BIOTIC AND ABIOTIC DRIVERS OF MACROINVERTEBRATE ASSEMBLAGES IN A SOUTH AFRICAN RIVER

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TERENCE ANDREW BELLINGAN

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Abstract

Aquatic insects are the most numerically abundant and diverse group of organisms found in lotic ecosystems in South Africa and the world over. They play vital roles in freshwater ecosystem functioning, processing nutrients and in turn forming integral links in stream food-webs. This thesis focussed on examining the macroinvertebrate fauna within three reaches of headwater streams of the Keiskamma River system: reaches that were considered to be fishless; reaches that were invaded by non-native salmonid species; and reaches that were dominated by native fish. I described the effects of predatory fish presence through detailed examination of macroinvertebrate assemblage composition; macroinvertebrate drift timing and density; and through niche utilisation determined from stable isotope data. Patterns in the macroinvertebrate assemblages of the headwaters of the Keiskamma River appear to be driven more strongly by flow rate and seasonal influences, but fish presence and biotope availability were also significant drivers. Niche shifts due to predator presence were not easy to detect and, while patterns of influence may have been evident, they were not found to be significant. However, I demonstrated that salmonids selectively feed on native fish species when the opportunity is presented, occupying significantly higher trophic levels when co-occurring with native fish than in invaded reaches where native fish are absent. Drift timing and density were demonstrated to be significantly different between reach for specific macroinvertebrate species from the Ephemeroptera and Plecoptera, under differing fish predation regimes, in agreement with what has been observed from studies in rivers elsewhere.

In freshwater ecosystems of South Africa and worldwide, mitigation of negative effects of alien fishes through their removal using piscicides may also affect non-target organisms. To better understand the effects of just such a removal operation, employed for the first time in the history of freshwater conservation in South Africa, macroinvertebrate communities were assessed for non-target effects of rotenone. The fish eradication operations were demonstrated to have a short-term negative effect on the macroinvertebrate assemblage, through water quality index measurements and alteration of densities of macroinvertebrate taxa collected from stone surfaces. However, no long-term detrimental impact was observed as macroinvertebrate faunas returned to a comparable pre-treatment state within a year of each rotenone application.

Declaration

This thesis forms the compilation of original research that I carried through the South African Institute for Aquatic Biodiversity (SAIAB) and the Department of Zoology and Entomology, Rhodes University, conducted between 2012 and 2016. It has not been submitted in whole or in part for a degree at any other academic institution. All data presented are original, and all assistance received is duly acknowledged.

Terence Andrew Bellingan

Date

This thesis and work is dedicated to my father, Andrew, my mother, Joy, and brother, Jonathan. I think I know why, we all being so different, ended up together, and I am glad we did! Thank you for your unwavering love and support!

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What a journey this has been, I don't think I was ready when it started, but I am ready for it to be finished!

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Chapter 1: General introduction

Rivers, along with their associated floodplains, wetlands and groundwaters, are in a state of crisis globally (Arthington 2012). Through the actions and inaction of mankind, they represent the most damaged ecosystems on the planet, characterized by a rate of biodiversity loss that surpasses any other habitat (Dudgeon et al. 2006). The comprehensive treatise on the threats to freshwater biodiversity by Vörösmarty et al. (2010) estimated that 10 000 - 20 000 freshwater species are threatened with extinction or are already lost, primarily as a result of anthropogenic factors, the rates of loss observed rivalling the transition between the Pleistocene and Holocene. In their assessment of the conservation status of South African rivers, Nel et al. (2007) found, rather alarmingly, that "the state of main river ecosystems in South Africa is dire: 84% of the ecosystems are threatened, with a disturbing 54% critically endangered, 18% endangered, and 12% vulnerable."

Dudgeon et al. (2006) listed the five major threats to aquatic biodiversity in natural freshwater ecosystems at a global scale as over-exploitation, pollution, habitat degradation, species invasion and flow modification. The threats facing rivers in South Africa are not dissimilar. In an assessment of the threats to fishes, molluscs, dragonflies and crabs in southern Africa, the most important immediate impacts were habitat degradation, flow modification, pollution and invasive alien species (Figure 1.1; Darwall et al. 2009).

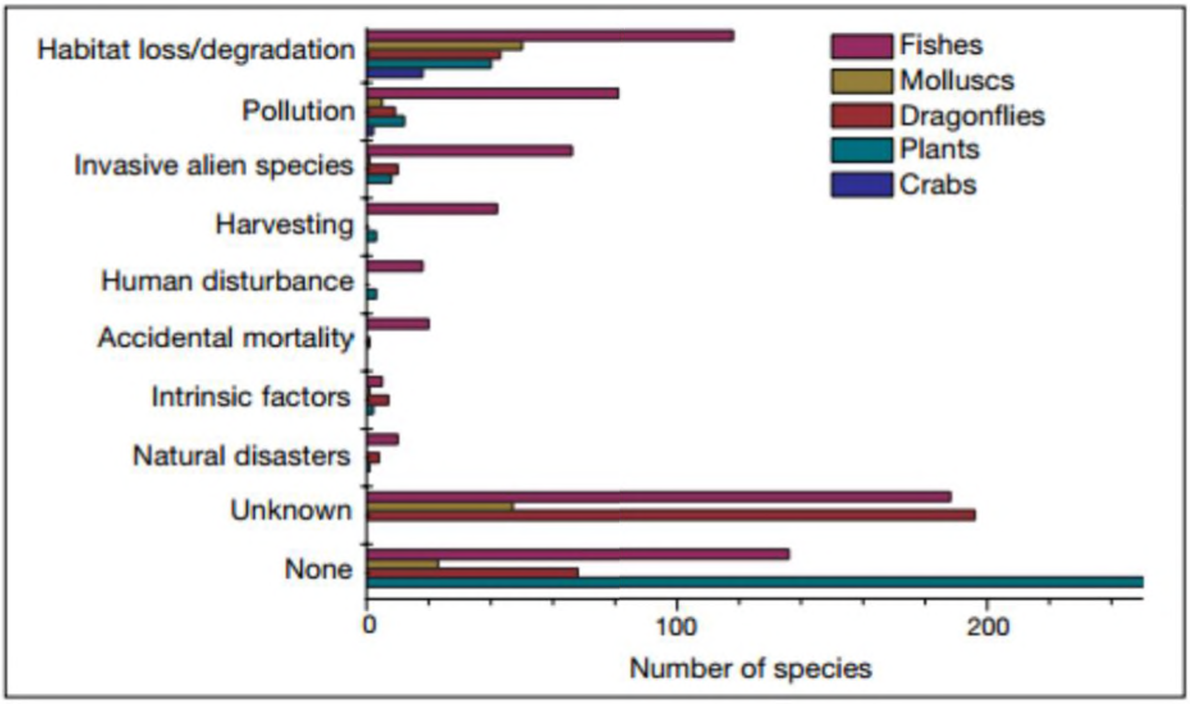

Figure 1.1: The number of freshwater species from each major organismal group estimated to be influenced by the corresponding threat, in natural ecosystems (from Darwall et al. 2009).

The introduction and spread of non-native invasive fishes has been recognised as the most pressing threat to native freshwater faunas within South Africa, particularly within the Cape Fold Ecoregion (Tweddle et al. 2009), a trend that has also been observed globally (Clavero & Garcia-Berthou 2005). The predominant driver for non-native fish introductions in South Africa, as in many other regions of the world, was the creation and establishment of recreational fisheries (Lomnicky et al. 2007, Keller et al. 2011, Olden et al. 2008, Ellender & Weyl 2014, Sanches et al. 2014, Azevedo-Santos et al. 2015). The human-mediated introduction and subsequent spread of non-native species has been called "one of the least reversible human-induced global changes" (Kolar & Lodge 2002). Non-native fish species have been introduced into lakes and rivers since the late 1800s for the creation of sport fishing opportunities (de Moor & Bruton 1988, van Rensburg et al. 2011, McCafferty et al. 2012, Ellender & Weyl 2014), and as a result, South Africa is regarded as one of six global invasion hotspots where non-native fish account for more than a quarter of freshwater fish species diversity (Leprieur et al. 2008).

The threats of alien invasive fish to native fishes include hybridisation and introgression, the introduction of associated parasites and disease, and direct predation and competition (Ellender & Weyl 2014). The latter impact extends to macroinvertebrates, where reductions in density (Albariño & Buria 2011), size-selective predation (Meissner & Muotka 2006) and alterations to the behaviour of stream insects may be observed (Feltmate & Williams 1989, 1991, McCutchen 2002). As non-native fishes reputedly sustain economically important fisheries despite potentially causing harm to their recipient ecosystems, the management of these species is often fraught with conflicts between stakeholders with different value sets (Ellender et al. 2014, Woodford et al. 2016). A heightened knowledge of the threats posed by non-native fish species to native biota is therefore required for better evidence-based protection, management and conservation of aquatic ecosystems (Lake et al. 2007). Headwater stream ecosystems present an opportunity to quantify these threats because the impacts of habitat degradation and pollution are generally considered of lesser importance than those of alien species, and particularly alien sport-fishes such as rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) and brown trout *Salmo trutta* L. 1758, which are considered the primary threats in this context (Ellender & Weyl 2014, Weyl et al. 2015).

Trout are not native to Africa (Crawford & Muir 2007). Both *S. trutta* and *O. mykiss* are listed in the top 100 of the World's worst invasive alien species (Lowe et al. 2000) due to their damaging effect on recipient ecosystems. Globally, *O. mykiss* is one of the most widely introduced fish species, rivalled only by the common carp *Cyprinus carpio* L. 1758 and the Nile perch *Lates niloticus* L. 1758 (Welcomme 1988, Fausch 2007). The habitat preferences and physiological tolerances of both *O. mykiss* and *S. trutta* limit their distribution within South Africa to cool, clear, perennial streams and higher altitude impoundments (Bjorn & Reiser 1991, Armstrong et al. 2003, Ellender et al. 2016). Worldwide, trout are reported to affect native faunas negatively through predation and competition

(McIntosh et al. 1992, McIntosh & Townsend 1995, Townsend & Crowl 1991, Flecker & Townsend 1994, Townsend 1996b, Lintermans 2000, Inoue et al. 2009, Baxter et al. 2004); by altering natural stream ecosystem functioning (Buria et al. 2007, Albariño et al. 2011); and through the disruption of reciprocal trophic subsidies (Matthews et al. 2002, Epanchin et al. 2010). In southern Africa, until recently, most work on the impacts of trout had been carried out on fish and amphibians (Woodford & Impson 2004, Karssing et al. 2012, Kadye et al. 2013). In a review of the current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa, Ellender & Weyl (2014) state that four studies had, to date, addressed the impacts of non-native fishes on invertebrate assemblages of the southern African region. Kadye & Booth (2012a, 2012b) examined macroinvertebrates' responses to the regionally native, but extralimital and invasive, African sharptooth catfish *Clarias gariepinus* (Burchell 1822). They attribute notable losses from the macroinvertebrate assemblage to a form of prey naïveté to an introduced predator. Kadye et al. (2013) demonstrated that an *O. mykiss* invasion in streams of the Eastern Zimbabwe Highlands Ecoregion (Abell et al. 2008) altered the distribution and abundance of macroinvertebrates and a native fish species. In a study aimed specifically at testing the effects of trout on macroinvertebrates, Rivers-Moore et al. (2013) could not conclusively demonstrate that *O. mykiss* and *S. trutta* negatively impacted the macroinvertebrate assemblages of Drakensberg-Maloti Highlands Ecoregion streams, though they suggest that effects may be present but masked by overriding environmental variables in comparing upstream and downstream reaches.

Subsequent to Ellender & Weyl's (2014) review, large-scale field experiments (Shelton et al. 2015a, 2015b) and an *in situ* mesocosm experiment (Shelton et al. 2016) examined the effects of *O. mykiss* on the fish and invertebrate faunas of a Cape Fold Ecoregion stream (CFE; *sensu* Abell et al. 2008). *Oncorhynchus mykiss* depleted the population of endemic native barb species through size-selective predation, while alleviating predation pressure on grazing invertebrates by usurping the native fish; native barb species were also found to be stronger regulators of the invertebrate community than trout, which were demonstrated to rely more heavily on terrestrial inputs for food. Outside the CFE, impacts on stream fishes and invertebrates remain poorly understood, particularly at species- and functional guild-level, and potential behavioural modification by aquatic insects is hitherto unexplored. Most recently, Jackson et al. (2016) used stable isotopes to demonstrate an effect of *O. mykiss* on riparian spiders, suggesting an interruption of reciprocal prey subsidies between the stream and predatory web-spinning spiders, with trout usurping emerging aquatic macroinvertebrate prey.

In a review paper entitled "Identification of 100 fundamental ecological questions", Sutherland et al. (2013) formulated 100 pressing, unanswered, questions in the field of ecology. Under the section "Ecosystems and functioning", Question 68 is: "*To what extent is biotic invasion and native species loss creating ecosystems with altered properties*?" In this context, this thesis aims to understand the extent to which trout invasions in some headwater streams have affected their macroinvertebrates' assemblages, behaviours and food-webs. The Amatolo-Winterberg Highlands Ecoregion (Abell et al. 2008) therefore provides a unique opportunity to study trout's impacts because the distribution of fishes within headwater tributaries of the region is know because of work carried out by Ellender (2013). Here, trout have been established for over a decade (de Moor & Bruton 1988).

During Ellender's (2013) study of the impacts of non-native fish and habitat degradation on an endangered headwater stream fish, the distribution patterns of the various fish species that occurred in the upper catchment was mapped in detail (Figure 1.2). Briefly, the study found that the distribution of fish fauna in the Keiskamma catchment showed a low degree of similarity between impoundment, main channel and headwater assemblages.

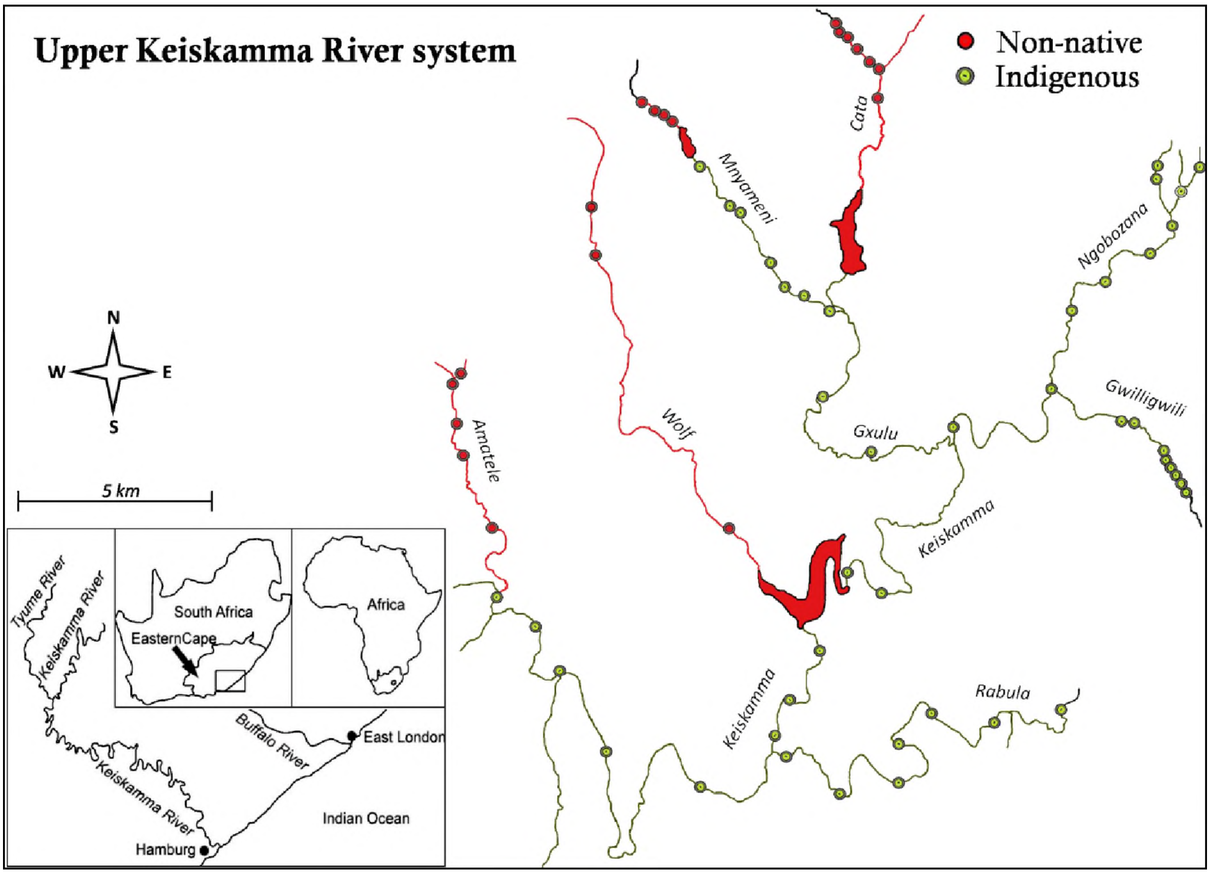

Figure 1.2: A map of the upper Keiskamma river system showing the distribution of native and non-native fish species described by Ellender's (2013) study of the system (modified from Ellender (2013)).

Among the drivers for the differences observed was a change in thermal regime downstream of the impoundments that made that habitat unsuitable for persistence of the non-native species (Ellender et al. 2016). Distinctly different fish communities were evident from the uppermost reaches of specific streams, where no fish occurred; within the impoundments and the reaches directly above the impoundments, where non-native fish proliferated; and the lower reaches below the impoundments, where native fish predominated (Figure 1.2). What remained unexplored were effects that *O. mykiss* and *S. trutta* might have on macroinvertebrate assemblages of the Keiskamma River headwaters.

These gaps are explored in this thesis, which contains detailed species-level assessment of the impacts of two salmonid species in South Africa, focussing specifically on using the full suite of macroinvertebrate assemblages that occur in the study streams. That the distribution of trout and native fish species along the headwaters of the Keiskamma River system was known prior to the start of this study presented an opportunity to test hypotheses relating to aspects of macroinvertebrate assemblages under differing fish predation regimes. A major component of this thesis was, therefore, to make a detailed examination of macroinvertebrate assemblage structure, macroinvertebrate behavior and food-web dynamics from reaches of stream that hosted no fish, trout, or predominantly native fishes.

The remediation of invasions of non-native fishes is commonly carried out using piscicides like rotenone (Finlayson et al. 2000, Cailteux et al. 2001), but this method poses risks to native faunas like invertebrates and amphibians (Dudgeon 1990, Vinson et al. 2010). Therefore, risks of the eradication method need to be weighed up carefully against the impacts of the invader. As such, the impacts of piscicides on aquatic insects need to be better understood, i.e. which is the lesser evil. A river treatment using rotenone presented an opportunity to better understand the response of invertebrate communities to the piscicide, and to evaluate the potential impact of a commonly-used remediation strategy on a macroinvertebrate community over the long term.

Therefore, the main aim of this thesis make a detailed examination of macroinvertebrate assemblage structure, macroinvertebrate behavior and food-web dynamics from reaches of headwater tributaries of the Keiskamma River that were fishless, invaded by trout, or contained predominantly native fishes. This requires an understanding of river and stream ecology, and the forces that drive of stream macroinvertebrate assemblages, a short review of which follows.

1.1 Background ecological theory on river and stream ecology

Studies examining interactions between organisms *in natura* should be grounded in ecological theory. Rivers and streams are in essence the result of a spatial and temporal equilibrium between the forces of erosion and resistance (Leopold 1962). The hydrology – the way that water flows and the characteristics of river and stream beds - is fundamental to most properties of freshwater habitats (Hynes 1970). The ecological theory of streams and their functioning is described in several seminal reference texts on stream ecology (e.g., Minshall 1988; Hynes 1970, 1976; Oglesby et al. 1972; Whitton 1975; Ward & Stanford 1979; Lock & Williams 1981; Barnes & Minshall 1983; Fontaine & Bartell 1983; Davies & Walker 1986). What follows here is a brief chronological summary of the major conceptual perspectives on lotic systems, as an introduction to the concepts underpinning our understanding of stream ecosystems and the theory addressed in this thesis.

On the whole, rivers may be thought of as four-dimensional systems, longitudinally by flow, laterally through interaction with the riparian zones, and vertically by interaction with the stream bed and hyporheic zone, with temporal change as the fourth dimension (Ward 1989, Poole 2002). Based on the way in which these dimensions change, rivers were initially categorised into major geomorphological zones, each zone naturally characterized by specific faunal elements and explained by physical changes from headwaters to floodplain (Huet 1959, Hawkes 1975, Bisson & Montgomery 1996). This way of thinking proved to be impractical as many rivers did not conform to the distinctness between zones that was envisaged, and evidence for dissimilar biological zonation was not always easily found; stream ecologists, having long recognized the gradual turnover and change of lotic processes down the gradient of rivers and streams, turned to viewing a continuum rather than a zonation (Arthington 2012).

The river continuum concept (RCC) was formalized by Vannote et al. (1980) and to this day remains a pervasive theme in conceptualizing the ecological and biological functioning of lotic ecosystems. The observation of the gradual change in physical attributes of lotic systems led Vannote et al. (1980) to describe rivers as intergrading linear networks characterized by a continuous gradient of water flow, predictable changes of physical conditions, and "consistent patterns of loading, transport, utilization, and storage of organic matter along the length of a river" (Arthington 2012). The RCC is underpinned by the importance of flow, as this is the primary agent for the transfer of materials, and ultimately energy, down the gradient of the river course.

The RCC model also relies heavily on the understanding that many of the processes taking place within a river are a function of what is found immediately outside it (Vannote et al. 1980). Along headwater reaches, inputs into the stream take the form of whole leaves and large particles of terrestrial origin, coarse particulate organic matter (CPOM). The composition of the invertebrate community leans towards groups able to process these inputs, taxa that are adapted to chewing CPOM (shredders) and harvesting algae, fungi and bacteria that break down large pieces of CPOM (scrapers) (Vannote et al. 1980, Merrit & Cummins 1996, Cummins et al. 2005). While this organic matter is physically and biochemically broken down, its smaller constituent parts are transported downstream as fine particulate organic matter (FPOM), restructuring the invertebrate communities to utilize this resource, where filtering collector and gathering collector taxa may predominate (Vannote et al. 1980).

Following the development of the RCC, Townsend (1989) suggested an alternative conceptual manner to view the functioning of lotic ecosystems, through the terrestrially-derived conceptual framework of changing patch dynamics. Instead of focussing on the changing resource base along the river continuum, which ultimately is assumed to be responsible for the organization of higher trophic levels, he suggested that the basis for comprehending lotic assemblage structure and dynamics should arise from holding the view that every section of stream bed is patchy on different scales, and "has its own kinds of disturbances, colonizers, colonist sources, and species interactions" (Townsend 1989). Thus, the concept of patch dynamics may have a wider applicability, as the assumptions of the RCC are not usually realized and lack world-wide generality (Townsend 1989). The concept of patch dynamics focuses explanation of the processes responsible for community organization on temporal and spatial phenomena, coupled with the history of the space in question. In essence, the patch dynamics concept emphasizes the fundamental role that disturbance plays in organising ecological processes within a stream (Resh et al. 1988). Poole (2002), based on Townsend’s (1996a) framework, suggested that rivers are in fact "formed as a patchy discontinuum from their headwaters to mouth", relying heavily on a hierarchical patch dynamics perspective.

It is important to recognise that the above approaches represent the respective stream ecologists' approach to streams and rivers as gradients, given the unidirectional flow of water from source to sink, in their pursuit to provide a unifying framework that explains observed patterns and processes in streams (Ward et al. 2002). Temporal succession and recolonization are products of disturbance (Minshall 1988). The effect of water flow is intricately related to the process of disturbance, as it facilitates the constant erosion and deposition of sediments, including inorganic and organic matter (Lake 2000). This movement, within the continuum down the stream length, facilitates the displacement of invertebrates between biotopes, and the microhabitats within those biotopes. This provides a conceptual model of why invertebrates are distributed in patches (Minshall & Petersen 1985). In lotic environments, it is largely accepted that headwater populations of macroinvertebrates are repopulated through winged, sexual life stages moving back upstream to breed followed by oviposition, following Müller's (1954) colonization cycle.

Further concepts that have been developed to account for the forces that shape and structure lotic ecosystems include the Flood Pulse Concept (Junk et al. 1989), the Hyporheic Corridor Concept (Stanford & Ward 1993) and the Serial Discontinuity Concept (Ward & Stanford 1983, 1995). However, these concepts are more useful in the context of providing insight into linkages between physical processes, like the movement, distribution, and quality of the water in large floodplain-type rivers. Poole (2002) notes that the RCC is most applicable to constrained streams and rivers where longitudinal linkages are strong, while the Flood Pulse and Hyporheic Corridor Concepts are more appropriately used in unconstrained reaches "where geomorphological structure facilitates lateral and vertical linkages" within and surrounding the lotic environment.

With the above concepts in mind, the RCC will be referred to throughout the course of this thesis because it provides the most appropriate background against which the effects of fish on macroinvertebrates can be considered in this study system, as the connectedness between stream reaches, biotopes within reaches, and the scale at which samples are collected remains conceivable. The RCC model provides a predictive framework for how invertebrate assemblages may be structured down the continuum of a stream, based on resource type and availability. While exceptions exist as local modifiers, like tributaries and naturally-formed impoundments (Ward 1989), the RCC is considered by many stream ecologists as the central organizing paradigm in fluvial ecology (Arthington 2012). As stated by Thorp et al. (2006), "the basic depiction of a river network as an ecological continuum is still largely accepted for headwaters through medium rivers" and thus is invoked here also. Having a firm grasp of these concepts is important and relevant to this work because it provides a framework within which observed patterns may be explained in headwaters.

Freshwater ecosystems are described as biogeographic islands, as a result of their specific organismal assemblages suffering from limited dispersal between neighbouring, functionally-similar systems (Leprieur et al. 2009). Through the formation of their biotic communities over geological time, islands are perceived to be species-poor refugia (Simberloff 1995), where competition between co-existing biota is limited (MacArthur et al. 1972). River and stream ecosystems are considered to be unsaturated in terms of niche space available for utilization by potential invaders (Leprieur et al. 2009, Kadye & Booth 2012a, Campbell & McIntosh 2013). The application of island biogeography theory is therefore also relevant for understanding stream ecosystems, particularly in the context of invertebrate assemblage recovery after disturbance events like alien fish removal operations (Minshall et al. 1983, Gore & Miller 1990, Lake 2000) and the impacts of biological invasions (Moyle & Light 1996, Townsend 1996b, Pyšek & Richardson 2006).

The framework of the RCC provides an understanding of how the natural histories of macroinvertebrate assemblages change along the gradient of a stream as physical conditions inherent to the stream reach change, providing a means of understanding and predicting the structure of invertebrate guild composition (Vannote et al. 1980). Within this gradually restructured assemblage, fundamental ecological forces work to regulate the properties of each functional organismal group through interspecific interactions like competition, predation and facilitation, carried out by the organisms that comprise the stream community, therein lying the balance of resource-driven bottom-up (Lindeman 1942) and predator-driven top-down (Hairston et al. 1960) population controls (Hunter & Price 1992). There is much debate as to which of the two types of control is more important and which plays the more ascending role in structuring communities (Strong 1992, Munkittrick & McCarty 1995, Chase 2000, Kitching 2001) but, when considering invasion by predatory fish that occupy the highest points in the trophic scale in recipient systems, headwater ecosystem effects are likely to be top-down (Eby et al. 2006, Terborgh & Estes 2010).

