Assessing community structure and trophic interrelationships in three differently impacted headwater streams in the Amathole-Winterberg freshwater ecoregion, South Africa

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ABSTRACT

Afromontane regions represent some of the highly threatened ecosystems on the planet as they harbour endemic and often relic freshwater fauna. These ecologically sensitive ecosystems have been altered by multiple impacts, including invasion by non-native fishes, replacement of diverse indigenous vegetation with monoculture plantations, agricultural and mining activities, hydrological modifications, and degradation of instream habitats, with potential detrimental effects on aquatic community structures and food web dynamics. The aim of the present study was to compare spatio-temporal patterns of macroinvertebrate and fish communities as well as food web dynamics in three differently impacted headwater tributaries of the Kat River in the Amathole-Winterberg freshwater ecoregion in the Eastern Cape Province of South Africa. The upper Kat River catchment was the ideal model for purposes of the present study as it contains streams with varying degrees of human impact. The streams considered in the present study were (i) the Eyre River which was considered to closely represent near-natural conditions as it is the least impacted stream in this catchment, with the riparian zone dominated by relatively intact and diverse native woody vegetation, (ii) the Elands River whose catchment has been altered by agricultural activities, and (iii) the Lushington River, whose riparian zone has been heavily invaded by black wattle. The three headwater streams were generally distinguished based on the physical-chemical variables. The Elands River was characterised by high conductivity and total dissolved solids (TDS). In addition, the Elands River was more alkaline and relatively warmer than the other two rivers. This suggested the negative influence of agriculture activities on the water quality in the Elands River. In general, the Lushington and Eyre rivers had comparable physical and chemical variables. However, the Lushington River was generally characterised by low streamflow, likely as a consequent of black wattle which is known for altering hydrological regimes of streams .The Eyre River and Lushington River were comparable in terms of macroinvertebrate richness and diversity, whereas the Elands River was characterised by low macroinvertebrate diversity and richness. Redundancy analysis indicated that the macroinvertebrate communities were mostly influenced by seasonality, with land-use accounting for a small but significant difference in community composition. In comparison, the generalised linear mixed-effects models (GLMMs) showed that chubbyhead barb abundance was significantly influenced by land-use patterns. Evaluation of food web dynamics using stable isotope analysis revealed that the food web structure in the three rivers differed substantially as a result of land-use. Specifically, the Eyre River was characterised by less variation in stable isotope values of basal food sources and consequently, the consumer groups had a narrow isotopic breadth. In contrast, the Elands River was characterised by a wide variation in basal food sources and therefore, a wider isotopic breadth for consumers. The Lushington River was spatially variable in terms of basal sources and isotopic breadth for consumers. The study concluded that food webs and trophic interrelationships were a more informative indicator of land-use than community structure was in evaluating the impact of land-use patterns on aquatic communities. In addition, future studies should seek to investigate food webs interrelationships in addition to community structure to infer a more conclusive river assessment.

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Ngiyabonga boGatsheni, oBoyabenyathi kanye noGombashe, oMhlahlandlela ngokuhlala nami kuko konke.

"Iso liwela umfula ugcwele"

CHAPTER 1

GENERAL INTRODUCTION

The Amathole-Winterberg ecoregion and its freshwater biota

The Amathole-Winterberg freshwater ecoregion in the Eastern Cape Province of South Africa is defined by highlands and mountain systems. It forms part of the African mountains and highlands system which includes the Drakensberg-Maloti Highlands (South Africa and Lesotho), the Eastern Zimbabwe Highlands (Zimbabwe), Mount Mulanje (Malawi), the Albertine Highlands (Democratic Republic of Congo), the Ethiopian Highlands (Ethopia), together with the Fouta-Djalon Highlands (Guinea), and Mount Nimba (Guinea and Côte d'lvoire) in West Africa (Thieme et al., 2005). Similar to these African highlands systems which harbour unique forms of aquatic fauna (Dudgeon et al. 2006, Mittermeier et al. 2011), the Amathole-Winterberg freshwater ecoregion is characterised by endemic macroinvertebrate and fish fauna (Darwall et al. 2010, Clausnitzer et al. 2012, Samways and Taylor 2004). The freshwater habitats of this region comprise both perennial and temporal rivers that are characterised by highly seasonal discharge (River-Moore et al. 2007). The high-altitude Afromontane region of the Amathole-Winterberg ecoregion is drained by a number of headwater tributaries of major river systems, including the Buffalo, the Keiskamma, the Great Fish and the Swart Kei rivers (Thieme et al., 2005).

The Amathole-Winterberg freshwater ecoregion harbours three highly threatened narrow-range endemic fishes, the anabantid *Sandelia bainsii*, and two cyprinids *Amatolacypris trevelyani* and *Enteromius amatolicus* (Skelton, 2001; Skelton et al., 2018). Both *S. bainsii* and *A. trevelyani* are currently listed by the IUCN as endangered, whereas *E. amatolicus* is listed as data deficient (Chakona, 2018), although evidence from *ad hoc* surveys suggest that there could be ongoing decline in the distribution range of this species. In addition to these freshwater fishes, this ecoregion hosts a wide range of aquatic macroinvertebrates, including two endemic odonates, namely *Chlorolestes tessellatus* and *C. apricans* that is currently listed as endangered on the IUCN Redlist (Samways and Taylor 2004, Samways 2004). Furthermore, this ecoregion is a hotspot for amphibians, hosting up to 19 species, two of which are endemic *(Sclerophrys pardalis* and *Vandijkophrynus amatolicus*), together with a wider range of other terrestrial invertebrates and flora (Channing 2001, Perera et al. 2011). Despite the occurrence of unique biota in this ecoregion, the headwater streams and their fauna are under severe threat from multiple impacts including invasion by both non-native fishes and plants, and agricultural activities that are likely to alter the integrity of riparian zones and flow regimes of these headwater streams.

One of the widely recognised primary concerns in the Amathole-Winterberg ecoregion's freshwater ecosystems is the presence of non-native piscivorous fishes, particularly rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, largemouth bass *Micropterus salmoides* and African sharptooth catfish *Clarias gariepinus* (Jubb 1979, Skelton 1993b, Cambray 2003, Kadye and Booth 2012). Although at present there have been no dedicated studies that have documented the specific impacts of these invasive fishes within the AWE, studies from the Cape Fold Ecoregion of South Africa have revealed changes in native fish and macroinvertebrate community structures in river sections invaded by trout (Woodford & Impson, 2004) and smallmouth bass species (Shelton et al., 2014). In particular, Shelton et al. (2015) reported that in the Breede River system, rainbow trout (*Oncorhynchus mykiss*) selectively preyed on small-sized minnows. In addition, rainbow trout was reported to have displaced minnows and limited them to small habitat patches that trout was unable to utilise (Shelton et al. 2017). The introduced black bass species were also found to influence habitat selection and abundances of Cape galaxias in the Witte River (Shelton et al., 2008). In the Olifants–Doorn River system, black bass was found to reduce abundances of small cyprinid minnows (Van der Walt et al., 2015). In the Maloti-Drakensberg ecoregion, non-native brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* have been reported to have caused range decline for the endemic *Pseudobarbus quathlambae* (Cambray 2003, Weyl et al. 2018). Similar studies in the Eastern Zimbabwe Highlands (Zimbabwe) and Nyika Plateau (Malawi) have also reported predation impacts by rainbow trout *O. mykiss* on the mountain catfishes and aquatic macroinvertebrates (Kadye et al., 2008; Kadye et al., 2013). Elsewhere, empirical evidence indicates that the establishment of these non-native fishes has led to either range reduction or local extirpation of native fishes (Cucherousset & Olde, 2011). In addition, non-native fishes have also been found to alter food webs by competing with and preying on native fishes (David et al., 2017).

Other impacts that have altered the integrity of Afromontane ecosystems include the spread of non-native invasive plants and modifications from arable farming (Ridgeley et al. 2012). In the Amathole-Winterberg ecoregion, research has shown that the temperate grass biome has been extensively invaded by non-native woody vegetation, primarily due to silviculture, which has led to uncontrolled spread of many invasive species (Henderson, 1992). Consequently, the riparian zones of many headwater streams of this Afromontane ecoregion have become invaded by non-native plant species. The common non-native invasive plant species in this region include different species of pine (*Pinus* sp), gumtree (*Eucalyptu*s sp), wattles (*Acacia* sp), and tickberry (*Lantana camara*) (Jimu, 2011). Previous research has shown that these non-native plant species are most prevalent in moist habitats. In particular, watercourses have been found to enable the dispersal of non-native plants, including *Acacia mearnsii*, *A. dealbata*, *Sesbania punicea* and *Ricinus communis* (Barney 2008; Van Wilgen et al., 2001). Some parts of the AWE have also been altered agricultural activities. There are serious conservation concerns as there has been no dedicated studies that have documented the specific effects of these impacts of the AWE’s endemic aquatic fauna.

General impacts of invasive plant species in aquatic systems.

Non-native species are taxa introduced from their natural range to a new region where they often spread rapidly and become established (Ricciardi & Cohen, 2007). In Afromontane ecoregions, both non-native flora and fauna have become established, raising concern on their potential negative impact on native biota (Darwall et al., 2010; Jimu, 2012). In these regions, non-native plant species have become established in both terrestrial and riverine habitats (McDougal et al., 2011). The impacts of non-native plant species in Afromontane ecoregions, which have been widely reported on terrestrial ecosystems, include altering native plant communities through competition for nutrients and water (Chamier, 2012) and by modifying fire regimes (Castro-Diez & Alonso, 2017). In riverine ecosystems, the impact of non-native plants has largely been associated with the alteration of stream hydrology through increased water loss (Cullis et al., 2007) and reduced stream flow (Chamier, 2012). The loss of streamflow and water loss is reported from non-native plants whose water requirements exceed those of the native vegetation. Other studies have also shown that non-native vegetation has impact aquatic ecosystems by influencing organic matter input and processing (McNeish et al., 2012; Mineau et al., 2012), the structure and composition of aquatic invertebrate communities (Ferreira et al., 2015) and the composition and productivity of fish communities (Strayer et al., 2010) together with the associated food web dynamics (Heleno et al., 2008; Stiers et al., 2011).

In forested headwater streams, organic input, in the form of leaves, is essential in supporting nutrient cycling and influencing the composition of aquatic communities (Moss, 2010). Several studies have shown that in invaded headwater streams, non-native vegetation alters both organic matter input and instream decomposition patterns (Sampaoi et al., 2001; Freund et al., 2013). In general, studies have reported slow decomposition of non-native plant matter in headwater streams, particularly for species of the genus *Eucalyptus* (Canhoto &

Graca, 1996; Graca et al., 2002). Slow decomposition of organic matter often has a negative impact on nutrient cycling, which ultimately influences stream productivity and the composition of both aquatic macroinvertebrates and fish communities (Wipfli et al 2007). This has been associated with factors such as low microbial and macroinvertebrate colonisation, particularly of the shredder community that are often responsible for breaking down of allochthonous matter (Medina-Villar et al., 2015). A few authors such as Dangles et al.,2004 and Leroy & Marks, 2006 have suggested that this may be associated with changes water chemistry, and intrinsic leaf litter characteristics such as lignin content and nitrogen levels (Martinez et al. 2013). In contrast, other studies have shown that native plants, such as alder (*Alnus* spp) have faster decomposition rates in invaded streams compared to uninvaded streams (Helsen et al., 2008; Leroy & Marks, 2006). In addition, some studies have shown that in headwater streams dominated by non-native riparian vegetation, the decomposition rates of native plant matter were slower compared to uninvaded streams (Harner et al. 2009; McNeish et al. 2012)

The impact of non-native plants on macroinvertebrates has been reported to be variable. For example, Stiers et al. (2001) reported that one of the biggest threats imposed by non-native plants was the inability of macroinvertebrates to colonise the non-native vegetation. Similarly, Whiles & Wallace (1997) identified that monoculture plantations of pine reduced the abundance and diversity of macro-invertebrates, particularly the shredder diversity due to slow leaf litter decomposition rates. In contrast, Strong et al. (1984) found that macroinvertebrates were often able to colonise non-native vegetation and reported that this depended on the type of non-native vegetation and the resilience of the macroinvertebrate fauna. In South Africa, however, empirical evidence seems to show that non-native vegetation is associated with negative consequences on aquatic biota. Specifically, Samways and Sharratt (2010) identified that non-native riparian vegetation reduced the biomass of sensitive and endemic invertebrate taxa. In addition, Samways (2004) found that odonates that utilise native riparian vegetation for reproduction were largely affected by the loss of native riparian vegetation, through the loss of perching and oviposition sites. The effects of non-native riparian vegetation often affect the rest of the ecosystem, including fish communities and consequently food web dynamics.

Similar to patterns on aquatic invertebrates, the impact of non-native riparian vegetation on the fish communities has been reported to be varied and often indirect. Research suggests that non-native riparian vegetation such as *Eucalyptus* release toxic metabolites in the form of leachates. For example, Morrongiello et al. (2011) reported that in the Castle Creek and the Broken River, female pygmy perch exposed to *Eucalyptus* leachates were unable to reproduce, either due to stress or found the habitat unsuitable. Other studies have also reported indirect impacts of non-native riparian vegetation, which has been linked to a reduction in terrestrial resource subsidies. For example, Roon et al. (2016) reported that European bird cherry (*Prunus padus*) led to reduced terrestrial macroinvertebrate subsidies which support juvenile Coho salmon (*Oncorhynchus kisutch*) which likely had negative effects on the fish. Additionally, the assemblage of warm-water fishes can be negatively impacted by non-native riparian vegetation, which has been found to provide constant shading and decreasing water temperature (Balts & Moyle, 1984). This often leads to dietary shifts in response to non-native riparian vegetation.

In addition to influencing aquatic community structure, the effect of non-native vegetation on food web dynamics is well documented. In particular, non-native riparian vegetation has been found to influence the timing, quality and quantity of leaf litter input (Going & Dudley, 2008). For example, Hladyz et al. (2011) reported that non-native common rhododendron (*Rhododendron ferrugineum)* impacted both allochthonous and autochthonous pathways. Specifically, rhododendron was found to negatively influence the autochthonous production by suppressing algal growth due to shading, whereas, the allochthonous-derived food web was negatively influenced by the common rhododendron's slow decomposition. In other studies, non-native riparian vegetation was found to negatively influence macroinvertebrate composition, with the invaded stream sections having a simpler macroinvertebrate community thereby reducing the size of the food web (Heleno et al., 2009). In contrast, Burkle et al. (2012) reported a positive relationship in stream sections invaded by purple loosestrife flower (*Lythrum salicaria*), as it increased visitation of terrestrial flying insects, thereby, creating a new resource and increasing trophic interaction. In addition to these, stable isotope-based studies have shown that in plant-invaded headwater streams the isotopic signatures of organic matter and consumers were depleted in $\delta^{13}C$ values and enriched in $\delta^{15}N$ values (Atwood et al., 2010). This was likely due to the non-native vegetation albizzia (*Falcataria moluccana*) displacing major contributor such as particulate organic matter and macroalgae to the lower-level consumers.

General impacts of agricultural land-use patterns on stream ecology

Afromontane ecoregions (Van der Merwe, 1998; Geldenhuys et al., 1986) are also used extensively for both crop and livestock farming (Geldenhuys & Van der Merwe, 1988). In general, small-scale crop farming is one of the common agricultural activities in Afromontane regions. Crop farming is generally associated with the alteration of the physical and chemical properties of the soil (Wanyama et al., 2018a, b), which in turn, often have profound negative effects on adjacent streams and rivers. Specifically, within Afromontane regions, the prevalent use of fertilizers in crop farming has been found to result in elevated concentration of nutrients, such as nitrogen (Royer et al., 2004), phosphorous (Dodds & Smith, 2016) and ammonia (Richardson, 1997). In addition, soil disturbance due to cultivation increases the susceptibility of soil to runoff into headwater streams, often leading to sedimentation and loss of habitats. Although few studies have explored the effects of agricultural land-use activities on Afromontane freshwater habitats, studies elsewhere have shown that runoff from agricultural lands influences the habitat structure and chemical composition of streams and rivers (Bullard 1966, Allan et al. 1997).

There exists a considerable body of literature on the impacts of agriculture, particularly on the impacts of agricultural runoff. Previous studies have emphasized that runoff from agricultural lands may affect the community composition, their structure and function in aquatic ecosystems. For instance, Liess & Ohe (2005) reported that runoff from pesticides led to a reduction in sensitive macroinvertebrate species due to pesticide toxicity, thereby favouring species with fast recovery time or the ability to migrate. Schafer et al.'s (2007) findings were that pesticide stress reduced taxonomic richness and abundance of macroinvertebrates, along with reducing leaf-litter decomposition rates. Similarly, Karr et al. (1985) noted that headwater streams impacted by agricultural runoff had low fish species richness and abundance due to poor water quality. Furthermore, Walser and Bart (1999) identified that agricultural runoff decreased fish biomass, specifically for species that use coarse substrate such as pool and riffles for spawning or feeding.

