on their own do not supply enough data to study with regression or multivariate analyses (e.g., *manyglm*) and their absence may be falsely recorded due to sampling error or low detectability. However, following from predictions 1, 2 and 4, if multiple species from certain functional groups are consistently excluded from certain river reaches, this may be reflected in different patterns of species richness among functional groups. Therefore, we used species accumulation curves to determine the species richness of each separate functional group of each river reach at 20 samples (to equalize sampling effort in the different river reaches).

Results

Species composition

Temperature was collinear with other predictors (variance inflation factor >20.03); without temperature, all remaining variables' variance inflation factors were <5.4. Forty-five of the

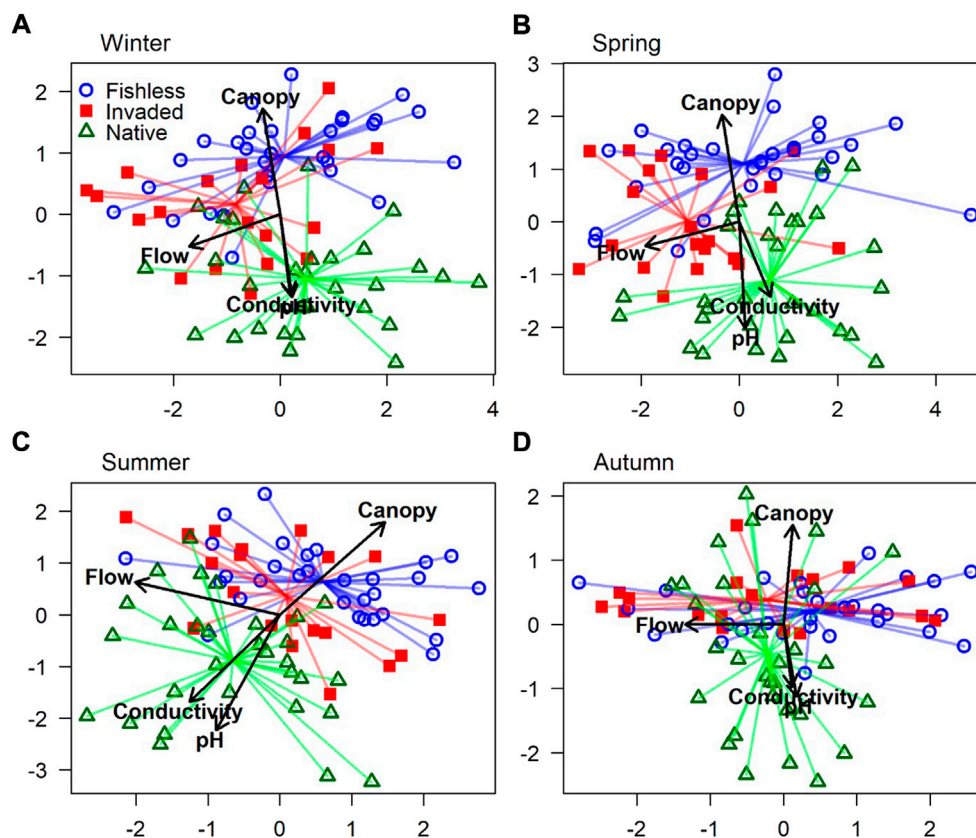


FIGURE 3

Bayesian ordination plots, for (A). Winter, (B). Spring, (C). Summer and (D). Autumn, based on latent variable 1 (x-axis) and 2 (y-axis) showing macroinvertebrate composition in river reaches without fish, invaded by trout, and inhabited by native fish. Ordered flow rate class, electroconductivity, percentage canopy cover and pH are illustrated with vectors.

104 taxa (or morphospecies) of macroinvertebrate identified from the samples taken were included in the species matrix for species composition analyses (Supplementary Table S1). Based on Akaike's information criterion values (lower indicating better model fit), the combination of predictor variables that best explain variation in macroinvertebrate composition was season, flow rate, river reach, biotope, canopy cover, conductivity and pH (Table 2). The Dunn-Smyth residuals plot and the quantile-quantile plot indicated that the model was appropriate for the data (Supplementary Figure S1). River reach was the third most important variable after season and flow rate (Table 2). Consequently, we created separate Bayesian ordinations for each season with river reach indicated, and we added flow rate as a vector (numerically ordered from no flow to fast flow), along with vectors for conductivity, canopy cover and pH (Figure 3). Biotope was not illustrated to prevent overcomplicating Figure 3. The bottom-up hypothesis was supported in all seasons, with regard to the influence of the upstream-downstream environmental changes in canopy cover, conductivity and