It is recognized that predators may exert strong regulatory forces on aquatic ecosystems (Chase 2000), sometimes resulting in cascading effects through the food web (Townsend 1996a, Lowe et al. 2008, Buria et al. 2010), in some instances even across ecosystem boundaries to the terrestrial environment (Matthews et al. 2002, Baxter et al. 2004, Knapp 2005, Epanchin et al. 2010, Terborgh & Estes 2010, Jackson et al. 2016, 2017). For these reasons the introduction of non-native fish into headwater stream ecosystems may modify the outcome of the forces that naturally regulate macroinvertebrate assemblages. The addition of a novel predator may directly affect prey, indirectly facilitating a reduction in density by altering their behaviour (predator avoidance), resulting in alteration of food acquisition for prey organisms (Estes et al. 2001, Jackson et al. 2017). A sound understanding of the ecological theory governing these biological processes enables better explanation of the ecological implications of invasions by non-native fishes. Examining the role that non-native fish have in influencing the regulation of these community processes is a key area of limnological research worldwide (e.g., Simon & Townsend 2003, Dudgeon et al. 2006, Strayer 2010), but the field is currently still in its infancy in South Africa (Ellender & Weyl 2014), in particular with respect to impacts on macroinvertebrate communities. Therefore, a better understanding is required because the impacts of trout have been demonstrated to be detrimental elsewhere in the world (Cambray 2003b), where they have invaded suitable habitat and become established and naturalized (Crawford & Muir 2008, Blackburn et al. 2011, 2014).

1.2 Thesis overview

This thesis aims to augment and improve the body of existing knowledge of the impacts of trout on macroinvertebrate assemblages in South African headwater streams. The leitmotif of this work is to place the effects of trout on macroinvertebrate communities in the upper Keiskamma River catchment within the context of the river continuum concept of Vannote et al. (1980). In addition, the effects of using rotenone for alien fish eradication on a South African aquatic macroinvertebrate community are evaluated.

To do this, the thesis is presented in seven chapters. Chapter 1 is a general introduction containing the background theory for this study, including fluvial ecological theory pertaining to rivers, streams and the forces that govern how they function, as well as biological invasions and how they are currently understood. The study area is described in Chapter 2. As the Keiskamma River is not well studied, this chapter synthesises important details of the physical and biological characteristics to provide supporting information upon which the contents of the remaining chapters rely. This chapter also contains much of the descriptive work undertaken during the course of the study because freshwater ecological work related to macroinvertebrates is still in its infancy in South Africa and Africa in general.

Chapter 3 explores the presence of native fish and salmonids as drivers of macroinvertebrate assemblage structure, taking into account environmental variables. Here macroinvertebrate density, functional feeding group composition and density, and assemblage structure are related to environmental and biotic variables.

Chapter 4 assesses the impact of trout on invertebrate behaviour by describing the drift activity of macroinvertebrates from fishless, invaded and native fish reaches in the system. Patterns of drift density are examined between groups of macroinvertebrates throughout the full day/night cycle, to test for changes in drift activity attributable to fish predation regime. The timing and density of macroinvertebrate drift of species for which a significant response to fish presence (as suggested by findings in Chapter 3) is examined, testing hypotheses relating to predator avoidance behaviour, as displayed elsewhere in the world.

Chapter 5 uses stable isotopes to explore whether differing predatory fish regimes resulted in niche shifts or alterations in niche width. The trophic position of each fish species within the Keiskamma headwaters, under the differing regimes from the three reaches, is examined.

Chapter 6 describes the effect of rotenone on the macroinvertebrate assemblage of the Rondegat River following its use by local nature conservation authority CapeNature. Parts of this chapter have been published (see Bellingan et al. 2015).

Finally, Chapter 7 provides a synthesis of what has been described in this thesis, outlining the observed effects of trout on macroinvertebrate communities from the Keiskamma River system, and the impacts of the fish eradication operations on the macroinvertebrate assemblage of the Rondegat River. Detailed avenues for future research in the areas of biological invasions by salmonids and their ecological effects with respect to macroinvertebrates are also provided, along with conservation and management recommendations to limit detrimental impacts. Future work towards the monitoring of non-native fish eradication programs is also suggested, along with recommendations for improving outcomes and reliability of results.

An extensive reference list containing all sources of literature cited within this thesis is included as a separate chapter to avoid the repetition of references between chapters. Appendices containing relevant macroinvertebrate species data sets for each chapter and supplementary information from analyses are also included.

1.3 Permitting for specimen collection and ethical clearance

To understand any biological system, it is extremely important to have an assiduous comprehension of the system itself, which is founded in turn on grasping the epistemological process employed in trying to understand it. For these reasons, I took it upon myself to carry out all field collection of data and specimens in person. This work included all of the identifications of invertebrates, and in relatively few instances fish too, from all sampling trips. I believe that the time spent collecting macroinvertebrates in person, or at the very least being physically present while they are collected, followed by making their identifications personally thereafter, lent a more intimate and intuitive understanding of their ecology, as not every observation of a system can be summarised on a graph, or in a table or spreadsheet.

The sampling methods and protocols for working with fish were approved by the ethics committee of the South African Institute for Aquatic Science (SAIAB) and Eastern Cape Parks Board's scientific services. Permits for the collection and transport of fish and invertebrates for research purposes were issued by the Department of Economic Development and Environmental Affairs (DEDEA) and are listed: Permit No's. CRO 1/12CR, CRO 2/12CR, CRO 3/12CR, CRO 4/12CR, CRO 14/12CR, 15/12 CR, CRO 16/13 CR and CRO 17/13 CR.

Chapter 2: General description of the study area: selected headwater tributaries of the Keiskamma River system, Eastern Cape Province, South Africa

2.1 Introduction

The Keiskamma River system is not well studied, this chapter contains important details of its physical and biological characteristics to provide supporting information on which the contents of the remaining chapters rely. Therefore, what follows in this Chapter is the descriptive and survey work carried out, including a brief description of the study region, macroinvertebrate characteristics of each reach sampled, and the important factors determining the differences between reaches, including trout densities.

The Keiskamma River begins as several perennial, oligosaprobic, headwater tributaries. The Gwiligwili, Cata, Mnyameni, Amatele, Thuyme and Wolf Rivers are found on the western edge of the Amatolo-Winterberg Ecoregion, Eastern Cape Province, South Africa (Kleynhans et al. 2005, Abell et al. 2008). The Keiskamma River rises in the Winterberg mountain range to the north, flowing in a south-easterly direction over the escarpment and through the Hogsback region of the Amathole Mountains. The Keiskamma River is approximately 263 kilometres long from source to mouth, and the catchment spans 2745 km^2 (Mhangara & Kakembo 2012). The average rainfall within the upper catchment is 600 mm.yr^{-1}, with the majority of this falling during early and mid summer (Mhangara & Kakembo 2012).

The basal geology of the upper escarpment catchment is Beaufort Group sedimentary rock, consisting primarily of fine-grained grey mudstone, intruded by doleritic dykes and inclined sheets of Jurassic age (Weaver 1991). The formation of the escarpment itself has been a topic of much debate for decades (*sensu* Clark et al. 2011), and it is postulated to be the passive erosional remnant of the continental margin, with its current position dating back to the end of the Cretaceous (McCarthy & Rubidge 2005; Clark et al. 2011). The vegetation of the upper Keiskamma catchment in the escarpment zone is predominantly healthy and consists of montane grassland on the slopes and indigenous mist belt forest in the valleys, lining the river course to the foothill reaches. The coastal plateau (middle) reach of the Keiskamma is lined with sour savannah grassland and is subject to communal land use for agriculture and is degraded as a result of poor land management (Mhangara & Kakembo 2012, Mhangara et al. 2012).

Long-term flow data for the Thyume River, a headwater tributary of the Keiskamma River system in the neighbouring valley to the west of the study area, was obtained from the Department of Water Affairs and Forestry hydrology section (DWAF 2017). Flow data for the period 1985/11-2016/09 were obtained from the gauging weir at Kwa Kayalethu (R1H014). Long-term rainfall data for the period 1985/11-2016/09 were obtained from station R1E003 at Sandile Dam, located on the mainstem of the Keiskamma River, below the confluence of the Cata, Gwiligwili and Mnyameni Rivers. These data were used to illustrate rainfall and flow variability in the headwaters of the Keiskamma River system. Like the Cata, Gwiligwili and Mnyameni Rivers, the Thuyme River is perennial (Figure 2.1).

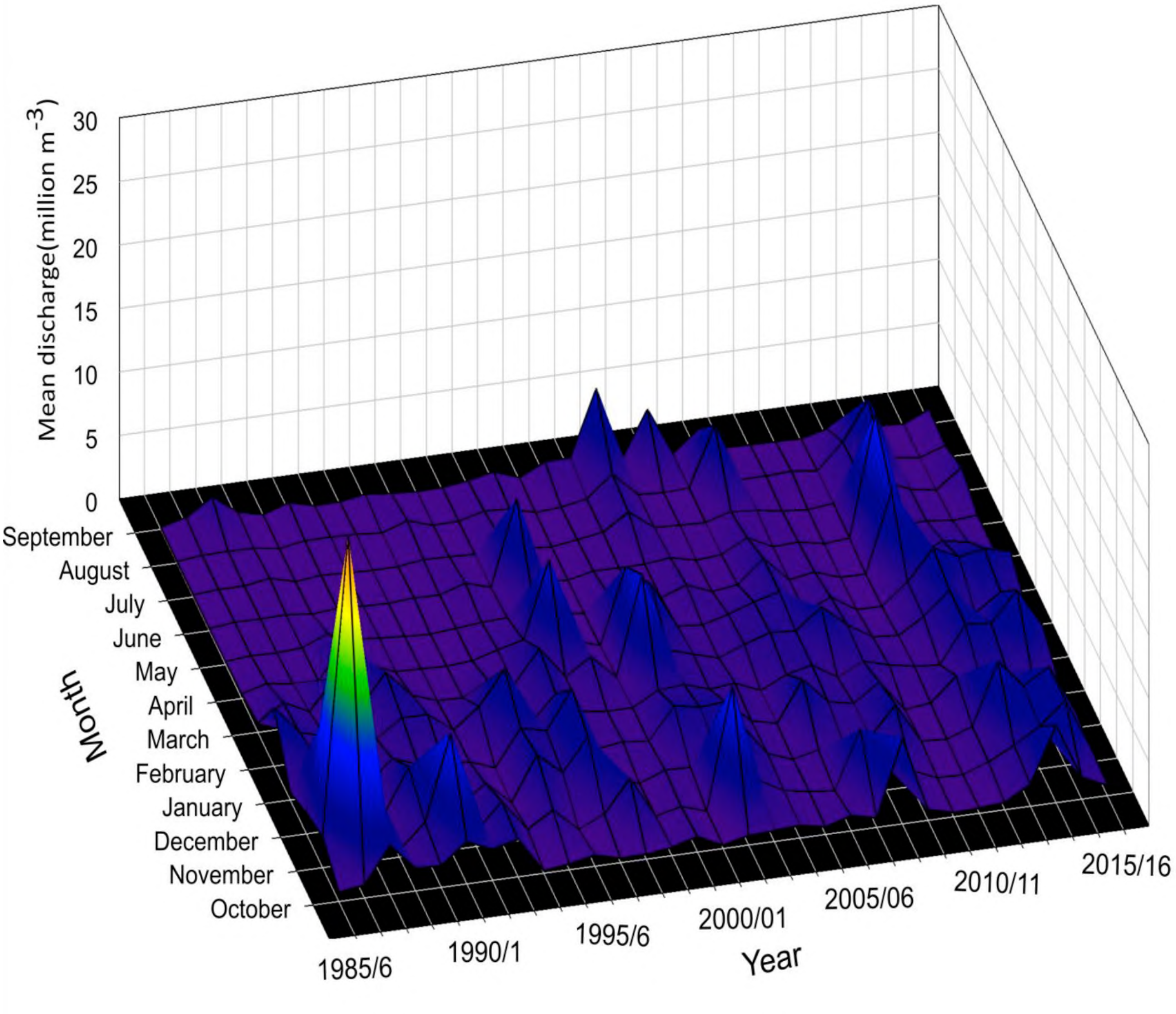

Figure 2.1: Mean monthly discharge in million m^3 obtained from the gauging weir (R1H014) at Kwa Kayalethu along the Thyume River, a headwater tributary of the Keiskamma River (1985/11-2016/09). These data are provided to represent the seasonal variability of flow in the upper Keiskamma River system, Eastern Cape, South Africa.

Peak flows occur predominantly leading to and during austral summer months, associated with higher rainfall along the escarpment during this time. Mean monthly discharge ranged from 0.6 ± 0.6 million m^3 for the month of July, to 3.1 ± 4.9 million m^3 for November for the period 1985/11-2016/09. Rainfall recorded at the meteorological station at Sandile Dam, approximately 15 km downstream from the confluence of the Cata and Mnyameni Rivers indicates peak rainfall between October and

March in the following year, during austral summer (Figure 2.2). Mean annual rainfall for the period 1985/11- 2016/09 was 715.1 ± 204.8 mm.

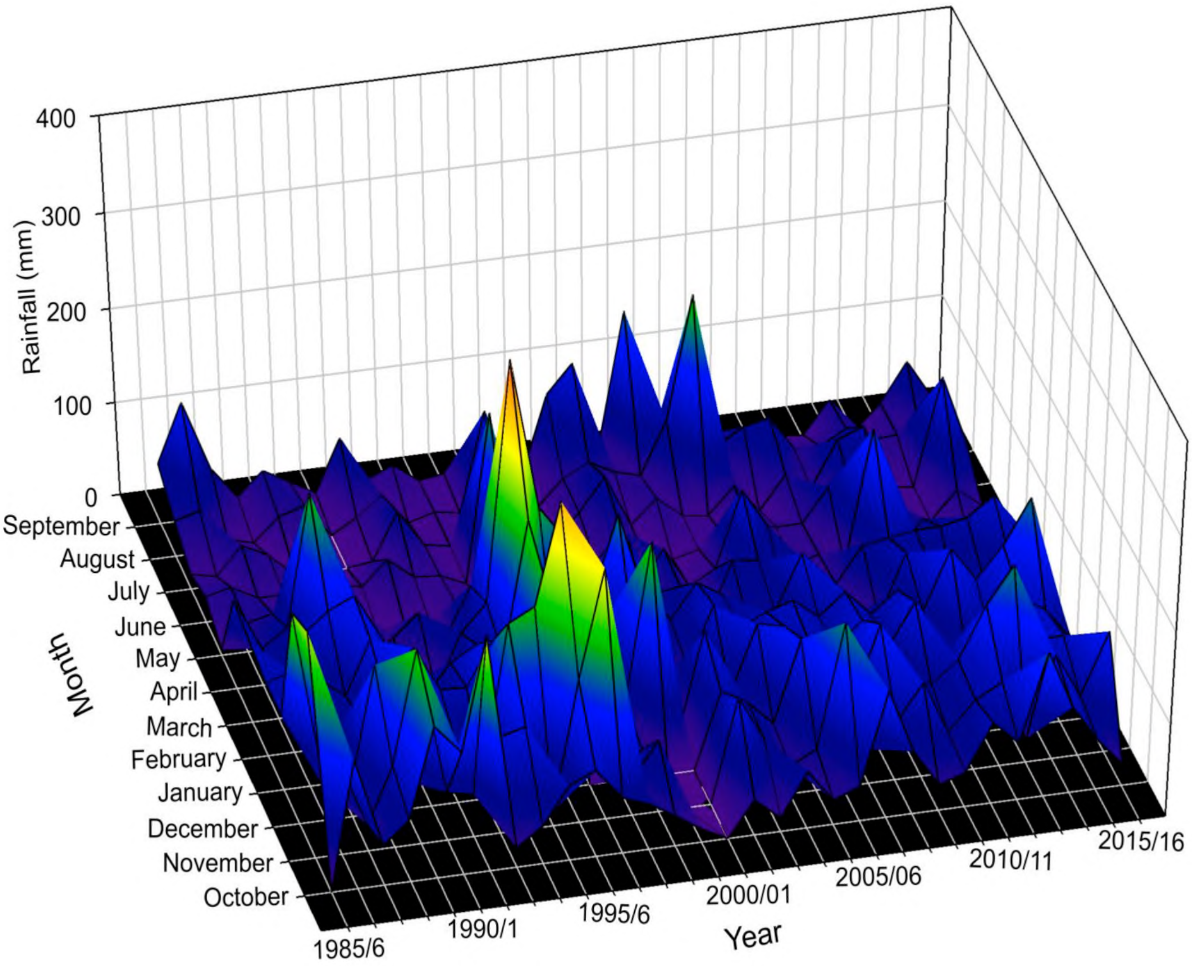

Figure 2.2: Total monthly rainfall (mm) from station R1E003 at Sandile Dam (1985/11- 2016/09), provided to represent the seasonal variability of rainfall in the upper Keiskamma River system, Eastern Cape, South Africa.

2.2 Macroinvertebrate study reaches

The riverine habitat consisted of three tributaries of the Keiskamma River: the Cata, Gwiligwili and Mnyameni Rivers (Figure 2.3). These are swift-flowing 3rd-, 2nd- and 2nd-order streams, respectively, derived from 1:50 000 scale maps of the region. As previously described, reaches selected for sampling macroinvertebrate assemblages were based on the distribution of fishes along headwaters of these streams, described first by Ellender (2013), and validated through electrofishing surveys. To identify suitable, comparable sampling areas for collecting macroinvertebrates, each stream was traversed along its length and areas of in-stream habitat were found that were representative of fishless, invaded and non-invaded reaches, allowing comparisons of the macroinvertebrate assemblages between these reaches to be made for each of these rivers.

The fishless sampling reach along the Mnyameni River was situated about 1013 meters above sea level (masl), the corresponding reach along the Cata River was slightly lower at 980 masl, while the Gwiligwili River fishless reach was situated at 776 masl. The downstream native fish reaches ranged from 836 masl along the Mnyameni River to 717 and 700 masl along the Cata and Gwiligwili Rivers respectively (Table 2.1). The fishless reach of the Mnyameni River was characterized by large, fairly deep, pools broken up by short riffles over cobbled beds. This is evidenced by this reach having the greatest average depth of 0.35 ± 0.16 m, and the highest proportion of pool-to-riffle ratio (Table 2.1). The Gwiligwili River was shallowest, ranging from 0.06 ± 0.03 to 0.07 ± 0.04 m deep along the fishless and native fish reaches, respectively. The fishless and invaded reaches along the Cata River were similar in substrate proportions, while the native fish reach contained the highest proportion of riffle compared to the remaining sites (Table 2.1). The mean stream width ranged from 7.80 ± 3.30 m at the fishless Mnyameni reach to 1.2 ± 0.4 m at the fishless Gwiligwili reach. The Gwiligwili River, being a narrower stream with a smaller catchment, flowed less strongly than the Cata and Mnyameni Rivers, with flow increasing downstream (Figure 2.4).

The river banks of the fishless reaches of all three rivers, and the invaded reaches of the Mnyameni and Cata Rivers, were completely shaded by indigenous mist-belt forest. Only small patches of direct sunlight reached the water surface. The riparian vegetation along the native fish reaches of the Gwiligwili, Cata and Mnyameni rivers was characterised by Savannah Thornveld and sparsely distributed, indigenous *Podocarpus* trees (Figure 2.4).

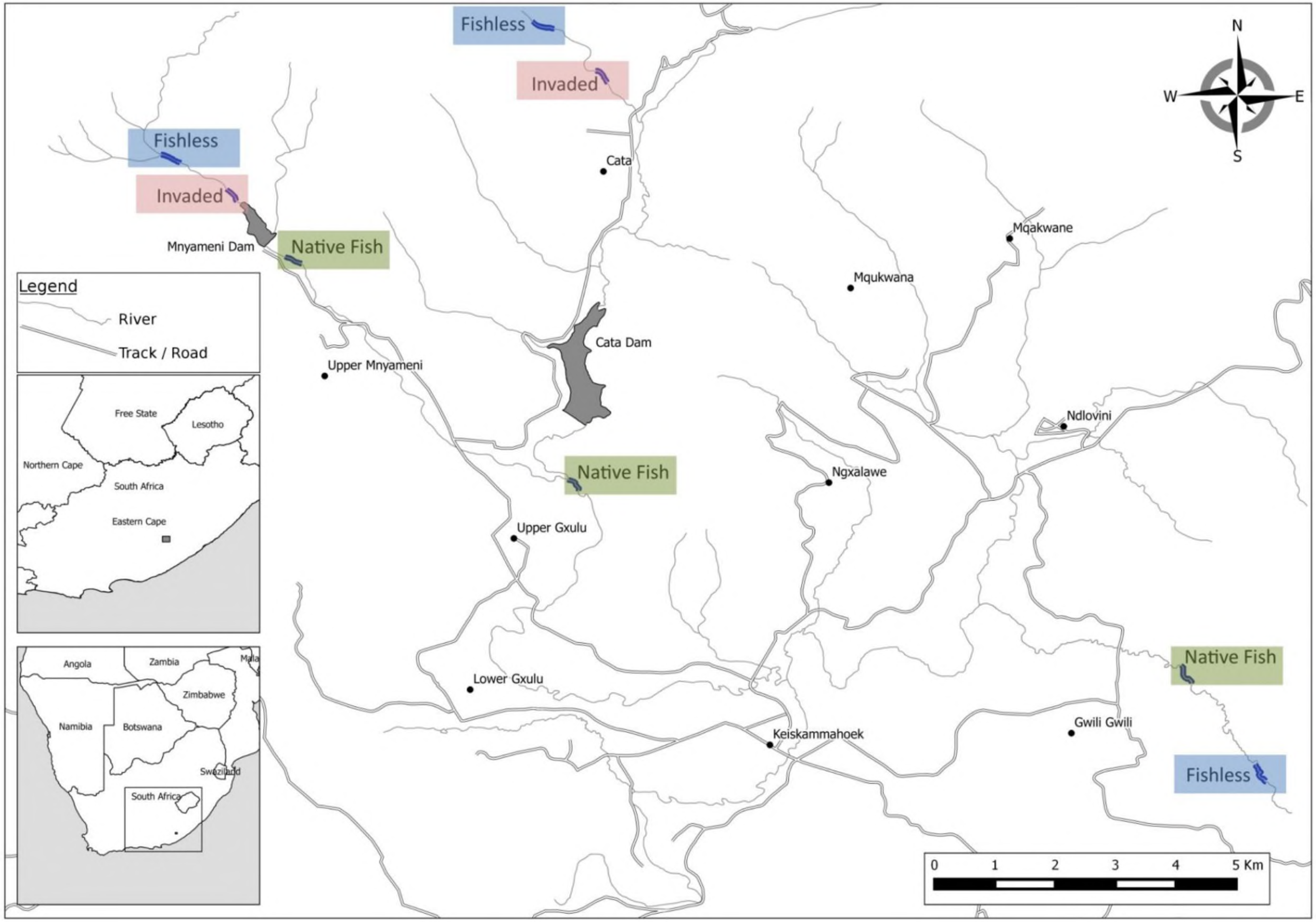

Figure 2.3: A map of the eastern tributary headwaters of the Keiskamma River system, Eastern Cape, South Africa, showing important landmarks and the position of fishless, invaded and native fish macroinvertebrate sampling reaches of the Cata, Gwiligwili and Mnyameni Rivers.

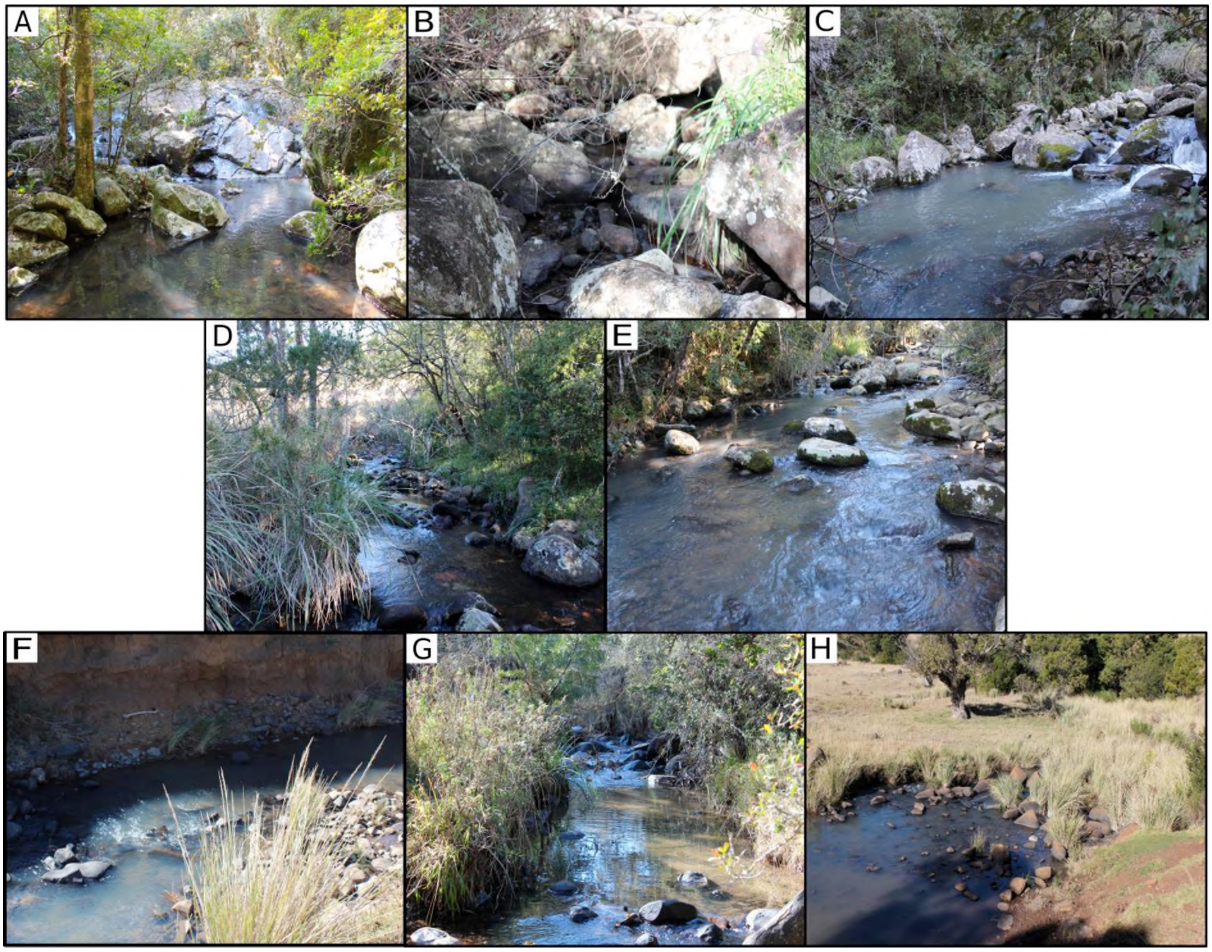

Figure 2.4: Photographs of sampling regions along fishless, invaded and native fish reaches of the Keiskamma headwaters, Eastern Cape, South Africa. (Fishless reach sites: A = Cata River, B = Gwiligwili River, C = Mnyameni River; Invaded reach sites: D = Cata River, E = Mnyameni River; Native fish reach sites: F = Cata River, G = Gwiligwili, H = Mnyameni River). Photographs were taken during July of 2012, when flow was at its lowest, in the case of the Gwiligwili River, flow can barely be seen between the boulders along the fishless reach (B).

2.2.1 Physico-chemical characteristics

Dissolved oxygen concentration was recorded during macroinvertebrate sampling events using a Cyberscan DO 300 dissolved oxygen meter (Oakton Instruments, Vernon Hills, IL, U.S.A.). Values decreased from the fishless to native fish reaches on the Cata River and the Gwiligwili River, while no change was observed between the fishless and invaded reaches of the Mnyameni River, an increase was observed along the native fish reach (Table 2.2). Dissolved oxygen concentration is highly dependent on local conditions, like rates of photosynthesis, respiration and advective transport (Kemp & Dodds 2001), and on abiotic factors such as altitude, temperature and atmospheric pressure (Dallas & Day 2004). The lowest dissolved oxygen concentration values recorded during this study were from the Mnyameni River native fish reach, but this value still exceeds the minimum allowable value for the target water quality value applicable to aquatic ecosystems of high conservation value (DWAF 1996).