Studies of agricultural impacts on streams are well documented, and it is well acknowledged that agricultural activities have a negative effect on stream food webs (Correll et al., 1995). For example, biocide leaching has been found to increase instream nutrients, which has profound effects on stream food webs (Harding et al., 1999). Ecosystem imbalances such as simplified food webs that are driven by grazing/herbivorous macro-invertebrates have been linked to increased instream nutrients (Wantzen & Mol, 2013). Prior stable isotope-based studies have shown that agricultural development correlated with high ^{15}N values for basal sources and consumers as a consequence of elevated stream nitrates concentrations (Bodey et al., 2011, Harrington et al., 1998).

Ecological attributes of the Kat River headwater streams

The Kat River is a major tributary of the Great Fish River, in the Eastern Cape, South Africa. Three headwater tributaries of the Kat River; the Lushington, Elands and Eyre rivers' catchments are part of the Amathole-Winterberg Afromontane ecoregion. These three headwater tributaries all drain into the Kat River Dam at Seymour town (Figure 1). The aquatic fauna of these headwater streams comprises two freshwater fishes, namely *Enteromius anoplus* and *Sandelia bainsii* and numerous aquatic macroinvertebrate species. Previous studies in the adjacent Koonap River and Buffalo River systems have showed that the aquatic macroinvertebrate fauna of this region is characterised by seasonal species assemblage in response to stream flow variability (Palmer et al., 1991, Kambikambi et al., 2019).

The headwater streams of the Kat River have different catchment characteristics. The Lushington River's riparian zone is extensively invaded by the non-native tree, black wattle (*Acacia maernsii)* (Figure 2). In addition, the upper catchment of this river is dominated by gumtree plantations (*Eucalyptus* spp). By comparison, the Elands River catchment is characterised by small scale subsistence crop farming. Although its riparian zone is generally unaltered, the Elands catchment is characterised by pronounced seasonal variation in flow (Figure 3). In contrast, the Eyre River catchment is sparsely populated, and its riparian zones are largely unaltered (Figure 4). The differences in the catchment characteristics of these Afromontane headwater streams raises questions on the structure and function of their southern temperate aquatic communities. In general, because of their small size, aquatic communities in headwater streams are influenced by the differential importance of allochthonous and autochthonous energy sources (Moss, 2010), which in turn influences the composition of higher trophic groups and their food web dynamics (Huxell & MacCann, 1998). These patterns are, however, likely to be related to catchment characteristics (e.g. forested vs grassland catchments) (Richardson & Dunhey, 2007) together with the nature of disturbances that occur within the catchments.

Within the relatively undisturbed Eyre River, it is likely that the aquatic communities would reflect spatial and temporal dynamics that are characteristic of forested temperate headwater streams. Studies elsewhere have shown that undisturbed temperate headwater streams are likely to rely on allochthonous litter input, promoting detritus-based food webs, and are therefore often maintained by a dominant shredder and collector community. Additionally, these food webs are predicted to have fewer insectivorous predators or piscivorous fish, often maintaining populations of small-sized fishes (Richardson & Dunhey, 2007). In comparison, it is likely that the Lushington and Elands rivers would have aquatic communities that reflect plant invasion and crop-farming related disturbances, respectively. Several studies have shown that headwater streams with non-native riparian plants are characterised by simplified food webs, often maintained by resilient macroinvertebrate taxa that have generalist diets and can adjust to potential physicochemical property shifts in water (Schirmel et al., 2016). Furthermore, non-native riparian vegetation such as wattle have been found to have deleterious effects on fishes, due to the secondary compounds such as saponins which are considered to be toxic to fish (Maerz et al., 2005). On the other hand, studies on headwater streams that are influenced by crop farming have reported altered physical-chemical characteristics, which in turn influence autotroph instream production (Griffiths et al., 2013). It is predicted that in such streams, both the communities and food webs are likely to be autochthonous based with a higher abundance of herbivorous macroinvertebrate taxa and a decline in shredder communities (Hladyz et al., 2001).

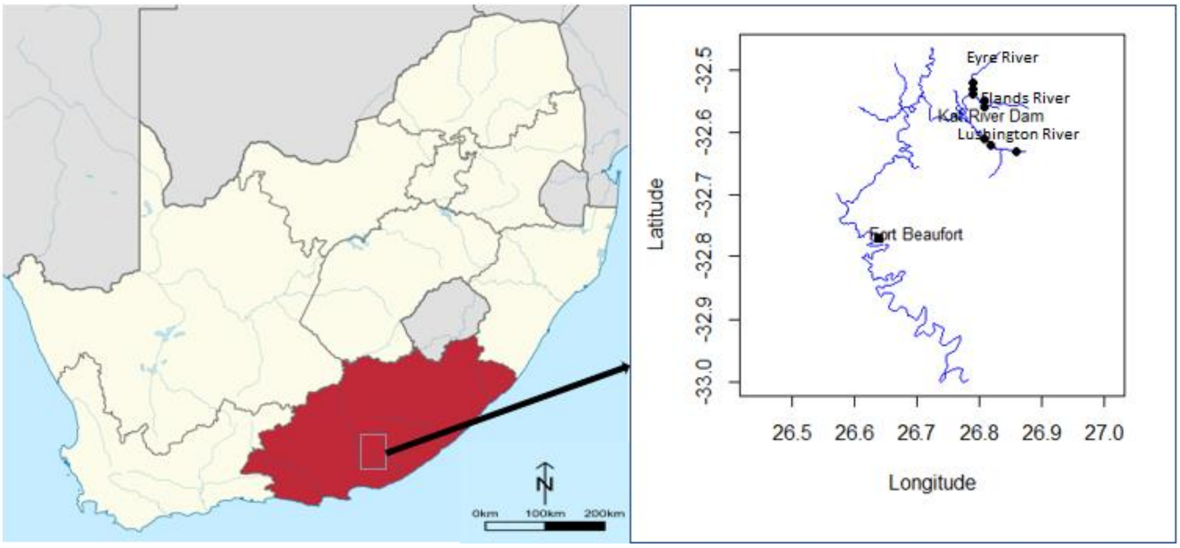

Figure 1: Topographic map of the head water tributaries of the Kat River, including the sampling sites in the Eyre, Elands and Lushington rivers.

Figure 2: The Lushington River, which is invaded by non-native vegetation, predominantly black wattle (*Acacia maernsii)*, although gabions are present in the picture they are not extensive.

Figure 3: The Elands River, which is impacted by agriculture, the picture depicts the low stream flow during the autumn season as a result of a water abstraction for agricultural purposes.

Figure 4: The Eyre River which is the least impacted of the three-study stream, is characterised by native vegetation, mainly dominated by *Vachellia karoo*, the picture was taken following a flood event.

Aims, objectives and key research questions.

The specific impacts of land use changes and invasion by non-native plants on aquatic ecosystems in the AWE have not been studied. This is despite the ecoregion's status as host to several endemic freshwater taxa, including endangered freshwater fishes and macroinvertebrates. Therefore, documenting the aquatic biodiversity and understanding the impacts associated with non-native plant invasions and agricultural land use disturbances is critical in order to mitigate further impacts and to inform conservation planning. Previous studies in this region have shown that, in general, within less disturbed habitats, the aquatic biota, including fish and macroinvertebrates, and their food webs are characterised by high seasonal variability (Kadye & Booth 2012; Kambikambi et al., 2019). It is therefore critical to understand the ecoregion's broad community structure and their trophic interrelationships, including in disturbed habitats.

The aim of the study was to assess responses of aquatic communities and evaluate changes in trophic structure related to replacement of riparian vegetation with non-native invasive plants and agricultural land use activities. The study focussed on three adjacent streams that are geographically situated in the same ecoregion, but with differences in the degree of impact from invasive plants and intensity of agricultural activities. The study was motivated by the fact that the upper Kat River catchment has been designated as a National Freshwater Ecosystem Priority Area (NFEPA) (Nel et al., 2011), because it represents one of the few remaining sanctuaries for the highly threatened anabantid fish, the Eastern Cape rocky (*Sandelia bainsii*) which has been a flagship species for aquatic biodiversity conservation in the Eastern Cape Province of South Africa.

The study addressed two key research questions:

1. To what extent have agricultural activities and replacement of natural and diverse riparian vegetation with invasive monoculture plantations influenced the spatio-temporal dynamics of macroinvertebrate and fish communities in the upper Kat River catchment?
2. To what extent have agricultural activities and replacement of natural and diverse riparian vegetation with invasive monoculture plantations altered aquatic food web dynamics in the upper Kat River catchment?

Study area

The Amathole-Winterberg freshwater ecoregion, is drained by three major river systems which are the Great Fish, the Keiskamma River, the Buffalo River and the Swart Kei. The study focussed on the headwaters of the Kat River, a major tributary of the Great Fish River system. The natural vegetation in this region is dominated by deciduous broad-leafed forests and grasslands (Mucina & Rutherford, 2006). *Vachellia karroo* and succulent thicket comprising *Euphorbia* spp. and *Diospyros dichrophylla* and *Olea europaea* are also important vegetation components of this catchment (Shackleton & Shackleton, 2006).

The rainfall in the catchment varies widely, with the upper catchment receiving the highest rainfall of approximately 1000 mm per annum (Jari & Fraser, 2009). The mean annual rainfall is approximately 500 mm, with the rainfall peaking around March and the lowest rainfall being recorded in winter months (June/July) (Mottaux, 2001). The climate is described as mild to semi-arid (Jari & Fraser, 2009), warm semi-arid in the Köppen-Geiger climate classification (Kottek et al., 2006). The maximum daily temperature varies from 30º C in summer (November to February) to 21º C in winter (June to August), while the minimum mean daily temperature varies from 17º C in summer to 8º C in winter (Lerotholi et al., 2004).

Thesis outline

This thesis has four chapters: (i) Chapter 1 which provides the background to the study and introduces that study area, (ii) Chapter 2 addresses the first aim of evaluating the spatial and temporal community patterns in differently impacted headwater streams, (iii) Chapter 3 addresses the second aim of examining trophic interrelationships within these headwater streams, and (iv) Chapter 4 provides a synthesis of the findings from this study.

CHAPTER 2

Comparative assessment of aquatic communities within three headwater streams influenced by different land-use patterns

Introduction

Despite their small size, headwater streams are critical both in nutrient cycling and in supporting locally adapted aquatic biota (Lowe and Likens 2005; Meyer et al. 2007). Because of their high connectivity with the surrounding landscape and close land-water interface (Richardson 2019; Moss 2010), headwater stream harbour communities that generally rely on locally available resources, such as the relatively shallow habitats together with allochthonous input and detrital-based organic matter (Vannote et al. 1985; Richardson 2019; Richardson and Danehy 2007). The biological communities associated with these headwater streams include macroinvertebrate groups that generally utilise particulate organic matter, such as shredders and collectors (Moss 2010), and few fish species that are either predators or small-sized insectivores (Richardson 2019). Due to the shallow habitats and the spatial isolation that characterise headwater streams, their aquatic communities are generally likely to reflect both natural changes associated with spatial and temporal scales (Richardson and Danehy 2007) and anthropogenic changes that are likely to occur within their surrounding landscape (Zhang et al. 2013). The spatial and temporal scales that are likely to influence

Generally, within near-pristine headwater streams, aquatic communities vary both spatially, due to the availability of a mosaic of habitats (Schlosser 1995; Kemenes and Forsberg 2014), and temporally, due to seasonal changes in physical conditions, such as temperature, stream flow and light (Cowell et al. 2004). Several studies have shown that headwater streams provide a mosaic of microhabitats, with the spatial structuring of organisms, such as macroinvertebrates being characterised by a general change in community composition along substratum and depth gradients (Rempel et al. 2000, Harvey and Stewart 1991). For example, Graça et al. (2004) found that macroinvertebrate diversity and abundance increased with substratum heterogeneity. In addition, increase in macroinvertebrate diversity and abundance has often been associated with upstream to downstream longitudinal changes, which is usually related to increase in habitat sizes (Vander Vorste et al. 2017), the availability of diverse habitats (Erman and Erman 1984) and increase in predator-prey interactions that minimises the dominance of certain taxa (Allan 1983). In comparison, temporal patterns in macroinvertebrates have been found to be related to hydrological stability and resource availability (Barbero et al. 2013). Collier (2008) found that extant taxa composition is often influenced by the persistence of the previous community composition. Similarly, Bradley and Merod (2001) found that this persistence was driven by environmental stability.

In contrast to near-pristine headwater streams, aquatic communities in anthropogenic-perturbed headwater streams are likely to vary in relation to the nature of the disturbances (Richardson and Danehy 2007). The common anthropogenic disturbances in headwater streams include agricultural activities, such as crop farming, which influences nutrient and sediment input into aquatic habitats (Richardson 2019) and alteration of natural riparian vegetation due to invasion by non-native plants, which influences allochthonous input and water chemistry (Wohl 2006; Maloney and Weller 2011). Several studies have shown that such disturbances have a negative effect on aquatic community structure and function. For example, agricultural activities have been found to alter water quality by increasing both organic and inorganic nutrient input (Harding et al. 1999). Increase in both organic and inorganic nutrients usually result in eutrophication and reduced water quality. Additionally, agriculture has been linked to reduced water quality leading to a decrease in the abundance and biomass of sensitive macroinvertebrate taxa and proliferation of disturbance-tolerant taxa (Sponseller et al. 2001). Furthermore, runoff from agricultural lands is often associated with sediment input within headwater streams, which often leads to habitat loss and the associated disappearance of stenotopic taxa in favour of eurytopic taxa (Kasangaki et al. 2007). In addition, Walser and Bart (1999) found that agricultural runoff reduced fish abundance and diversity in headwater streams. Similarly, Staufer et al. (2000) found that agricultural runoff reduced fish richness in impacted sites.

In comparison, invasion by non-native vegetation in riparian zones of streams has been associated with the alteration of aspects such as organic matter input, light availability, thermal regimes and stream flow, which ultimately alters the composition of aquatic communities (Stiers et al. 2011; Vilà et al. 2011). For example, non-native riparian vegetation has been found to either decrease both the quantity and quality of organic matter input (Heleno et al. 2009). In addition, by outcompeting native woody vegetation and grasses (Hager 2004), the non-native evergreen riparian vegetation alters instream seasonality through continuous shading, thereby changing the thermal regimes and inhibiting autotrophic productivity (Thiébaut et al. 2018). Furthermore, Roon et al (2006) found that non-native riparian vegetation reduced prey input for headwater stream fishes. Lastly, Casatti (2005) found that rocky substrate fishes were impacted mostly by non-native riparian vegetation, as non-native vegetation was found to deteriorate and reduce habitat for fish.

The Amathole-Winterberg ecoregion is drained by several major rivers whose headwater streams vary in their catchment characteristics, ranging from near-pristine to highly disturbed. Previous ecological studies in this ecoregion have given insight on the general spatial and temporal dynamics of both aquatic macroinvertebrates (Palmer et al. 2014; Eady et al. 2013; Mabidi et al. 2017) and fishes (Kadye and Booth 2012, 2014, Kambikambi et al. 2019) in less disturbed habitats. There is, however, little information on the broad scale comparison of different headwater streams in relation to different disturbance regimes. This study focussed on the spatial and temporal dynamics of aquatic communities in three differently impacted headwater tributaries of the Kat River. The three headwater streams are the Eyre River, which is the least impacted stream and thus closely represented the reference conditions, the Lushington River, whose riparian zone has been invaded by non-native black wattle (*Acacia maernsii*), and the Elands River, whose catchment was characterised by subsistence agricultural faming. Specifically, this study evaluated the spatial and temporal patterns of aquatic macroinvertebrates and the chubbyhead barb *Enteromius anoplus*, which was the common fish species, within these headwater streams.

It was hypothesized that the macroinverbrate communities, together with the chubbyhead barb populations would exhibit pronounced spatial variation that reflected the differences in the disturbance patterns of the three headwater streams. Specifically, it was predicted that, within the least impacted Eyre River, the macroinvertebrates would be characterised by a diverse community whose abundance and composition would reflect high spatial and temporal variability. In addition, it was predicted that the chubbyhead barb's abundance and size structure would exhibit a pronounced seasonal variability influenced by the reproductive timing. In contrast, it was predicted that the Lushington River, which was invaded by black wattle, would be characterised by a less diverse community with less pronounced seasonality due to extensive stream shading, which likely altered both the stream temperature regimes and the timing of allochthonous organic matter input. Furthermore, it was predicted that due to the likely influence of extensive riparian shading on stream temperature regimes and aquatic macroinvertebrate community, which are the general prey for fish (Kambikambi et al. 2019), this river would be characterised by low macroinvertebrate taxonomic diversity. It was further predicted that the chubbyhead barb's population would be small due to the altered low macroinvertebrate diversity. By comparison, it was hypothesized that for the Elands River, whose catchment was influenced by subsistence crop farming, the macroinvertebrate community would be characterised by high abundance with low taxonomic diversity. The chubbyhead barb's population was predicted to be characterised by low abundances likely due to habitat loss from high sediment input.