pH on macroinvertebrate composition. River reach remained a significant predictor of macroinvertebrate composition after all environmental variables have been taken into account, suggesting support for the top-down hypothesis where different river reaches largely represents different predation regimes. However, the strength of this effect varied among seasons, e.g., with macroinvertebrate composition overlapping more in autumn and being more differentiated in spring (Figure 3). Further, macroinvertebrate composition may vary among reaches due to environmental variables that have not been included in this study, necessitating the further supporting univariate analyses.

A closer look at the individual species with significant relationships with one or more predictor variables indicated four gathering collectors, two predators, and seven scrapers that varied significantly with river reach after other predictors have been taken into account (Table 3, see complete univariate results in Supplementary Table S1). Supplementary Figure S2 illustrates how each of these 13 macroinvertebrates varied among river reaches in each season—predicted abundance values were

TABLE 3 Univariate results (marginal analysis of variance) for those species that had a significant relationship with one or more of the predictor variables. Rao's score test statistics are given, and *p*-value, adjusted for multiple testing, is symbolised using asterisks. Coverage indicates the number of samples (out of 320) each species was present in, while abundance reflects the total count of individuals across all samples.

Species	River reach	Season	Conductivity	pH	Flow	Biotope	Canopy cover	Coverage	Abundance
Filtering collectors									
<i>Cheumatopsyche afra</i>					21.92**			70	187
<i>Pisidium ovampicum</i>					31.38**			53	275
<i>Simulium vorax</i>					17.36*			66	363
Gathering collectors									
<i>Afroptilum sudafricanum</i>	14.97*	42.42****						223	2,893
<i>Bezzia</i> sp. 1					27.34**			33	124
<i>Caenis</i> sp. 1					40.02****			81	1,650
<i>Caenis</i> sp. 2		32.82**			20.43*			97	1,019
<i>Cheleocloeon excisum</i>	14.28*							33	170
<i>Chironiminae</i> sp. 1	14.76*	24.55**				16.40*		222	1,607
<i>Cloeodes</i> sp. 1	16.86*							22	70
<i>Oligochaete</i> sp. 1						18.48*		106	435
Scirtidae sp. 1		36.97**	19.68*				17.53*	73	208
Predators									
<i>Aeshna</i> sp. 1	22.04*							36	43
<i>Cheumatopsyche thomasseti</i>					22.03**			89	258
<i>Dugesia</i> sp. 1	17.92*						17.87*	162	595
Scrapers									
<i>Adenophlebia</i> sp. 1	14.37*				21.51*			180	1,339
<i>Afronurus harrisoni</i>	15.63*	23.06**		14.78*				132	583
<i>Baetis harrisoni</i>	19.21*	26.79**			21.95**			123	959
<i>Burnupia</i> sp. 1	13.38*							141	531
<i>Castanophlebia</i> sp. 1	42.46**	48.81****			26.14**		45.51**	158	1872
<i>Demoreptus capensis</i>					24.19**			63	225
<i>Demoreptus monticola</i>		21.14*	26.65***	†16.07*	23.14**		24.85**	99	645
<i>Euthraulus</i> sp. 1	21.56*	17.88*						73	296
<i>Lestagella penicillata</i>	20.14*		†25.05**	15.65*				57	220
<i>Trichorythus</i> sp. 1					29.79**			97	867
Shredder									
<i>Goerodes cafrariae</i>		18.13*			19.30*	33.47**		116	819

*Negative relationship with continuous variable. *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001; **** *p* < 0.0001.

extracted from the multivariate generalised linear model where all predictors except river reach (i.e., insectivorous fish regime) and season were kept constant. *Cheleocloeon excisum* and *Baetis harrisoni* (Ephemeroptera: Baetidae), *Castanophlebia* sp. 1 (Ephemeroptera: Leptophlebiidae), *Chironominae* sp. 1 (Diptera: Chironomidae), *Aeshna* sp. 1 (Odonata: Aeshnidae), and *Dugesia* sp. 1 (Platyhelminthes: Dugesidae) appeared to be more abundant in the native fish reach. *Afroptilum sudafricanum* (Ephemeroptera: Baetidae), *Adenophlebia* sp. 1 (Ephemeroptera: Leptophlebiidae), *Afronurus harrisoni* (Ephemeroptera: Heptageniidae), and *Lestagella penicillata* (Ephemeroptera: Teloganodidae) were less abundant in the reaches with invasive and native fish. *Adenophlebia* sp. 1 and *Cloeodes* sp. 1