Electrical conductivity (EC) and pH were recorded using a Hanna HI98219 Combo pH and electrical conductivity meter (HANNA Instruments Inc., Woonsocket, U.S.A). Electrical conductivity showed the same trend on both the Cata and Mnyameni Rivers, increasing from upper fishless sites to lower native fish reach sites (Table 2.1). This is to be expected as the sediment load increases down the length of a river (Hart et al. 1991, Dallas & Day 2004). pH values recorded remained relatively uniform between 6.85 ± 0.26 and 7.46 ± 0.24 across all sites (Table 2.1). The directly averaged pH values corresponded those recorded from the pristine reaches along the neighbouring Buffalo River, upstream of impoundments, during times of high and low flow (Palmer & O'Keeffe 1990), but were all lower than those (8.2 ± 0.2 to 9.2 ± 0.2) recorded by Ellender (2013) from the same streams.

Temperatures recorded at each site using HOBO Pendant® temperature/light data loggers, set to record temperatures every three hours, are summarized in Table 2.2 and represented in Figure 2.5 for each river separately, and median values for fishless, invaded and native fish reaches are presented in Figure 2.6. The HOBO units are waterproof and are widely used in recording water temperatures (Rivers-Moore et al. 2005, Eady et al. 2013, Ellender et al. 2016). The seven-day moving average temperature profiles in Figure 2.5 illustrate how the temperature at each sampling reach, within each reach, fluctuates during the course of nearly a full annual cycle. Stream temperatures displayed a seasonal sinusoidal pattern with the lowest recordings corresponding to austral winter, between June and July, and the warmest during summer, between December and January (Figures 2.5, 2.6). The Gwiligwili fishless reach is noticeably warmer than the Cata and Mnyameni River fishless sites, the invaded reaches along the Mnyameni River and Cata River are very similar, to each other and their respective upstream fishless sites. The native fish reach along the Gwiligwili is similar in temperature profile to the native fish reach along the Mnyameni River, except that the absolute minimum is lower along the Gwiligwili at 5.76°C, and the absolute maximum is higher along the Mnyameni River, at 26.23°C. The temperature profile of the native fish Mnyameni River site, when compared to the upstream invaded and fishless reach sites, is 2-3°C warmer at any point in time (Figure 2.5). This is due to the influence of the surface-release impoundment upstream of the native fish reach, were water is warmed as an epilimnetic layer exposed to direct sunlight before flowing down stream, described in Ellender et al. (2016). The lowest average temperatures (11.8 ± 3.4°C) were recorded from the fishless reaches along the Mnyameni River; this site also contained the narrowest absolute range of 5.0 - 20.3°C (Table 2.2). The native fish reach along the Mnyameni River yielded the highest average temperatures, 15.03 ± 3.96°C, nearly 4°C higher than the fishless reach along the same river. The fishless Cata River reach and the invaded Cata and Mnyameni River reaches displayed very similar average temperatures, ranging from 12.2 ± 3.3°C along the fishless Cata reach to 12.9 ± 3.2°C along the invaded Cata reach (Table 2.2).

Table 2.1: Treatment reach, stream, altitude, GPS location, absolute range and mean temperatures recorded from eight HOBO loggers recoding temperature every three hours, deployed along the headwaters of the Keiskamma River system, Eastern Cape, South Africa (Figures 2.5, 2.6).

	Cata	Mnyameni	Gwiligwili
Fishless reach			
Altitude (m above sea level)	947	990	755
GPS Location (°)	32.57062 S 27.11572 E	32.58825 S 27.04997 E	32.68772 S 27.22811 E
Logging period	27/01/2013 - 17/01/2014	27/01/2013 - 17/01/2014	27/01/2013 - 17/01/2014
Absolute range (°C)	5.75 - 20.81	5.04 - 20.33	6.17 - 23.00
Mean (± SD)	12.18 ± 3.33	11.78 ± 3.36	13.83 ± 2.98
Invaded reach			
Altitude (m above sea level)	866	923	
GPS Location (°)	32.57931 S 27.12684 E	32.59314 S 27.05935 E	
Logging period	27/01/2013 - 17/01/2014	27/01/2013 - 17/01/2014	
Absolute range (°C)	5.57 - 21.76	5.35 - 22.62	
Mean (± SD)	12.90 ± 3.24	12.37 ± 3.37	
Native fish reach			
Altitude (m above sea level)	717	695	826
GPS Location (°)	32.64091 S 27.11200 E	32.67663 S 27.22104 E	32.61202 S 27.07418 E
Logging period	18/07/2011- 10/01/2012	27/01/2013 - 17/01/2014	27/01/2013 - 17/01/2014
Absolute range (°C)	6.67 - 29.45	5.76 - 24.06	6.47 - 26.23
Mean (± SD)	16.02 ± 4.35	13.98 ± 3.53	15.03 ± 3.96

Table 2.2: Summary of the habitat and physicochemical characteristics (mean ± SD) for eight reaches sampled quarterly on three headwater tributaries of the Keiskamma River from July 2012 - March 2013. "Fishless", "Invaded" and "Native fish" refer to the reach of stream sampled (Figures 2.3, 2.4). Values marked with an asterisk (*) indicate qualitative estimations.

	Cata River			Mnyameni River			Gwiligwili River	
Parameter	Fishless	Invaded	Native fish	Fishless	Invaded	Native fish	Fishless	Native fish
Altitude (meters above sea level)	980	889	717	1013	944	836	776	700
GPS waypoint (°)	32.57062 S 27.11572 E	32.57727 S 27.12372 E	32.64091 S 27.11200 E	32.58825 S 27.04997 E	32.59314 S 27.05935 E	32.61202 S 27.07418 E	32.68772 S 27.22811 E	32.67373S 27.21777 E
Length (m)	27.0	30.0	30.0	22.0	26.0	30	28.0	30.0
Mean width (m)	2.83 ± 0.45	3.83 ± 1.55	5.20 ± 1.83	7.80 ± 3.30	4.03 ± 0.68	7.30 ± 1.47	1.2 ± 0.41	1.76 ± 0.49
Depth (m)	0.20 ± 0.12	0.20 ± 0.10	0.23 ± 0.10	0.35 ± 0.16	0.25 ± 0.10	0.24 ± 0.15	0.06 ± 0.03	0.07 ± 0.04
Surface Area (m^2)	76.5	115	156	171.6	104.8	219	53	34.5
Canopy cover (%)	90*	70*	0	87*	80*	0	70*	76*
Cobbles (%)	30	42	66	16	26	23	8	12
Leaf litter (%)	34	30	0	39	16	24	52	18
Bedrock (%)	24	24	6	26	37	6	9	2
Sand/Gravel (%)	0	0	20	7	19	9	3	63
Mud (%)	12	4	8	12	2	38	28	5
Pool (%)	62	54	28	68	38	62	55	59
Riffle/Run (%)	38	46	72	32	62	38	45	41
pH (n=5)(direct average)	7.19 ± 0.68	6.85 ± 0.26	7.88 ± 0.29	6.98 ± 0.20	7.15 ± 0.25	7.46 ± 0.24	7.09 ± 0.30	6.99 ± 0.14
Oxygen concentration ($mg.l^{-1}$) (n=3)	12.9 ± 0.05	12.6 ± 0.06	11.5 ± 0.45	12.4 ± 0.04	12.4 ± 0.50	14.9 ± 4.16	12.5 ± 0.33	12.1 ± 0.11
Conductivity ($\mu S.cm^{-1}$) (n=2)	17.3 ± 4.60	25.0 ± 2.76	106.8 ± 31.90	34.3 ± 11.19	37.7 ± 8.46	71.8 ± 12.50	75.4 ± 44.69	69.4 ± 20.64

Temperature readings taken along the Gwiligwili River fishless reach were warmer on average than the remaining fishless reaches, more than 2°C warmer when compared to the fishless Mnyameni River reaches. Using the statistical program R (R Core Team 2017), the temperature data recorded from the 27/01/2013 to the 17/01/2014 was compared using a Generalised Additive Model (GAM) using the R package mgcv (Mixed GAM Computation Vehicle with GCV/AIC/REML Smoothness Estimation). General additive models are used to fit nonlinear curves to data (Wood 2011), in this case to distinguish between nonlinear variation in the data from diel and seasonal effects on temperature, while accounting for river reach differences.

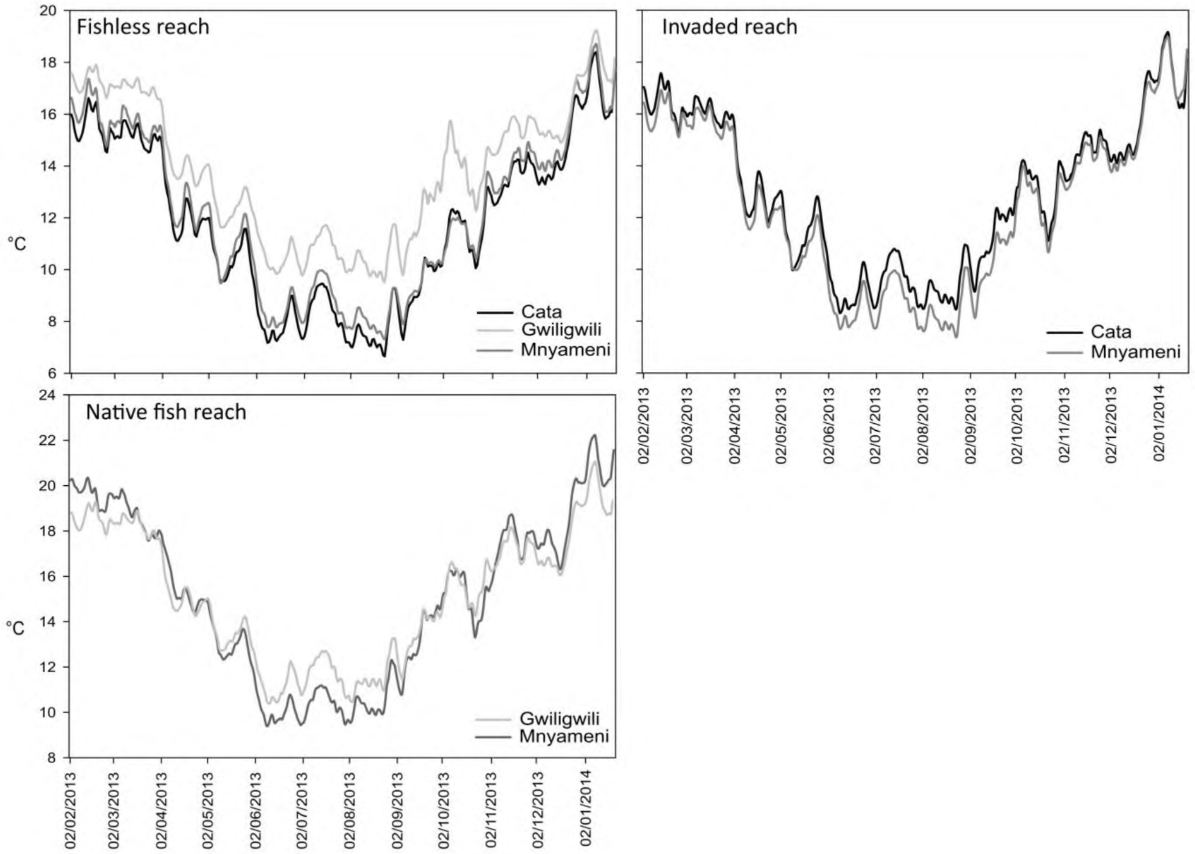

Figure 2.5: Seven-day moving average plots of temperature recorded along fishless, invaded and native fish reaches of the Cata, Gwiligwili and Mnyameni Rivers, headwater tributaries of the Keiskamma River system, Eastern Cape Province, South Africa.

Because temperature is a continuous variable, the Gaussian distribution family was specified in the model. Time of day and month were specified as smooth terms, and river reach specified as the categorical variable. Median monthly values are presented in Figure 2.6 and parametric coefficients and the significance values for the smooth terms in Table 2.3. The R-squared value obtained from the GAM was 0.794, therefore, 79.4% of the deviance in the data was explained by the model. Table 2.3

details comparisons between native fish reaches and fishless reaches, compared to invaded reaches. Fishless and invaded reaches were not significantly different from one another, but both reaches were significantly cooler than the native fish reaches ($F = 2779.65$, df = 2, $P < 0.01$).

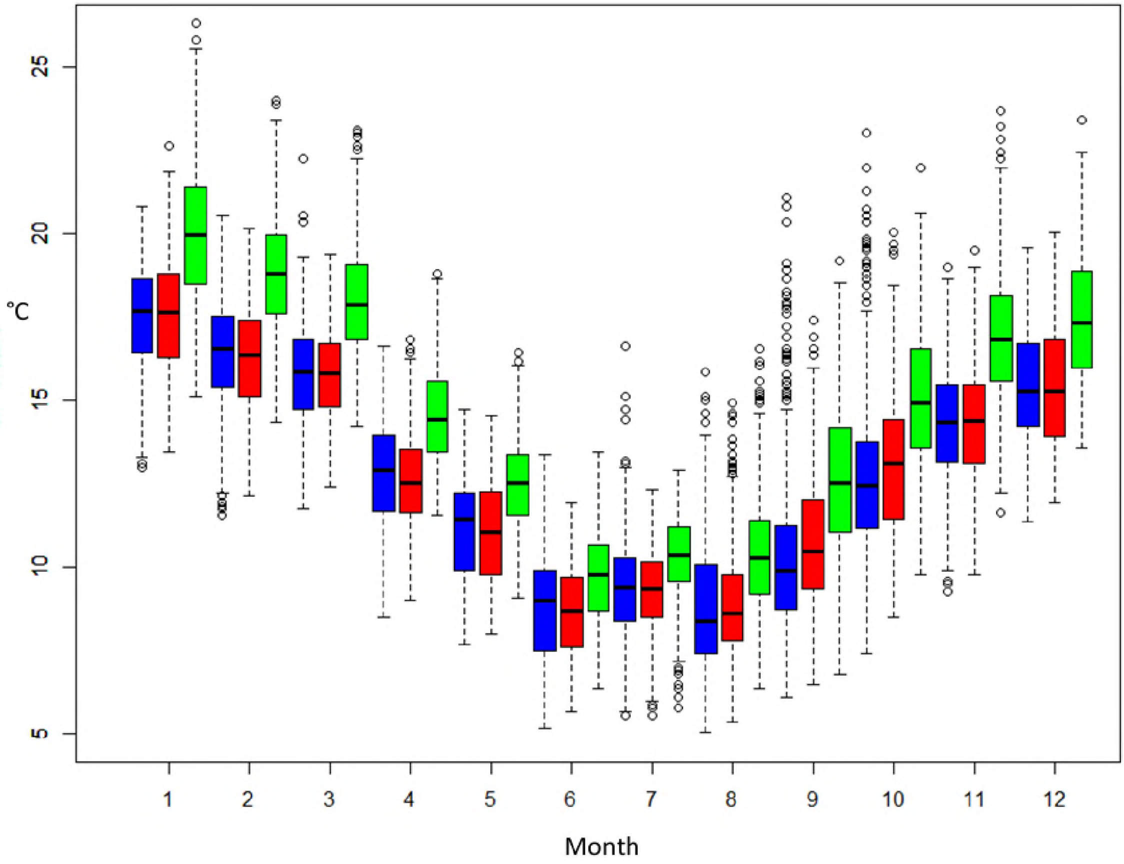

Figure 2.6: Median monthly temperature values for fishless (blue), invaded (red) and native fish (green) reaches from the Keiskamma River headwaters, Eastern Cape, South Africa.

Table 2.3: Parametric coefficient results from the General Additive Model (GAM) testing for differences in temperature between fishless, invaded and native fish reaches along headwater tributaries of the Keiskamma River system, Eastern Cape, South Africa.

Stream reach	Mean temperature	Standard error	T value	*P* - value
Fishless	12.57	0.03	-1.56	$P > 0.05$
Invaded	12.62	0.02	585.76	$P < 0.01$
Native fish	14.49	0.03	61.51	$P < 0.01$

2.3 Macroinvertebrate assemblage characteristics of the headwaters of the Keiskamma River system

As no published studies of the macroinvertebrates of the Keiskamma River headwaters exist, species inventories needed to be compiled from the Surber samples and drift samples collected and analysed in Chapters 3 and 4 respectively. As a result, brief species-level descriptions from the current surveys are included here, while each sampling method is described in detail in the respective chapter. These species level assessments are presented in Appendix 1, Table 3, and Appendix 2, Table 1, and contain the macroinvertebrate taxa identified from the 320 Surber samples and 72 drift samples respectively, collected from the same headwater reaches of the Keiskamma River. The following section contains a descriptive characterisation of these reaches, based on the taxa sampled from each reach. Thereafter, the findings are compared to research that was conducted on the neighboring Buffalo River (Palmer & O'Keeffe 1990, Palmer 1991, Palmer et al. 1991, 1993a, 1993b, 1994, 1996, Hill & O'Keeffe 1992), and elsewhere (Madikizela et al. 2001, Madikizela & Dye 2003, Odume et al. 2012).

2.3.1 Assemblage characteristics of sampling reaches

The fishless Cata River reach was characterised by a waterfall at the top, followed by several deep pools interspersed with cobbled riffles and another waterfall at the bottom, forming the barrier to upstream movement of *S. trutta* (Figure 2.4 A). A large amount of leaf litter and other terrestrially-derived vegetable debris existed in the pools, which accounted for the high numbers of out-of-current taxa like *Pisidium ovampicum* Ancey, 1890 (Mollusca: Sphaeriidae), *Aprionyx tricuspidatus* Crass (Ephemeroptera: Leptophlebiidae) and *Goerodes caffrariae* (Barnard 1934) (Trichoptera: Lepidostomatidae). The proportion of *A. tricuspidatus* and *G. caffrariae* that occurred along the fishless Mnyameni reach (Figure 2.4 C) was similar to the fishless Cata, but for unknown reasons, the density of *Caenis* sp 1 and *Caenis* sp 2 (Ephemeroptera: Caenidae) was almost an order of magnitude higher, while *P. ovampicum*, the mayflies *Afroptilum sudafricanum* (Lestage) (Ephemeroptera: Baetidae), *Lestagella penicillata* (Barnard 1932) (Ephemeroptera: Teloganodidae) and *Castanophlebia calida* Barnard, 1932 (Ephemeroptera: Leptophlebiidae) all occurred at almost twice the density found in the fishless Cata reach. The fishless Gwiligwili reach (Figure 2.4 B) had the highest observed density for the minnow mayfly *Acanthiops erepens* (Gillies) (Ephemeroptera: Baetidae), which is reported as favouring swift-flowing mountain streams (Lugo-Ortiz et al. 2001) yet was sampled from the site, and river, with the least and lowest amount of flow observed throughout this study. The fishless Gwiligwili reach also had the highest density of the forest malachite *C. tessellatus* (Burmeister, 1839) (Odonata: Synlestidae), the goldtail damselfly *Allocnemis leucosticta* Sélys, 1836 (Odonata: Platycnemididae) and unusually high numbers of larval beetles of the family Scirtidae. The caddisfly *Leptonema natalense* Mosely, 1933 (Trichoptera: Hydropsychidae) was found only at this site throughout this study, and was not previously known from this region (de Moor & Scott 2003).

The invaded reaches along the Cata and Mnyameni Rivers consisted primarily of knee-deep runs between boulders and cobble and gravel bottom riffles (Figure 2.4 D, E), and supported the highest abundances of the ancylid limpet snail *Burnupia* sp1, the mayfly *Tricorythus discolor* (Burmeister 1839) (Ephemeroptera: Tricorythidae), and low numbers of "out-of-current" taxa like *L. penicillata* and *Afronemoura* sp 1 (Plecoptera: Notonemouridae) found along the fishless reaches. The invaded Mnyameni reach was characterized by the highest abundances of Oligochaeta, the tricladid flatworm *Dugesia* sp1, the net-weaving caddisfly *Cheumatopsyche thomasseti* (Ulmer, 1931) (Trichoptera: Hydropsychidae) and the rare silk-tube-constructing caddis *Paduniella* sp1 (Trichoptera: Psychomyiidae). The invaded Cata reach was characterized by comparatively low numbers of Oligochaeta, *Dugesia* sp1 and *P. ovampicum*, and had the highest densities of the two-tailed minnow mayflies *Demoreptus monticla* (Crass, 1947) and *Demoreptus capensis* (Barnard, 1932) (Ephemeroptera: Baetidae) that both favoured fast–flowing, shallow, cobbled riffles. The blackfly *Simulium vorax* Pomeroy, 1920 (Diptera: Simuliidae) was up to three times more abundant along the invaded Cata reach than anywhere else, attributable to the prevalence of faster flowing instream habitat at this site.

The native fish Gwiligwili reach (Figure 2.4 G) contained by far the highest densities of the mayflies *Pseudocloeon* sp 1 (Ephemeroptera: Baetidae) and the southern African endemic *C. calida* (Ephemeroptera: Leptophlebiidae); *Cheleocloen excisum* (Barnard, 1932) (Ephemeroptera: Baetidae) was also most abundant at this site, along with the highest abundance of larval Odonata, particularly amongst the Aeshnidae, Coenagrionidae and Gomphidae; it was also the only site were mosquito larvae were found (*Anopheles* sp 1). The Mnyameni River native fish reach (Figure 2.4 H) contained several taxa unique to this reach within this study. These species include the water mite *Arrenura* sp 1 (Acarina: Arrenuridae); the mayflies *Choroterpes nigrescens* Barnard, 1932 (Ephemeroptera: Leptophlebiidae), *Oligoneuriopsis* sp 1 (Ephemeroptera: Oligoneuriidae); the aquatic beetles *Berosus* sp 1 (Coleoptera: Dytiscidae), *Helminthopsis* sp 1 (Coleoptera: Elmidae), ptylodactylid sp 1 (Coleoptera: Ptylodactylidae) and the fly larva *Limnophora* sp 1 (Diptera: Muscidae). The native fish Cata reach (Figure 2.4 F) did not contain any unique taxa, but several species were shared with the native fish Mnyameni reach and occurred at this altitudinal reach at higher densities than elsewhere. The minnow mayflies *Baetis harrisoni* Barnard, 1932, *Dabulamanzia* sp 1, and *Nigrobaetis bethunae* Lugo-Ortiz & de Moor, 2000 (Ephemeroptera: Baetidae) occurred at densities of double, quintuple and an order of magnitude higher, respectively, than along the invaded and fishless reaches. The mayfly *Euthraulus elegans* Barnard, 1932 (Ephemeroptera: Leptophlebiidae) and the blackfly *S. damnosum* were particularly abundant along the native fish Cata and Mnyameni reaches, while the creeping water bug *Laccocoris* sp1 (Hemiptera: Naucoridae) was only recorded from the lower native fish reaches.

The current assessment of diversity of macroinvertebrate taxa from the headwaters of the Keiskamma River represents the closest examination of a catchment at this taxonomic resolution within the Amatolo-Winterberg Highlands Ecoregion in the last two decades. Previous work on the neighbouring Buffalo River (Palmer 1991, Palmer et al. 1991, 1993a, 1993b, 1994, 1996) represents the previous intensive macroinvertebrate study, where the effects of impoundments, flow regimes and stream gradient on macroinvertebrate communities were examined. This was preceded by work in the Cape Fold Ecoregion (King 1981, 1983; King et al. 1979, 1987a, 1987b, 1988). Studies performed within the Eastern Cape in the interim include those of Madikizela et al. (2001), who examined the Mollusca, Ephemeroptera, Plecoptera, Trichoptera and Simuliidae to species level, and Madikizela & Dye (2003), who used the familial level, both from the Umzimvubu River and selected tributaries, within the Drakensberg-Maloti Highlands Ecoregion (Abell et al. 2008). Odume et al. (2012) examined water quality in relation to macroinvertebrate community composition from the Swartkops River, Eastern Cape, and also used a familial resolution.

To make meaningful comparisons of diversity between different studies, it is important to take into account the type of sampling employed, and the effort made towards sampling the particular assemblage (Gotelli & Collwell 2001). When comparing alpha diversity, the study carried out in the neighbouring Buffalo River (Palmer 1991) collected 119 taxa, 15 more than are represented here. This is to be expected as the sampling technique employed by Palmer (1991) was qualitative and specific hydraulic habitats were located and sampled to be representative of the change in river gradient with a mesh size more than 6 times finer than was used in this study (80µm compared to 500µm). For example, the biotope termed "waterfall" was sampled specifically and the invertebrate assemblage compared to downstream assemblages where no such biotope was sampled, while the inverse was true for the biotope "marginal vegetation" (Palmer et al. 1991). Differences in level of identification between the studies also make direct comparisons difficult: while the Chironomidae are treated here as one group, Palmer (1991) identify to subfamilial and in some instances generic level; similarities do exist though, as both studies treat the Oligochaeta and the Nematoda as collective taxonomic units. A family of invertebrates not recorded by Palmer (1991) and represented here is the Psychomyiidae (Trichoptera), which was sampled from the middle and lower Mnyameni sites, which is an interesting difference from a conservation point of view as they are rarely collected, with this study locating more than an order of magnitude more than are held in the National Invertebrate Collection along with the type specimens (H. Barber-James, F. de Moor, Albany Museum, pers. comm.).

A qualitative species-level survey of the Ephemeroptera from 35 sites along six rivers in the northern parts of the Drakensberg-Maloti Highlands Ecoregion (Barber-James 1995), revealed 41 species of mayfly from 16 genera compared to the 22 species from 20 genera found in this study. The greater diversity of species from Barber-James (1995) may again be attributed to the qualitative nature of this study, along with the higher number of rivers and sites, spread across a larger area. It is interesting to note that at a generic level, the headwaters of the Keiskamma are more diverse, an indication of the pristine condition of the upper catchment. A study of macroinvertebrate community under the presence/absence of trout upstream and downstream of waterfalls along three rivers of the Northern Drakensberg, KwaZulu-Natal province, reported "63 taxa from an excess of 1500 aquatic macroinvertebrates sampled" (Rivers-Moore et al. 2013). Qualitative SASS sampling (Dickens & Graham 2002) and quantitative Surber sampling were both employed for examining the macroinvertebrate community. Despite better taxonomic resolution of the Chironomidae and employing two sampling methods, Rivers-Moore et al. (2013) did not find the high levels of species diversity of invertebrates found in the headwaters of the Keiskamma.

The macroinvertebrate assemblage of the Cape Fold Ecoregion (Abell et al. 2008) is known to contain high levels of endemism as high as the familial level within the Trichoptera, postulated to be a remnant of an ancient Gondwanaland fauna (Sæther & Ekrem 2003, Barber-James et al. 2008, Simaika & Samways 2009). Shelton (2013), examining the effects of *O. mykiss* presence on macroinvertebrate communities from 24 headwater streams in the Cape Fold Ecoregion (Breede River), reported a total of 92 taxa sampled quantitatively from in- and out-of-current biotopes. Although the total number of taxa found is comparable to those recorded in this study, the densities of invertebrates were considerably lower, which is expected given the low nutrient, oligotrophic nature of these streams resulting in lower stream productivity (de Moor & Day 2013). A possible reason for a comparatively lower number of taxa having been found due to specific groups like the Elmidae and Hydraenidae (Coleoptera) not receiving species-level focus.