Methods

Ecological and physical characteristics of study streams

A total of nine sites (three sites in each of the headwater tributaries) were sampled once every season. The seasons sampled were, April 2018 (autumn), August 2018 (winter), October 2018 (spring) and February 2019 (summer). At each sampling site, altitude and geographical co-ordinates were recorded using a global positioning system (GPS, Garmin etrex Ltd) unit to an accuracy of 5 m, landscape variables such as land-use patterns, local micro-habitat and physical-chemical variables were characterised. Land use was noted from the riverbank and categorised as either near-natural, invaded (all sizes) or agricultural. Local micro-habitat variables included the substratum composition, which was categorised as bedrock (> 500 mm), boulder (250 – 500 mm), cobble (50 -250 mm), gravel (2- 50 mm), sand (1-2 mm) and silt (< 1 mm) according to the Udden-Wentworth scale (Wentworth 1922). Substratum composition was visually assessed within a radius of 25 cm at 30 random points within each sampling site. Similarly, depth was measured using a graduated pole from the 30 random measurements. In order to evaluate stream width, 5 to 10 transects were set across the sampled area perpendicular to flow, and each transect was measured using a graduated tape. The length of the entire sampled stretch was also measured from upstream to downstream. The environmental variables were measured from every site including, temperature (°C), pH, dissolved oxygen (mg/L) using a YSI Pro20 portable meter, while total dissolved solids (ppm) and conductivity ($\mu S.cm^{-1}$) were measured using the Hanna HI98129 Combo meter, and turbidity (NTU) was measured using the Hanna ISO Turbidity Portable Meter (HI98713).

Macroinvertebrate and fish sampling

Macroinvertebrates were collected from vegetation and in-stream substrate following the South African Scoring System (SASS) procedure based on the guidelines provided by Dickens and Graham (2002). A sample of macroinvertebrates was collected from vegetation hanging onto or growing at the edge of the riverbank, by rigorously pushing the net into the vegetation and sweeping it back and forth, covering a 2 m stretch over 2 minutes. Instream, a sample of macroinvertebrates was collected by disturbing the stream substrate by kicking and shuffling feet whilst positioning the SASS net downstream of river flow to collect dislodged macroinvertebrates for 2 minutes. Each sample was transferred to a collection tray, after which the macroinvertebrates were picked and transferred to collection bottles with 70% alcohol and transported to the laboratory.

Fish were sampled from all the nine sites in all seasons from the three headwater streams. Fish were captured using a backpack electro-fisher (Samus 725MP) that was powered by a 12 V battery with a standardised frequency of 90 Hz and sampling duration of approximately 15 minutes per site. The fish were transferred to a 10-litre bucket with aerated river water. Using a measuring board, total length and standard length for 30 individuals were measured following procedure outlined by Laevastu et al. (1965) which was defined below. Standard length (SL) was measured from the tip of the snout to the caudal flexure. Total length (TL) was measured from the tip of the snout to the tip of the longest caudal fin ray. A sample of 15 random individuals was retained for stable isotope analysis (see Chapter 3). The rest of the fish were counted and released to the river alive.

In the laboratory, the macroinvertebrates were identified to either genus or lowest practical taxonomic level using a compound light microscope (Olympus, magnification × 100) and local identification guides for freshwater invertebrates (Day et al. 2001, Day and de Moor

2002a,b, Day et al 2002, Day and de Moor 2003a,b). Lastly, the macroinvertebrates were counted for each genera or lowest taxonomic level for each site.

Data analysis

The relative importance of land use categories, which depicted different disturbances, and temporal patterns was evaluated by comparing macroinvertebrate communities and chubbyhead barb abundance among rivers and seasons. To evaluate whether there were any differences in macroinvertebrate communities among rivers and seasons, both taxonomic richness and diversity were compared. Taxonomic richness was evaluated based on the number of macroinvertebrate taxa per site, whereas taxonomic diversity per site was calculated using the Shannon-Weiner index, given as $H = -\sum_{i=1}^{s} p_i \ln p_i$, where H is the Shannon-Weiner index, s is the total number of genera in a sample and p_i is the number of individuals of one genera in relation to the number of individuals in the population. Analysis of variance (ANOVA) was used to compare the Shannon-Weiner index for the three headwater streams.

A hierarchical cluster analysis (Anderberg, 1973) was used to explore whether the three rivers could be distinguishable based on their macroinvertebrate community structure. For the cluster analysis, Ward’s minimum variance (Ward, 1986) was used to construct a dendrogram to depict the correlations between macroinvertebrate assemblages at each site, land-use and season. The clustering is designed to define groups in a way that minimises within-group sum of squares based on Euclidean dissimilarities. The macroinvertebrate data were presented as $n \times p$ (sample × species) matrix, where sample represented the site and season, and species data were based on abundance of the individual macroinvertebrate taxa. Lastly, to investigate the influence of both spatial (land use patterns) and seasonal (physical-chemical variables) factors, canonical ordination, redundancy analysis (RDA) was used (Rao 1964). Redundancy analysis is a direct gradient multivariate analysis that summarises the variation between a set of response variables and explanatory variables. The RDA was based on both macroinvertebrate data ($n \times p$ matrix) and environmental data that were presented as $n \times q$ (sample × environmental variable), where environmental data included land use variable (categorical data), season (categorical data) and physical-chemical variables (continuous data). The species data were Hellinger transformed using the decostand function within the vegan package (Oksanen et al. 2013) in R (R Development Core team, 2019). The Hellinger transformation was expressed as $y'_{ij} = \sqrt{\frac{y_{ij}}{y_i}}$, where y' represents the transformed value, i represents the sample and j represents the species (Legendre and Gallagher 2001). Partial ordination was used to test the hypotheses on the differences in the importance of spatial, temporal and physical-chemical factors in influencing macroinvertebrate communities, with the significance testing being evaluated using Monte Carlo permutations (× 999 permutations). To examine specific macroinvertebrate taxa abundances across the different seasons and the associated environmental factors, RDA-based contours were plotted.

The partial ordination analysis was done under the hypothesis that land use was the primary driver of the differences in macroinvertebrate communities compared to season and physical-chemical variables in the three headwater streams. The first partial ordination tested the statistical significance of land use, with season and physical-chemical variables being co-variates. The second partial ordination tested the statistical significance of season, with land-use and physical-chemical variables being co-variates. The third partial ordination tested the statistical significance of physical-chemical, with land-use and season being co-variates.

A generalised linear mixed effect model (GLMM) was used to evaluate the relationship between chubbyhead barb abundance and environmental factors. In the GLMM, the chubbyhead barb count data were used as the response variable, whereas land use, season and physical-chemical variables were used as predictor variables. The model was analysed based on Poisson distribution with a logit-link and Laplace approximations for the parameter estimations (Bolker et al. 2009). The best predictor variables were evaluated using multi-model inference, which was conducted from a fully saturated model with all environmental variables (Bolker et al., 2009). The most parsimonious model was selected based on the lowest Akaike weight (Burnham & Anderson 2002). Multi-model inference was conducted in R using the package *MuMIn* (Burton, 2011). Length data from each site were pooled for each river in order to create length-frequency distributions in order to compare the population structures among rivers and across seasons. For the Eyre and Elands rivers that had large populations, statistical tests between seasons for length-frequency distributions were done using randomisation permutation tests based on kernel density estimates (KDEs) following (Langlois et al. 2012). Under the null hypothesis of no difference between two length-frequency distributions for the respective seasons, the KDEs were used to compare the shapes of two length-frequencies. To test the differences in shapes, first, each of the length-frequency data were standardised based on median and variance (i.e. yi = (xi – $median / sd$), where y_i is the standardised value, x_i is the individual length-frequency value and sd is the standard deviation of the data, and second, the equality in distributions were compared using random permutations (100 000 permutations). Comparisons between pairs of length-frequency distributions were presented using kernel smoother splines and a grey band that represented the null model between two length-frequencies. The grey band centred on the mean KDE with a bandwidth of the standard error.

Results

Spatio-temporal patterns of environmental factors and macroinvertebrates

The three headwater streams were generally distinguished based on the physical-chemical variables (Table 1).The Elands River had the highest conductivity (range 98 – 236 µS/cm across seasons), followed by the Eyre River (range 63 – 130 µS/cm), whereas the Lushington River had the lowest conductivity (range 50 – 122 µS/cm). A similar pattern was observed for TDS, which was high in the Elands River (range 97.67- 236.33 ppm), intermediate in the Lushington River (range 39.67- 76.99 ppm) and low in the Eyre River (range 36 – 75.67). Water pH was more alkaline in the Elands River, ranging from 8.01-8.27 in spring to 8.12-9.26 during summer than in the Eyre (7.26- 9.50) and Lushington (7.63- 8.90) rivers. Dissolved oxygen was variable across all three rivers. Seasonally, dissolved oxygen was higher in spring (range 11.18- 13.23 mg/l) and lowest in summer (range 7.52-9.30 mg/l). Although there was a pronounced seasonal variation in water temperature, the Elands River was relatively warmer (range 11- 23.77°C) than both the Eyre (range 9.13-21.90°C) and Lushington (9.07-19.07°C) rivers.

A total of 46 families of macroinvertebrates with 62 genera were identified in these headwater streams. The Elands River had the lowest number of macroinvertebrate genera (38), followed by the Eyre River (44), whereas the Lushington River had the highest number of macroinvertebrate genera (47). Based on the Shannon-Weiner index, macroinvertebrate diversity was comparable between seasons (ANOVA, F = 2.34, P = 0.12) and between rivers (ANOVA, F = 2.28, P = 0.11). However, the general pattern showed that diversity was highest in the Lushington River in most seasons, followed by the Eyre River, whereas the Elands River had the lowest diversity in all seasons (Figure 5). Cluster analysis revealed that optimal number of clusters according to matrix correlation was 7. The hierarchical cluster analysis revealed that the macroinvertebrates were grouped based on seasons (Figure 6). These groups showed similarities among most sites in summer and spring, which was due to the common occurrence of *Trichorythus*. By comparison, similarities of most sites in autumn was due to the common occurrence of *Baetis*, whereas similarities of most sites in winter due to the common occurrence of *Cheumatopsyche* (Figure 7). In addition, the Redundancy analysis (RDA) revealed that the macroinvertebrate communities were significantly influenced by environmental factors (Monte Carlo permutation test, P = 0.001). The constrained ordination explained 61.79% of the variance in the data. In addition, both RDA axes 1 and 2 were significant in explaining the species-environment relationships (Monte Carlo permutation, P < 0.001), and accounted for 42.81 and 18.98%, respectively, in explaining the variation in species data (Table 2). The RDA axis 1 showed a gradient that reflected seasonal patterns in macroinvertebrate communities and the associated environmental factors that included pH, turbidity, temperature and conductivity (Figure 8). On the other hand, RDA axis 2 revealed a gradient that reflected land use patterns. The partial ordination revealed that of the variance explained, season explained 19.5 % and thus significantly (Monte Carlo permutation test, P = 0.001) accounted for temporal patterns in the macroinvertebrate communities (Table 3). By comparison, land use explained a small (5 %) but significant (Monte Carlo permutation test, P = 0.003) variation in macroinvertebrate communities among the three headwater streams. In contrast, physical-chemical factors explained a small (2%) variation that was not significant in influencing macroinvertebrate communities in these headwater streams (Monte Carlo permutation test, P =0.26). The taxa triplot revealed that six macroinvertebrate taxa were important in explaining the seasonal patterns that were observed (Figure 9).These taxa included *Dicentroptelum* of the order

Ephemeroptera, which was most abundant during spring, and *Trichorythus* of the order Ephemeroptera , which was commonly abundant in spring, summer and autumn (Figure 9). On the other hand, *Burnupia* of the order Gastropoda and *Cheumatopsyche* of the order Trichoroptera were most abundant in winter, which was characterised by low temperature (Figure 9). *Potamonautes* of the order Decapoda was most abundant in spring, which was characterised by high dissolved oxygen, and *Baetis* was abundant in autumn (Figure 9).

Table 1:Water quality parameters, including pH, dissolved oxygen (DO), conductivity, total dissolved solids (TDS), turbidity and temperature that were measured in the Eyre, Elands and Lushington rivers in winter, spring, summer and autumn.

Site	Season	pH	DO (mg/l)	Conductivity (µS/cm)	TDS(ppm)	Turbidity (NTU)	Temperature (ºC)
Eyre	Winter	7.80-9.50	11.79±0.45	129.67±6.11	75.67±0.51	5.92±1.54	9.13±1.34
	Spring	7.30-7.75	12.96±2.62	63.00±1.00	36.00±0.00	79.60±2.55	13.00±2.41
	Summer	7.94-8.15	7.52±0.41	108.33±5.86	61.67±1.15	52.57±5.80	21.90±2.97
	Autumn	7.26-8.16	8.51±0.00	123.00±4.36	72.67±1.53	13.4±3.58	18.03±1.46
Elands	Winter	7.80-9.14	10.23±0.51	162.33±8.08	94.33±5.03	9.10±2.48	11.00±3.55
	Spring	8.01-8.27	11.18±2.64	97.67±9.45	58.00±6.24	35.7±22.36	17.43±1.17
	Summer	8.12-9.26	9.30±3.56	236.33±64.69	141.00±34.51	30.03±23.88	23.77±2.71
	Autumn	7.99-8.16	8.6±0.43	99.67±5.13	58.33±3.06	36.43±3.60	16.97±1.01
Lushington	Winter	8.65-8.90	6.54±3.01	122.33±12.50	76.00±2.00	8.45±1.46	9.07±0.93
	Spring	7.63-8.08	13.23±0.74	49.67±9.29	29.67±4.93	37.60±11.74	11.90±2.01
	Summer	7.85-8.53	7.56±0.89	70.00±14.73	40.67±8.39	25.13±3.60	19.07±1.63
	Autumn	7.90-8.15	9.22±0.28	68.67±10.4	39.67±6.11	13.53±2.81	14.93±0.38

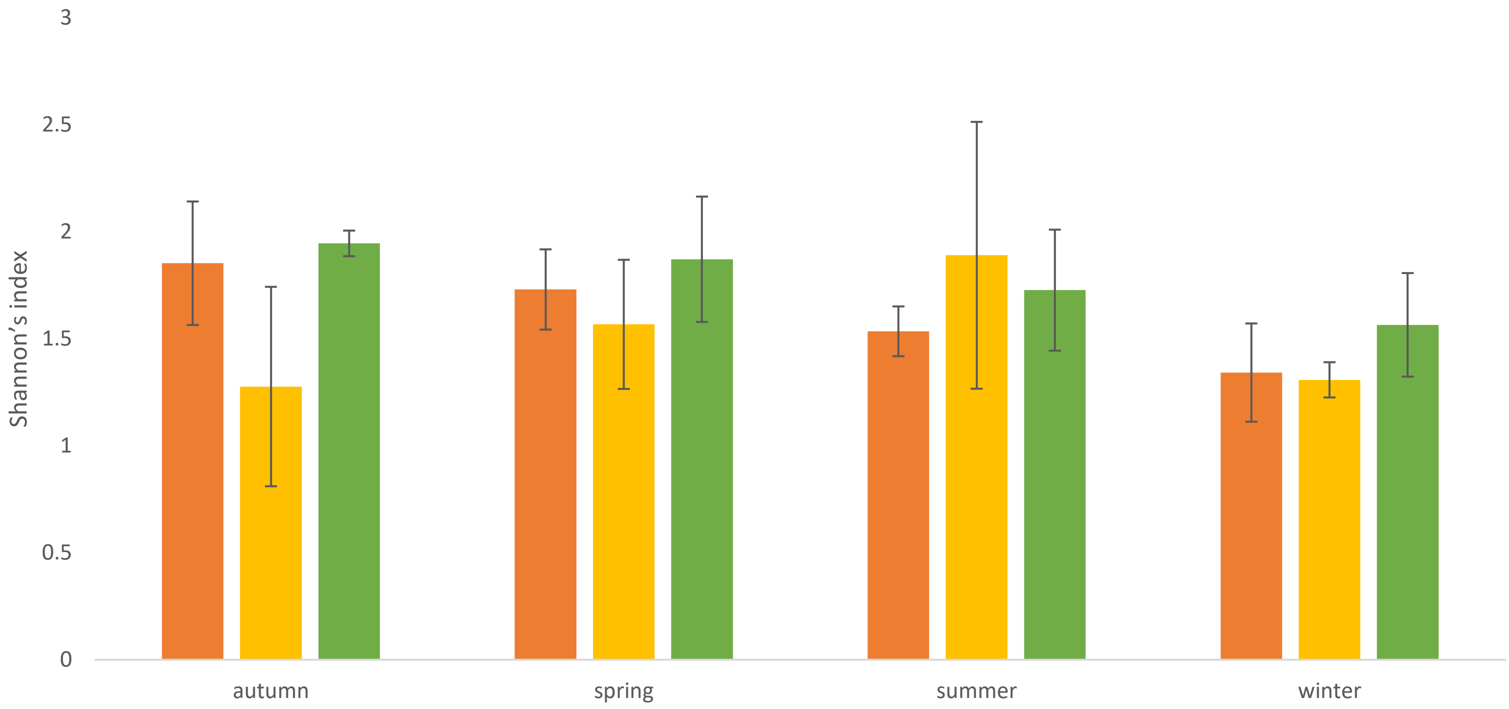

Figure 5: Seasonal differences in the diversity (Shannon-Wiener diversity indices) of macroinvertebrates among the Eyre River (orange), Elands River (yellow) and Lushington River (green).