(Ephemeroptera: Baetidae) were less abundant in the invaded reach than in the native fish reach. Conversely, *Burnupia* sp. 1 (Mollusca: Ancyliidae) and *Euthraulus* sp. 1 (Ephemeroptera: Leptophlebiidae) were more abundant in the invaded fish reach.

Species richness and diversity

Following the strong seasonal influence found for species composition and abundance, species richness and Shannon diversity in river reaches with different predation regimes were examined separately for different seasons (Table 4; Figures 4, 5 and Supplementary Figure S3). Support for the bottom-up and top-

down hypotheses were found; however strength of support varied among season as both species richness and diversity fluctuated over seasons, with differences among river reaches especially prominent in summer (Table 4; Figures 4, 5). Species richness and diversity in the native fish reach were their lowest out of the three reaches in winter, intermediate between the other reaches in spring, and highest of the three reaches in summer (Table 4; Figures 4, 5). Species richness in the invaded reach was lowest of the three reaches in all seasons except winter (Table 4; Figure 4). Species diversity in the invaded reach was highest in winter and spring, intermediate between the other reaches in summer, and lowest in autumn (Table 4; Figure 5).

A strong seasonal influence on patterns of variation in species richness was also observed within functional groups (Figure 6). In the fishless reach, species richness was greatest for filtering collectors in autumn, gathering collectors in winter and spring, predators in winter, scrapers in spring and summer and shredders in winter and spring (Figure 6). In the native fish reach, species richness was greatest for filtering collectors in summer, gathering collectors in summer and autumn, predators in spring and summer, and scrapers in autumn (Figure 6). In the invaded reach, species richness was lowest for filtering collectors in summer, gathering collectors in spring and predators in winter, summer and autumn (Figure 6).

Discussion

The RCC predicts that the structure of macroinvertebrate assemblages changes along the gradient of streams (*cf.* Figure 1), particularly when longitudinal linkages are strong, as is the case for pristine forested headwater streams moving towards foothill reaches (Vannote et al., 1980; Poole 2002; Arthington 2012). Based on variation in species composition, abundance of certain common species, species richness and diversity, and contributions from different functional groups, our findings generally indicate that environmental and seasonal variation are dominant drivers of macroinvertebrate community structure in these headwater tributaries of the Keiskamma River—the differences observed between upper fishless, middle trout-invaded and lower reaches with native fish can largely be attributed to the upper-to-lower environmental gradient (favouring predictions 1, 2, and 3).

Nevertheless, although it was difficult to disentangle the effects of the cohabiting fish fauna from the environmental gradient, we found some compelling patterns of variation in abundance and presence of specific species and functional groups that suggest both detractive and augmentative effects of fish presence. We found some evidence in support of both the bottom-up and top-down hypotheses, depending on the species or functional group considered, and strongly modulated by season.

Season and, secondarily, flow rate were the most important predictors of variation in species composition. Seasonal changes can be expected in the macroinvertebrates comprising the species matrix, which includes many univoltine species for which abundance