Nine sites along six headwater reaches of the Olifants River system in the Cedarberg, Western Cape, were qualitatively surveyed for Trichoptera (de Moor 2011), resulting in 44 taxa from 19 genera catalogued. The objective of this study was a survey of diversity to make the best representation of the caddisfly fauna possible. By comparison, a study of the effects of land use on Trichoptera from two regions within eastern and North eastern Zimbabwe (Chakona et al. 2009), where Trichoptera were sampled quantitatively to gauge their usefulness as biological indicators, revealed 11 genera from 32 sites along eight rivers (Chakona et al. 2009), a number comparable to the 14 genera collected during this study. Both studies pale in comparison to the Trichopteran diversity found by de Moor (2011). An important consideration is that when working with highly diverse taxa, like stream invertebrates, it is important to factor in the objective of the study when making comparisons. The conservation- and management-orientated river length studies of the Orange River (Palmer 1996) and Cunene River (de Moor et al. 2000), which produced 207 and 251 taxa respectively, are further examples of the high levels of diversity found within the lotic waters of southern Africa.

The species diversity described from the headwaters of the Keiskamma, given that quantifying diversity was not the primary objective, is thus noteworthy on a regional and national scale, further qualitative study would undoubtedly reveal an even greater wealth of macroinvertebrate diversity.

2.4 Trout abundance in Keiskamma River headwaters

The headwater tributaries of the Keiskamma River were surveyed in detail by Ellender (2013) using various gears, to determine the distribution of fish species within the catchment. These included fyke nets, gill nets, underwater video analysis, hook and line angling, and electrofishing. Localities along the Thuyme, Amatele, Wolf, Rabula, Gxulu, Mnyameni River Cata and Gwiligwili Rivers were surveyed, with a total 15 species of fish reported (Ellender 2013).

Ellender (2013) noted differences in patterns of distribution between native and non-native fish species along the Cata and Mnyameni Rivers compared to the remaining rivers surveyed. Along the Cata and Mnyameni Rivers, non-native rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) and brown trout *Salmo trutta* Linnaeus 1758 were reported at densities of 0.06 fish m^{-3} and 0.01 fish m^{-3}, respectively, upstream of impoundments, and at extremely low densities within the impoundments.

The reaches surveyed downstream of impoundments contained only native species, dominated by the Border barb *Pseudobarbus trevelyani* (Günther 1877) and chubbyhead barb *Enteromius anoplus* (Weber 1897), at densities of 0.2 fish m^{-3} and 0.004 fish m^{-3} respectively along the Mnyameni River, and at 0.2 fish m^{-3} downstream of the Cata Dam for *P. trevelyani* only (Ellender 2013).

To reaffirm the distribution of trout along the Cata and Mnyameni Rivers in more detail, and to obtain estimates of their abundance, *S. trutta* and *O. mykiss* were sampled from 17 sites along the Cata River and 15 sites along the Mnyameni River, upstream of impoundments (Figure 2.7).

Sampling of *S. trutta* took place along the Cata River on 27 and 28 March 2013; 17 and 30 July 2013 and 26 and 27 May 2014. Sampling of *O. mykiss* took place along the Mnyameni River on 16 July 2013 and 28 May 2014. Sampling sites ranged between 22 m and 53 m in length along the Cata River and 18 m and 26 m in length along the Mnyameni River (Tables 2.4, 2.5).

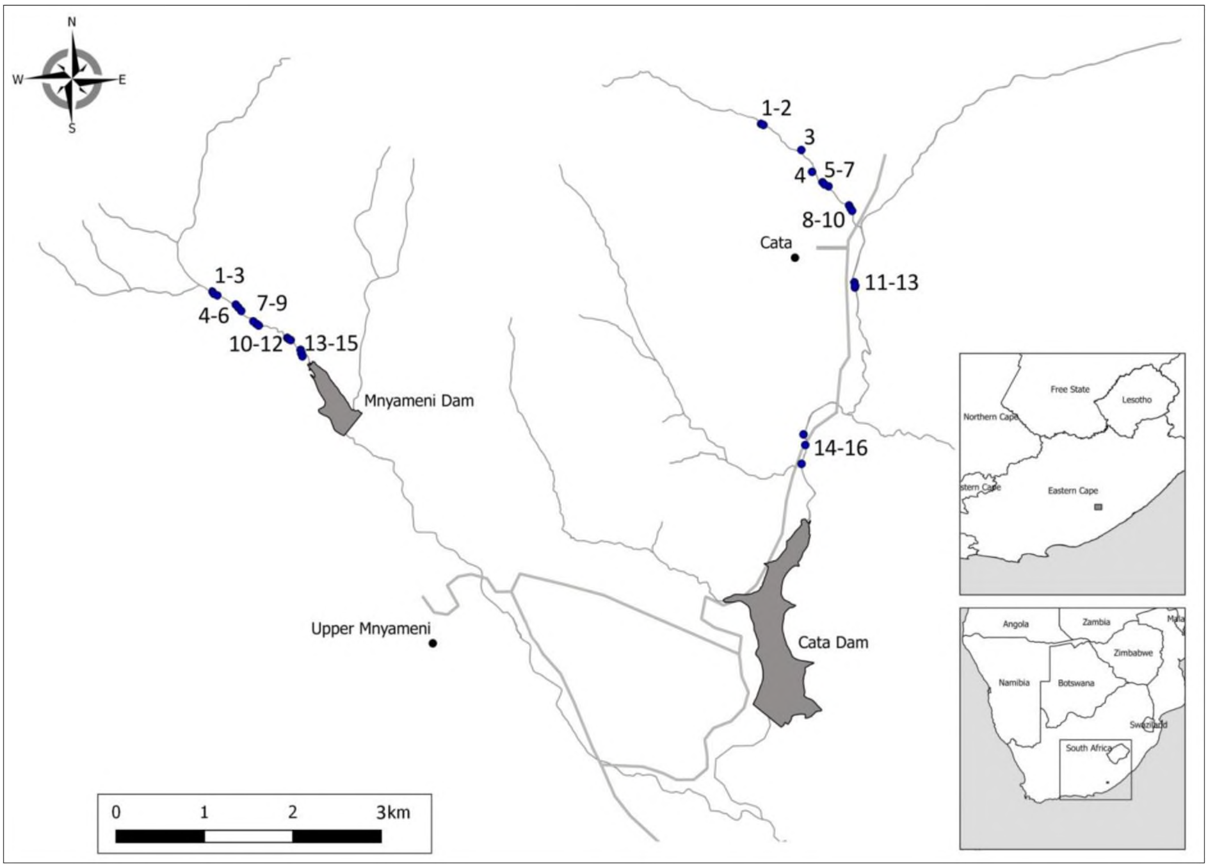

Figure 2.7: A map of the headwaters of the Keiskamma River system, Eastern Cape, South Africa, denoting the position of non-native fish sampling sites, as blue dots, on the Mnyameni and Cata Rivers.

Sites were selected from reaches of stream where a natural constriction point could be found at the start and end, for example: a chute, small waterfall, or narrowing of the stream from bedrock intruding concomitantly from either side. Block-nets were placed at the bottom and top ends of each sampling site prior to the start of electrofishing, ensuring that the site was completely cordoned off. Each site was sampled by three-pass electrofishing employed by Ellender (2013) using a Samus 725G backpack electrofisher. Settings were standardized at the duration of 0.3 ms and at a frequency of 80 Hz. Electrofishing is commonly used to assess the abundance and biomass of salmonid fishes (Bohlin et al. 1989, Riley & Fausch 1992, Peterson et al. 2004).

Table 2.4: Physical descriptions of each non-native fish sampling site along the Cata River, Eastern Cape, South Africa; site numbers correspond to those of Figure 2.7.

Site number	1	2	3	4	5	6	7	8
Altitude (masl)	950.0	941.0	912.0	889.0	879.0	876.0	876	863.0
GPS waypoint (°)	32.57130 S	32.57141 S	32.57394 S	32.57615 S	32.57720 S	32.57742 S	32.57761 S	32.57954 S
	27.11624 E	27.11653 E	27.12110 E	27.12240 E	27.12367 E	27.12391 E	27.12439 E	27.12687 E
Length (m)	22.5	24.5	34.1	24.0	26.0	30.0	34.2	26.5
Mean width (m ±SD)	6.00 ± 3.16	3.93 ± 0.40	4.10 ± 1.60	4.23 ± 1.44	1.60 ± 1.51	3.83 ± 1.55	4.20 ± 2.00	4.17 ± 1.21
Mean depth (m ±SD)	0.17 ± 0.12	0.15 ± 0.10	0.19 ± 0.10	0.16 ± 0.09	0.18 ± 0.09	0.20 ± 0.10	0.14 ± 0.07	0.15 ± 0.10
Surface Area (m^2)	135	96.37	139.81	101.6	41.51	115	143.64	110.42
Volume (m^3)	23.49	14.65	26.76	16.59	7.63	23.3	19.73	16.49
Pool (%)	73.0	57.0	40.0	37.0	45.0	44.0	72.0	30.0
Riffle/Run (%)	27.0	43.0	60.0	63.0	55.0	56.0	28.0	70.0
Site number	9	10	11	12	13	14	15	16
Altitude (masl)	862.0	861.0	835.0	834.0	834.0	788.0	787.0	782.0
GPS waypoint (°)	32.57986 S	32.58009 S	32.58732 S	32.58784 S	32.58767 S	32.60271 S	32.60378 S	32.60569 S
	27.12706 E	27.12724 E	27.12753 E	27.12759 E	27.12763 E	27.1214 E	27.12162 E	27.12117 E
Length (m)	29.0	24.3	34.0	26.1	23.0	40.6	34.5	53.0
Mean width (m ±SD)	3.83 ± 0.60	3.13 ± 1.29	6.67 ± 3.16	3.90 ± 1.39	4.33 ± 1.56	3.60 ± 1.77	4.34 ± 1.30	4.10 ± 0.96
Mean depth (m ±SD)	0.13 ± 0.05	0.11 ± 0.04	0.17 ± 0.09	0.24 ± 0.14	0.18 ± 0.10	0.17 ± 0.06	0.20 ± 0.11	0.24 ± 0.09
Surface Area (m^2)	111.17	76.14	226.67	101.79	99.67	146.16	149.5	217.3
Volume (m^3)	14.82	8.78	39.29	25.1	18.73	25.14	30.5	51.87
Pool (%)	60.0	64.0	35.0	44.0	54.0	8.0	40.0	47.0
Riffle/Run (%)	40.0	36.0	65.0	56.0	46.0	92.0	60.0	53.0

Table 2.5: Physical descriptions of each non-native fish sampling site along the Mnyameni River, Eastern Cape, South Africa; site numbers correspond to those of Figure 2.7.

Site number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Altitude (masl)	987.0	986.0	982.0	966.0	964.0	958.0	944.0	944.0	943.0	928.0	926.0	925.0	921.0	915.0	912.0
GPS waypoint (°)	32.58833 S	32.58853 S	32.25578 S	32.58963 S	32.58992 S	32.59026 S	32.59132 S	32.59158 S	32.59174 S	32.59299 S	32.59313 S	32.59321 S	32.5942 S	32.59453 S	32.59485 S
	27.05005 E	27.05021 E	27.05044 E	27.05286 E	27.05316 E	27.05352 E	27.05498 E	27.05534 E	27.05564 E	27.05908 E	27.05929 E	27.05949 E	27.06069 E	27.06082 E	27.06091 E
Length (m)	22.0	18.0	22.0	22.0	24.0	24.0	26.0	21.0	23.0	26.0	26.0	23.0	21.0	24.5	25.0
Mean width (m ±SD)	7.80 ± 3.31	4.30 ± 1.15	6.00 ± 0.52	5.73 ± 2.04	4.80 ± 2.34	4.03 ± 1.41	5.27 ± 1.20	5.07 ± 0.30	3.87 ± 1.26	5.37 ± 2.75	4.03 ± 0.68	4.63 ± 1.27	5.63 ± 1.71	4.60 ± 2.08	4.17 ± 0.76
Mean depth (m ±SD)	0.36 ± 0.17	0.28 ± 0.11	0.27 ± 0.23	0.23 ± 0.12	0.25 ± 0.13	0.33 ± 0.17	0.22 ± 0.11	0.22 ± 0.09	0.24 ± 0.08	0.16 ± 0.05	0.25 ± 0.10	0.16 ± 0.07	0.22 ± 0.11	0.20 ± 0.08	0.21 ± 0.06
Surface Area (m^2)	171.60	77.40	132.00	126.13	115.20	96.90	136.93	106.40	88.93	139.53	104.87	106.57	118.30	112.70	104.17
Volume (m^3)	60.86	41.03	35.11	28.67	28.64	31.75	29.76	23.34	21.28	22.70	26.22	17.26	26.02	22.39	22.08
Pool (%)	74.0	57.0	57.0	53.0	43.0	100.0	43.0	45.0	68.0	32.0	60.0	42.0	77.0	38.0	67.0
Riffle/Run (%)	26.0	43.0	43.0	47.0	57.0	0.0	57.0	55.0	32.0	68.0	40.0	58.0	23.0	62.0	33.0

The fish collected from each pass were placed in the bucket corresponding to the pass they were collected from, counted, and fork length (FL) measured to the nearest millimetre on the stream bank. Trout density was estimated using Leslie depletion estimates using pass data (Leslie & Davis 1939) and are provided in Table 2.6, with density of fish per site and per 100 m of wetted river length. Using this data, graphs of non-native fish density vs. altitude are presented (Figures 2.8, 2.9) along with densities of native fish reported from Ellender (2013), downstream of impoundments.

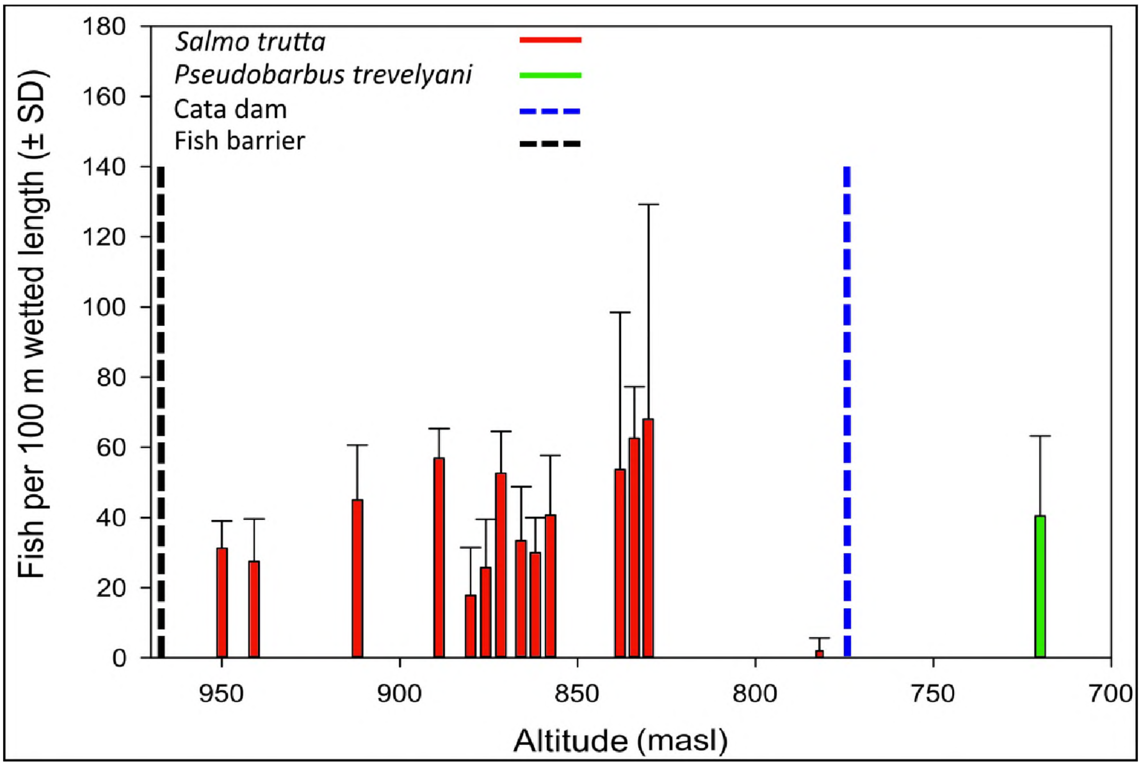

Figure 2.8: A bar graph of the number of brown trout *Salmo trutta* per 100 m wetted length of stream (± SD) derived from Leslie depletion estimates, and the number of Border barb *Pseudobarbus trevelyani* reported by Ellender (2013), against altitude along the Cata River, Eastern Cape, South Africa. The black dotted line signifies the barrier to upstream movement of trout at 970 masl, representing the start of the fishless reach; the blue dotted line at 775 masl signifies the position of the Cata Dam.

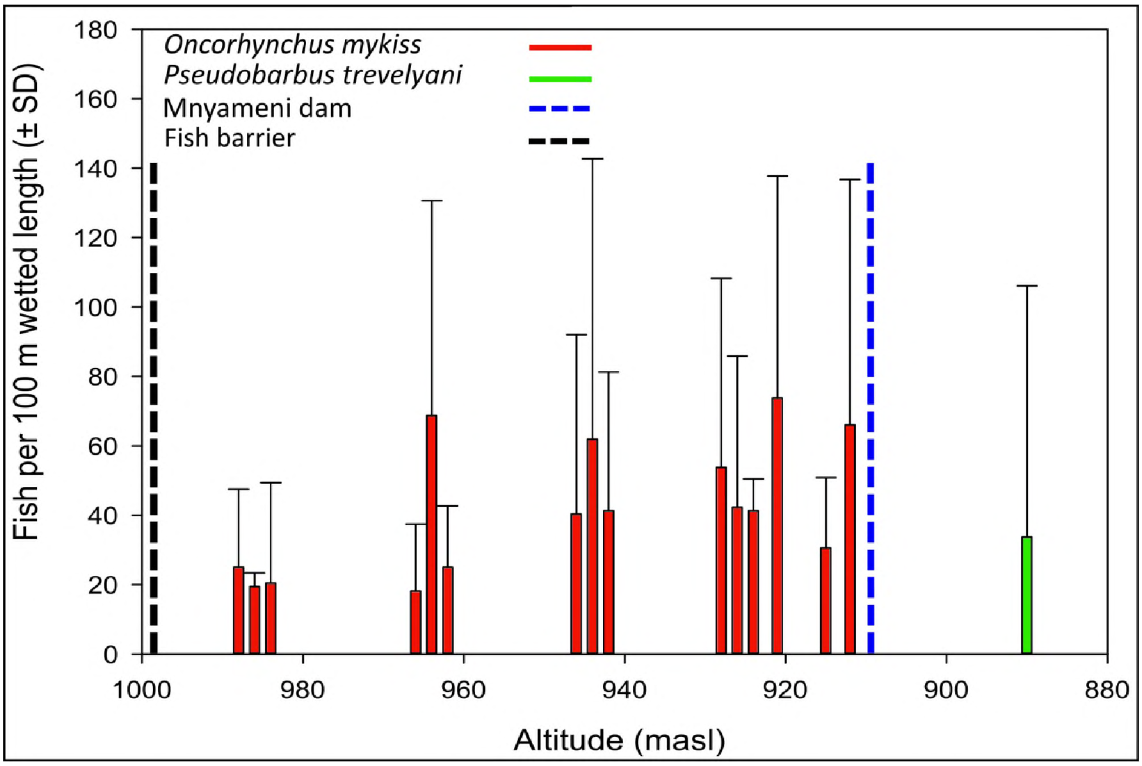

Figure 2.9: A bar graph of the number of rainbow trout *Oncorhynchus mykiss* per 100 m wetted length of stream (± SD) derived from Leslie depletion estimates, and the number of Border barb *Pseudobarbus trevelyani* reported in Ellender (2013), against altitude along the Mnyameni River, Eastern Cape, South Africa. The black dotted line signifies a waterfall barrier preventing upstream movement of trout, at 990 masl representing the start of the fishless reach; the blue dotted line at 915 masl signifies the position of the Mnyameni Dam.

To make population estimates by a mark-recapture method, *S. trutta* were marked during the first sampling event by clipping off the top half of their adipose fin (Johnsen & Ugedal 1988), and using the Lincoln-Petersen Estimator:

$$N\text{-}_{lp} = \frac{Kn}{k} \qquad (1)$$

and the Chapman Estimator:

$$N_c = \frac{(K+1)(n+1)}{k+1} - 1 \qquad (2)$$

For equations (1) and (2), N is the estimated population of trout, n is the number of trout marked during the first sampling event, K is the number of trout captured during the second sampling event and k the number of marked trout recaptured (Chapman 1953, Nordwall 1999, Southwood & Hendersen 2000, Rosenberger & Dunham 2005). These data are presented in Table 2.6.

Table 2.6: Population estimates, and density estimates, for brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* along the Cata River and Mnyameni River respectively.

Cata River - *Salmo trutta*								
Petersen estimator	328							
Chapman estimator	324							
Leslie depletion estimates								
Site number	1	2	3	4	5	6	7	8
Altitude (masl)	950	941	912	889	879	876	876	863
Number of fish (±SD) n = 3	24.4 ± 30.3	6.7 ± 3.0	14.5 ± 5.3	13.6 ± 2.0	4.7 ± 3.5	7.7 ± 4.1	18.0 ± 4.1	8.9 ± 4.0
Number of fish per 100 m wetted length	30.5 ± 7.6	27.5 ± 12.1	45.1 ± 15.5	56.8 ± 8.5	17.9 ± 13.5	25.8 ± 3.7	52.7 ± 11.9	33.5 ± 15.3
Site number	9	10	11	12	13	14	15	16
Altitude (masl)	862	861	835	834	834	788	787	782
Number of fish (±SD) n = 3	8.7 ± 2.9	9.9 ± 4.1	18.3 ± 15.2	16.4 ± 3.8	15.6 ± 14.1	0	0	1.1 ± 1.9
Number of fish per 100 m wetted length	30.1 ± 9.8	40.9 ± 16.8	53.7 ± 44.8	62.7 ± 14.6	68.0 ± 61.2	0	0	2.1 ± 3.6
Mnyameni River - *Oncorhynchus mykiss*								
Leslie depletion estimates								
Site number	1	2	3	4	5	6	7	8
Altitude (masl)	987	986	982	966	964	958	944	944
Number of fish (±SD) n = 2	5.5 ± 4.9	3.5 ± 0.7	4.5 ± 6.4	4.0 ± 4.2	16.5 ± 14.8	6.0 ± 4.2	10.5 ± 13.4	13.0 ± 17.0
Number of fish per 100 m wetted length	25.0 ± 22.5	19.4 ± 3.9	20.5 ± 28.9	18.2 ± 19.3	68.8 ± 61.9	25.0 ± 17.7	40.4 ± 51.7	61.9 ± 80.8
Site number	9	10	11	12	13	14	15	
Altitude (masl)	943	928	926	925	921	915	912	
Number of fish (±SD) n = 2	9.5 ± 9.2	14.0 ± 14.1	11.0 ± 11.3	9.5 ± 2.1	15.5 ± 13.4	7.5 ± 4.9	16.5 ± 17.7	
Number of fish per 100 m wetted length	41.3 ± 39.9	53.8 ± 54.4	42.3 ± 43.5	41.3 ± 9.2	73.8 ± 63.9	30.6 ± 20.2	66.0 ± 70.7	

The results from electrofishing surveys along the Keiskamma headwater stream reaches revealed that *S. trutta* in the Cata River were more abundant on average than *O. mykiss* from the Mnyameni River, but this difference was not significant (t-test, t-value = 1.25, df = 29, $P > 0.05$). On average, across all sampling sites and events, *S. trutta* occurred 0.09 ± 0.05 (SD) fish per m^2, and 34 ± 22 (SD) fish per 100 m of wetted length (Table 2.6). Size class distribution for *S. trutta* is provided in Figure 2.10, for the May 2014 sampling period. The population structure of *S. trutta* represented suggests an established population consisting of several year classes of fish.

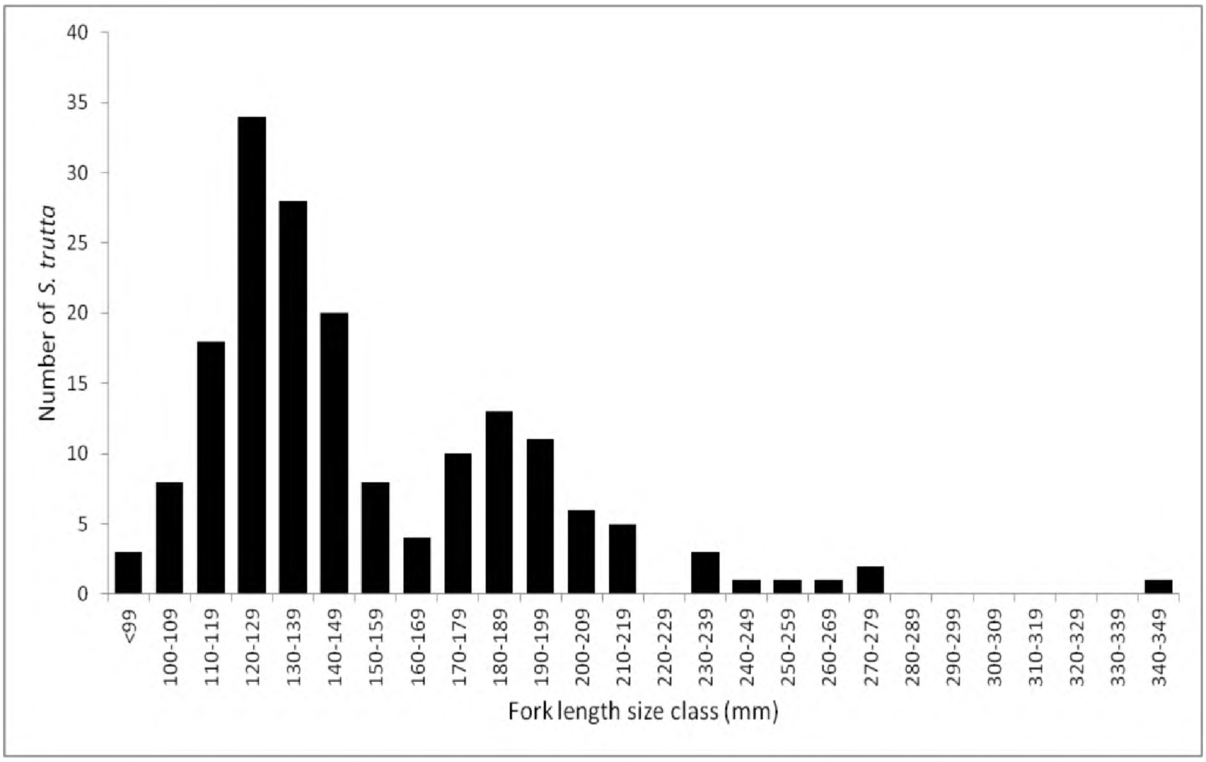

Figure 2.10: Size class distribution of brown trout *Salmo trutta* collected during May 2014 from the Cata River, Eastern Cape, South Africa.

On average, across all sampling sites and events, *O. mykiss* occurred at an average of 0.09 ± 0.04 (SD) fish per m^2, and 42 ± 19 (SD) fish per 100 m of wetted length (Table 2.6). The size class distribution for *O. mykiss* is provided in Figure 2.11 for May 2014 sampling period. Again, the length frequency distribution demonstrates establishment with several age classes.

The population structure of both trout species confirms the findings of Ellender (2013), that *S. trutta* and *O. mykiss* consist of self-sustaining populations within these headwater streams, where spawning and recruitment occur successfully.