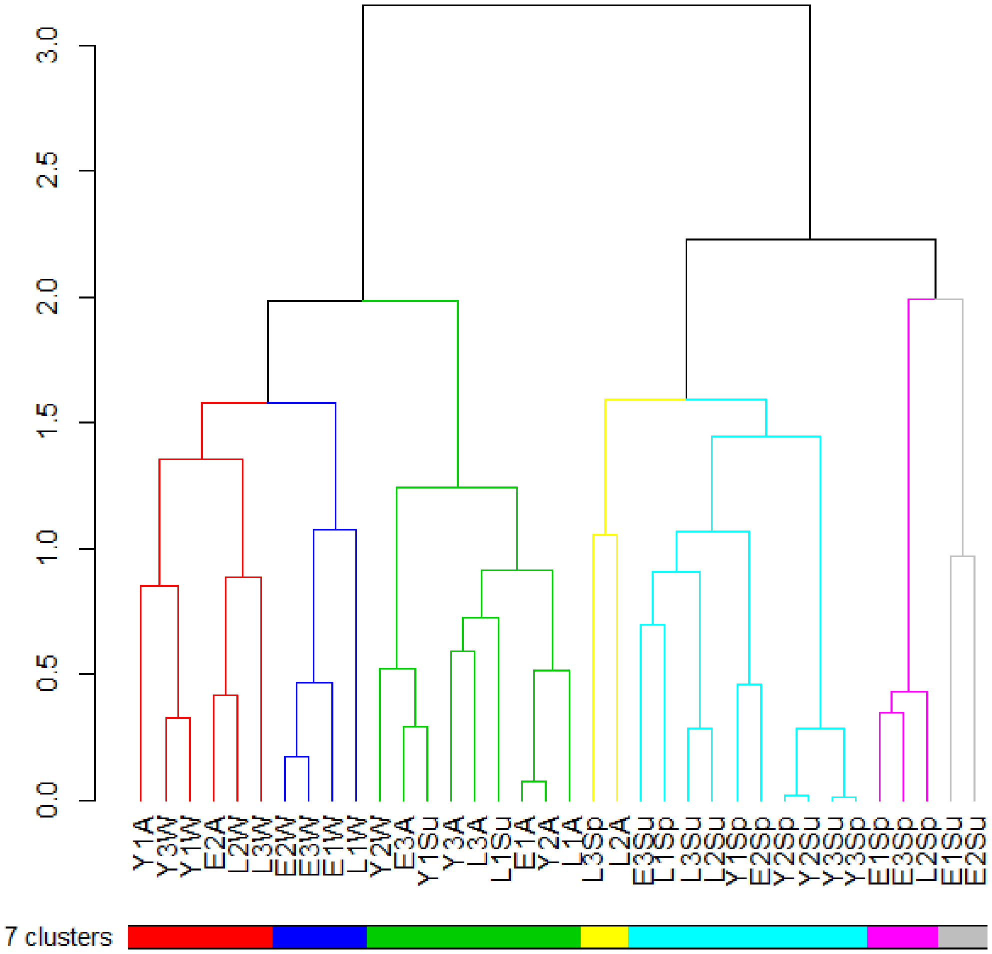

Figure 6: Cluster analysis dendrogram depicting the macroinvertebrates and the seasons they were associated with. The clustering was based on the Euclidean distance measure of standardised macroinvertebrate community abundance data (i.e., chord distance). Each colour indicated different clusters. The rivers were abbreviated as: Eyre River (Y), Elands River (E) and Lushington River (L) and the seasons were abbreviated as winter (W), summer (Su), autumn (A) and spring (Sp).

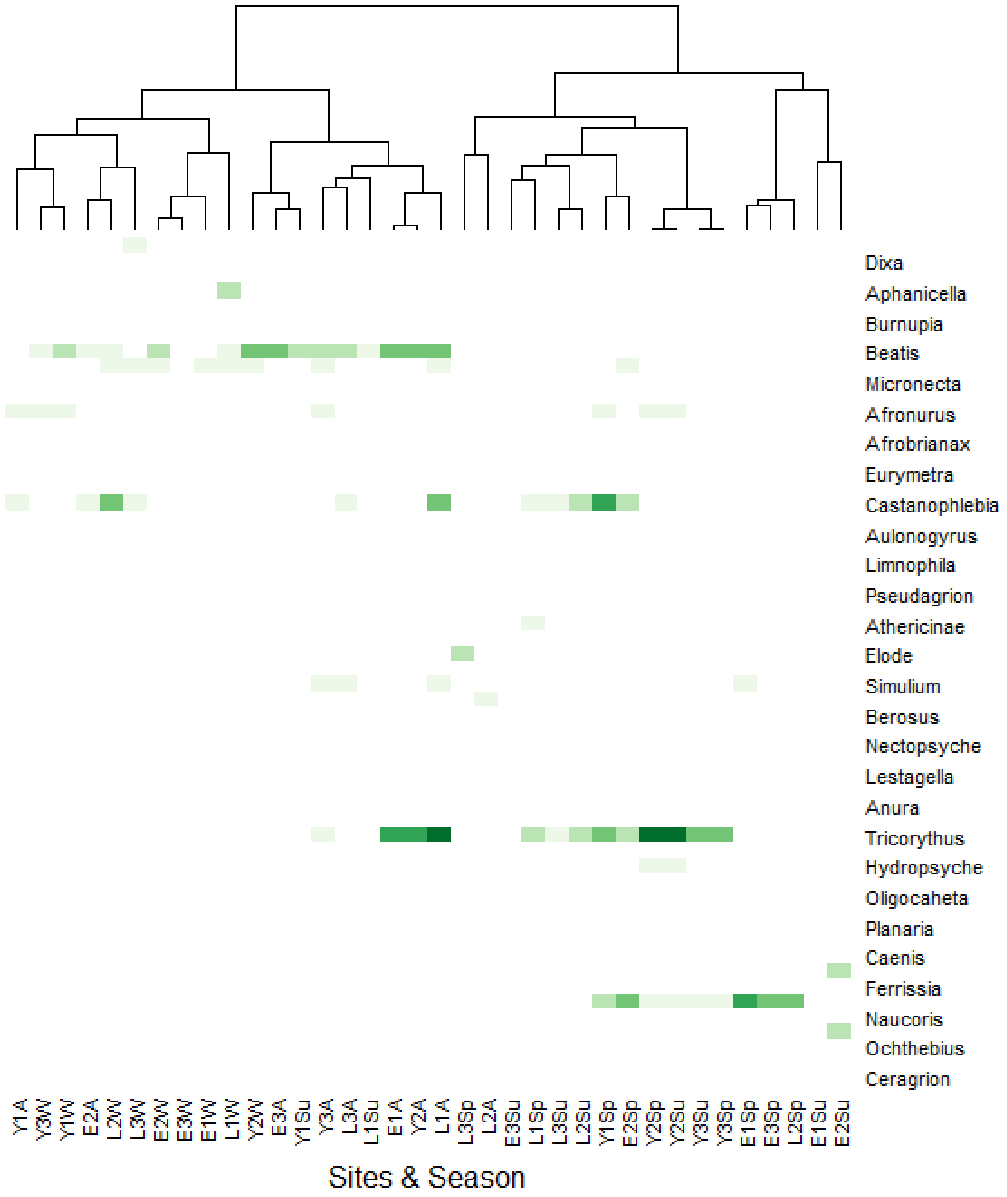

Figure 7: Cluster analysis dendrogram depicting the macroinvertebrates and the clusters they were associated with. The clustering was based on the Euclidean distance measure of standardised macroinvertebrate community abundance data (i.e., chord distance). The green indicated high macroinvertebrate abundances while white indicated low abundances. The rivers were abbreviated as: Eyre River (Y), Elands River (E) and Lushington River (L) and the seasons were abbreviated as winter (W), summer (Su), autumn (A) and spring (Sp).

Table 2: Summary statistics of the redundancy analysis, showing the eigenvalues for main axes, the proportion of variance explained and eigenvectors for predictor variables of the relationship between macroinvertebrate communities and environmental factors in the three headwater streams, the Eyre, Elands and Lushington rivers.

	RDA axis 1	RDA axis 2
Eigenvalue	0.13	0.05
Proportion explained (%)	0.43	0.19
Cumulative proportion (%)	0.43	0.62
Land use		
Agriculture	-0.04	-0.11
Invaded	-0.01	-0.02
Natural	0.05	-0.13
Season		
Autumn	-0.06	0.39
Spring	0.40	-0.32
Summer	0.16	0.16
Winter	-0.50	-0.24
Physical-chemical factors		
pH	-0.53	-0.14
Conductivity	-0.40	-0.12
Turbidity	0.75	-0.09
Temperature	0.53	-0.40
Dissolved oxygen	0.21	-0.17

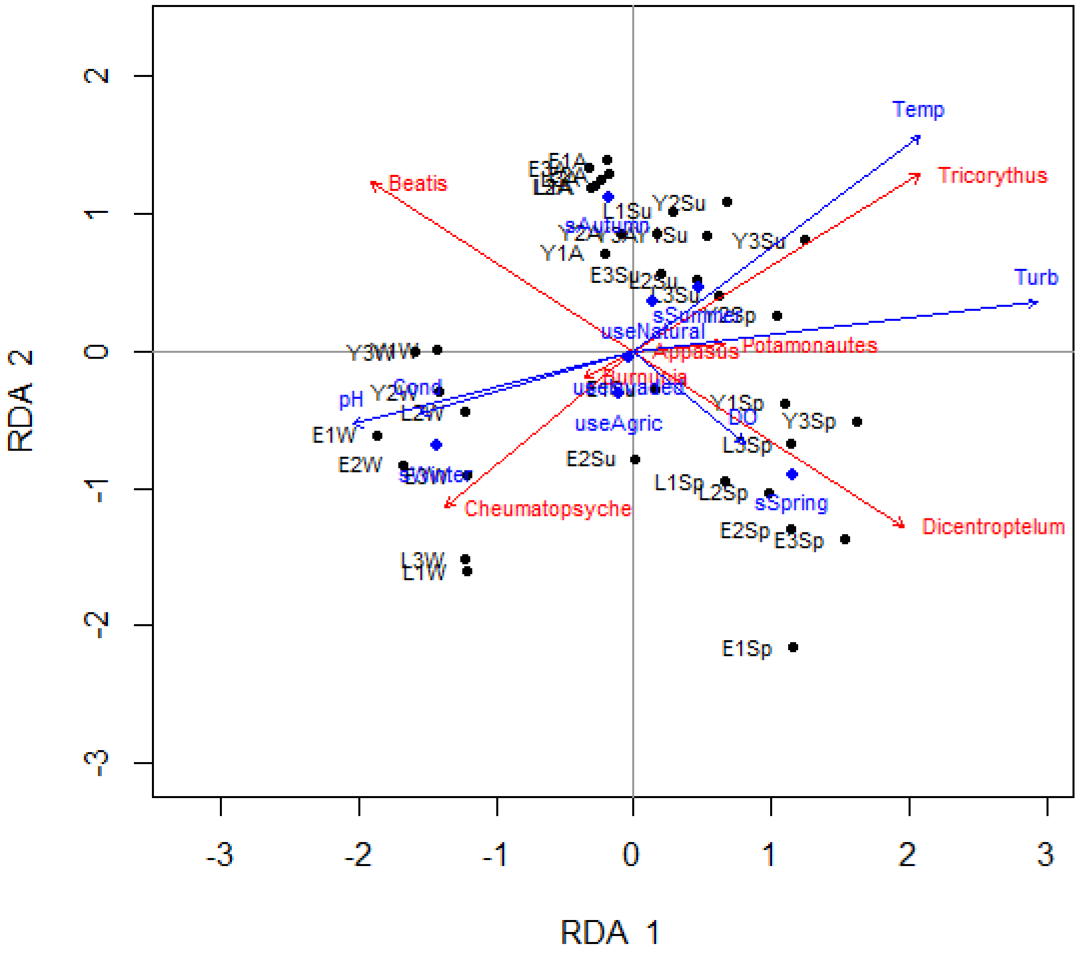

Figure 8: The Redundancy analysis (RDA) ordination triplot indicating the macroinvertebrate communities and the environmental factors influencing them. The blue arrows represent the significant (p =< 0.05) environmental variables explaining variation in the macroinvertebrate assemblage and red arrows representing the seven major macroinvertebrates. The black dots represented sampling sites together with seasons, which was abbreviated as follows; winter (W), spring (Sp), summer (Su) and autumn (A) and the rivers were abbreviated as, Eyre River (Y), Elands River (E) and Lushington River (L). The abiotic variables that were abbreviated were Temp for temperature, Cond for conductivity, DO for dissolved oxygen.

Table 3: Summary statistics for redundancy analysis (RDA) showing the variance explained (inertia) and the proportion explained by the constrained ordination and the partial ordinations testing the significance of land use, season and physical-chemical factors on macroinvertebrate communities in three headwater streams

	Inertia	Proportion (%)	F value	P-value
Total	0.533	1.000	3.290	0.001
Constrained	0.284	0.533		
Unconstrained	0.249	0.468		
Land use	0.041	0.077	2.111	0.003
Season	0.104	0.195	3.573	0.001
Physical-chemical factors	0.034	0.063	1.159	0.258

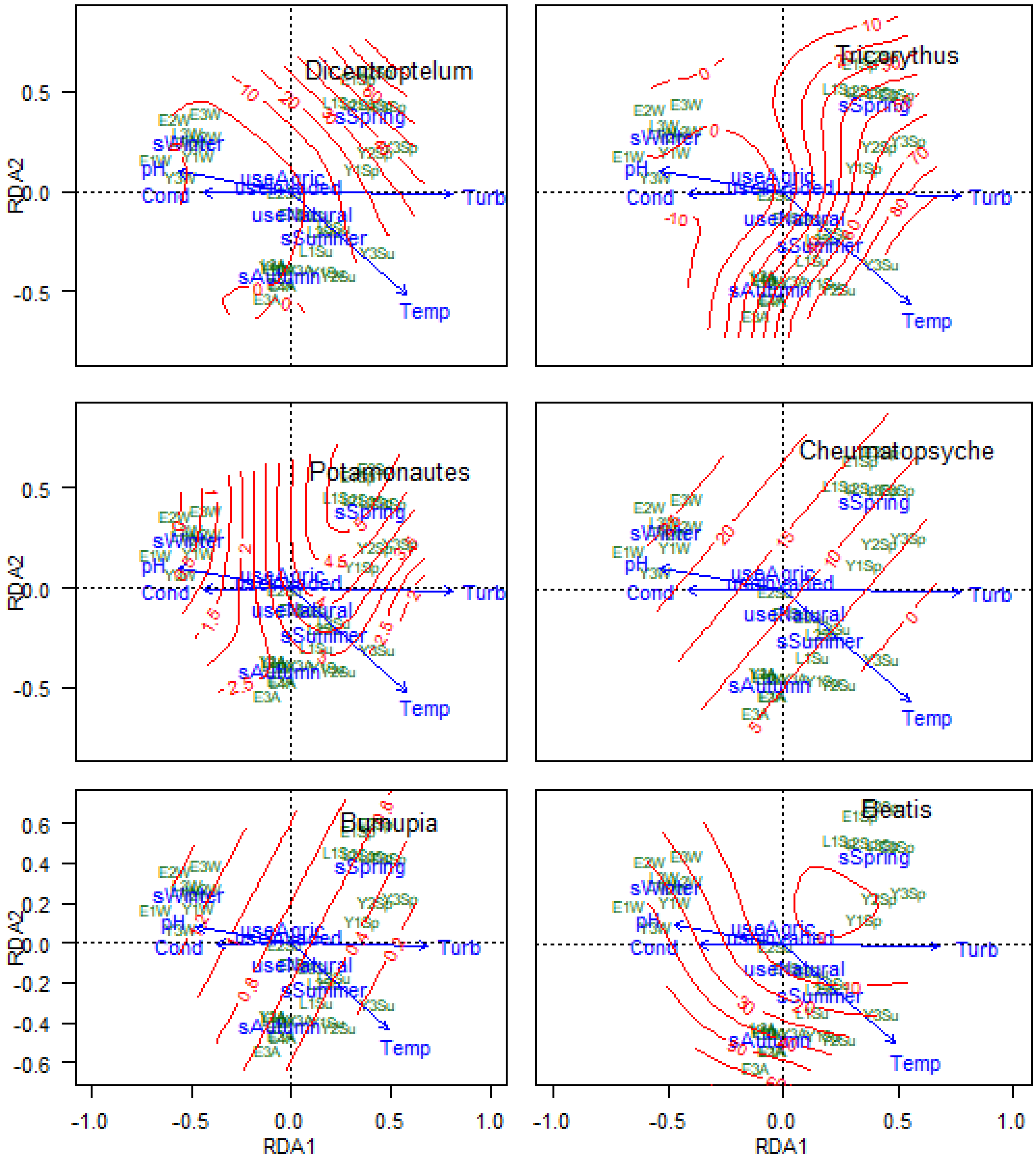

Figure 9: RDA analysis plots of the macroinvertebrate assemblage across all seasons, with contour lines (red) indicating the abundance. The blue arrows represented the environmental variables explaining variation in the macroinvertebrate assemblage. The green represented season and sites, which was abbreviated as follows; winter (W), spring (Sp), summer (Su) and autumn (A) and the rivers were abbreviated as, Eyre River (Y), Elands River (E) and Lushington River (L).