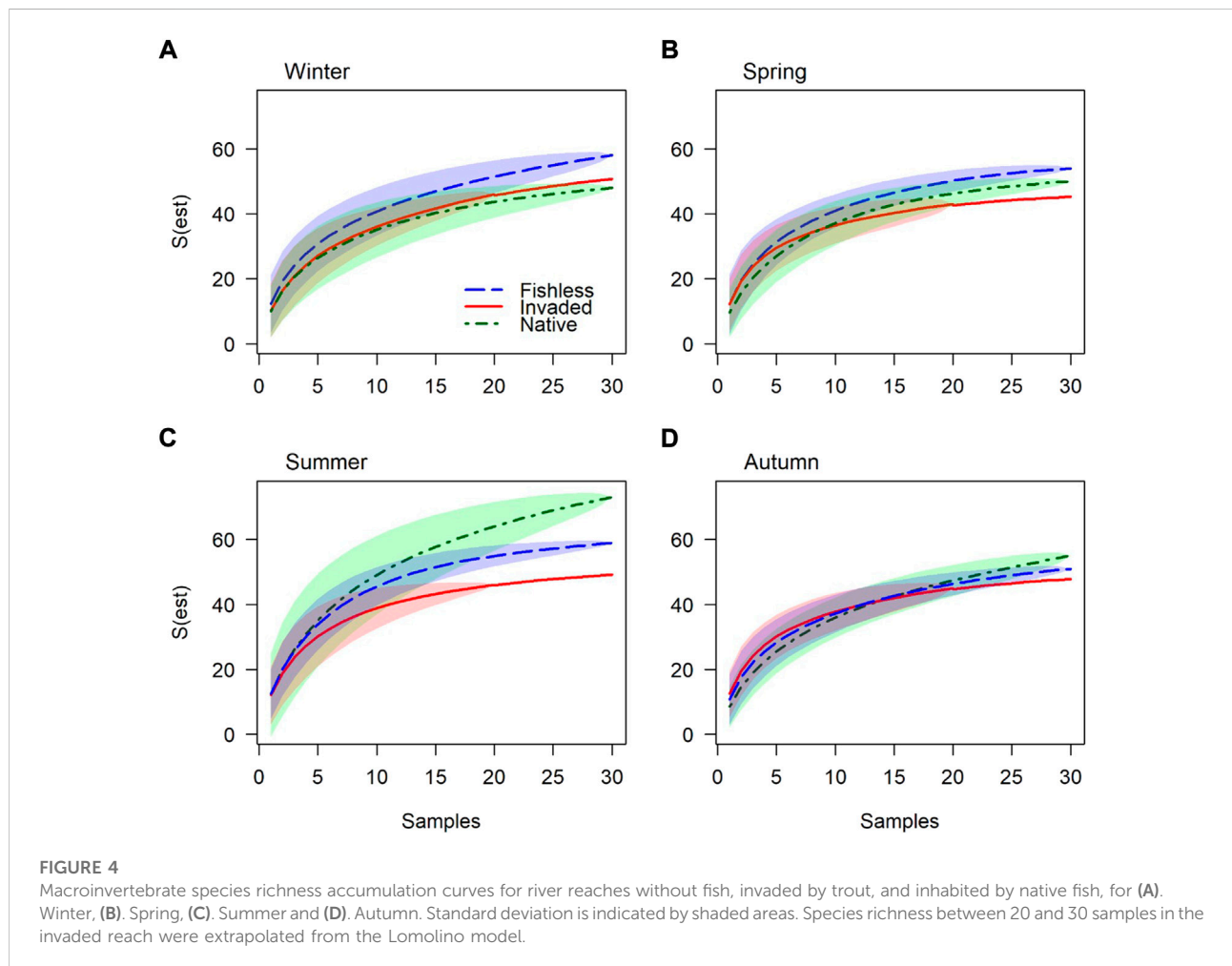
and biomass may vary over orders of magnitude during the year. The degree of overlap between macroinvertebrate composition of the different reaches varied among seasons, e.g. with a greater overlap between reaches observed in autumn and clearer differences between reaches observed in spring (Figure 3). Flow rate interacts with river morphology and substrate and affects the distribution of food particles and dissolved oxygen, so its influence on species composition is expected (Brittain & Eikeland, 1988). Flow rate is expected to decrease along the river continuum, but in the Keiskamma tributaries flow rate varied within river reaches instead of across them (Figure 3).

After season and flow rate, river reach was the third most important variable, followed by biotope, canopy cover, pH and conductivity. The effect of biotope may be due to the different degrees of protection that are offered to inhabitants by different biotopes over and above spatial, phylogenetic and predation effects (Clarke et al., 2008). Canopy cover, pH and conductivity all changed along the upper-lower environmental gradient (Table 1; Figure 3); canopy cover also represents where shredders' predominant food sources enters the river. River reach, a proxy for fish presence, remains an important predictor after bottom-up environmental variables have been taken into account. However, river reach may still cover other environmental gradient variables that were not explicitly measured in the current study, like availability and variety of particulate food and interactions with other invertebrates (Richardson 1991). Therefore, interpreting the possible effect of fish presence requires a closer look at the responses of specific species to identify mechanisms of action.