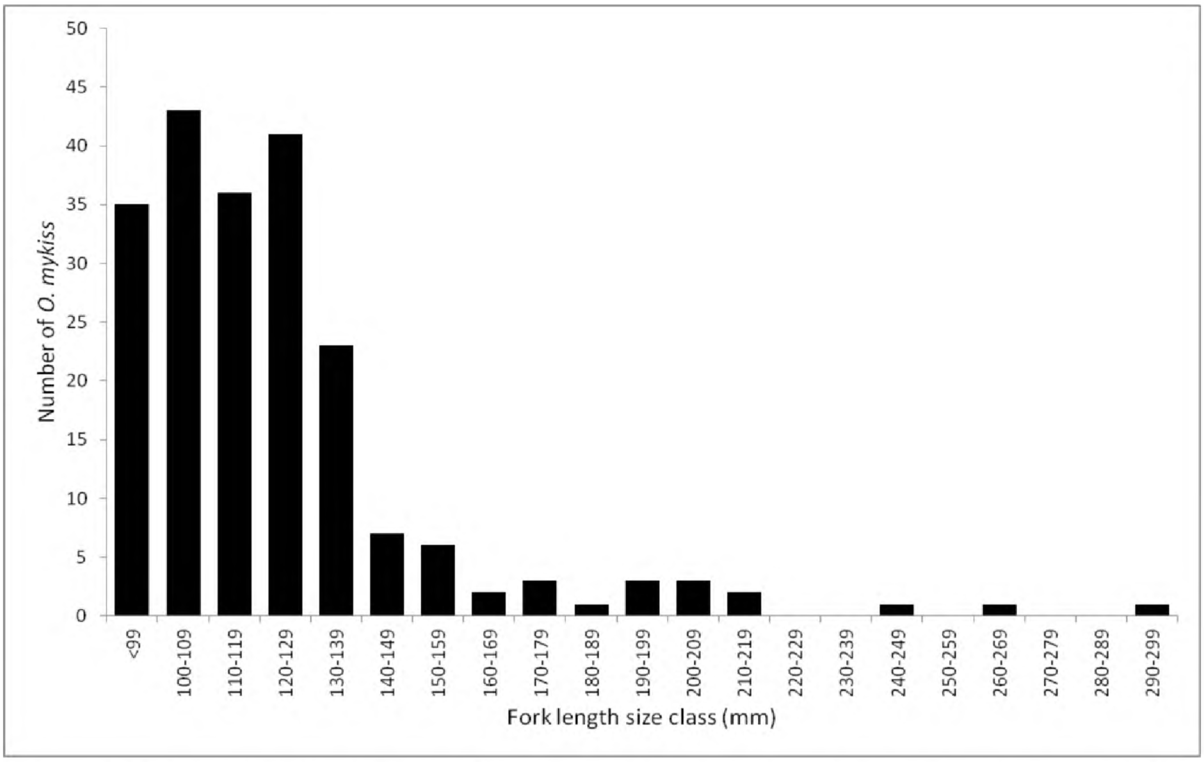

Figure 2.11: Size class distribution of brown trout *Oncorhynchus mykiss* collected during May 2014 from the Mnyameni River, Eastern Cape, South Africa.

In comparison with other studies (Table 2.7), the headwaters of the Keiskamma River host some of the highest densities of trout reported from South Africa, where studies have examined the effects of trout with respect to native biota (Rivers-Moore et al. 2013, Shelton 2013, Shelton et al. 2015a, 2015b, Jackson et al. 2016). These differences may be indicative of a higher levels of productivity capable of supporting higher densities of trout in streams from the Amatolo-Winterberg Highlands Ecoregion, compared to oligotrophic CFE streams (de Moor & Day 2013) and the findings described in Shelton et al. (2015a, 2015b).

Table 2.7: Densities of brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* recorded from studies examining their effects on macroinvertebrates in river systems across southern Africa. Trout densities are reported in the same units used in the original study ± SD, with the exception of Shelton (2013) who reported density ± SE. Ecoregion abbreviations correspond to: Drakensberg-Maloti Highlands (DMH); Cape Fold Ecoregion (CFE); Amatolo-Winterberg Highlands (AWH); *sensu* Abell et al. (2008).

River	Ecoregion	*O. mykiss* density	*S. trutta* density	Study
Cata	AWH		0.01 ± 0.01 fish m^{-3}	Ellender (2013)
Amatele	AWH	0.03 ± 0.01 fish m^{-3}		
Mnyameni	AWH	0.1 ± 0.03 fish m^{-3}		
Thyume	AWH	0.04 ± 0.02 fish m^{-3}		
Groothoek	CFE	102.32 ± 7.36 fish/100m^2		Shelton (2013)
Hartmanskloof	CFE	7.85 ± 3.72 fish/100m^2		
Houtboskloof	CFE	40.79 ± 5.90 fish/100m^2		
Jan du Toit	CFE	0.85 ± 0.17 fish/100m^2		
Kaaimansgat	CFE	0.72 ± 0.14 fish/100m^2		
Klip	CFE	61.71 ± 5.13 fish/100m^2		
Kraalstroom	CFE	21.51 ± 1.62 fish/100m^2		
Krom	CFE	1.01 ± 0.00 fish/100m^2		
Mol trib.	CFE	3.51 ± 0.40 fish/100m^2		
Morainekloof	CFE	1.93 ± 0.50 fish/100m^2		
Raaswater	CFE	1.3 ± 0.86 fish/100m^2		
Sterkspruit, Lotheni, Mooi	DMH		2.0 ± 1.0 "per 100m reach"	Rivers-Moore et al. (2013)
Sterkspruit	DMH		3.25 fish/100m^2	Jackson et al. (2016)
Lotheni	DMH		7.25 fish/100m^2	
Mooi	DMH		7.66 fish/100m^2	

Chapter 3: Drivers of macroinvertebrate stream assemblages under differing fish predator regimes from the headwaters of the Keiskamma River system, Eastern Cape, South Africa

3.1 Introduction

Macroinvertebratesare reliable indicators of the ecological processes within a river reach as a result of their ubiquity, abundance, rapid life-cycle turnover and biological importance in the food chain (Palmer 1996). Rempel et al. (2000) for example, examined the spatial distribution of macroinvertebrates in relation to environmental variables in the Frazer River in Canada and found that the distribution of invertebrates was most closely correlated to hydraulic conditions; and Eady et al. (2013) demonstrated that aquatic macroinvertebrates communities responded to water temperature in a predictable manner, suggesting that macroinvertebrates are useful environmental indicators. In South Africa, Rivers-Moore (2012) studied patterns of species richness and turnover along the length of nine rivers, comparing macroinvertebrates and fish as indicators of environmental gradients and found that, unlike fish, macroinvertebrate assemblages displayed predictable turnover patterns that were useful for exploring responses to environmental gradients (Rivers-Moore 2012). Earlier studies by King (1981, 1983) focusing on the diversity, distribution and abundance of macroinvertebrates in two Cape Fold Ecoregion (CFE; *sensu* Abel et al. 2008) rivers found that the distribution and density of macroinvertebrates along the length of the rivers was seasonally predictable and changed along the gradient of the stream and with deteriorating water quality, partially reflecting the expectations of the river continuum concept (Vannote et al. 1980). Trends of changing invertebrate assemblage composition as a response to the natural longitudinal gradients of the river, including responses to anthropogenically derived influences, have been described from within the Amatolo-Winterberg Ecoregion (Palmer et al. 1991, 1993a, 1993b, 1994) and elsewhere in South Africa (King 1981) and the world (Wiberg-Larsen et al. 2000). Therefore, by studying patterns of macroinvertebrate abundance, density and assemblage composition, inferences can be made about the ecological forces shaping macroinvertebrate assemblages.

The characterization of streams using macroinvertebrate communities can be done in two ways, either by following a taxonomic approach that describes the fauna with sufficient resolution that an index of richness or diversity can be obtained (Rosenberg & Resh 1993), or by assigning the fauna to guilds based on their function within the ecosystem (Cummins et al. 2005). The former method is time-consuming as it relies heavily on correct separation of taxa at species or genus level, while the latter is often simpler as it relies on easily recognizable morphological or behavioral traits of the invertebrates that are related to fundamental aspects of their life history, e.g. the acquisition of resources (Hauer &

Resh 1996, Merrit & Cummins 1996, Cummins et al. 2005). However, Dalu et al. (2017) suggested that "for certain ecological questions, a more detailed taxonomic approach may be required to adequately understand the ecology of aquatic macroinvertebrates within river systems". In this chapter I therefore examined macroinvertebrate assemblages of headwater tributaries of the Keiskamma River system to assess for impacts of introduced salmonids by studying assemblage characteristics from invaded and non-invaded reaches employing both a taxonomic and a functional feeding group approach.

3.1.1 Effects of salmonid predation on macroinvertebrate assemblages

When salmonids have been introduced outside their native range, predation on and competition with native fauna have been shown to: extirpate native vertebrates and invertebrates in New Zealand (McIntosh et al. 1992, McIntosh & Townsend 1995, Townsend & Crowl 1991, Flecker & Townsend 1994, Townsend 1996b) and Australia (Lintermans 2000); alter invertebrate community structure and function in Patagonia (Buria et al. 2007, Albariño & Buria 2011); alter invertebrate communities (Herbst et al. 2009) and disrupt reciprocal subsidies in North America (Mathews et al. 2002, Epanchin et al. 2010); and exclude native fish (Inoue et al. 2009) and disrupt trophic subsidies in Japan (Baxter et al. 2004).

In a study aimed specifically at testing for the effects of non-native salmonids on macroinvertebrates from the Drakensberg-Maloti Highlands Ecoregion, Rivers-Moore et al. (2013) could not conclusively demonstrate that *O. mykiss* and *S. trutta* negatively affected the macroinvertebrate assemblage of the Sterkspruit, Lotheni and Mooi Rivers, suggesting that negative predation effects may be masked by overriding environmental variables in comparing upstream and downstream reaches. Recently, studies in the CFE have examined the effects of non-native *O. mykiss* on native fish and macroinvertebrate communities (Shelton et al. 2015a, 2015b), and compared the effects of non-native *O. mykiss* and native barb species on macroinvertebrate communities (Shelton et al. 2016). *Oncorhynchus mykiss* depleted endemic native fish species through size-selective predation, thus alleviating predation pressure on grazing invertebrates by usurping native fishes; native barb species were found to be stronger regulators of the invertebrate assemblage than non-native *O. mykiss*, in an *in situ* mesocosm experiment.

3.1.2 The Keiskamma headwaters: model systems for studying invasion impacts

The headwaters of the Keiskamma River, described in Chapter 2, represent a particularly interesting entity and were chosen for this study as a result of findings by Ellender (2013), who described the distribution patterns of the various fish species in the upper catchment in detail (see Chapter 2). The fish communities were distinctly different between the uppermost reaches, where no fish occurred; within the impoundments and reaches directly above them, where non-native fish occurred; and the lower reaches below the impoundments, where native fish occurred (Ellender 2013, Ellender et al.

2016). This formed what can be considered to be a "natural experiment" *sensu* Diamond (1983), where the effects of fish predator regime on macroinvertebrate assemblages from selected headwater tributaries could be tested.

3.1.3 Aims and hypotheses

The aim for this study was to use quantitative benthic macroinvertebrate samples from fishless, invaded and native fish reaches of headwater tributaries of the Keiskamma River, to test if non-native fishes were significant drivers of macroinvertebrate assemblage structure, when the influence of environmental variables (e.g., flow, season and water chemistry) is taken into account. The working null-hypothesis was that non-native salmonids would not have a significant effect on macroinvertebrate assemblages from these streams, but rather that the macroinvertebrate assemblage would be structured by biotope availability and flow, as proposed by the river continuum concept (Vannote et al. 1980).

3.2 Materials and methods

3.2.1 Sampling reach selection

The riverine habitats consisted of fishless, invaded and native fish reaches of three headwater tributaries of the Keiskamma River: the Cata, Mnyameni and Gwiligwili Rivers (Chapter 2, Figures 2.3, 2.4). Chapter 2, Section 2.3, contains detailed descriptions and habitat characteristics of the study reaches. Sampling reach selection took place during March, 2012. Each stream was visited, traversed along its length, and comparable areas of in-stream habitat found in which benthic sampling could take place. This limited bias towards any particular biotope along a particular reach, while ensuring that stretches of stream sampled were still reasonably representative of each stream as a whole. Sampling reaches consisted of stretches of stream roughly 30 m long (Chapter 2, Table 2.2).

3.2.2 Sampling strategy

Quantitative invertebrate samples were collected with a modified Surber sampling technique (Hauer & Resh 1996). Two squares of 300 mm x 300 mm were constructed from polyvinyl chloride (PVC) tubing and supported 120 mm apart by clear Perspex panes, to allow a channel of water flow from upstream to downstream through the Surber box and into the net (Figure 3.1). A long-handled water net with frame dimensions of 300 mm x 300 mm and mesh size 500 µm, known commonly as a "SASS" net (Dickens & Graham 2002), was held flush behind the box, supported by the investigator's legs while sampling was carried out. While the net was held in place, the substrate within the box area was disturbed vigorously by hand for one minute to dislodge any invertebrates. The water flowing through the box carried the invertebrates into the net (Figure 3.1).

When samples were collected from areas of stream with low or no flow, a current was created whilst agitating the substrate by flushing water by hand through the box sampler and into the net downstream of it (arrowed in Figure 3.1). The sample was then emptied into a sturdy white tray filled with water, dipping the SASS net into the tray repeatedly to wash off insects, followed by a visual inspection for invertebrates still clinging or stuck to the net. This was done to ensure that all invertebrates, in particular blackfly larvae, were collected from the area within the Surber sampler. The content of the tray was then poured through a 100 µm mesh size sieve, and the residue transferred into the sample bottle via a funnel. The sample bottle lid was then labelled using an ethanol-proof paint marker and preserved using 95% ethanol, at a ratio of one part sample to three parts ethanol.

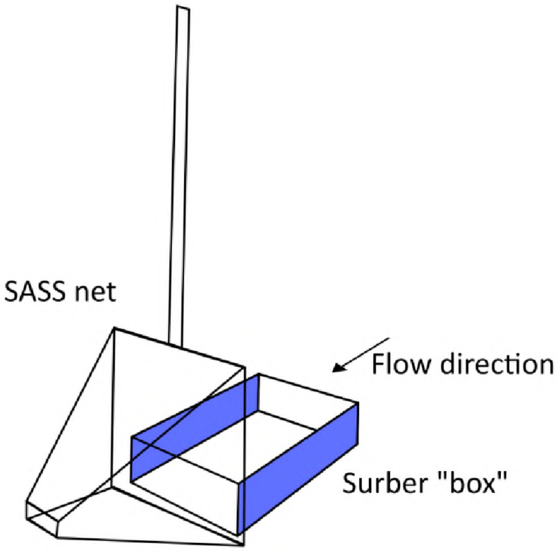

Figure 3.1: Simplified schematic diagram of sampling equipment used to collect Surber samples. The direction of water flow is illustrated by the arrow; the area inside the box between the shaded walls was disturbed by hand for 60 seconds to dislodge macroinvertebrates which were then caught in the net downstream and held flush to the box.

Sampling was carried out quarterly, during July and October of 2012, and January and April of 2013. Ten replicates were collected from each of the eight reaches (three fishless, two invaded and three native fish reaches), repeated across four seasons (4 seasons × 8 reaches × 10 replicates = 320 samples). For each sample, the flow from the area of stream was recorded using a Marsh McBirney, Inc. Flo-Mate (Model 2000) portable electromagnetic flow meter. Flow was categorised, based on current speed, as no flow (nf = 0 m/s), slow flow (sf = 0.05-0.3 m/s), medium flow (mf = 0.3-0.5 m/s) and fast flow (ff ≥ 0.5 m/s). The instream biotopes were also recorded at each replicate, following the descriptions of primary stream biotopes standardised in Wadeson & Rowntree (1998) and Dickens & Graham (2002). At each sampling reach for each sampling event, temperature, pH and conductivity were measured using a Hanna HI98219 Combo pH and electrical conductivity meter (HANNA Instruments Inc., Woonsocket, U.S.A), Dissolved oxygen was measured using a Cyberscan DO 300 dissolved oxygen meter (Oakton Instruments, Vernon Hills, IL, U.S.A.).

3.2.3 Sample processing and identification

Samples were sorted and macroinvertebrates identified in the laboratory under a Wild M5 dissecting microscope (up to 50× magnification) using the identification keys of Appleton (2002a, 2002b); Coetzee (2002); de Meillon & Wirth 2002, de Moor 2002, Harrison et al. 2002a, Harrison et al. 2002b, Jansen van Rensburg & Day 2002, van Hoven & Day 2002, Barber-James & Lugo-Ortiz 2003, de Moor & Scott 2003, Mansell, 2003, Samways & Wilmot 2003, Stevens & Picker 2003, Reavell 2003, Biström 2007, de Moor 2007, Endrödy-Younga & Stals 2007, Nelson 2007, Perkins 2007, Stals 2007, Stals & Endrödy-Younga 2007, Villet & Endrödy-Younga 2007, Shepard & Lee 2007). Blackfly larvae were identified using the key and illustrations in Freeman & de Meillon (1953). These identifications were made to species level where possible but, where literature was unavailable, morphospecies identifications were made to differentiate between taxa and the abbreviation "sp" and occurrence number was assigned to the morphospecies. An example of this is the aquatic larvae of the family Ptylodactylidae for which there are no keys to species or genera. The identification of earthworms (Oligochaeta) and roundworms (Nematoda) remained at the level of subclass and phylum, respectively, as identification of these groups to lower levels was considered impractical. A full species list with taxonomic authorities is provided in Appendix 1, Table 1.

Functional feeding group (FFG) allocations for the Insecta were made following Cummins & Klug (1979) and the key to FFGs of Merritt & Cummins (1996). Where published data were available, they were used to categorise individual taxa. For example, the Palmer et al. (1993b) FFGs of the invertebrates of the neighbouring Buffalo River was used for species found in common and the categorisation of the baetid *Centroptiloides bifasciata* (Esben-Petersen, 1913) as a benthic predator whereas other species in this family are scrapers and gathering-collectors followed Agnew (1962). The micro-caddis (Trichoptera: Hydroptilidae) were assigned from expert opinion (Ferdinand de Moor, pers. comm.); Oligochaeta and Nematoda were classified as gathering collectors (Cummins & Klug 1979, Bongers & Bongers 1998); *Dugesia* species (Dugesiidae: Tricladida) were classified as predators (Aditya & Mahapatra 1991); Hydracarina were classified as predators (Smock et al. 1985) and the river crab *Potamonautes sidneyi* (Rathbun, 1904) (Potamonautidae: Decapoda) as a gathering collector (Hill & O'Keeffe 1992). Appendix 1, Table 1 contains the FFG designation for each species incorporated in further analyses.

3.2.4 Analyses

3.2.4.1 Sampling assessment and functional feeding group characterisation

To gauge whether the species collected represented the total assemblage, a species accumulation plot was constructed using PRIMER Version 6.1.15 (Clarke & Gorley 2006, Plymouth, UK). Sample order was randomized and permutated 999 times to produce a plot of species count vs. number of samples collected. This provided a measure of sampling effort at the level of each river reach as a function of the rate at which species are observed (Colwell & Coddington 1994, Colwell et al. 2004). The number of species observed "S_{obs}" and the functions "Chao1", "Jacknife1" and "Michaelis - Menten" were fitted to the samples to produce resampled curves (Clarke & Warwick 2001, Gotelli & Collwell 2001, Colwell et al. 2004, Clarke & Gorley 2006).

To compare the densities of invertebrates between fishless, invaded and native fish reaches, a repeated-measures ANOVA was employed using the software package Statistica. A repeated measures ANOVA was used because replicate samples were taken repeatedly along each of these reaches, and each replicate was resampled seasonally. The density of invertebrates collected from each sample was square-root-transformed to conform to the assumptions of parametric analysis (Zuur 2007). Tukey's HSD post hoc tests were carried out to determine differences between factors for each sampling season.

To visualize proportional differences at the FFG level, tributary reaches were represented by a plot of the proportion of each FFG summed across sampling season, graphed using Microsoft Excel (2007). To test for proportional difference between the densities of each FFG per sample, a permutational MANOVA (PERMANOVA) was run using the PERMANOVA+ package in PRIMER (Anderson et al. 2008a, 2008b). The proportional density of each FFG was calculated for each sample by area of stream substrate, and the PERMANOVA run with season and reach as factors, Euclidean distance was used when creating the resemblance matrix, and the model was run under 9999 permutations. To represent any observable change in FFG structure between the fishless, invaded, and native fish reaches, the FFG allocations were averaged by season per reach and graphed using Statistica. The data did not conform to parametric analysis, therefore Kruskal-Wallis and F-tests were carried out to test for significant differences ($\alpha = 0.05$) in mean number of macroinvertebrates per FFG from each sample between reach. Prior to graphing, the data was square-root-transformed to reduce the difference in magnitude between the abundant functional guild taxa and depauperate guilds.

3.2.4.2 Multivariate analyses

To compare invertebrate assemblages sampled from different reaches that vary according to environmental or physical parameters, it is common place to use multivariate analyses (Kilgour & Barton 1999, Baptista et al. 2001, Pinder et al. 2004, Stewart & Samways 2008). PCA is one of the oldest and most commonly used ordination methods because of its simplicity and the wide variety of statistical packages that offer it as tool for analyzing multivariate data (Zuur et al. 2007). Principal Components Analysis (PCA) attempts to characterize the variation within a multi-dimensional data set by constructing axes to pass "through" the data, with the largest amount of variation described or accounted for by the first axis; the second axis accounting for the next largest amount of variation and so forth (Zuur et al. 2007). To determine whether sampling sites differed between reach of river based on invertebrate assemblage, invertebrate species data collected from each site was first analysed by PCA using CANOCO for Windows version 4.5 (ter Braak & Šmilauer 2002). The species data were square-root-transformed prior to analysis (Zar 1999, O'Hara & Kotze 2010); scaling was focussed on inter-species correlations and species scores were divided by standard deviation. Ordination diagrams were plotted using CanoDraw (ter Braak & Šmilauer 2002).

Thereafter, recently developed multivariate modelling analyses were employed to test if macroinvertebrate species composition is influenced by the presence of predatory fishes, while taking into account environmental variables such as biotope and current flow rate (Table 3.1). The relationship between macroinvertebrate species assemblage and the various environmental variables was modelled through a multinomial generalised linear model (GLM) for multivariate abundance data (i.e. species-location datasets), using the function 'manyglm' from R package 'mvabund' (Moorhouse et al. 2014; Barrett et al. 2017; R Core Team, 2017; Wang et al. 2017). Multivariate GLM is equivalent to older, more widely used Analysis of Similarity (ANOSIM) and Permuted Multivariate Analysis of Variance (PERMANOVA), but has several benefits over older methods (Warton et al. 2015). Ecological data often violates assumptions of ANOSIM and PERMANOVA (e.g. normality of the data and particularly homogeneity of variances), whereas multivariate GLMS are more flexible (Wang et al. 2012; Warton et al. 2012). Unbalanced experimental designs are acceptable and data characterized by non-normally distributed residuals can be analysed without transformation. Multivariate GLMs do not depend on a distance matrix, as the whole species matrix is the response variable in a regression that takes into account the effect of correlation among species, and the univariate test results for each species are also provided as output (Wang et al. 2012). *P*-values are obtained via resampling, thereby controlling for the errors that may occur during multiple tests (Westfall & Young 1993; Wang et al. 2012). Finally, multivariate GLMs are not over-influenced by a few of the most dominant species, and less-abundant species also have an effect (the mean-variance relationship: Warton et al. 2015).

Table 3.1: Predictor choices made for the "manyglm" function employed to model for the effects of non-native and native fishes on macroinvertebrates in headwater tributaries of the Keiskamma River, Eastern Cape, South Africa.

Predictor	Type	Levels if categorical
Season	Categorical	Spring, Summer, Autumn, Winter
Biotope	Categorical	Stones, Gravel, Litter
Conductivity	Continuous	
pH	Continuous	
Flow rate	Categorical	None, Slow, Medium, Fast
Fish presence	Categorical	Absent, Non-native, Native

Species that were present in more than 5% of the samples were included in the response matrix (44 species; Appendix 1, Table 2). Although 'manyglm' also takes into account less abundant species, as mentioned, the rarest species are still poorly modelled. The predictors that were included in the model were season, biotope, electrical conductivity, pH, flow rate, and fish presence or type (Table 3.2). The model was fitted to the negative binomial distribution (for species count data), as Dunn-Smyth residuals and quantile-quantile plots derived from null models (i.e. response ~ 1) suggest that the negative binomial distribution fits the data better than the Poisson distribution (Dunn & Smyth 1996, Warton 2005; Appendix 1, Figure 1). The model fit was evaluated by systematically omitting each predictor from the model and comparing the resulting Akaike's Information Criterion (AIC) value with the AIC of the whole model. AIC penalises model complexity, i.e. redundant terms decrease model fit. The lowest AIC value was obtained when all predictors were used, and all were therefore included in the model.

Table 3.2: Model selection by comparing AIC (Akaike's Information Criterion) values when each predictor was omitted from the model. A lower AIC model indicates better fit.

Predictor dropped	df	AIC
None (whole model)		24353
Season	132	24761
Biotope	88	24448
Conductivity	44	24473
pH	44	24572
Flow rate	132	24928
Fish presence	88	24680

An Analysis of Variance (ANOVA) table was then derived from the model with the function 'anova.manyglm' (R package mvabund). This is a sequential sum-of-squares test, which tests the last predictor entered into the model after taking all other predictors into account. Therefore, several models were constructed by reordering the predictors so that each predictor was modelled as the last term, to obtain a marginal sum-of-squares test. Collinearity among factors was tested with the function "vif" (Lin 2015) using R package "car" (Fox & Weisberg 2017). "Vif" produces Generalised Variance Inflation Factors (GVIF) when the model has categorical variables, and the formula $GVIF^{1/(2.df)}$ makes the values comparable across different numbers of parameters. The values obtained were low and therefore collinearity was negligible, so all of the predictor variables were included in the model (Appendix 1, Table 4).

Variation in species composition among samples was visually represented by a Bayesian ordination (R function 'boral' from package 'boral'; Hui 2016, 2017) which is equivalent to non-metric multidimensional scaling. Like multivariate GLM, Bayesian ordination is a modelling-based method that accommodates count data without transformation or building a distance matrix, which accounts for the data type (Hui et al. 2015, Hui 2016). The main difference compared to multivariate GLM is that no predictors variables are used, and instead two latent variables are estimated from the response matrix and used to represent the ordination axes of a two-dimensional ordination (Ovaskainen et al. 2016). Latent variable values are estimated for each sample through Markov Chain Monte Carlo (MCMC) methods via the programme JAGS (Just Another Gibbs Sampler, v. 4.2.0, Plummer 2003), implemented by function 'boral'. Medians of the possible range of estimated values (Bayesian posterior distributions) for each sample were plotted as a two-dimensional ordination plot to depict the relationship between samples in terms of species composition.

3.3 Results

3.3.1 Sampling assessment and basic invertebrate data

In total, 103 species of invertebrate were identified from the 320 Surber samples (20 741 individuals determined to species/morphospecies), collected from 60 families and 15 orders (Appendix 1, Table 3). This richness is comparable to headwater stream studies elsewhere in the world (Hauer & Lamberti 1996, Lenat & Resh 2001) and locally (Palmer 1991, Barber-James 1995, Uys & O'Keeffe 1997, Madikizela et al. 2001, Madikizela & Dye 2003, Rivers-Moore et al. 2007). The species accumulation curve suggests that, given the method of sampling, the invertebrate assemblage has been adequately sampled at the level of tributary reach, and the data are representative of the respective assemblages because saturation was reached within the first 150 samples and sampling continued to 320 samples in total (Figure 3.2).