Community structure and spatio-temporal pattern of Enteromius anoplus

Chubbyhead barb exhibited seasonal variation in abundance, but these patterns differed among the three rivers (Figure 10). In the Eyre River, chubbyhead barbs were more abundant in winter and spring than in summer and autumn. In comparison, in the Elands River, chubbyhead barbs were more abundant in winter and had the lowest abundance in autumn. In the Lushington River, chubbyhead barb abundance was the lowest in all seasons compared to other rivers, with fish not being caught in spring.

The GLMMs for chubbyhead barb abundance explained between 84 and 98% of the variation in the data. Comparison of chubbyhead barb abundances showed significant effects of land used patterns (Wald's χ^2= 2566800, P < 0.001), with significantly low abundances being observed in the Lushington River (GLMM, z-value = -1602.11, P < 0.001). Chubbyhead barb also exhibited significant seasonal variability (Wald's χ^2= 371350, P < 0.001), with significantly high abundance (GLMM, z-value = 462.89, P < 0.001) being observed in the Elands River during winter compared to other rivers. In addition, chubbyhead barb appeared to be abundant in deeper habitat (GLMM, z-value = 449, P < 0.001).

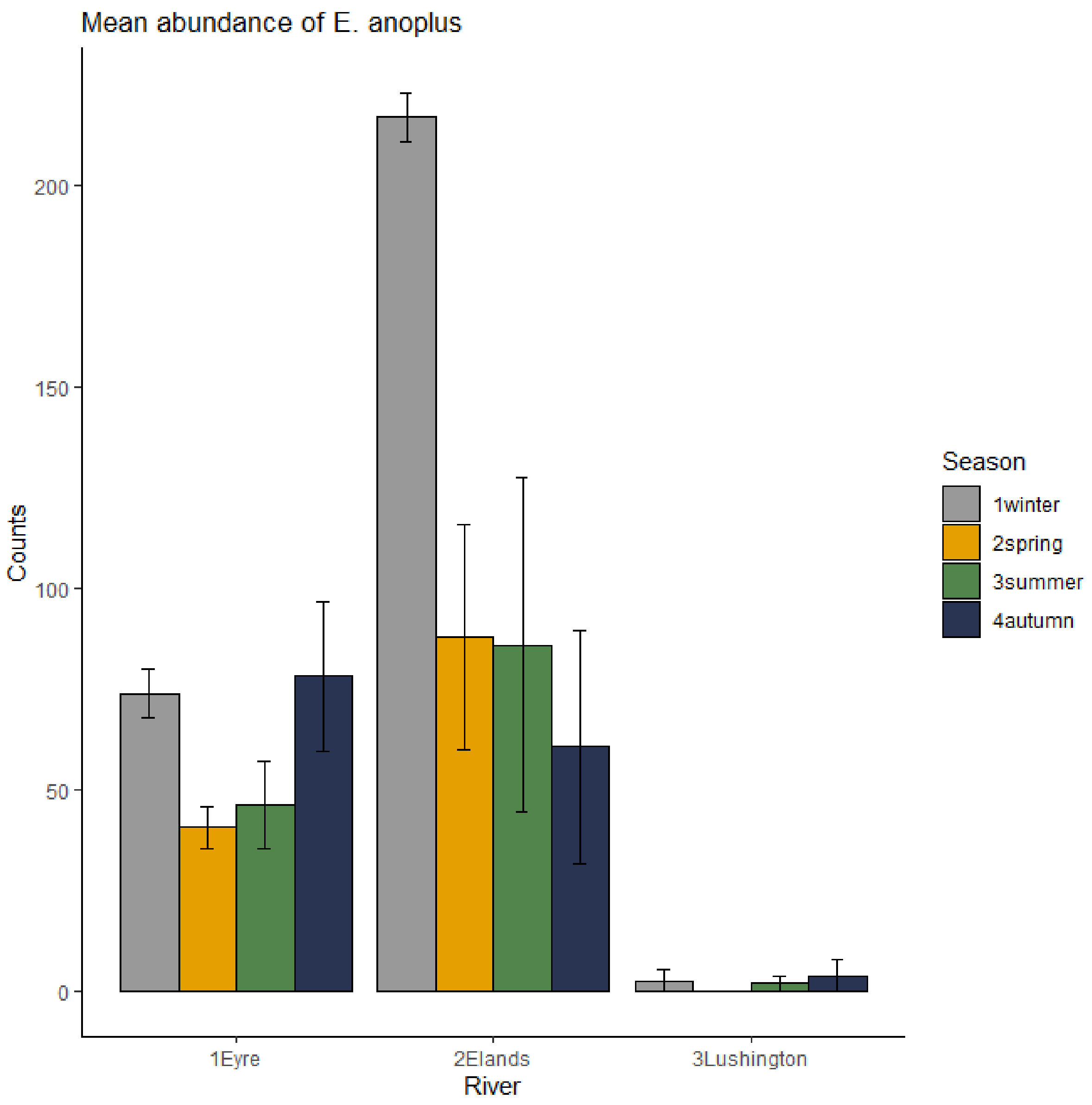

Figure 10: Mean seasonal abundance of chubbyhead barb in the three headwater streams, namely, the Eyre, Elands and Lushington rivers. The seasons were graphed as follows, winter (grey), spring (yellow) summer (green) and autumn (blue).

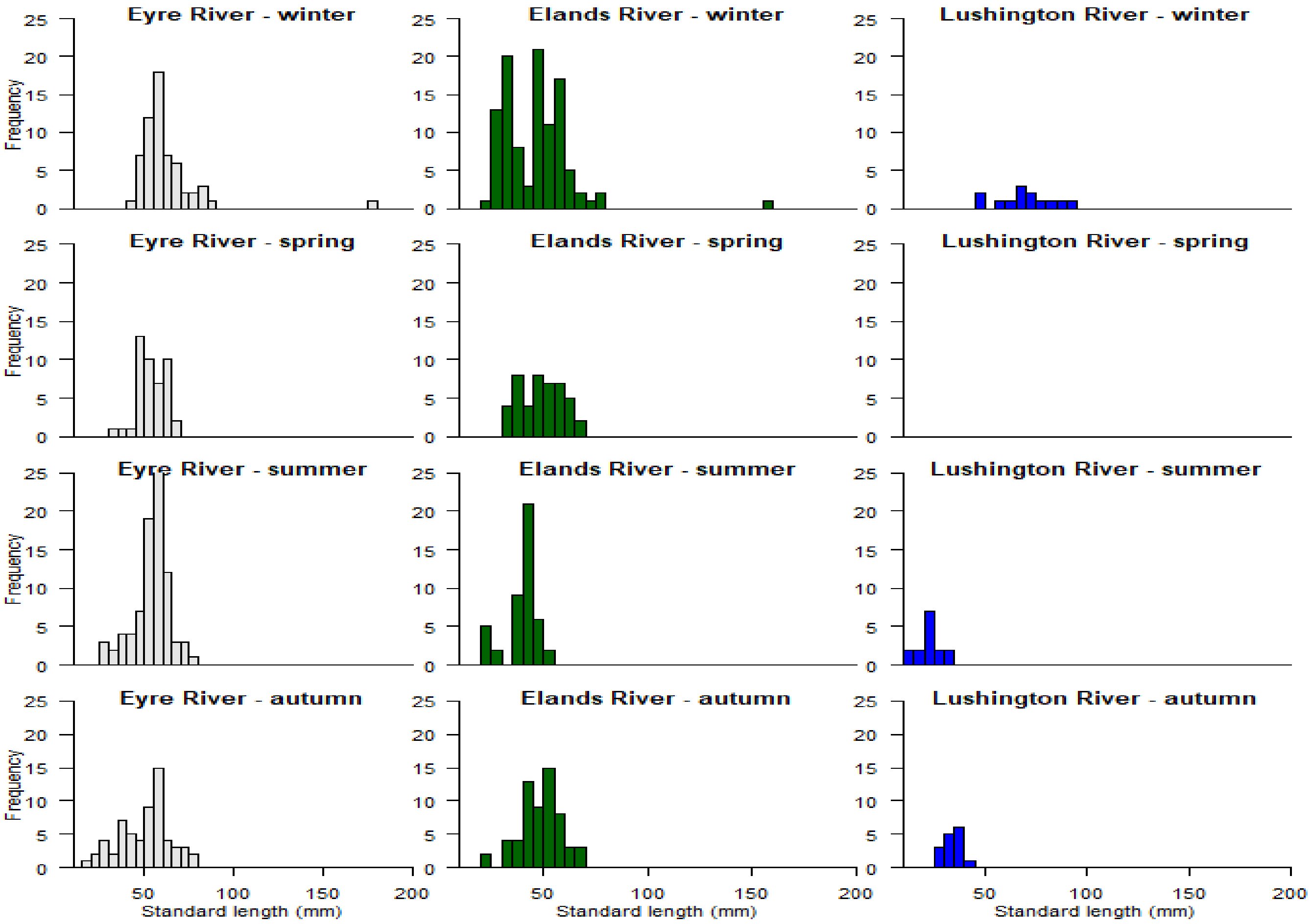

Figure 11: Length frequency bar graphs depicting the seasonal population structure of the chubbyhead barb in the three headwater streams, namely, the Eyre River (white), Elands River (green) and Lushington River (blue).

Table 4: Analysis of GLMMs indicating the null and best-fit models describing the mean abundance patterns of chubbyhead barb in relation to environmental and land-use patterns.

Enteromius anoplus	Intercept	AIC	Δ_i	w_i	R^2
	Intercept +landuse +season +pH +temperature +conductivity +maximum depth +boulder+cobble+silt +vegetation	328.5	0.00	0.24	0.99
	Intercept +landuse +season +pH +temperature + conductivity +maximum depth +boulder+cobble+silt +vegetation	328.5	0.02	0.023	0.84

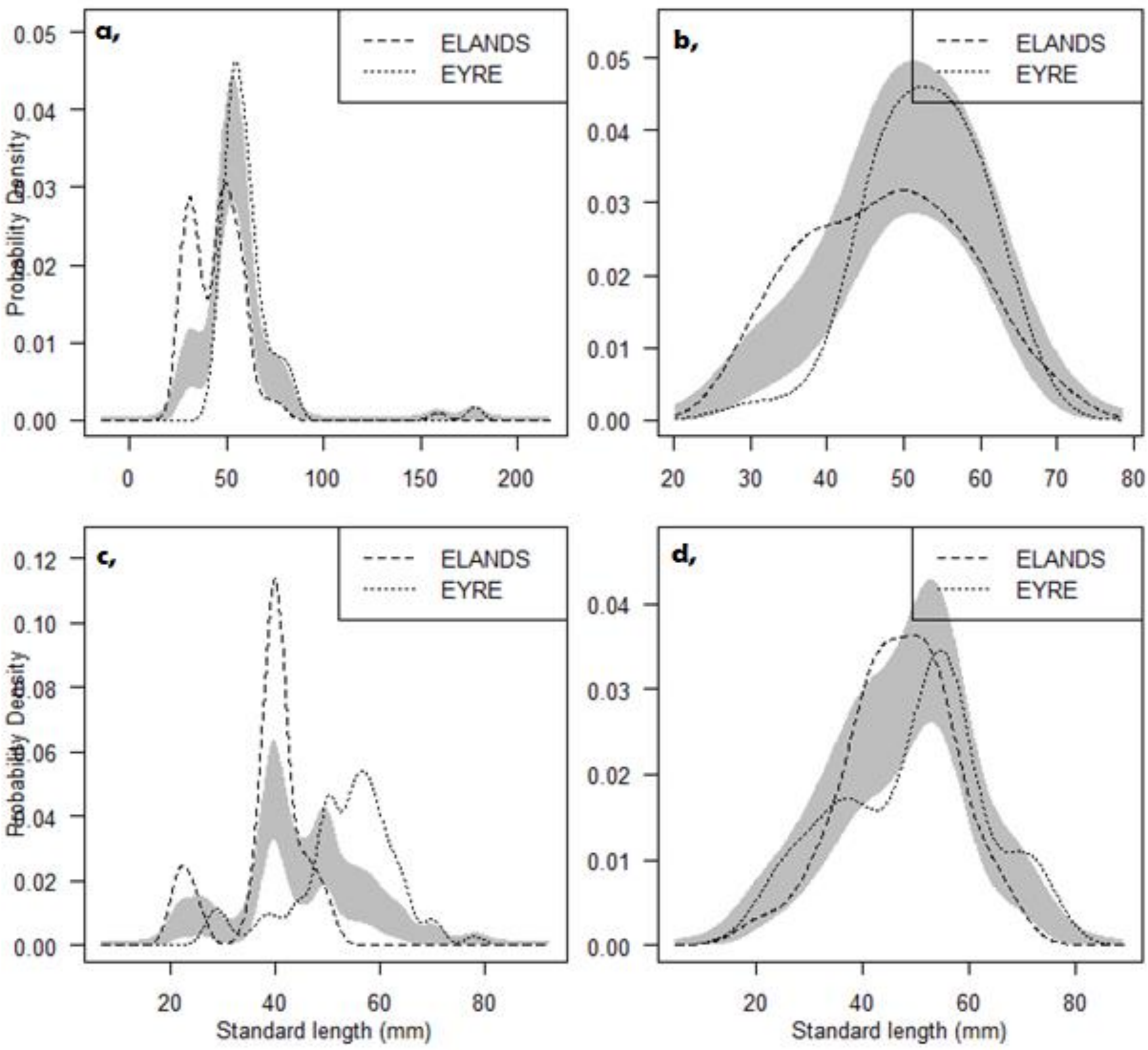

Figure 12: Kernel density estimates (KDEs) probability densities comparing the length-frequencies of chubbyhead barb minnows between seasons in the Eyre and Elands rivers. The seasons are as follows, winter (a), spring (b), summer (c) and autumn (d).

Comparison of chubbyhead barb's length-frequency distribution indicated that the Eyre and Elands river populations differed significantly in all seasons (randomisation permutation tests, $P < 0.05$). During winter, the chubbyhead barb population in the Eyre River was characterised a by unimodal distribution with a modal length of approximately 50 mm, whereas in the Elands River, the chubbyhead barb had a bimodal distribution with modal length of approximately 40 and 60 (Figure 11& Figure 12). During spring, the chubbyhead barb population in the Eyre River was characterised a unimodal distribution with a modal length of approximately 55 mm, whereas in the Elands River, the chubbyhead barb had a unimodal distribution with modal length of approximately 50 mm. During summer, the chubbyhead barb population in the in the Eyre River was characterised a multimodal distribution with a modal length of approximately 30, 40, 50 and 60 mm, whereas in the Elands River, the chubbyhead barb had a bimodal distribution with modal length of approximately 25 and 40mm.

Discussion

Macroinvertebrates spatio-temporal patterns

This study hypothesized that different land use patterns, which depicted different disturbance regimes in the study streams, would represent the major influence on the variations in the aquatic communities. The results of this study, however, showed that variations in the macroinvertebrate communities were largely explained by seasonal changes, with land use patterns representing a small, albeit significant, role in the differences among the rivers. These observations were thus generally inconsistent with the study hypothesis. In particular, the results showed that diversity and richness were generally comparable among the three headwater streams, with little variation between the Eyre River, which was least impacted, and the Lushington and Elands rivers, which were subjected to non-native plant invasion and agricultural crop farming, respectively. The observed patterns for the Lushington River were in contrast with the study hypothesis because this river represented invasion disturbance due to the presence of black wattle within its riparian zone. This pattern was inconsistent with other studies which have shown that rivers whose riparian zones are invaded by non-native plants, such as black wattle, would be characterised by low macroinvertebrate diversity and richness due to habitat loss as a result of altered hydrological regimes (Lowe et al., 2008). Other studies have, however, reported consistent patterns as those in this study whereby invasion of riparian zones by non-native plant was found to have little impact on instream communities (Chakona and Marshall 2007, Reinhart and Van deVoort 2006). Although macroinvertebrate diversity and richness were lowest in the Elands River, the pattern in community composition was generally comparable to that found in the other rivers, with seasonality playing a major role in community composition in this river. These observations suggest that agricultural land use through crop farming had little impact on community composition in this river. This is in contrast with studies elsewhere which have shown that crop farming usually has profound negative impact on macroinvertebrate communities due the effects of agricultural practices on habitat stability and heterogeneity (Wang et al 2019) and negative impact on water quality (Lenat 1989, Genito et al 2002).