Thirteen macroinvertebrate taxa were found to vary significantly among river reaches, with an apparent seasonal influence on degree of variation, after all other environmental predictors have been taken into account (Supplementary Figure S2). Nine of these species were mayflies, which are known to be influenced by predatory fish. For example, mayflies may show avoidance behaviour in response to predatory fish (Culp et al., 1991; Cowan & Peckarsky 1994; McIntosh & Townsend 1994; Huhta et al., 2000), abundance of *Baetis* mayflies was reduced in streams occupied by *S. trutta* (Meissner & Muotka 2006), and rainbow trout have altered mayfly distributions (Albariño & Buria 2011). Nevertheless, even for these thirteen species, effects from fish predation were difficult to confirm. For example, the mayfly *A. sudafricanum*, a gathering collector, decreased in abundance from upper to lower reaches—such a pattern is not necessarily due to suppression by predatory fish, as it could be expected from the river continuum concept (*cf.* Figure 1). Further, some species, like *C. excisum* (gathering collector), *Chironominae* sp. 1 (gathering collector), *Aeshna* sp. 1 (predator) and *Baetis harrisoni* (scraper), were more abundant in the native fish reaches, possibly due to a stronger predation effect by trout compared to native fish, but more likely because of a greater variety and abundance of food in the native fish reaches (Ellender et al., 2016). The possible influence of insectivorous fish presence was clearer in *Cloeodes* sp. 1 (gathering collector), and *Adenophlebia* sp. 1 (scraper), which were least abundant in the invaded reach, whereas the limpet mollusc *Burnupia* sp. 1 (scraper) and the mayfly

TABLE 4 Asymptotes of Lomolino models fitted to species accumulation and Tsallis's diversity accumulation curves, represent estimated species richness and Shannon diversity, respectively, under condition of complete sampling. Values in brackets indicate the 95% confidence interval.

Season	Fishless	Invaded	Native fish
Species richness			
winter	92.54 (89.29, 95.80)	73.85 (69.83, 77.87)	65.53 (64.60, 66.46)
spring	69.50 (68.62, 70.38)	53.31 (51.87, 54.75)	62.91 (62.07, 63.75)
summer	70.78 (70.05, 71.51)	58.88 (58.62, 59.14)	113.63 (111.02, 116.24)
autumn	70.19 (68.87, 71.51)	58.26 (56.74, 59.78)	91.29 (87.06, 95.52)
Shannon diversity			
winter	16.39 (16.30, 16.48)	22.37 (22.27, 22.47)	15.35 (15.30, 15.40)
spring	16.50 (16.38, 16.62)	25.99 (25.69, 26.29)	24.42 (23.94, 24.90)
summer	13.37 (13.21, 13.53)	18.44 (18.38, 18.50)	24.28 (24.15, 24.41)
autumn	21.95 (21.91, 21.99)	18.66 (18.59, 18.73)	23.46 (23.44, 23.49)



Euthraulus sp. 1 (scraper) seem to thrive in the presence of trout, perhaps because of a trophic cascade involving the absence of native fish species and certain other competing invertebrates (cf. Figure 1).

Patterns of variation in species richness and diversity among river reaches also generally varied with season (Table 4); in some cases, patterns even reversed in different seasons, e.g., for gathering collectors in spring and autumn (Figure 6). Some observed