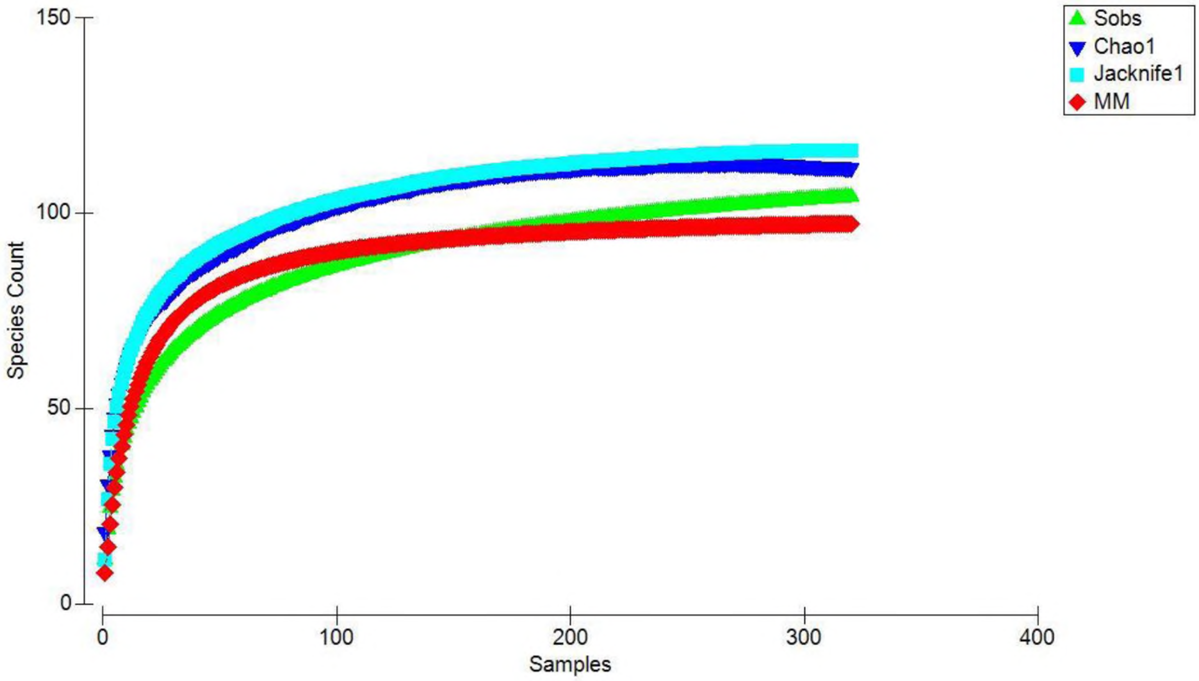

Figure 3.2: Species accumulation curves constructed using PRIMER from a matrix of 320 Surber samples containing 103 species in total.

The most diverse order of invertebrates was the Diptera (25 species), followed by the Ephemeroptera (22 species) and the Coleoptera and Trichoptera (15 and 16 species, respectively). The reaches showed considerable variation in their numbers of taxa. The lowest number of species was found along the native fish reaches of the Cata River (49 species) and the highest along the cognate reach of the Gwiligwili River (67 species). The fishless Mnyameni reach had the highest absolute and average density of invertebrates per square meter; the Mnyameni native fish reach had the least (Table 3.3).

Table 3.3: Basic diversity and density information from the reaches of stream sampled in the headwaters of the Keiskamma River, Eastern Cape, South Africa.

Reach	River	Number of species (s)	Number of individuals (n)	Density of invertebrates (m^2 of substrate)	Mean seasonal density (±SD) of invertebrates (m^2 of substrate)
Fishless	Cata	56	2591	28589	7197 ± 2801
	Mnyameni	57	5192	57689	14442 ± 7869
	Gwiligwili	61	1807	20078	5019 ± 2121
Invaded	Cata	49	2234	24822	6206 ± 1241
	Mnyameni	58	2653	29478	7369 ± 1672
Native fish	Cata	50	1766	19622	4906 ± 2126
	Mnyameni	63	1737	19300	4825 ± 1560
	Gwiligwili	67	2761	30878	7669 ± 3705

The overall densities of invertebrates from each reach of each river are presented in Figure 3.3. Invertebrate densities were suggested to differ significantly between localities in autumn and spring. Tukey's post hoc tests revealed that these differences were between the native fish and invaded reaches for Autumn, and the fishless and native fish reaches for Spring (Table 3.4). No significant difference was observed in the density of invertebrates for the remaining seasons, and average macroinvertebrate density was higher along fishless reaches (Figure 3.3), no significant difference was observed between the fishless and invaded reaches.

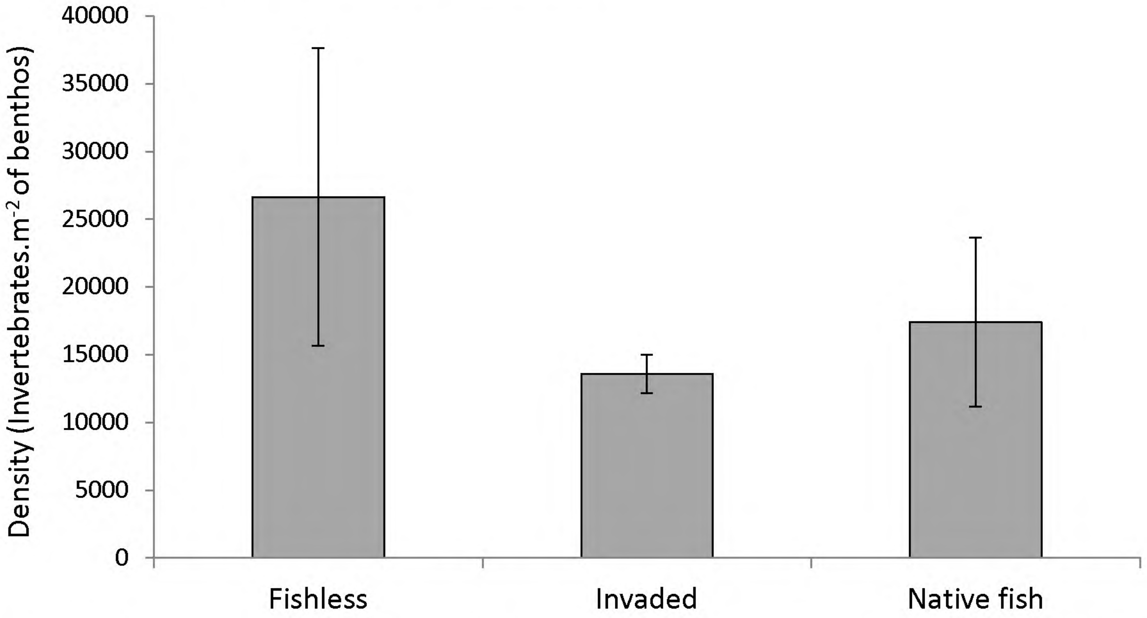

Figure 3.3: Bar graph of the mean density of macroinvertebrates (±SD) summed and averaged across season from fishless, invaded, and native fish reaches of headwater tributaries of the Keiskamma River, Eastern Cape, South Africa.

Table 3.4: Tukey's post hoc tests of a repeated measures ANOVA comparing density of macroinvertebrates from fishless, invaded, and native fish reaches of headwater tributaries of the Keiskamma River, Eastern Cape, South Africa. Significant differences are set in bold.

Variable: Autumn			
Approximate Probabilities for Post Hoc Tests Error: Between MS = 3.73, df = 77.0			
Reach	Fishless	Invaded	Native
Fishless		0.07	0.14
Invaded	0.07		**< 0.01**
Native	0.14	**< 0.01**	
Variable: Spring			
Approximate Probabilities for Post Hoc Tests Error: Between MS = 15.41, df = 77.0			
Reach	Fishless	Invaded	Native
Fishless		0.39	**0.01**
Invaded	0.39		0.44
Native	**0.01**	0.44	

3.3.2 Functional feeding group composition

Of the 103 species, the greatest proportion were gathering collectors (41.5%), followed by scrapers (37.3%), filtering collectors (9.1%), predators (6.8%) and shredders (5.3%). The predominant FFG along the fishless reaches of all three rivers was gathering collectors followed by scrapers, while the invaded reaches were predominated by scrapers followed by gathering collectors (Figure 3.4). The native fish reaches along the Mnyameni and Cata Rivers were characterized by similar proportions of scrapers, which predominated, followed by gathering collectors, while the Gwiligwili native fish reach was characterized mainly by gathering collectors.

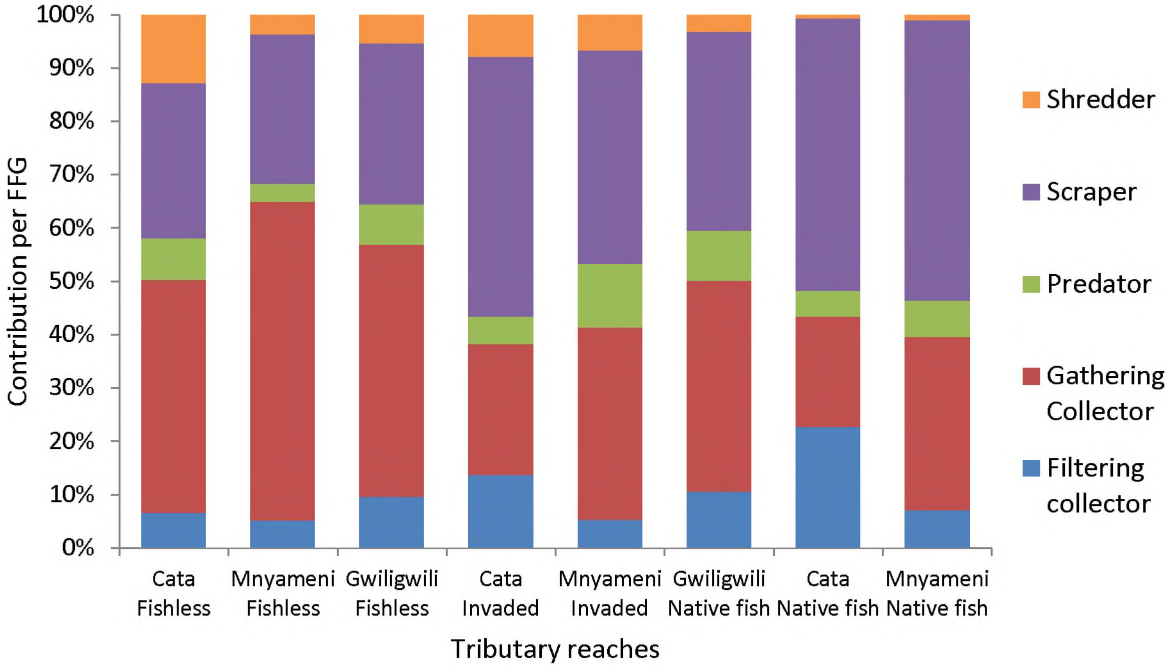

Figure 3.4: Stacked column plot of the percentage contribution of each functional feeding group (FFG) allocated from invertebrate species collected from the headwaters of the Keiskamma River, Eastern Cape, South Africa. Reaches are placed from left to right to reflect the change in river gradient with altitude, from fishless to invaded to native fish reaches.

The change in FFG structure from the upper fishless reaches down the gradient of the river to the lower native fish reaches is illustrated in Figure 3.5. Each FFG is treated separately, for simpler comparison between reach. The mean number of shredders per sample was significantly lower along the native fish reaches, compared to upstream invaded and fishless reaches, suggesting the lower native fish reaches do not contain the necessary resources to support comparable numbers of this group. The mean number of scrapers, predators and filtering collectors showed very little variation between reaches, while the gathering collectors were observed to progressively decrease in number per sample from the fishless to the native fish reaches, but this decrease was not significant.

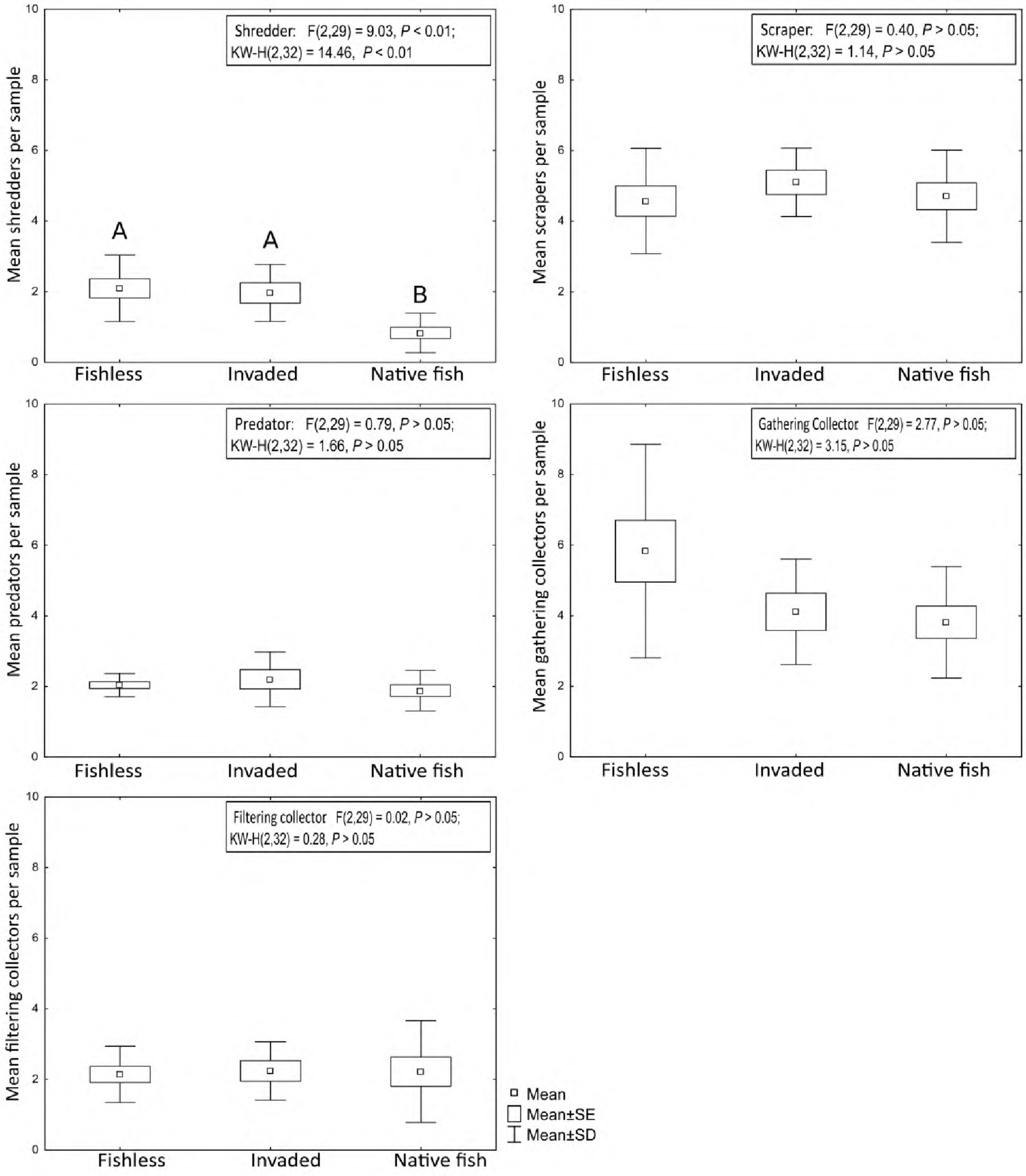

Figure 3.5: Box and whisker plot representing the mean number of invertebrates per functional feeding group (FFG) per sample, averaged across season, from the fishless, invaded and native fish reaches of the Mnyameni, Cata and Gwiligwili Rivers, Eastern Cape, South Africa. Prior to graphing, the data was square-root-transformed to reduce the amount of difference between the larger and smaller guilds. Statistically significant ($p < 0.05$) differences were suggested by Kruskal-Wallis and F-tests comparing the density of invertebrates in each FFG between reach (see Flecker (1984) and Flecker & Allan (1984)). Only shredders differed significantly between reach of river; When significant differences were observed between reach, these are denoted by differing letters.

While significant differences were observed for shredding taxa (Figure 3.5), the PERMANOVA comparing the proportional density of each FFG per sample suggested no significant difference between reaches.

3.3.3 Macroinvertebrate assemblage structure

Figure 3.6 is an ordination plot produced from a PCA based on benthic sample data from each of the reaches, summed across sampling event and season. Reaches that are grouped more closely together share species assemblages and of similar abundance. The PCA suggests a grouping of the native fish reaches that fall below dams, along the Cata and Mnyameni Rivers, apart from a central cluster. This cluster consists of both invaded reaches, the native fish reach and fishless reach along Gwiligwili River and the fishless reach along the Cata River. The fishless Mnyameni reach is situated apart from the remaining reach, while occupying a similar position along the PCA axis 1 to the Cata and Mnyameni River native fish reaches, its position along PCA axis 1 is unique.

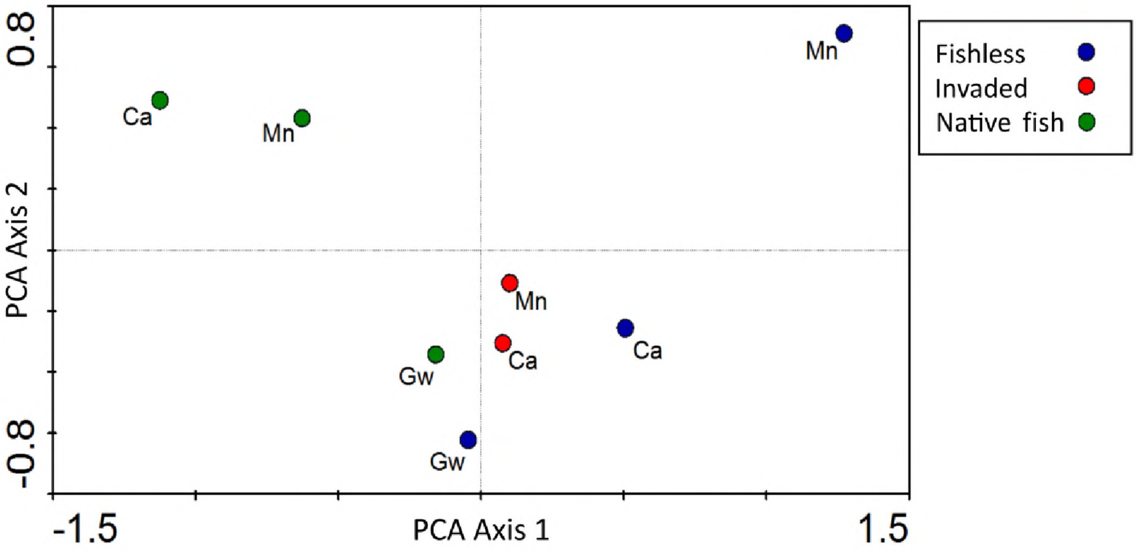

Figure 3.6: Ordination biplot produced from a Principal Components Analysis (PCA) of species assemblage data collected from headwater tributaries of the Keiskamma River, Eastern Cape Province, South Africa. Reaches of stream are colour coded and the river names abbreviated as follows: Ca: Cata River; Gw: Gwiligwili River; Mn: Mnyameni River.

Nearly 50% of the variation within the data is described by the first axis, with the second and third axes contributing a further 20% and 14% respectively (Table 3.5). This suggests that there was a reasonably strong signal within the data that is explained primarily by the first two axes, and that the Mnyameni and Cata native fish reaches share a similar invertebrate assemblage that is different from the remaining sites, and most different from the fishless Mnyameni River reach.

Table 3.5: Summary statistics from Principal Components Analysis (PCA) for species assemblage data sampled quarterly from eight headwater reaches in the upper Keiskamma River, Eastern Cape Province, South Africa.

Statistic	Axis 1	Axis 2	
Eigenvalues	0.48	0.20	
Cumulative percentage variance of species data	44.70	64.70	
Total sum of squares in species data			5193.97
Total standard deviation in species data TAU			2.50
Number of observations per reach			40

All environmental variables were found to be significantly related to variation in macroinvertebrate species composition (Table 3.6). Season was the most important driver of macroinvertebrate assemblage structure, having the highest Rao's test statistic value, followed by flow rate and then fish presence (Table 3.6). This suggests that these three predictors, in that order, account for macroinvertebrate assemblage structure best.

Table 3.6: Summary of results from the type II ANOVA (marginal test) showing the influence of each environmental variable on the macroinvertebrate assemblage after all other predictors are taken into account. Rao's test score statistic, *P*-values, and degrees of freedom (df) and residual df are provided.

Predictor	Res. df	df	Rao's test Score	*P*-value
Season	307	3	372.09	< 0.05
Flow rate	307	3	330.76	< 0.05
Fish presence	307	2	280.12	< 0.05
Biotope	307	2	193.46	< 0.05
pH	307	1	151.51	< 0.05
Conductivity	307	1	128.63	< 0.05

This is supported by Bayesian ordination plots, where flow rate and fish presence were highlighted (Figures 3.7 and 3.8). These figures represent plots of the median posterior distributions of the Surber samples, plotted in a low-dimensional plot. Latent variables, as mentioned earlier, account for unknown gradients within the species matrix, similar to the axes constructed during Principle Components Analysis (Walker & Jackson 2011, Warton et al. 2015). The ordination functions in the same way as a traditional nMDS, in illustrating patterns accounted for in the ANOVA run under a general linear model.

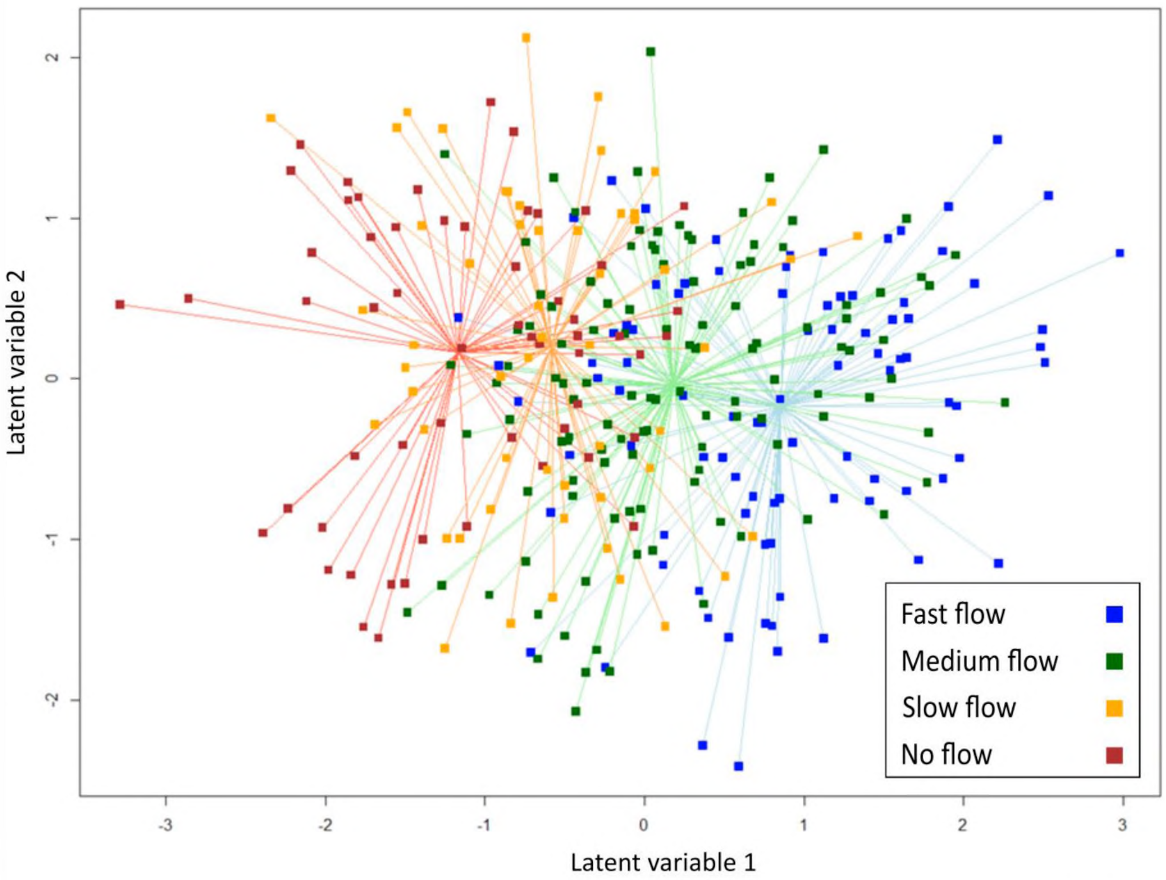

Figure 3.7: Latent variable plots produced from an unconstrained Bayesian ordination representing the categorical classes of flow rate for each sample. Individual data points represent median values of Bayesian posterior distributions of Surber samples, and corresponding flow rate category for each sample collected along headwater tributaries of the Keiskamma River system, Eastern Cape, South Africa. As with nMDS or PCA, samples that are closer in ordination space have more similar species compositions. Spider diagrams for each category were added with function ‘ordispider’ from R package ‘vegan’ (Oksanen et al. 2017).

Although there is considerable overlap of the groups, Figure 3.7 indicates a gradient in species composition with flow rate, and Figure 3.8 shows a difference especially between sampling points in upper reaches without fish and lower reaches with non-native fish. The associated univariate tests suggested that a total of 16 macroinvertebrate species demonstrated a significant response to the predictor "flow rate", while fish presence accounted for 13 out of a total of 44 species used in the analysis. The remaining predictors "season", "pH" accounted for significant responses from seven species each, while "biotope type" and "electrical conductivity" accounted for significant responses from three and four species, respectively. The macroinvertebrate species that demonstrated a significant response to the predictor variable "flow rate" for the most part include those that either have a preference for hydraulic habitats of "no flow" or "fast flow". Table 3.7 provides the names of these species and the associated predictor variable for which a significant association was suggested.