Comparison of macroinvertebrate community patterns in the study streams suggest that although land-use patterns exert substantial stress upon streams and stream biota, for macroinvertebrates in these streams, seasonality was the major driver. This is due to season influencing the most fundamental needs for macroinvertebrate survival including stream flow, temperature (Yurtseven et al 2016) and resource abundance (Richardson 1991).

Enteromius anoplus spatio-temporal pattern

The study hypothesised that different land-use patterns would influence the instream biota spatio-temporal patterns, including the chubbyhead barb population which occurred throughout the three headwater streams. The results of the study are in accordance with the study hypothesis because the population structure of chubbyhead barb was influenced by land-use.

Specifically, the chubbyhead barb abundance in the Eyre River had a temporal pattern, which seemed to be timed with reproduction, as hypothesised. Chubbyhead barb breeds in late summer (Cambray and Bruton 1984), therefore, the population structure is indicative of this as the size classes in autumn are of the smaller age classes. The reproductive timing of chubbyhead barb is linked to seasonal fluctuations in precipitation, with the breeding season being linked to summer rainfall (Cambray 1983). In contrast, the Elands River was hypothesised to have low chubbyhead barb abundance as a result of crop farming. However, the Elands River had the highest chubbyhead barb abundance overall, particularly in winter. The results contradict the conclusions of numerous studies which have suggested that streams impacted by agriculture have low fish populations as a result of high sediment input (Talmage et al. 2002) and habitat loss (Walser and Bart 1999). The Elands had an intact riparian buffer unlike most of the streams, which was consistent with the findings of Ferreira et al. (2012) wherein they noted that riparian buffers limit the impact of land-use, particularly the effects of agriculture. In line with the hypothesis, the Lushington River had the lowest chubbyhead barb mean abundance, with no fish being caught in spring. The results suggest that the population of chubbyhead barb was likely diminished as a result of altered stream flow as this stream was characterised by low stream flow. Although these streams are subject to episodic low stream flow, this river had the lowest stream flow continually. Black wattle is known to alter the hydrological regime of streams (Le Maitre et al. 2000, Clulow et al. 2011) by reducing stream flow and this is often uninhabitable for fishes. In addition, biotic interactions were not examined in this study, however, it is worth noting that chubbyhead barb co-occurs with the Eastern Cape rocky (*Sandelia bainsii*) in this river. Biotic interactions such as competition would likely play a significant role in structuring the fish population in this river.

Conclusion

The study concludes that community structure in these headwater streams may not be an informative method to evaluate the impacts of land-use. The results indicated that seasonality was a significant driver of the macroinvertebrate population assemblage. Although there were notable differences in land-use patterns, these appeared to play an insignificant role in the structure the macroinvertebrate community assemblage. In contrast the chubbyhead barb populations appeared to respond to different land-use patterns. This suggests that chubbyhead barb may be more sensitive to land-use changes than macroinvertebrate populations were. The contrasting patterns between macroinvertebrates and the chubbyhead barb on their response to land-use patterns prompts the need to explore alternative approaches. In particular, evaluating the food web dynamics of these communities provide an approach that could provide community-wide effects on energy transfer (see Chapter 3).

CHAPTER 3

Examining the trophic interrelationships and food web dynamics of aquatic communities in three differently impacted headwater streams.

Introduction

Food webs can be defined as the transfer of matter and energy in ecosystems, and they depict the connectivity of one organism to the other (Elton 1927). In addition to depicting predator-prey interactions through trophic relationships, food webs provide a mechanism for predicting responses to either natural or human-mediated changes in ecosystems (Morin and Lawler 1995). In Afromontane headwater streams, understanding trophic relationships is important because of the ecological dynamics of both natural and anthropogenic changes. Lotic environments, especially in these mountain streams are governed by several factors, particularly those affecting trophic interrelationships, including land-use patterns which play a major role in energy input dynamics and higher trophic level interactions (García et al. 2017).

Despite the growing focus on African stream ecology, Afromontane streams are still largely unexplored, particularly in relation to trophic ecology, taxonomy and systematics. The prior limited interest on the ecology of Afromontane headwater streams is largely because these systems are considered to be species poor (Darwall et al. 2010). However, there is emerging evidence on the hidden diversity within these Afromontane streams, which has highlighted taxonomic interest and biodiversity challenges. In addition to documenting the spatial and temporal patterns associated with aquatic biodiversity within Afromontane headwater streams, there is need to understand the ecological processes that are likely to explain ecosystem structure in both natural and human-mediated systems. From a food web perspective, headwater streams, including those in the Afromontane ecoregions, tend to be variable, with riparian vegetation-shaded headwater streams being characterised by heterotrophic communities that are supported by terrestrial-derived organic matter (Moss 2010, Richardson 2019), whereas unshaded headwater streams are often characterised by autotrophic communities that are supported by in-stream primary production (Allan and Castillo 2007). Despite the small size of many headwater streams, their communities and food web structure appear to be susceptible to anthropogenic activities. In Afromontane streams, the common anthropogenic activities include invasion of riparian zone by non-native vegetation, and alteration of riparian zones due to small scale crop farming, which ultimately has an impact the adjacent streams.

The common impacts of invasion by non-native vegetation on aquatic food webs include altering the allochthonous matter input into the streams. The food web structure in plant-invaded headwater streams have therefore been observed to be more simplified when species evenness increases on all trophic levels (Carvalheiro et al., 2010). In comparison, agricultural activities have been shown to alter aquatic food webs through altering basal sources, through increased autochthonous production through fertiliser input. In addition, the prevalent use of fertilisers has been linked to increased nutrient load, particularly nitrogen, which is often incorporated into the food web (Walser and Bart 1999).

In stream ecology, the use of stable isotope analysis is a common tool of evaluating food web dynamics, stream processes and potential food sources that drive stream ecosystems (Gannes et al. 1997). Stable isotope analysis is generally used to examine animal migration (Hansson et al. 1997, Trueman et al. 2012), host-parasite interactions (Stapp and Salkeld 2009, Fritts et al. 2013), ecosystem fluxes in nutrients (Uhlig et al. 2017), tracing anthropogenic nutrient inputs (Ulseth and Hershey 2005) and trophic ecology (Kelly 2000). In trophic ecology, stable isotope analysis is used to reconstruct diets, elucidate patterns of resource acquisition and allocation and characterise niche properties (Fry 2011). The application of stable isotope analysis in trophic ecology studies is often appropriate because it is considered to provide a time-integrated overview of trophic dynamics within ecosystems (Boecklen et al. 2011). In general, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes are the most commonly used in trophic ecology studies where they are used to infer the energy sources of consumers (Samelius et al. 2007) and their trophic positions (Post 2002), respectively. Several studies have used stable isotopes to evaluate food web dynamics in headwater streams (Lau and Lueng 2009, McNeely et al., 2007), including those that have been impacted by anthropogenic factors (Milanovich et al., 2014, García et al., 2017). However, few studies have evaluated the food webs of the Afromontane streams despite these systems both harbouring cryptic diversity (Chakona et al. 2018) and being susceptible to a wide range of impacts (Thieme et al., 2005). This is particularly so for the Amathole-Winterberg Afromontane Ecoregion which comprises of headwater streams that are characterised by different catchment characteristics. This study, thus, intends to bridge the ecological gap, by not only focusing on the use of stable isotope analysis to evaluate trophic interrelationships into this region, but also evaluating the impact of land-use patterns have on biodiversity. The impacts of agricultural activities and non-native plants are well documented as biodiversity threats (Walser and Bart 1999, Harding et al. 1999, Burghardt et al. 2010). However, they are not well documented in this ecoregion, and the effects they have may have on food web dynamics and species distribution is also not well understood

This study examined the food webs of three differently impacted headwater streams in the Amathole-Winterberg ecoregions. The three streams had different land-use patterns. The Eyre River was characterised by native vegetation and is closest to pristine with intact riparian zone. The Elands River was impacted by small-scale crop farming and was relatively densely populated. The Lushington River was invaded by non-native black wattle (*Acacia maernsii*) which was the most dominant vegetation in the riparian zone of this river. The study hypothesized that the Eyre River would have a food web primarily supported by allochthonous matter input. The stream would likely have an abundance of allochthonous-dependent macroinvertebrates. In addition, it was predicted that the trophic niche breadth of consumer groups would be narrow as a result of the availability allochthonous matter as the only major basal food source. The Elands River, on the other hand, was hypothesized to have a wide range of food sources due to the availability of both allochthonous matter and autochthonous-driven basal sources due to the influence of agricultural activities as a result of runoff of agrochemicals. It was, however, predicted that this stream would support few tolerant taxa that persisted within this disturbed ecosystem. In addition, as a result of basal source availability, the trophic niche breadth of consumer groups was predicted to be wide. The Lushington River was hypothesized to have a simplified food web with generalist macroinvertebrate groups and a narrow trophic niche breadth for consumer groups due to the dominance of black wattle as the major source of allochthonous matter. In addition, it was predicted that stream would be characterised by less seasonal variation due to in-stream shading by wattle, resulting in the food web that would be characterised by tolerant taxa able to utilise black wattle as a food source.

Methods

Data collection

Sampling was done in three headwater streams of the Kat River, a major tributary of the Great Fish River, located in the Eastern Cape, South Africa. The headwater streams had different land-use patterns namely; the Eyre River which had natural riparian vegetation and was closest to pristine, the Elands River was influenced by small-scale crop farming and the Lushington

Rivers was invaded by black wattle (*Acacia maernsii*). Sampling was done in August 2018. A total of nine sites (three sites per river) were sampled for the study. Potential basal food sources such as - CPOM (coarse particulate organic matter), FPOM (fine particulate organic matter), epilithic algae, and bryophytes were collected. The FPOM was collected by filtering 25 liters of stream water through a 100 µm net, three replicates were collected and stored in Eppendorf tubes. The CPOM was collected by dislodging organic matter through a 250 µm net and replicated three times and stored in Eppendorf tubes. Epilithic algae, macrophytes and bryophytes found instream were collected by hand and stored in Eppendorf tubes. The consumer groups in the rivers included macroinvertebrates and chubbyhead barb *Enteromius anoplus*, which was the most common fish in the three headwater streams. Macroinvertebrates were collected from both instream substrates and vegetation along the riverbanks. Instream, the substrate was disturbed by kicking the substrate and the macroinvertebrates were collected using a 250 µm net for one minute. A 250 µm net was used to collect macroinvertebrates by sweeping the net repeatedly over the vegetation for a one minute. The macroinvertebrates were identified down to genera level and stored in ice. Chubbyhead barb was captured in shallow riffles and pools using a SAMUS backpack electrofisher for 10 minutes. Fifteen individuals were euthanised using clove oil. Upon rigor mortis, both anal fin clip and white muscle was dissected from each of the 15 individuals. White muscle as collected from the posterior region of the dorsal fin, stored in Eppendorf tubes and kept on ice in the field. All the samples were then transported to the freshwater ecology laboratory at the Department of Ichthyology and Fisheries Science at Rhodes University, Grahamstown, South Africa.

The macroinvertebrate groups were assigned to functional feeding groups following Palmer et al. (1993, 1996) and Cummins et al. (2005). The macroinvertebrate functional feeding groups were filterers, gatherers, predators, omnivores, scrapers, brushers and collectors (Appendix 2). Filterers were defined as macroinvertebrates that are suspension feeders, whereas gatherers were assigned to deposit feeders. Predators were defined as macroinvertebrates that feed on live prey, whereas omnivores consume both live prey and plant material. Scrapers were macroinvertebrates that graze on attached non-filamentous algae on surfaces. Brushers were macroinvertebrates that fed on particles adhered to substrate and collectors fed on fine particulate organic matter.

Stable isotope processing and analysis

In the laboratory, the macroinvertebrates were identified to genera level using a light microscope (Olympus, magnification × 100). The samples of basal food sources, macroinvertebrates and chubbyhead barb muscle tissues were dried at 60basal°C for 48 hours, the samples were then ground into a homogenized homogenised powder using a mortar and pestle. The samples were packaged in separate Eppendorf tubes and sent for stable isotope analysis (SIA) at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria, Hatfield, Pretoria, South Africa. Aliquots of approximately 0.6-0.7 mg of muscle and fin tissue or 1-1.2 mg for plant materials were weighed into tin capsules that were cleaned with toluene. The isotopic analysis was done on a Flash SEA 1112 series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via ConFlo IV system (ThermoFischer, Bremen, Germany). The Flash EA 1112 series analyser is based on Dynamic Flash Combustion technology, which produces complete combustion of the sample followed by an accurate and precise determination of the elemental gases produced by a wide variety of materials. The ConFlo IV system expands the range of experiments that can be conducted by stable isotope ratio mass spectrometer (IRMS). The ratios of the stable isotopes were measured against the reference standards, PeeDee belemite for $\delta^{13}C$ (Craig, 1957) and the atmospheric nitrogen gas for $\delta^{15}N$ (Ehleringer & Rundel, 1989) (Equation 1). A laboratory running standard (Merck Gel: $\delta^{13}C$ = -20.26‰, $\delta^{15}N$ = 7.89‰ and DL-Valine: $\delta^{13}C$ = -10.57‰, $\delta^{15}N$ = 6.15‰) and a blank sample was run after every 11 unknown samples. The delta notation (δ), the difference (‰, parts per thousand) between the isotopic ration of the sample and the standard was calculated as:

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

where X = $\delta^{15}N$ or $\delta^{13}C$ and R represents $^{15}N/^{14}N$ or $^{12}C/\ ^{13}C$, respectively. Analytical precision was < 0.08‰ for $\delta^{13}C$ and <0.13‰ for $\delta^{15}N$.

Data analysis

Biplots of the carbon and nitrogen stable isotopes were used to depict the food web structure of the Sample size-correlated standard ellipse areas (SEAc) (Jackson et al. 2011) in the R package *stable isotope Bayesian Ellipses in R* (SIBER) (R Development Core Team 2019) were used to estimate isotopic niche *Enteromius anoplus*. The SEA is constructed using variance and covariance of the isotope biplot to contain only 40% of the data, which represents the core isotopic niche that is not affected by sample size, unlike the convex hull area (Syväranta et al. 2013). The resultant SEA is corrected to minimise bias caused by small sample sizes (SEAc) using the following correction factor:

$$SEAc = SEA\ \times [(n-1)/\ (n-2)]$$

Results

Isotopic values of basal sources and consumers

The $\delta^{13}C$ values for the basal food sources, CPOM, FPOM and C_3 plant matter, were generally comparable among the three rivers (Table 5). Both CPOM and FPOM had higher $\delta^{13}C$ values, which ranged between approximately -22 ‰ and -25‰, compared to C_3 plant matter which had lower $\delta^{13}C$ values that ranged between -29 to -32 among the three rivers. The three rivers, however, differed based on $\delta^{13}C$ for algae, which had the lowest value in the Eyre River ($\delta^{13}C$ = -31.21±3.4) compared to the Elands River ($\delta^{13}C$ = -17.63±3.18) and the Lushington River ($\delta^{13}C$ = -15.01±1.00) where the highest values were recorded (Table 5). The three rivers further differed based on the $\delta^{13}C$ values of C_4 plant matter that was collected in stream. Specifically, the Elands River had higher $\delta^{13}C$ values ($\delta^{13}C$ = -21.94±9.37) compared to the Lushington River ($\delta^{13}C$ = -28.50±1.26) and the Eyre River ($\delta^{13}C$ =-30.45±1.17). For $\delta^{15}N$, the Elands River was most distinguished based on high values for CPOM, FPOM and algae, whereas the Lushington River had the lowest values for these basal food sources (Table 5). In comparison, the $\delta^{15}N$ values for these basal food sources in the Eyre River were intermediate.

The macroinvertebrate functional feeding groups within the Eyre River were characterised by more taxa (Figure 13). In general, the other two rivers, the Eyre and Elands were both characterised by the dominance of predators, gatherers and filterers functional feeding groups. However, the Eyre River showed less variation in $\delta^{13}C$ values for the functional feeding groups, which ranged from -20 ‰ to -25 ‰, whereas those for the Elands River showed more variation ranging from -19 ‰ to -27 ‰ (Table 6&Table 7). In addition, the $\delta^{15}N$ values of the functional feeding groups were lower in the Eyre River (6.47 to 8.58) than those for the Elands River (6.57 to 9.78). In contrast, the Lushington River was characterised by more taxa for the brushers and collectors functional feeding groups compared to the Eyre and Elands rivers (Figure 13). In general, the $\delta^{13}C$ values for most functional feeding groups were lower (-18.62 to -27.32) compared to those in the Eyre and Elands rivers. Similarly, the $\delta^{15}N$ values for most functional feeding groups were lower (5.44 to 7.04) compared to those in the Eyre and Elands rivers. The stable isotope values for the chubbyhead barb were consistent with those for the macroinvertebrates, with the $\delta^{13}C$ values being high in the Lushington River ($\delta^{13}C$ = -22.66±0.86), followed by Eyre River ($\delta^{13}C$ = -23.66±0.93) and being low in the Elands River

($\delta^{13}C$ = -23.07±1.65) (Table 7). For the $\delta^{15}N$, chubbyhead barb had high values in the Elands River ($\delta^{15}N$ = 12.19±1.31), followed by the Eyre River ($\delta^{15}N$ = 10.96±0.54) and being low in the Lushington River ($\delta^{15}N$ = 10.36±0.36).