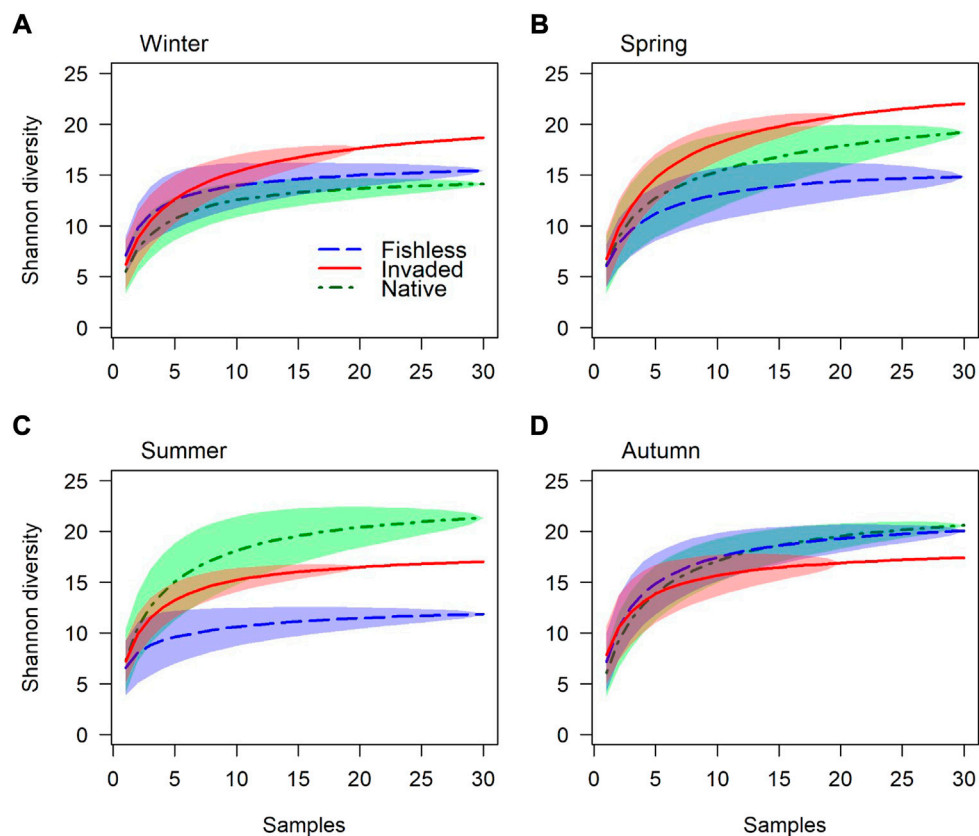


FIGURE 5

Macroinvertebrate Tsallis's accumulation curves equivalent to Shannon diversity, for river reaches without fish, invaded by trout, and inhabited by native fish, for (A) Winter, (B) Spring, (C) Summer and (D) Autumn. Standard deviation is indicated by shaded areas. Species richness between 20 and 30 samples in the invaded reach were extrapolated from the Lomolino model.

patterns suggest bottom-up environmental drivers of aquatic macroinvertebrate faunas; for example, higher species richness and diversity in the native reach in summer and autumn (Table 4; Figures 4–6), perhaps due to greater quantities and variety of food resources and greater primary productivity at higher temperatures (Ellender et al., 2016). In contrast, lower species richness and diversity in the invaded and native reaches may, in some cases, suggest a suppressing impact from fish predation. Species richness and diversity of all species together in winter, and filtering collector species richness in autumn, was lowest in the native reach, suggesting predation by native fish (Figures 4–6). Further, species richness in spring, summer and autumn, species diversity in autumn, and species richness of predators (winter, summer and autumn), gathering collectors (spring) and filtering collectors (summer), was lowest in the invaded reach, suggesting a greater negative impact on macroinvertebrates by trout compared to native fish (Figures 4–6). However, a decrease in species richness from the upper to lower reaches for gathering collectors (winter), scrapers (spring) and shredders (all seasons) is expected from the RCC and the detractive effect of fish predation could not be established or ruled out where such patterns were found (Figure 6).

Conclusion

Although multiple bottom-up and top-down drivers and interactions structure the macroinvertebrate assemblages in the Keiskamma River headwaters (Figure 1), a few consistent effects could be identified when synthesising the outcomes of the species composition, abundance, richness and diversity analyses.

First, environmental variables appear the strongest drivers of the aquatic macroinvertebrate assemblages of the Keiskamma River headwaters—favouring predictions 1–3; however, patterns of abundance and presence of certain species and functional groups suggest that predation by introduced trout and native fishes could significantly influence the structure and dynamics of the macroinvertebrate community—favouring predictions 4–6. Evidence of an effect of fish predation on macroinvertebrate communities were similarly subtle in other studies. For example, Herbst et al. (2009) found no difference in invertebrate densities in fishless streams compared to paired trout streams, but they reported lower invertebrate richness and diversity from invaded streams. Flecker & Allan (1984) found that substrate type determined the abundance, richness or density of stream