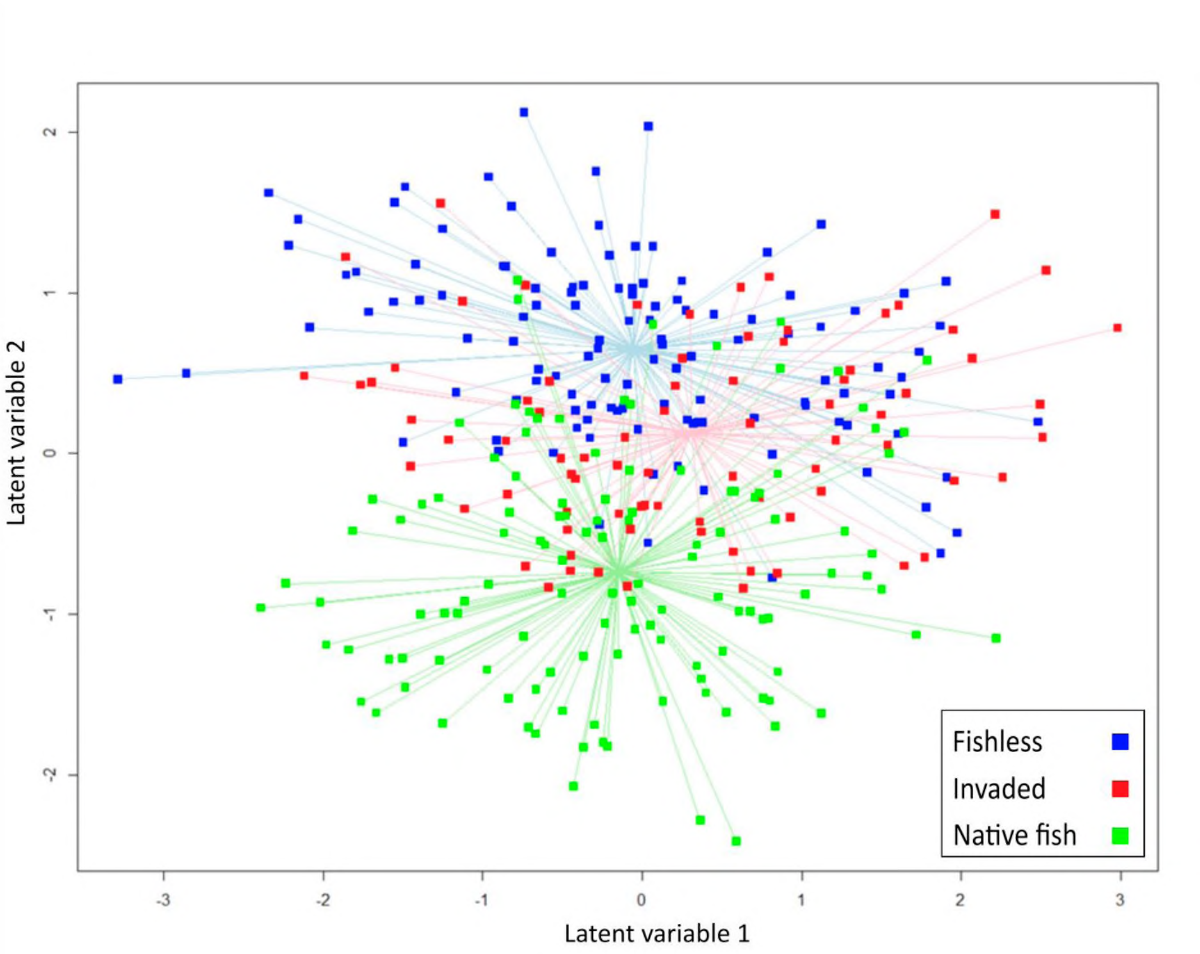

Figure 3.8: Latent variable plot produced from an unconstrained Bayesian ordination representing samples from fishless, invaded and native fish reaches of headwater tributaries of the Keiskamma River system, Eastern Cape, South Africa. Spider diagrams for each category were added with function ‘ordispider’ from R package ‘vegan’ (Oksanen et al. 2017).

The pill clam *Pisidium ovampicum* Ancey, 1890 (Mollusca: Sphaeriidae), the two species of square-gilled mayflies *Caenis* sp 1, *Caenis* sp 2 (Ephemeroptera: Caenidae), larvae of the biting midge *Bezzia* sp 1 (Diptera: Ceratopogonidae), and the caddisfly *Pisulia austrina* Morse, 1974 (Trichoptera: Pisuliidae) all occurred almost exclusively in backwaters out of current (Appleton, 2002a; de Meillon & Wirth 2002; Barber-James & Lugo-Ortiz 2003, de Moor & Scott 2003). With the exception of the minnow mayfly *Baetis harrisoni* Barnard, 1932, the remaining species (predominantly mayflies) for which flow accounted significantly, all prefer quick-flowing riffle and chute in-stream habitats (Palmer et al. 1991). Eight of the 13 species for which "fish presence" is as significant predictor are mayflies, a group known to reflect the effects of predatory fish (Culp et al. 1991, Culp & Scrimgeour 1993, Cowan & Peckarsky 1994, McIntosh & Townsend 1994, 1995, 1998, Huhta et al. 1999, 2000, Albariño & Buria 2011).

Table 3.7: Macroinvertebrate taxa that displayed a significant ($P < 0.05$) response to predictor variables. Rao's test statistic is supplied along with the univariate test P-value from the relationship between the species and predictor, in parentheses. The total number of taxa accounted for per predictor variable is displayed in the final row. Missing values represent non-significant effects for that particular predictor/taxon relationship.

Species	Flow rate	Fish presence	Season	pH	Biotope	Electrical Conductivity
Oligochaete sp 1					18.86 (0.02)	
Burnupia sp 1		12.56 (0.05)				
Pisidium ovampicum	36.37 (0.01)					
Acanthiops erepens				14.08 (0.01)		
Afroptilum sudafricanum		28.50 (0.01)	39.95 (0.01)			
Baetis harrisoni	20.93 (0.01)	21.92 (0.01)	27.40 (0.01)			
Cheleocloeon excisum		13.32 (0.04)				
Demoreptus monticola	24.01 (0.01)		21.40 (0.01)			16.29 (0.01)
Demoreptus capensis	23.77 (0.01)					
Afronurus harrisoni	16.77 (0.04)			22.15 (0.01)		
Tricorythus discolor	29.02 (0.01)	13.61 (0.04)				
Lestagella penicillata		21.39 (0.01)				
Caenis sp 1	39.36 (0.01)					
Caenis sp 2	21.30 (0.01)		27.45 (0.01)			
Adenophlebia sp 1	20.69 (0.01)	24.34 (0.01)				
Castanophlebia sp 1	20.61 (0.01)	15.43 (0.02)	54.80 (0.01)	27.36 (0.01)		14.58 (0.02)
Euthraulus sp 1		29.69 (0.01)	28.46 (0.01)	14.96 (0.01)		
Aeshna sp 1		13.31 (0.04)				
Afronemoura sp 1		24.11 (0.01)				
Cheumatopsyche afra	20.45 (0.01)					
Cheumatopsyche thomasseti	22.56 (0.01)					
Goerodes caffrariae				16.8 (0.01)	38.94 (0.01)	
Pisulia austrina	17.37 (0.03)					
Scirtid sp 1		28.08 (0.01)	33.06 (0.01)	18.84 (0.01)		
Pericoma sp 1		22.61 (0.01)				
Bezzia sp 1	29.16 (0.01)					
Simulium rutherfoordi	16.31 (0.05)			22.41 (0.01)		
Simulium vorax	21.13 (0.01)					
Atherix sp 1					18.08 (0.02)	
Number of taxa	16	13	7	7	3	2

The remaining macroinvertebrates for which the predictor "fish presence" accounts for a significant effect include the limpet snail *Burnupia* sp 1 (Mollusca: Ancylidae), the dragonfly *Aeshna* sp 1 (Odonata: Aeshnidae), the stonefly *Afronemoura* sp 1 (Plecoptera: Notonemouridae), the marsh beetle Scirtid sp 1 (Coleoptera: Scirtidae) and the diminutive moth fly larvae *Pericoma* sp 1 (Diptera: Psychodidae).

3.4 Discussion

The main findings of this study were two-fold, while predatory fish did not appear to account for significant differences in density or FFG composition of macroinvertebrates from invaded and uninvaded headwater tributaries, fish presence was found to be a significant predictor of overall insect assemblage structure, subordinate only to flow rate and season. Therefore, the hypothesis that the macroinvertebrate assemblage would be structured through flow and biotope availability as predicted by the river continuum concept, in favour of top-down predation pressure by salmonids, was partly accepted because the null hypothesis that predatory fish would have no effect on macroinvertebrate assemblage could not be rejected. While the effect of the predictor "biotope" was not as important as the effect of "fish presence", abiotic effects of "season" and "flow rate" were demonstrated to outweigh the effect of fish presence. However, this predictor still accounted for a significant proportion of the variation within the data, and elicited significant responses from 13 out of 44 taxa (Tables 3.6, 3.7). The river continuum concept predicts that macroinvertebrate assemblage structure changes along the gradient of streams, particularly when longitudinal linkages are strong as is the case for pristine forested headwater streams moving towards foothill reaches (Vannote et al. 1980, Poole 2002, Arthington 2012). Bottom-up, resource driven, structuring of the macroinvertebrate assemblages of the Keiskamma River headwaters is thus favoured in explaining patterns of macroinvertebrate density, FFG structure and overall assemblage structure, balanced against a top-down driver like fish predation.

3.4.1 Macroinvertebrate density and functional feeding group characteristics under differing fish predation regimes

Comparisons of the density of macroinvertebrates between reaches suggested that they only differed significantly between lower native fish reaches (intermediate average density) and the upper fishless reaches (highest average density); and between the lower native fish reaches and the middle invaded reaches (lowest average density), for particular seasons. While macroinvertebrate densities along the invaded reaches were on average lowest, significant differences were not observed between this reach and the fishless reaches for any of the sampling events. This suggests that either the spatial difference between the upper fishless reaches and the lower native reaches, or the presence of native fish may be responsible for the differences observed in macroinvertebrate density, as the invaded reaches were not significantly different from the fishless reaches for any sampling season.

Significant differences in macroinvertebrate density were reported from above and below barriers to invasion by trout along headwater Patagonian streams (Buria et al. 2007, Albariño & Buria 2011). Similarly, *O. mykiss* were reported to reduce the density of water striders from pools in Californian streams, while also resulting in altered behaviour and in-stream distribution, negatively affecting water strider fitness (Cooper 1984). Meissner & Muotka (2006) reported a reduced abundance of

Baetis mayflies, and significantly lower densities of larger predatory macroinvertebrates in streams occupied by *S. trutta*, compared to paired streams without trout and suggested a very strong size-dependent effect of trout on benthic macroinvertebrates in forested streams. Contrary to these findings, Herbst et al. (2009) found no difference in invertebrate densities in fishless streams compared to paired trout streams, but they reported lower invertebrate richness and diversity from invaded streams. Flecker & Allan (1984) found that fish predation had no significant effect on stream macroinvertebrate abundance, richness or density, while stream substrate type was found to affect all of these three measures of macroinvertebrate assemblage structure significantly. However, fish predation was found to affect species richness negatively for the largest macroinvertebrate size class (>8mm). Allan (1982) reported that a reduction of trout from reaches of stream by 80-90% of normal levels produced no observable difference in the macroinvertebrate community. A study conducted in headwater streams on the island of Kauai, Hawaii, examining stomach contents of introduced trout suggested that they do not negatively impact endemic damselfly populations, concluding that trout feed opportunistically, exploiting drifting prey of both aquatic and terrestrial origin (Englund & Polhemus 2001). As the density of invertebrates is independent of taxonomic or functional guild, findings here suggest that the differences observed between reaches, when they existed, are most likely attributable to the combined effects of the dams on the Mnyameni and Cata Rivers and the change in environmental attributes inherent to the lower foothill reaches of the streams, predicted by the river continuum concept.

The FFG compositions observed from the Cata, Mnyameni and Gwiligwili Rivers tend to be dominated by either gathering collectors or scrapers across all reaches. The fishless and invaded reaches hosted higher proportions of gathering collectors than scrapers, with the converse occurring along native fish reaches. No trends were evident for the predator FFG; while published studies suggest that larger predatory invertebrates may be affected through predation by trout (Flecker 1984, Meissner & Muotka 2006), these effects were not detected here. The highest proportional abundance of filtering collectors was observed from the native fish reaches of the Cata and Gwiligwili Rivers, suggesting an increase in availability of the fine organic matter that these organisms eat. Along with the shredders, these groups conform best to the expected change in FFG assemblage along the gradient of the Keiskamma River, following an application of the river continuum concept (Vannote et al. 1980). The change in food particle size, predicted by the river continuum concept, dictates that larger food objects would occur in higher abundance in the headwaters of any river and would gradually decrease in size as the gradient of the river decreases (Cummins & Klug 1979), and any variation in ecosystem condition that influences the nutritional resource condition should be evident in a change in the proportion of FFGs (Cummins et al. 2005). The findings here agree with this statement, and the descriptions by Palmer (1991) from the neighbouring Buffalo River, with higher proportions of shredders and gathering collectors at upper sites followed by a change in proportional composition of these groups to scrapers and filtering collectors at downstream sites, rather than the influence of predatory fish species. As no significant difference was observed through a PERMANOVA comparing densities of each FFG between fishless, invaded and native fish reaches, the hypothesis that no influence of predatory fishes would be observed on the proportional macroinvertebrate FFG composition could not be rejected.

3.4.2 Drivers of macroinvertebrate assemblage composition

The results of the invertebrate assemblage PCA suggests that the fishless Mnyameni reach and the native fish Cata and Mnyameni reaches have differing assemblage structures from the remaining reaches studied. An explanation for the former case is the very high abundance of specific taxa along this particular reach, while the latter can be explained by the high levels of "endemism" among taxa along the lower reaches compared to upstream invaded and fishless reaches. Bredenhand & Samways (2008) reported a "quantum decline" in the diversity of aquatic fauna as a result of a dam on a small Cape Fold Ecoregion stream; de Jalon et al. (1994) report significant decreases in taxonomic richness, total density and total biomass of macroinvertebrates downstream of a dam, compared to what was present in the Rio Tera, Spain prior to the dam's construction. An impoundment effect may be responsible for the separation observed by the native fish Cata and Mnyameni River reaches, whilst the native fish Gwiligwili River reach grouped with the remaining reaches, apart from the fishless Mnyameni River reach.

The multivariate modelling analysis suggested that macroinvertebrate assemblage was driven mainly by season, flow rate, and fish presence, followed by pH, biotope and electrical conductivity. Therefore, the hypothesis that flow and biotope availability would be the most important drivers of macroinvertebrate assemblage structure was only partly accepted. This is because both predictors were suggested to be significant and accounted for significant responses from the highest proportion of taxa. While flow rate was the most important predictor, the presence of fish was also important, therefore the hypothesis that fish would not have a significant effect in structuring macroinvertebrate assemblages from these streams is rejected as the ANOVA run under a GLM suggested that fish presence is a significant predictor of assemblage structure.

The species that were demonstrated to be affected by fish, with the exception of the single beetle and true fly species, are representatives of groups for which fish have already been demonstrated to have an effect elsewhere. For example, salmonids are known to affect the density and behaviour of stoneflies through a response to predation risk (Feltmate et al. 1986, Feltmate & Williams 1989, 1991, McCutchen 2002), while mayflies are also known to respond to predatory fish as a predator avoidance mechanism (Culp et al. 1991, Culp & Scrimgeour 1993, Cowan & Peckarsky 1994, McIntosh & Townsend 1994, 1995, 1998, Huhta et al. 1999). Flecker (1984) studying the effects of fish predation vs. substrate composition in headwater streams in Virginia, U.S.A, found that fish predation played an important role in structuring the stream insect community. He also found that total macroinvertebrate abundance and diversity was correlated to the presence of plant detritus, and this relationship was taxon-specific, suggesting that the combined effects of fish predation and habitat availability affected specific taxa more than others. Rivers-More et al. (2013) examined macroinvertebrate assemblages for the effects of trout specifically. They concluded that overriding environmental factors made detecting the effects of non-native salmonids on macroinvertebrates difficult. The findings here corroborate these studies, in particular those of Flecker & Allan (1984), in that abiotic factors like season and flow play a more important role in structuring macroinvertebrate assemblages than the presence of insectivorous fish.

3.5 Conclusion

The data collected during this study suggest that the fishless and invaded reaches within the Keiskamma River headwaters shared similar densities and functionally similar macroinvertebrate assemblage compositions, while the reaches occupied predominantly by native fishes were characterised by significantly lower insect densities in autumn compared to the fishless reaches and in spring, compared to the invaded reaches. The native fish reaches also displayed significantly lower densities of shredding taxa, compared to both the invaded and fishless reaches. Therefore, effects of non-native trout do not appear to be evident on the density or FFG composition of macroinvertebrates from the study streams.

Using macroinvertebrates as a tool for assessing the strengths of environmental predictors, flow, season and the presence of fish were found to be the most important factors in determining assemblage structure. Therefore, it is concluded that patterns of effect by the presence of trout, and native fishes, in concert with abiotic drivers, structure the macroinvertebrate assemblage. This is similar to the findings by Shelton et al. (2015a, 2015b, 2016) who demonstrated through a broad-scale comparative study of invaded CFE headwater tributaries that non-native trout suppress native fishes through direct predation and release macroinvertebrates from predation pressure resulting in a trophic cascade, with decreases in algal biomass observed in streams containing trout (Shelton et al. 2015b). An *in situ* mesocosm field experiment examining top-down predation pressure of fishes on stream invertebrates, demonstrated that macroinvertebrates and detritus were more important in the diets of native cyprinids, while trout fed predominantly on terrestrially-derived invertebrate prey (Shelton et al. 2016). The overall conclusion of these studies was that in the CFE, *O. mykiss* had differing effects on stream macroinvertebrates compared to native cyprinids, meaning that two different "types" of predatory effect were present. Unfortunately, the study by Shelton et al. (2015b) did not examine fishless reaches of these streams, but in "no fish" mesocosm treatments, macroinvertebrate density and composition were almost indistinguishable from treatments containing trout, while treatments containing native cyprinids were significantly different (Shelton et al. 2016). These results are similar to the findings here, where fishless and invaded reaches of stream are more similar in their macroinvertebrate composition when both are compared to native fish reaches.

Englund (1997) suggested that, in caged experiments like that of Shelton et al. (2016), scale of the experimental design is crucial as predator effects are scale-dependant. Therefore, any hypothesis erected surrounding predator effects cannot be extrapolated from an assemblage scale, to population scale, and are specific to the spatial scales that the experiments are capable of detecting. A meta-analysis by Cooper et al. (1990) examining published studies on the predatory effects of fish on stream invertebrates found a significant positive relationship between decreasing mesh size, and a significant predatory effect, concluding that a highly significant correlation existed between predator impact and mesh size across 52 studies. Furthermore, they suggest that, in large-scale experiments employing benthic and drift samples from pools with or without fish, the magnitude of perceived predator effects on prey may still be determined by prey exchange rates. The experimental design employed during this study, in the form of a natural experiment (*sensu* Diamond 1983), examines predation by salmonids under "natural" circumstances, where neither the density of prey nor predator is experimentally manipulated.

The impacts of salmonids have been quantified in recipient ecosystems through measures of macroinvertebrate diversity, abundance, density and overall assemblage structure in streams were salmonids have invaded (Buria et al. 2007, Herbst et al. 2009, Albariño & Buria 2011). The review of studies of the effects of predatory fish on macroinvertebrates by Dahl & Greenberg (1996) for example, concluded that the method by which the fish fed (e.g., within the drift or in a benthivorous way) was the strongest determining driver of benthic prey density, when an effect was observed. While this chapter examined measurements made from benthic samples, a response to drift-feeding fish may be expected from prey species, where avoidance behaviour towards drift-feeding salmonids may be demonstrated through changes in timing and density of drift (Brittain & Eiekeland 1988, Wooster & Sih 1995). The following chapter will examine behavioural aspects of the macroinvertebrate assemblage, focussing on species that have been identified from this chapter for which the presence of fish was a significant predictor. This will be done through the examination of drift density and drift periodicity between fishless, invaded and native fish reaches.

Chapter 4: Exploring macroinvertebrate drift behaviour in headwater reaches of the Keiskamma River, Eastern Cape, South Africa

4.1 Introduction

An important part of lotic ecosystem functioning is the phenomenon of invertebrate drift (Townsend & Hildrew 1976, Brittain & Eikeland 1988). It can be defined as the downstream transport of any aquatic organism in the water column, and is of paramount importance in structuring invertebrate assemblages in rivers and streams (Waters 1965, 1972, Hynes 1970, Brittain & Eikeland 1988). Drift functions dually in facilitating the displacement of invertebrates from the benthos and transporting them downstream to more favourable biotopes, thereby assisting the process of (re)colonization from upstream sources (Townsend & Hildrew 1976). Living invertebrates are theorized to drift not very far, approximate estimates suggesting that 60% of invertebrates entering the current and sampled by a drift net, originate from less than 10 m upstream of the net (Townsend & Hildrew 1976).

The factors suggested to be important in determining the timing and intensity of drift activity of stream invertebrates are reviewed by Brittain & Eikeland (1988) and summarised in Figure 4.1. Drift has been classified into various forms, the reason for entering the water current being the primary distinguishing criterion. These are constant drift, behavioural drift, active drift, distributional drift, and catastrophic drift (Brittain & Eikeland 1988). Catastrophic drift, which was not observed during the course of this study along the Keiskamma River, is defined by an extreme behavioural response by entire invertebrate communities to a physical or chemical cue, such as severe flooding, thermal stress, or chemical pollution (Waters 1972, Dudgeon 1990). Woodford et al. (2013) observed drift at nearly two orders of magnitude above baseline levels, following the application of rotenone along the Rondegat River, a Cape Fold Ecoregion (CFE) stream (Abell et al. 2008). Constant drift is characterized by the displacement of low and irregular densities of invertebrates, attributable to "accidental" removal of individuals from the stream benthos (Schreiber 1995). Active and distributional drift can be considered as subcategories of behavioural drift, as both involve the deliberate but presumably temporary displacement of an organism into the water column from its point of origin (Waters 1975, Brittain & Eikeland 1988, McIntosh & Townsend 1998). With the exception of catastrophic drift, separating continuous and behavioural drift is not entirely possible in a fundamental sense (Huhta et al. 2000), but a multitude of studies have shown that the latter is an extremely important part of stream ecology, and the evidence for it is derived from studying diel or circadian drift rhythms of macroinvertebrates (Flecker 1992, Brewin & Ormerod 1994, Schreiber 1995, Grzybkowska et al. 2006).

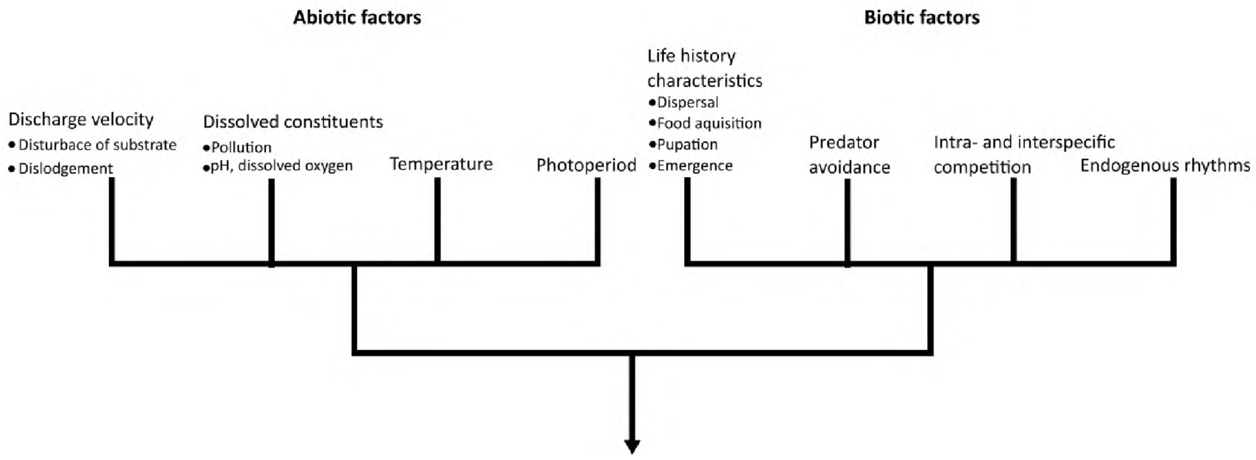

Figure 4.1: A conceptual model of the biotic and abiotic factors contributing to the drifting of invertebrates in lotic ecosystems (modified from Brittain & Eikeland 1988)

Strong seasonal effects are evident for cold temperate streams where drift is limited during winter while higher densities occur in summer (Clifford 1972). A seasonal signal was detectable for some taxa in a temperate Australian stream and not for others (Schreiber 1995), while tropical streams tend to lack strong seasonal invertebrate drift patterns (Ramírez & Pringle 1998). Drift as a function of chronobiological changes due to the day/night cycle are reviewed in detail by Müller (1974), who suggested photoperiod to be an important driver.

High altitude fishless streams, like those of the Nepalese Himalayas falling between 2000 - 3305 m, appear not to show the same drift patterns of lower altitude reaches, where drift of the majority of taxa was aperiodic, having neither diurnal or nocturnal preference (Brewin & Ormerod 1994). Studies of invertebrate drift in fishless, high-altitude, Andean streams yielded similar results, with a lack of any particular diel pattern, and where they existed, they were weak with a tendency for invertebrates to drift during the day rather than at night (Turcotte & Harper 1982). While abiotic factors may be strong drivers of drift, it has been suggested that the diel patterns in drift of invertebrates may have evolved as a "hard-wired" predator avoidance mechanism (Allan 1978, Flecker 1992).

Not all invertebrates drift equally readily; active swimmers like *Baetis harrisoni* (Ephemeroptera: Baetidae) enter the water column readily and may easily be observed swimming in an erratic yet purposeful manner within any of their respective, favoured biotopes, while *Lestagella penicillata* (Ephemeroptera: Teloganodidae) do not swim as effectively (Barber-James & Lugo-Ortiz 2003). For this reason, Rader (1997) suggested a functional classification of invertebrates based on this varying propensity to drift.

Figure 4.2: A drift sample being collected at midday along the invaded reach of the Mnyameni River, Eastern Cape, South Africa, where rainbow trout *Oncorhynchus mykiss* proliferate. The position of the net is indicated by the red arrow, and can be seen just above the surface of the water anchored in place by two iron standards.

Fish predation has been hypothesised as a causative mechanism for shifting drift behaviour from a diurnal pattern to a nocturnal one in response to visual predators like trout (Brittain & Eikeland 1988). This predator avoidance mechanism may have evolved due to the relationship between higher light intensity and fishes' ability to feed effectively (Allan 1978, Rader 1997, Huhta et al. 2000). For example, Pringle & Ramírez (1998) reported a higher abundance of drifting invertebrates at night where fish were present at lower altitudes in a stream in Costa Rica, while along reaches where fish were absent there was no diel pattern. McIntosh et al. (2002) found that mayfly drift in the Rocky Mountains, Colorado, U.S.A., was aperiodic along five fishless streams and predominantly nocturnal along five streams that contained brook trout *Salvelinus fontinalis* (Mitchill 1814). Under experimental conditions Williams & Moore (1982) observed that drift into the water column by the freshwater shrimp *Gammarus pseudolimnaeus* (Gammaridae: Amphipoda) was almost completely absent when rainbow trout *Oncorhynchus mykiss* were added to the system. Douglas et al. (1994) found that in streams of Southern California, drift-feeding rates of *O. mykiss* alone could not account for the observed difference in the drift density of a baetid mayflies, and concluded that the difference between diurnal and nocturnal drift density must have been as a result of a behavioural response from the mayfly nymphs. Adding trout, kept in cages, to previously fishless reaches of the same streams precipitated an immediate change from a predominantly diurnal drift to nocturnal drift, suggesting predator avoidance behaviour (Douglas et al. 1994). Studies in multi-predator environments (present in most natural lotic scenarios), suggest that the presence of invertebrate predators that forage nocturnally may drive increased drift density at night in prey species (Peckarsky 1980, Huhta et al. 1999).

Lintermans (2004) suggests that headwater stream habitats are particularly vulnerable to invasion by non-native fish species because these environments contain few native species, leaving "unoccupied ecological space" for novel invaders to take up (Ricciardi & Atkinson 2004; Ricciardi et al. 2013). In the CFE, Shelton et al. (2016) recently demonstrated via an *in situ* field experiment that *O. mykiss* have differing effects on macroinvertebrate assemblages, compared to a redfin, *Pseudobarbus burchelli* (Smith 1841), suggesting differences in their feeding mechanism compared to the native cyprinid. In the Keiskamma River system both *Salmo trutta* and *Oncorhynchus mykiss* represent a phylogenetically distinct predatory archetype not otherwise known from streams in the Amatolo-Winterberg Highlands Ecoregion (Ellender 2013, Ricciardi et al. 2013). Both species have been documented to have serious effects on recipient ecosystems elsewhere (Simon & Townsend 2003, Ellender & Weyl 2014), and may be demonstrated to leave patterns of influence within the invertebrate community through predation pressure (Crowder & Cooper 1982, Buria et al. 2007). The findings thus far from the Keiskamma River headwaters suggest limited impact by trout on the macroinvertebrate assemblages within study streams, based on measures of density, functional feeding group composition and assemblage structure. However, an aspect of macroinvertebrate assemblages from these streams not yet studied is drift, where the behaviour of macroinvertebrates between invaded and uninvaded reaches of stream may be explained as an avoidance of fish predation, inferring some resistance to possible predatory affects by salmonids.