Table 5: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for all basal sources that occurred in the Eyre, Elands and Lushington rivers.

	Eyre River		Elands River		Lushington River	
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
CPOM	-24.92±0.87	2.42±0.56	-25.64±0.49	4.92±2.99	-24.09±0.22	0.49±1.14
FPOM	-24.96±1.17	3.06±1.95	-22.33±1.35	4.41±1.22	-24.94±0.58	2.94±1.18
C_3	-32.55±0.6	6.40±2.04	-30.14±1.64	3.92±2.99	-29.83±0.36	1.93±1.74
C_4	-30.45±1.17	5.72±1.67	-21.94±9.37	3.81±2.47	-28.50±1.26	4.10±1.44
Algae	-31.21±3.4	4.36±0.49	-17.63±3.18	7.33±1.69	-15.01±1.00	3.04±1.83

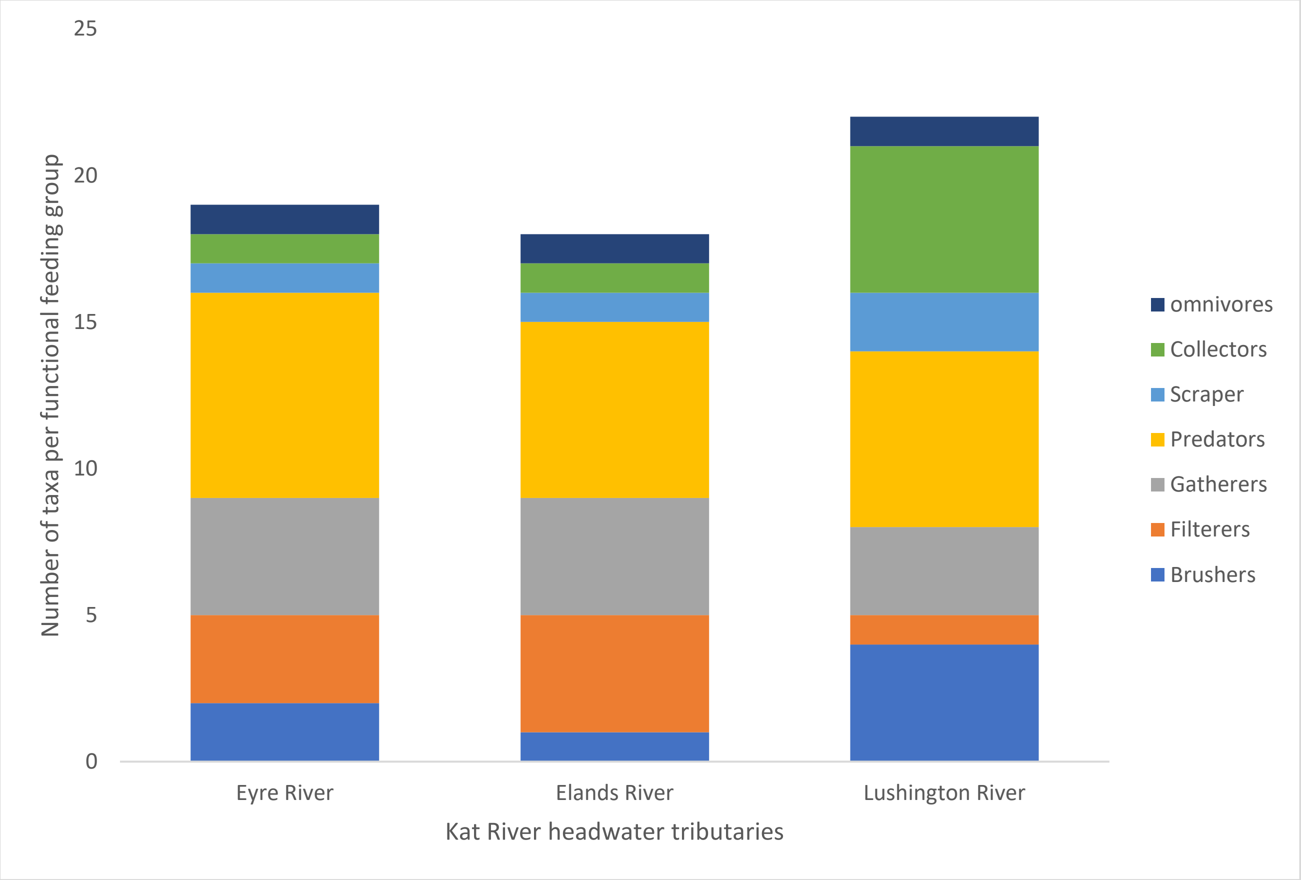

Figure 13: Number of taxa per functional feeding group in the three headwater streams of the Kat River, namely the Eyre, Elands and Lushington rivers. The functional feeding groups were based on Palmer et al. (1993, 1996) and Cummins et al. (2005).

Table 6: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for aquatic macroinvertebrates that were collected from the Kat River headwater tributaries

			Eyre River		Elands River		Lushington River		
Order	Family	Genus	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	FFG
Ephemeroptera	Baetidae	*Baetis*	-25.54±0.76	6.80±0.27			-26.32±0.71	6.01±0.76	collector
	Oligoneurid	*Elassoneuria*			-22.72±0.00	7.16±0.00			filterer
	Heptaginiidae	*Afronurus*	-25.88±1.51	7.68±0.31	-27.44±0.38	8.63±0.27	-24.68±0.32	7.06±0.44	brusher
	Trycorythidae	*Trichorythus*	-24.63±0.92	6.47±0.68	-23.92±0.34	6.90±0.6	-27.32±2.66	4.96±0.72	gatherer
	Leptophlebidae	*Castanophlebid*	-24.67±0.43	7.13±0.36	-25.26±0.75	7.37±0.6	-24.58±0.79	6.25±0.66	Gatherer
Diptera	Simuliidae	*Simulium*	-24.29±0.34	6.49±0.57	-23.58±1.04	7.48±0.56	-23.00±0.00	5.71±0.00	filterer
		Rhabdiomastix	-26.11±0.00	1.70±0.00			-26.03±0.00	1.66±0.00	shredder
		Limnophila					-23.37±0.00	7.68±0.00	predator
Odonata	Synlestidae	*Chlorostes*	-24.55±0.00	8.28±0.00			-24.42±0.42	7.89±0.68	predator
	Ashnidae	*Aeshna*	-24.82±0.02	8.15±0.25	-24.76±0.76	8.39±0.43	-25.88±1.05	7.31±0.7	predator
	Lestidae	*Lestes*	-24.07±0.00	8.58±0.00			-25.76±0.00	6.98±0.00	predator
Coleoptera	Gyrinidae	*Orectogyrus*	-24.09±0.00	6.10±0.00			-24.11±2.5	7.47±0.29	predator
Trichoptera	Hydropsychidae	*Cheumatopsyche*	-25.29±1.26	7.87±1.17	-23.34±1.26	8.76±0.24	-25.13±0.64	7.06±0.5	gatherer
	Philopotamidae	*Chimarra*					-24.93±0.48	6.75±0.11	filterer
Hemiptera	Sialidae	*Leptosialis*	-24.41±1.82	6.54±0.62	-22.50±7.34	6.57±0.6	-26.29±1.13	4.77±1.55	predator
Decapoda	Potamonautidae	*Potamonautes*	-20.20±1.26	7.47±0.4	-19.84±1.59	7.59±0.95	-20.36±0.7	5.44±0.8	omnivore
Gastropoda		*Burnupia*	-20.52±0.00	6.00±00	-23.03 ±0.00	9.78±0.00	-18.62±0.00	4.18±0.00	scraper
Plecoptera		*Afronemoura*	-24.82±1.92	7.91±2.54			-25.29±0.00	4.42±0.00	scraper
Oligochaeta							-22.55±0.00	7.66±0.00	gathering collector

Table 7: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for consumer groups including macroinvertebrates and chubbyhead barb *Enteromius anoplus* that occurred in the Eyre, Elands and Lushington rivers.

	Eyre River		Elands River		Lushington River	
	δ^{13}C (‰)	δ^{15}N (‰)	δ^{13}C (‰)	δ^{15}N (‰)	δ^{13}C (‰)	δ^{15}N (‰)
Filterers	-24.27±0.69	6.41±0.47	-23.45±1.00	7.44±0.52	-24.54±0.95	6.54±0.48
Scrapers	-24.82±1.91	7.91±2.54	-	-	-	-
Collectors	-24.83±0.82	6.80±0.27	-25.71±0.96	7.74±0.54	-26.32±0.72	6.01±0.76
Brushers	-25.21±2.35	7.47±0.66	-27.44±0.39	8.63±0.27	-23.90±2.35	6.28±1.39
Predators	-23.39±1.95	7.34±2.15	-24.07±0.55	8.23±0.69	-25.15±1.65	7.04±1.53
Omnivores	-19.63±0.76	7.45±0.42	-19.84±1.59	7.59±0.96	-20.36±0.7	5.44±0.80
Gatherers	-	-	-24.34±1.23	7.57±1.11	-25.64±1.57	5.80±1.43
Chubbyhead barb	-23.66±0.93	10.96±0.54	-23.07±1.65	12.19±1.31	-22.66±0.86	10.36±0.36

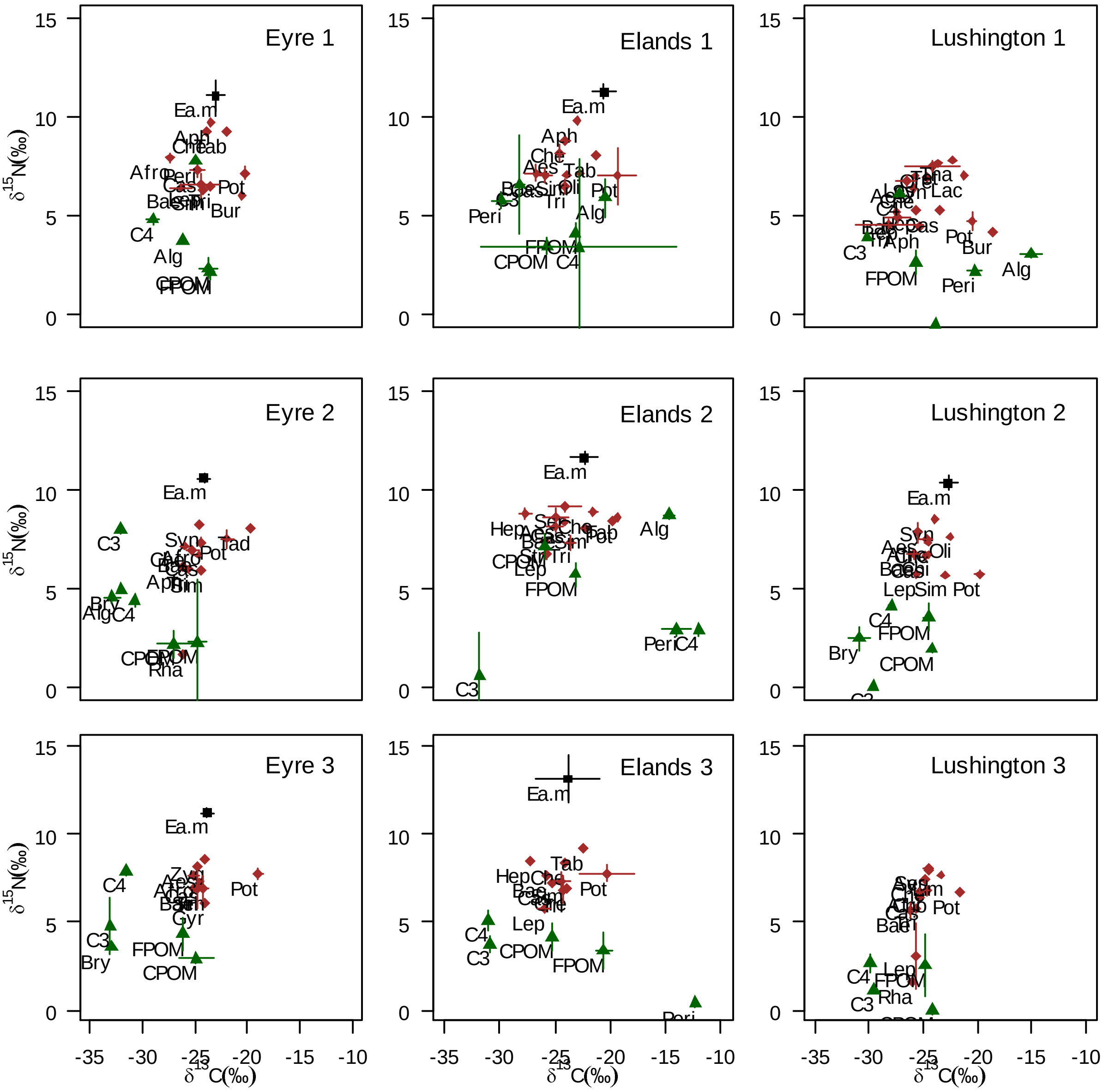

Figure 14: Stable isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ for chubbyhead barb (*Enteromius anoplus*) using muscle tissue, macroinvertebrates and basal sources for all sampled sites in the Eyre, Elands and Lushington Rivers. The abbreviations are as follows; *abbre*

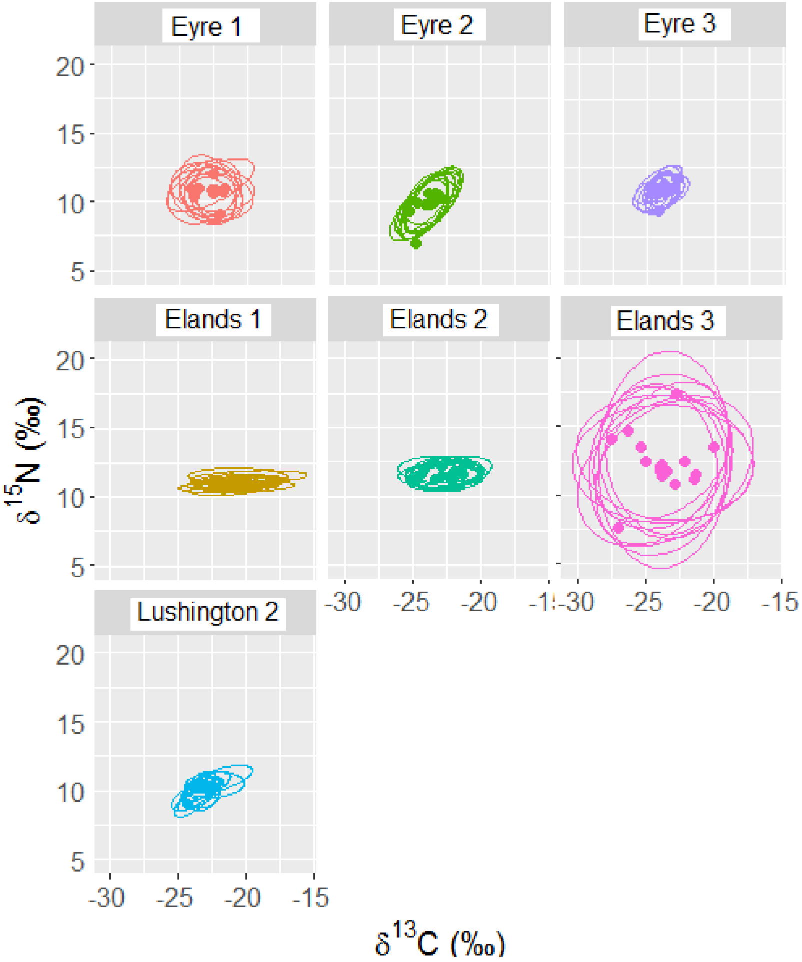

Figure 15: Isotopic niche size for chubbyhead barb (*Enteromius anoplus*) using sample size-corrected standard ellipse areas (SEAc) based on muscle tissues for the different rivers (Eyre, Elands and Lushington Rivers).

Isotopic niche and chubbyhead barb trophic niche

In all the three sites, the Eyre River was characterised by less variation in the δ^{13}C values of basal food sources, which coincided with a narrow breadth of the food web sizes (Figure 14). The isotopic niche for *E. anoplus*, however, varied among site because it was large at site 1 (SEAc = 2.18 ‰), intermediate at site 2 (SEAc = 1.98 ‰) and small at site 3 (SEAc = 0.73 ‰) (Figure 15). In contrast, the Elands River was characterised by a wide variation in in the δ^{13}C values of basal sources, which included both allochthonous and autochthonous matter (Figure

14). Consequently, the consumer groups (macroinvertebrates and *E. anoplus*) had broad isotopic niches. The isotopic niche size of *E. anoplus* varied among sites, it was small at site 1 (SEAc = 0.92 ‰), intermediate at site 2 (SEAc = 1.54 ‰) and large at site 3 (SEAc = 14.58 ‰) (Figure 15). By comparison, the Lushington River was characterised by a wide spatial variation in its food web structure, with both autochthonous and allochthonous sources contributing to the food web (Figure 14). Specifically, site 1, which was the most upstream, was characterised by a wide breadth in δ^{13}C values for both basal food sources and the consumer community, whereas site 1, the most downstream, was characterised by a narrower breadth. The isotopic niche of *E. anoplus*, which was captured at only one site, was the smallest (SEAc = 0.70 ‰) compared to those for the other rivers (Figure 15).