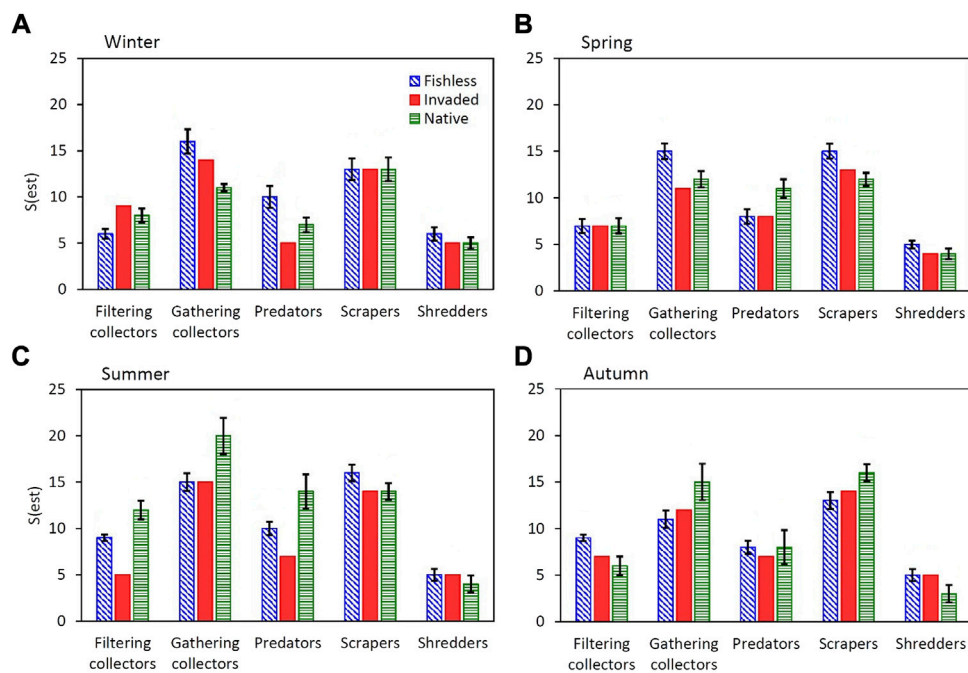


FIGURE 6

Species richness from accumulation curves at 20 samples for (A). Winter, (B). Spring, (C). Summer and (D). Autumn, for separate functional groups per fishless, invaded and native fish river reaches. Error bars indicate standard deviations of $S(\text{est})$. See [Supplementary Table S2](#) for further detail.

macroinvertebrates, while only the largest macroinvertebrates were affected by predation. Locally, [Rivers-Moore et al. \(2013\)](#) examined macroinvertebrate assemblages for the effects of trout specifically and concluded that overriding environmental factors related to upstream/downstream effects made detecting the effects of introduced salmonids on macroinvertebrates difficult.

Second, although introduced predators have been shown to have a greater impact on prey species than natives (e.g., [Alexander et al., 2014](#)), we could not confirm in this study that trout had a greater detractive influence (through predation) on macroinvertebrates, as it may simply be easier to distinguish unexpectedly higher or lower values in the middle reach from the background environmental gradient.

Third, patterns of species composition, richness and diversity varied strongly with season, probably due to large variations in abundance of univoltine macroinvertebrates. However, there may be other possible explanations, including competition among macroinvertebrates due to seasonal variation in food resources (e.g., [Richardson 1991](#)), and seasonal changes in predation intensity, particularly by trout, which may switch seasonally between terrestrial and aquatic food sources ([Li et al., 2016](#); [Milardi et al., 2016](#)).

The experimental design employed during this study examined predation by salmonids under “natural” circumstances (a natural experiment sensu [DiNardo 2008](#)), where the densities of neither

prey nor predator were experimentally manipulated, in systems where trout have formed self-sustaining, wild, populations. Further scope exists for exploring the relative strengths of top-down predation and bottom-up environmental effects in these streams, particularly investigating intra-annual variation in food resources and competitive interactions for both macroinvertebrates and fish. This may only be achievable through manipulative experiments, e.g., by exclusion of trout within invaded reaches (e.g., [Allan 1982](#)), or through the use of stable isotopes. Moreover, as shown in this study, more insight can be provided by studies recording intra- and inter-annual variation, compared to single-season studies.

Data availability statement

The original contributions presented in the study are included in the [Supplementary Material](#), further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by South African Institute for Aquatic Biodiversity (SAIAB) Animal Ethics Committee.

Author contributions

TB conceptualized the study, collected the field and laboratory data, and drafted and finalized the documentation. SH conducted the analyses and drafted and finalized the documentation. MV supervised, drafted and finalized the documentation. OW conceptualized and funded the study and drafted the documentation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2022.1004939/full#supplementary-material>

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