Discussion

This study revealed that the food web structure in the three rivers differed substantially, which indicating that the different land use patterns had a role in the structuring of the trophic dynamics. Specifically, the Eyre River was distinguished by less variation in the stable isotope values of basal food sources, which coincided with a narrow breadth in the food web sizes. This pattern was similar among all sites in this river. Although the macroinvertebrate functional feeding groups were generally similar to those in the Elands River, the consumer community in the Eyre River showed little variation in both carbon and nitrogen stable isotope values. This appeared to be consistent with the hypothesis for this river, which suggest the likely importance of few basal food sources in supporting the food webs. In general, the observed patterns in this river suggest the likely importance of allochthonous matter, particularly CPOM and FPOM as a consequence of shading from the native vegetation. These finding are in support of other studies on food web structure in near natural streams, which have shown that autotrophs were not a significant contributor of carbon in these streams (Iversen et al., 1982, Hicks 1997, Tanks et al., 2010). The results were consistent with the findings of de Castro et al. (2016) who identified that in near natural streams, consumers have narrow isotopic niche as a consequence of specialised feeding habits.

Consistent with the study hypothesis, the food webs within the Elands River were characterised by a wide variation in basal food sources, which resulted in the wide breadth of the consumer isotope niche size. Specifically, there was a wide variation in the isotope values of allochthonous (CPOM and FPOM) and autochthonous (algae) food sources. The wide variation in the stable isotope values of the consumers suggest that that both allochthonous and autochthonous matter were likely important in supporting the food webs in this river. Studies on streams that are influenced by agriculture have shown that agriculture significantly increases primary production (Griffiths et al 2013). Lee et al. (2018) demonstrated that agriculture stimulated primary production as a result of high nutrient loads from nitrate fertilisers and animal waste. A similar pattern was reported by Allen et al. (2009) who found that agriculturally impacted streams showed greater variability in consumer isotopic signatures as a consequence of wider variability in basal sources.

Although the Lushington River was predicted to have a simplified food web due to the likely negative influence of non-native wattle, this river was found to show spatial variability in food web structure. This spatial variability appeared to coincide with the occurrence of many macroinvertebrate taxa, and a high number of functional feeding groups, particularly collectors and brushers compared to the Eyre and Elands rivers. In addition, the stable isotope values depicted wide variability for both basal food sources and consumer communities for the most upstream site, whereas the most downstream site showed less variability and was narrower. The observed patterns in this river were thus inconsistent with the study hypothesis. The possible reasons for the observed patterns were resource availability, spatial heterogeneity and the presence/absence of fish. The results are in line with the finding of Thompson and Townsend (2005) wherein they identified that resource availability and spatial heterogeneity influence the food web dynamics within streams. From the results it is evident that the presence or absence of chubbyhead barb has an influence on the food webs in this river. Pace et al (1999) found that the absence of a top predator leads to trophic cascades, which likely explains the wider variability in food sources and wider isotopic niche for consumers in the site where chubbyhead barb was absent.

The isotopic niche patterns of the chubbyhead barb reflected contrasting patterns among the three rivers as a result of land-use. In particular, in the Eyre River, the chubbyhead barb isotopic niche varied from each site. More specifically, the isotopic niche was for chubbyhead barb was wider downstream as a result of a more generalised feeding habit, indicating there were fewer food sources downstream. In contrast, in the Eyre River, the chubbyhead barb isotopic niche was narrower downstream than upstream, indicating a more selective feeding habit as a result of wider availability in food sources. Similarly, in the Lushington River, the isotopic niche of chubbyhead barb was narrow where chubbyhead barb was present. This river was characterised by higher diversity and richness of macroinvertebrate groups (see Chapter 2). Consequently, chubbyhead barb had a selective feeding habit as a result of a wider availability of food sources.

To conclude, the results demonstrated a strong negative relationship between land-use and trophic interrelationships in these headwater streams. More specifically, the impacts of land-use were more evident in the Elands River which was impacted by agriculture. This is in support of the growing body of evidence that has shown that the impacts of agriculture are quite detrimental in food web dynamics. Despite the limitations, this study has highlighted valuable information about the state of these Afromontane headwater streams. Future research is needed to explore the role of isotopic carbon as an indicator of land-use patterns in food webs. Further research is indeed needed in these headwater streams, particularly considering the various anthropogenic threats that these headwater streams are facing.

CHAPTER 4

GENERAL DISCUSSION

Evaluating the impacts of land-use in lotic systems has been investigated extensively. However, few studied have investigated the same pattern in southern temperate headwater streams, which is of concern as these streams are increasingly being impacted by anthropogenic effects, particularly non-native vegetation and agriculture. This study evaluated the impacts of land-use patterns on stream structure and food web dynamics in differently impacted headwater streams.

Spatio-temporal community patterns provide insight on community dynamics and the influence that biotic interactions have on community structure. The key research question for the first chapter was that, given the differences in the catchment characteristics of these headwater streams as a consequence of differences in land-use patterns, would land-use patterns such as non-native riparian vegetation and agriculture play a significant role in the spatio-temporal patterns of the fishes and macro-invertebrates inhabiting these streams? Based on the results, macroinvertebrate communities were structured and influenced by seasonality more than land-use patterns. Several studies (Giller and Twomey 1993; Bêche et al. 2006; Johnson et al. 2012) have reported that despite the impact that land-use undoubtedly has on stream communities, season plays a far more significant role on structuring macroinvertebrate communities. This was unsurprising, however, as macroinvertebrates have strategies and species traits that facilitate their survival in dynamic environments such as aerial adult stages (Thorat and Nath 2018). In addition, Junker and Cross (2014) suggested that seasonality has a significant impact on macroinvertebrates communities as it affects the timing of food availability. By comparison, Gage et al. (2004) and

Zhang et al. (2013) found land-use to be more influential than seasonality. However, studies that found contrasting results included major differences in the level of invasions (Sponseller et al. 2001) or the type of system investigated such as tropical streams (Bücker et al. 2010) or boreal streams (Jonsson et al. 2017).

The study results indicate that chubbyhead barb population structure was impacted by land-use patterns. This can be attributed to the reports that land-use alters resource availability (Garcia et al. 2017), habitat (Walser and Bart 1999), and stream hydrology (Nagasaka and Nakamura 1999), which has a holistic impact on fish. Fish are unable to employ the same strategies as macroinvertebrates, consequently, fish communities are more impacted by land-use. Stable isotope analysis was used to investigate the second research objective, which was to evaluate whether differences in land-use patterns such as non-native riparian vegetation and agriculture had a significant impact on the aquatic food web dynamics. The findings in this study suggest that land-use critically affected trophic interrelationships in these headwater streams. The results showed that resource availability and basal sources were significantly altered as a consequent of land-use. The findings are in line with most studies on similar research (Price et al., 2019, de Castro et al., 2016, Harrington et al., 1998) which have found that land-use patterns alter the composition and availability of resources as well as altering the flow of energy in stream food webs. Contrastingly, a previous study by Milanovich et al. (2014) found that land-use did not predictably influence consumer groups due to isotopic variation across functional feeding groups or families, nor did land-use alter the quality of basal sources. These findings cannot be considered as conclusive because this study evaluated land-use patterns that were not identical to the land-use patterns investigated in the current study.

Precautions to take when interpreting findings from the present study include, firstly, the potential impacts of biotic interaction that the presence of *Sandelia bainsii* might impose on both the spatio-temporal patterns and the food web dynamics in the Lushington River. The presence of *Sandelia bainsii* which is an invertivore and of a larger size than chubbyhead barb, *Sandelia bainsii* might create competition for chubbyhead barb both spatially and in terms of resource utilisation. Secondly, sampling for the second chapter consisted of a single sampling event. Trophic interrelationships vary both spatially and temporally, therefore, the results in this study may not translate across seasons.

Although the current study did not focus on the methods of bioassessment, it, however, has brought forward an important addition to the current body of knowledge that other methods have not. There have been several rapid bioassessment methods available, including the local South African Scoring System (SASS), to assess the condition or health of a river, and often to assess the impacts of land-use (Dickens and Graham 2002). In addition, the development of the Headwater Index of Biotic Integrity (HIBI) which unlike the most indices investigates trophic aspects of a headwater streams has progressed the state of bioassessment methods (Lyons 2006). The current study indicates that evaluating the impacts of land-use requires more than assessing community structure as it often is not conclusive or not sensitive enough as a stand-alone method. Future studies should investigate assessing food web dynamics as an addition to community structure to provide a more robust bioassessment. In conclusion, the results of the current study suggest that evaluating community structure is not a sensitive bio indicator for land-use impacts. By contrast, food web interrelationships provided a better indication of the impacts of land-use on these headwater streams.

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Appendix 1: The functional feeding groups of the macroinvertebrates that were found in the headwater streams of the Kat River, namely the Eyre, Elands and the Lushington Rivers. The functional feeding groups (FFG) were based on Palmer et al. (1993, 1996) and Cummins et al. (2005).

	Family	Genera	FFG
Ephemeroptera	Baetidae	*Baetis*	Collector
		Cleodes	Gatherer
		Dicentroptelum	Collector
	Oligoneurid	*Elassoneuria*	Filterer
	Caenidae	*Caenis*	Gatherer
	Telagonidae	*Lestagella*	Brusher
	Heptaginiidae	*Afronurus*	Brusher
	Trycorythidae	*Trichorythus*	Gatherer
	Leptophlebidae	*Castanophlebid*	Gatherer
Diptera	Tabanidae	*Tabanus*	Filterer
	Chironomidae	*Tanypus*	Predator
	Chironomoidae	*Ceratopogonidae*	Scraper
	Athericidae	*Trichacantha*	Gatherer
		Atherix	Predator
	Tipulidae	*Tipula*	Predator
		Antocha	Collector
		Limonia	Shredder
		Rhabdomastix	Collector
	Muscidae	*Limnophora*	Predator
		Limnophila	Predator
	Dixidae	*Dixa*	Predator
	Culicidae	*Anopheles*	Filtering collector
Odonata	Gomphidae	*Microgomphus*	Filterer
		Ceratogomphus	Predator
	Synlestidae	*Chlorostes*	Predator
	Ashnidae	*Aeshna*	Predator
	Lestidae	*Lestes*	Predator
	Coenagrionidae	*Ceragrion*	Predator
		Psuedagrion	Predator
Coleoptera	Scirtidae	*Elodes*	Predator
	Psephenidae	*Afrobrianax*	Scraper
	Hydrophilidae	*Berosus*	Scraper
	Hydraenidae	*Ochthebius*	Gatherer

	Gyrinidae	*Orectogyrus*	Predator
		Aulogyrus	Predator
Trichoptera	Dytisticidae	*Hydaticus*	Predator
	Stenopsychidae	*Stenopsyche*	Predator
	Hydropsychidae	*Cheumatopsyche*	Gatherer
		Hydropsyche	Gatherer
	Leptoceridae	*Nectopsyche*	Gatherer
	Philopotamidae	*Chimarra*	Filterer
	Hydroptilidae	*Stactobia*	Algal piercer
Araneae	Pisauridaee	*Thalassius*	Predator
	Tetragnathidae	*Tetragnatha*	Predator
Hemiptera	Notonectidae	*Enithares*	Predator
	Pleidae	*Plea*	Predator
	Veliidae	*Rhagovelia*	Predator
	Gerridae	*Eurymatra*	Predator
	Corixidae	*Micronecta*	Predator
Megaloptera	Sialidae	*Leptosialis*	Predator
Tricladida	Planariidae	*Planaria*	Collector
Decapoda	Potamonautidae	*Potamonautes*	Omnivore
Gastropoda	Ancylidae	*Ferrissia*	Scraper
		Burnupia	Scraper
Plecoptera	Notonemouridae	*Desmonemoura*	Scraper
		Afronemoura	Scraper
	Oligochaeta		Gatherer Collector

Appendix 2: The average number and standard deviation of the macroinvertebrates identified from the headwater streams of the Kat River, namely, the Eyre, Elands, and Lushington Rivers, the macroinvertebrates were identified down to genera level.

Order	Family	Genera		Sites	
			Eyre River	Elands River	Lushington River
Ephemeroptera	Baetidae	*Acanthiops*	30.42±27.64	22.33±28.04	18.42±22.55
		Cleodes	0.67±1.56		0.75±1.76
		Dicentroptelum	15.08±20.36	20.58±37.97	6.75±20.03
	Oligoneurid	*Elassoneuria*	0.08±0.29		
	Caenidae	*Caenis*	0.75±1.54	0.33±0.65	0.92±2.57
	Telagonidae	*Lestagella*			0.08±0.29
	Heptageniidae	*Afronurus*	17.83±10.34	2.00±2.73	1.67±2.87
	Trycorythidae	*Trichorythus*	48±43.54	14.08±25.57	26.00±35.44
	Leptophlebidae	*Castanophlebid*	13.92±23.76	11.67±13.75	30.58±24.60
Diptera	Simuliidae	*Simullium*	6.17±11.39	7.42±11.56	6.00±12.61
	Tabanidae	*Tabanus*	0.42±0.51		
	Stenopsychidae	*Stenopsyche*	0.33±0.78		0.67±1.61
	Chironomidae	*Tanypus*	0.83±1.11	0.50±1.17	0.33±0.89
	Chironomoidae	*Ceratopogonidae*	0.008±0.29	0.08±0.29	
	Athericidae	*Trichacantha*		4.83±13.86	0.08±0.29
		Atherix	1.50±3.15	1.92±3.89	3.75±9.27
	Tipulidae	*Tipula*	0.33±0.78		0.33±0.49
		Antocha	0.25±0.45		0.17±0.39
		Limonia	0.08±0.29		0.17±0.58
		Rhabdiomastix	0.39±0.17		0.39±0.17
	Muscidae	*Limnophora*		0.45±0.25	1.5±3.45
		Limnophila	0.08±0.29	0.17±0.58	1.50±3.45
	Dixidae	*Dixa*		0.08±0.291	

	Culicidae	*Anopheles*	0.08±0.29		
Odonata	Gomphidae	*Microgomphus*	0.33±0.65	3.58±12.10	
		Ceratogomphus	0.08±0.29	0.08±0.29	
	Synlestidae	*Chlorostes*	1.42±2.50	1.08±3.15	2.08±3.37
	Ashnidae	*Aeshna*	0.42±0.67	1.58±2.57	3.92±5.23
	Lestidae	*Lestes*	0.08±0.29	0.17±0.39	0.33±1.15
	Coenagrionidae	*Ceragrion*		1.17±0.5	
		Psuedagrion			0.08±0.29
Coleoptera	Scirtidae	*Elodes*	0.25±0.62		5.92±17.18
	Psephenidae	*Afrobrianax*			0.42±0.51
	Hydrophilidae	*Berosus*		0.08±0.29	1.50±4.89
	Hydraenidae	*Ochthebius*		0.17±0.58	
	Gyrinidae	*Orectogyrus*	0.33±0.65	0.75±2.00	
		Aulogyrus	0.42±1.44	0.42±1.44	0.08±.29
	Dytisticidae	*Hydaticus*			0.08±0.29
Trichoptera	Hydropsychidae	*Cheumatopsyche*	8.83±11.75	12.08±14.85	13.75±13.19
		Hydropsyche	6.58±8.01		
	Leptoceridae	*Nectopsyche*	0.17±0.39		0.25±0.65
	Philopotamidae	*Chimarra*			2.50±6.37
	Hydroptilidae	*Stactobia*			0.08±0.29
Hemiptera	Notonectidae	*Enithares*	0.83±2.89	4.83±13.84	1.33±3.77
	Pledae	*Plea*		0.33±1.16	
	Veliidae	*Rhagovelia*	0.17±0.39	0.08±0.29	
	Gerridae	*Eurymatra*	0.50±1.17	0.33±1.57	
	Corixidae	*Micronecta*	0.33±1.15		
Megaloptera	Sialidae	*Leptosialis*			0.08±0.29
Tricladida	Planariidae	*Planaria*		0.17±0.39	0.08±0.29
Decapoda	Potamonautidae	*Potamonautes*	1.67±2.57	4.33±4.42	1.92±1.56
Gastropoda	Ancylidae	*Ferrissia*		0.67±1.15	0.17±0.58

		Burnupia	0.42±0.99	0.17±0.39	1.50±3.61
Plecoptera	Notonemouridae	*Desmonemoura*	0.08±0.29	0.50±1.00	11.08±2.19
		Afronemoura			2.75±6.70
		Aphanicella	0.50±1.17	0.08±0.29	4.67±14.64
Hemiptera	Corixidae	*Micronecta*	0.33±1.15		
		Sigara			0.08±0.29
	Naucoridae	*Naucoris*	0.08±0.29	1.08±2.53	
		Laccocoris			0.08±0.29