4.1.1 Aims and hypotheses

The aim of this chapter was therefore to determine if there were differences in macroinvertebrate drift behaviour between fishless, invaded and native fish reaches of headwater tributaries of the Keiskamma River system, Eastern Cape, South Africa. The working hypothesis was that a significant difference in the timing of drift density would be evident upon comparison of drift collected from these reaches, but that no difference would be observed in the absolute drift density across reaches, following the predator avoidance hypothesis of Allan (1978).

4.2 Materials and methods

The lotic habitat studied for this chapter consists of two headwater tributaries of the Keiskamma River, the Cata and Mnyameni Rivers (Figure 4.3), described in Chapter 2.

4.2.1 Invertebrate drift sampling

Data for this chapter consisted of 72 drift samples. These constituted four samples, collected every six hours over a 24-hour cycle, from fishless, invaded and native fish reaches, across three seasons, (spring, summer, autumn) for each of the two study streams. The drift net consisted of a square metal frame, 40 cm x 40 cm, supporting a 250 µm mesh bag, approximately 1 m long, with a circular cod-end. The long bag allowed for a large surface area of net through which water could pass, preventing backflow if clogging occurred. Samples were preserved on site using 90% ethanol.

Drift samples were collected at the same sampling locations, and at the same times, as the Surber samples collected for Chapter 3 (Figure 4.3). In each instance, as the collecting of drifting invertebrates is dependent upon their natural, spontaneous displacement and movement along the continuum of a stream, drift samples were always collected prior to entering the stream to collect Surber samples analyzed in Chapter 3.

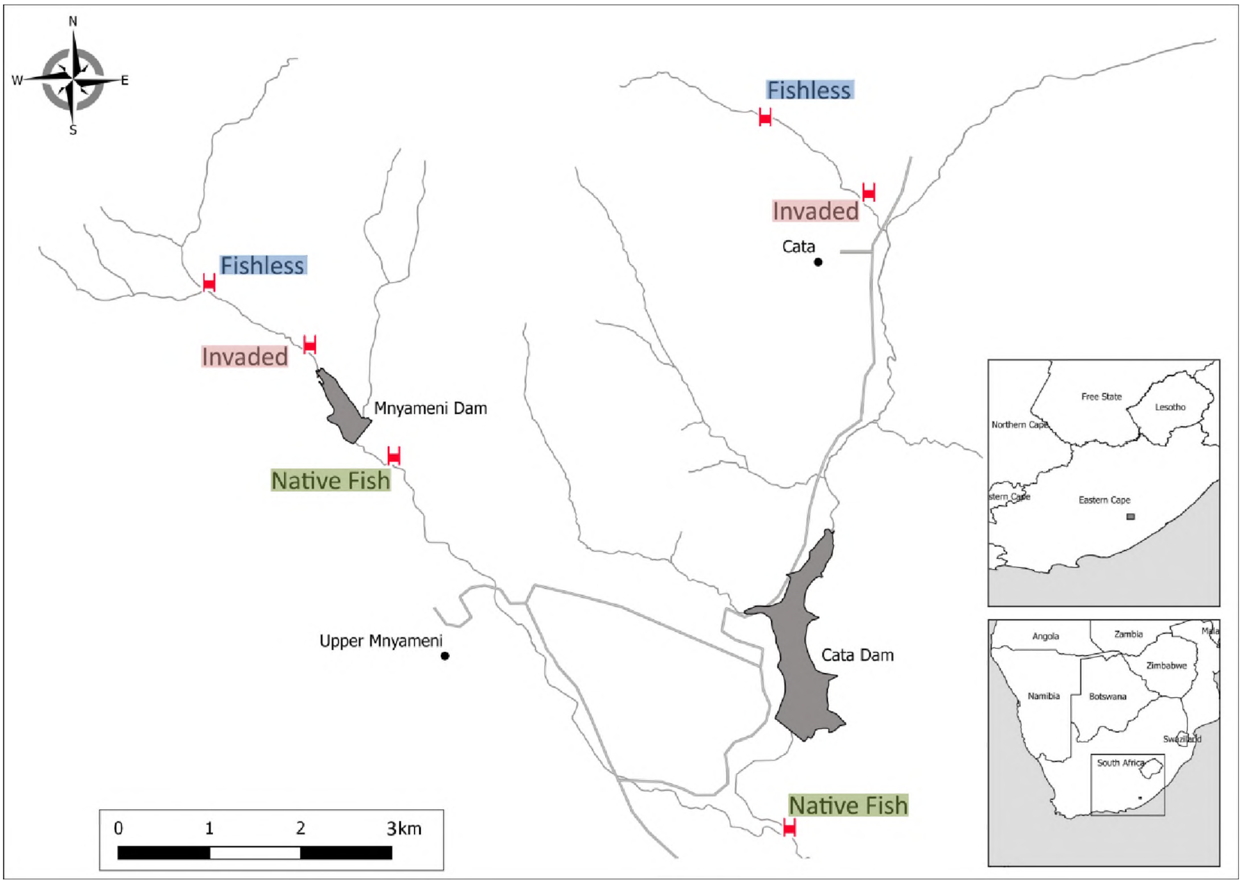

Figure 4.3: Drift sampling stations, represented by the red "H" drift net icons, along fishless, invaded and native fish reaches of the Mnyameni and Cata Rivers, headwater tributaries of the Keiskamma River system, Eastern Cape, South Africa. Drift sampling stations correspond to the sampling reaches described in Chapter 2.

Upon arrival at the reach where drift sample was to be collected, an appropriate place in the stream was selected where the net could be secured allowing a steady flow of water through it. This was typically in a riffled reach, next to or between two large boulders (Figure 4.2). The net was anchored firmly in place, flush along the stream bed. The depth and flow at the mouth of the net was recorded using a Marsh McBirney Flo-Mate (Model 2000) portable electromagnetic flow meter. On each occasion the drift net was installed such that it was not completely submerged, allowing macroinvertebrates trapped in the meniscus to be collected. Each drift sample was collected for 1 hour, and, with the recording of water flow and depth of the water at the mouth of the net, the volume of water sampled per unit of time was calculated. Four drift samples were collected over 24 h, timed to coincide with daylight conditions at midday, total darkness at midnight and crepuscular conditions at dusk and dawn because literature on drifting invertebrates suggests that crepuscular periods may be important for some groups of drifting and emerging invertebrates (Dudgeon 1983, Brewin & Ormerod 1994, Schreiber 1995).

4.2.2 Sample processing and identification

The sorting and identification of the contents of the drift samples followed the procedure outlined in Section 3.2.3 of Chapter 3. However, extra attention was required for the drift samples both in sorting and for identifications, as the mesh size of the drift net was a quarter of that of the sampling net used in Chapter 3. The drift samples also contained a multitude of diverse adult invertebrates from a wide variety of taxa. These were treated as allochthonous invertebrate rain (Appendix 2, Table 1). While some, such as adult mayflies or stoneflies, may have originated from the stream, they were not treated as part of the stream drift because their occurrence in the drift net was from falling on the waters' surface rather originating from the stream substrate. Exception was made for surface-dwelling macroinvertebrates (Veliidae (Hemiptera: water crickets), Gerridae (Hemiptera: pond skaters) and Gyrinidae (Coleoptera: whirligig beetles)) whose preferred biotope is the meniscus of the river, and they were not classified as invertebrate rain. From the earliest studies on drifting invertebrates, it has been know that terrestrially-derived inputs of invertebrates (and vertebrates) are an important food resource for fish (Needham 1928, Shelton et al. 2016), and for that reason they were included here for analysis.

4.2.3 Analyses

The data accumulated through sorting and identifying invertebrates from the drift samples produced slightly over 47 000 specimens and 118 species (Appendix 2, Table 1). To visualize patterns in drift per sampling reach over the 24-h period, invertebrate taxa were grouped to ordinal level (terrestrial input was categorised and assessed as invertebrate rain) and expressed as individuals per m^3 by dividing the number of taxa collected by the flow volume passed through the net. Tests for normality of residuals were carried out in Statistica (Statsoft Ltd, V.13.0), and suggested that the residuals were not normally distributed (Shapiro-Wilk's W, $P < 0.01$). Therefore, to test the strength of the patterns observed between orders of insect, between reach, densities were compared using non-parametric Kruskal-Wallis ANOVA, as reaches were considered mutually independent (3 seasons x 2 river reaches x 4 times of day = n of 24).

Multivariate analysis of the drift samples was conducted in the same way as for the Surber samples in Chapter 3 (Section 3.2.4.2), using a multinomial generalised linear model (GLM) for multivariate abundance data, using the function 'manyglm' from R package 'mvabund' (Moorhouse et al. 2014; Barrett et al. 2017; R Core Team, 2017; Wang et al. 2017), in R (R Core Team, 2017). This was done to test which factors where important in explaining the drift patterns between observed reaches. Species that were present in more than 5% of the drift samples were included in the response matrix (82 species; Appendix 2, Table 2). This excluded extremely rare taxa (e.g., *Securiops* sp 1 and *Oligoneuriopsis* sp 1; Appendix 2, Table 1) that occurred as single specimens from single samples. Macroinvertebrates that were too small to identify to species level were also included in analyses as "tiny Baetidae" and "tiny Odonata" (Appendix 2, Table 2). Microinvertebrates, including Daphniidae: Cladocera, Cyclopodidae: Copepoda and cypridid Ostracoda were excluded as these rarely occurred. The drift sample model was fitted with negative binomial distribution (for species count data), and as with the Surber sample analysis, the model fit was evaluated by systematically omitting each predictor from the model and comparing the resulting Akaike's Information Criterion (AIC) value with the AIC of the whole model. Predictors used included "Volume filtered", "Season", "Time of day" and "Reach" (Table 4.1). An Analysis of Variance (ANOVA) table was then derived from the model with the function 'anova.manyglm' (R package mvabund), suggesting which predictors were most important in explaining variation in macroinvertebrate drift patterns.

Table 4.1: Model selection by comparing AIC (Akaike's Information Criterion) values when each predictor was successively omitted from the model. A lower AIC model indicates better fit, suggesting the whole model be employed.

Predictor dropped	df	AIC
None (whole model)		12171
Time of day	246	12332
Season	164	12625
Reach	164	12885
Volume filtered	82	12426

Thereafter, drift timing and density were examined for the species for which a significant proportion of variation between reach as explained by the predictor variable "fish presence", based on the results from Chapter 3. Following the procedure outlined by Buria et al. (2007), taxa that either contributed to less than 0.5% of the total observed counts or contributed to less than 0.1% of total specimens sampled, were excluded. Thus analyses were limited to the mayfly species *Afroptilum sudafricanum*, *Baetis harrisoni* (Ephemeroptera: Baetidae), *Tricorythus discolor* (Ephemeroptera: Tricorythidae), *Adenophlebia* sp 1, *Castanophlebia* sp 1. (Ephemeroptera: Leptophlebiidae), the stonefly *Afronemoura amatolae* (Plecoptera: Notonemouridae) and marsh beetle *Scirtid* sp 1 (Coleoptera: Scirtidae).

Differences in timing and drift density for the species included were assessed within reach i.e. comparison of different times of day, same reach, using a Friedman's ANOVA, the non-parametric equivalent of the repeated measures ANOVA (Zuur et al. 2007). "Reach" was specified as the grouping variable, season was treated as a replicate (3 seasons x 2 river reaches = n of 6, per species). Comparisons between reach i.e. same time of day, different reach, were made using a Kruskal-Wallis ANOVA by ranks. Multiple comparisons between mean ranks were used to test differences between groups, season was treated as a replicate (3 seasons x 2 river reaches = n of 6, per time period). Statistical tests were carried out, and graphs plotted, in Statistica. Non-parametric analysis was employed as drift data did not conform to the assumptions of parametric analysis, residuals within the data being non-normally distributed (Shapiro-Wilk's W, $P < 0.01$). Drift density data was square-root transformed prior to plotting in Statistica; Inkscape v0.91 (Free Software Foundation, Inc. Boston, U.S.A.) was used to combine multiple graphs into figures.

4.3 Results

4.3.1 Invertebrate drift patterns in the headwaters of the Keiskamma River

Patterns of drift density (individuals/m^3) for each aquatic order of macroinvertebrate, along with the allochthonous invertebrate rain, are presented in Figure 4.4. Invertebrate rain density was highest along the native fish reaches, followed by the invaded reach, with the lowest proportion being observed along the fishless reach. Similarly, Ephemeroptera drift densities were highest along the native fish reaches, and the invaded reaches contained slightly fewer individuals per m^3 on average than the fishless reaches (Figure 4.4). Odonata were uncommon in drift samples, with the highest number observed drifting along the invaded reach, followed by the native fish reach and lastly the fishless reach. Plecoptera drift density was significantly lower along native fish fish reaches (n = 24, H = 32.68, $P < 0.01$), compared to upstream invaded and fishless reaches, which contained comparable densities of drifting stoneflies (Figure 4.4). Aquatic Hemiptera were most common along the slower flowing native fish reaches, their numbers here dominated by Corixidae (water boatmen), Notonectidae (backswimmers), Naucoridae (creeping water bugs) and Veliidae (water crickets). Megaloptera were entirely absent from drift samples collected along the native fish reaches, and were most common from the fishless reaches, compared to reaches invaded by trout (Figure 4.4). Trichoptera were most commonly observed drifting along the invaded reaches, which contained significantly higher densities along the invaded than the native fish reaches (Kruskal-Wallis ANOVA, n = 24, H = 11.18, $P < 0.05$), but were not significantly different from the fishless reaches. Coleoptera exhibited the inverse distribution, drifting at a lower density where trout were present compared to fishless and native fish reaches. Diptera drifted in progressively higher densities from fishless to native fish reaches in a downstream order, with the lowest and highest numbers observed from the fishless and native fish reaches respectively (Figure 4.4).

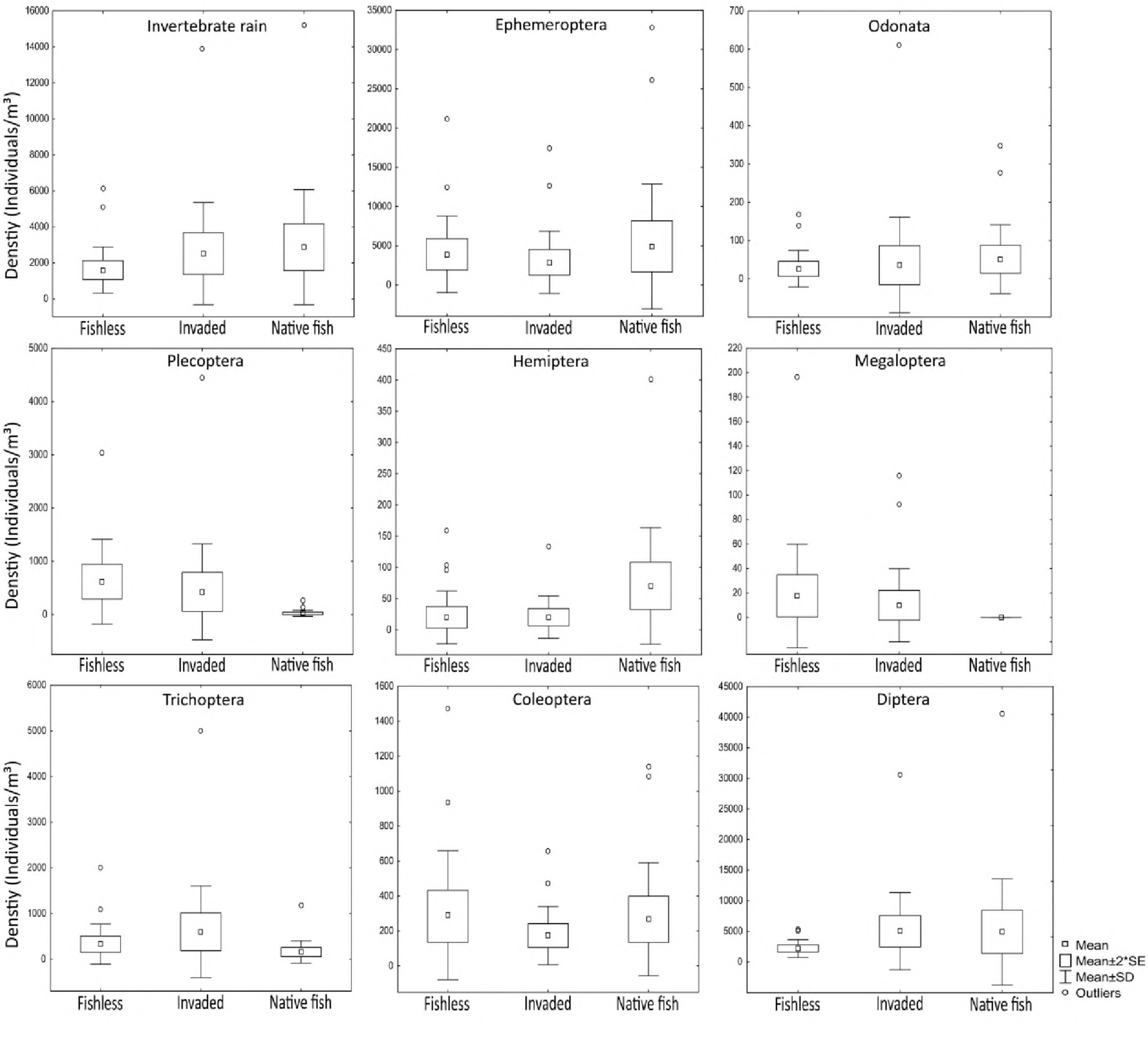

Figure 4.4: Mean drift density from each order of aquatic invertebrate and invertebrate rain per m³ of flow from the fishless, invaded and native fish reaches of the Keiskamma River, Eastern Cape, South Africa. Care should be taken when comparing different orders of invertebrate as the scale on the y-axis is different for each plot.

The results of the ANOVA testing the significance of each predictor variable in explaining drift variation between reaches are presented in Table 4.2. The most important predictor in explaining drift pattern was "time of day", followed by "season" and then "reach". Table 4.3 provides the names of these species and the associated predictor variable for which a significant association was suggested.

Table 4.2: Summary of results from the type II ANOVA (marginal test) showing the influence of each environmental variable on the macroinvertebrate assemblage after all other predictors are taken into account. Rao's score test statistic, *P*-values, and degrees of freedom (df) and residual df are provided

Predictor	Res. df	df	Rao's test Score	*P*
Time of day	63	3	227.48	< 0.01
Season	63	2	203.23	< 0.01
Reach	63	1	197.24	< 0.01
Volume filtered	63	1	100.65	< 0.01

Table 4.3: Macroinvertebrate taxa that displayed a significant ($P < 0.05$) response to predictor variables. Rao's test statistic is supplied along with the univariate test *P*-value from the relationship between the species and predictor, in parentheses. The total number of taxa accounted for per predictor variable is displayed in the final row. Missing values represent non-significant effects for that particular predictor/taxon relationship.

Species/Class	Reach	Season	Time of day	Volume filtered
Tiny Baetidae			18.54 (0.02)	
Afroptilum sudafricanum			17.52 (0.03)	
Baetis harrisoni	23.44 (0.01)		15.46 (0.05)	17.99 (0.01)
Cheleocloeon excisum	15.58 (0.02)			
Pseudocloeon sp 1	18.87 (0.01)			
Afronurus sp 1		17.84 (0.01)		
Tricorythus discolor				12.68 (0.01)
Lestagella penicillata	19.18 (0.01)	17.77 (0.01)		
Castanophlebia sp 1	13.53 (0.03)	19.78 (0.01)		14.13 (0.01)
Afronemoura amatolae	17.79 (0.01)	15.29 (0.03)	34.11 (0.01)	11.53 (0.01)
Plea sp 1	17.53 (0.01)			
Taeniochauliodes attenuatus	12.65 (0.05)			
Goerodes caffrariae	16.93 (0.01)	17.09 (0.02)	20.15 (0.01)	
Mesoceration sp 1			33.17 (0.01)	
Scirtid sp 1	17.18 (0.01)	22.67 (0.01)	16.93 (0.03)	
Pericoma sp 1	15.89 (0.01)			
Simulium albivirgulatum	17.01 (0.01)			
Simulium medusaeforme	13.59 (0.03)			
Simulium impukane		15.86 (0.03)		
Simulium rutherfoordi	16.14 (0.01)			
Simulium vorax	14.66 (0.02)			15.05 (0.01)
Number of taxa	15	7	7	5

The univariate test results suggest that while "time of day" was observed to have the highest Rao's test statistic, accounting for the most variation in drift pattern for the species assemblage sampled, reach of stream accounted for significant responses from the highest number of taxa, double that of the predictors "season" and "time of day" (Table 4.2).

Figures 4.5 and 4.6 represent plots of drift density of the species for which a significant difference was observed for comparisons within reach by time of day (Figure 4.5) and between reach, by time of day (Figure 4.6). Of the species that were examined on an individual basis, based on findings from Chapter 3, significant differences in timing of drift over a 24-h period within the fishless reach were observed for *A. amatolae*, for which drift densities were significantly higher at midnight, compared to dawn and midday (Friedman ANOVA, n = 6, df = 3, $\chi^2 = 8.80$, $P < 0.05$). Within the invaded reach, *B. harrisoni* was observed to drift at significantly higher densities at midnight compared to midday and dusk (Friedman ANOVA, n = 6, df = 3, $\chi^2 = 8.60$, $P < 0.05$), as was *A. amatolae* compared to dawn, midday and dusk (Friedman ANOVA, n = 6, df = 3, $\chi^2 = 9.88$, $P < 0.05$). Along the native fish reach, no significant difference in drift density between time of day was observed for any of the species examined, but all three species exhibited higher mean drift densities at night.

Afroptilum sudafricanum drift densities at dawn were significantly higher in the native fish reach compared to the invaded and fishless reaches (Kruskal-Wallis ANOVA by ranks, n = 18, H = 6.36, $P < 0.05$). For *B. harrisoni*, significant differences were observed in drift density at midnight (Kruskal-Wallis ANOVA by ranks, n = 18; H =6.88, $P < 0.05$), and dawn (Kruskal-Wallis ANOVA by ranks, n = 18, H = 7.27, $P < 0.05$) between the fishless and native fish reaches, and at dusk (Kruskal-Wallis ANOVA by ranks, n = 18, H = 7.27, $P < 0.05$) between the invaded and native fish reaches. In all instances, *B. harrisoni* drift densities were greatest along the native fish reaches. For *A. amatolae*, significant differences in drift density were observed between the fishless reaches and native fish reaches for dawn (Kruskal-Wallis ANOVA by ranks, n = 18,H = 9.28, $P < 0.05$), midday (Kruskal-Wallis ANOVA by ranks, n = 18, H = 9.73, $P < 0.05$), dusk (Kruskal-Wallis ANOVA by ranks, n = 18, H =8.67, $P < 0.05$) and midnight (Kruskal-Wallis ANOVA by ranks, n = 18, H =11.91, $P < 0.05$) and between the invaded and native fish reaches for midnight (Kruskal-Wallis ANOVA by ranks, n = 18, H =11.91, $P < 0.05$), and dawn (Kruskal-Wallis ANOVA by ranks, n = 18,H = 9.28, $P < 0.05$). In each instance *A. amatolae* drift density was lower along the native fish reaches. No significant difference in drift density was observed for comparison of time of day between the invaded and fishless reaches.

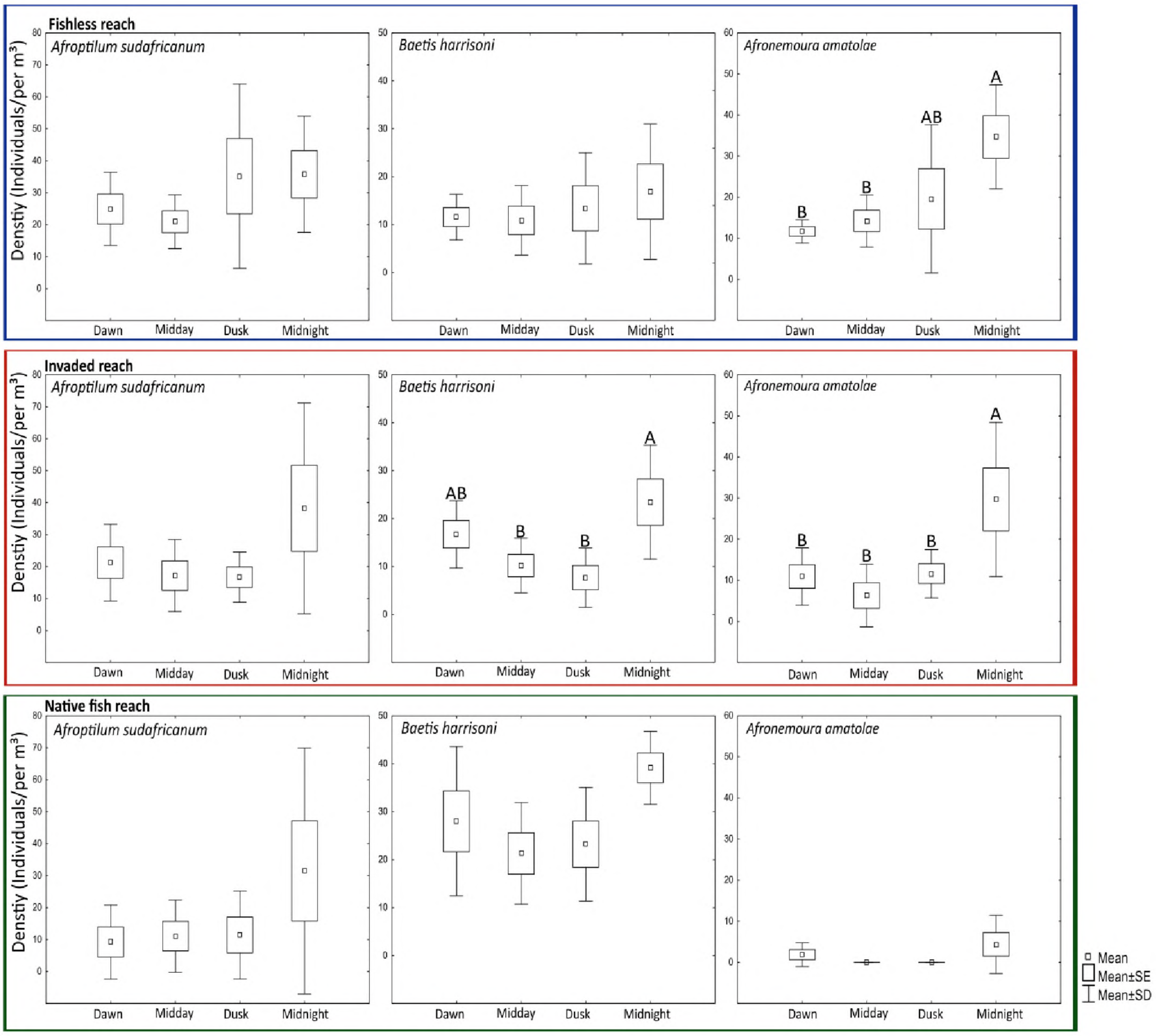

Figure 4.5: Square root transformation of drift density of individuals per m^3 of the species *Afroptilum sudafricanum*, *Baetis harrisoni* (Ephemeroptera: Baetidae) and *Afronemoura amatolae* (Plecoptera: Notonemouridae) from fishless, invaded and native fish reaches of headwater tributaries of the Keiskamma River system, Eastern Cape, South Africa. The blue border encompasses drift across time of day recorded from fishless reaches, red across invaded reaches and green across native fish reaches. Differing letters above the drift density correspond to significant differences, where this was observed only.

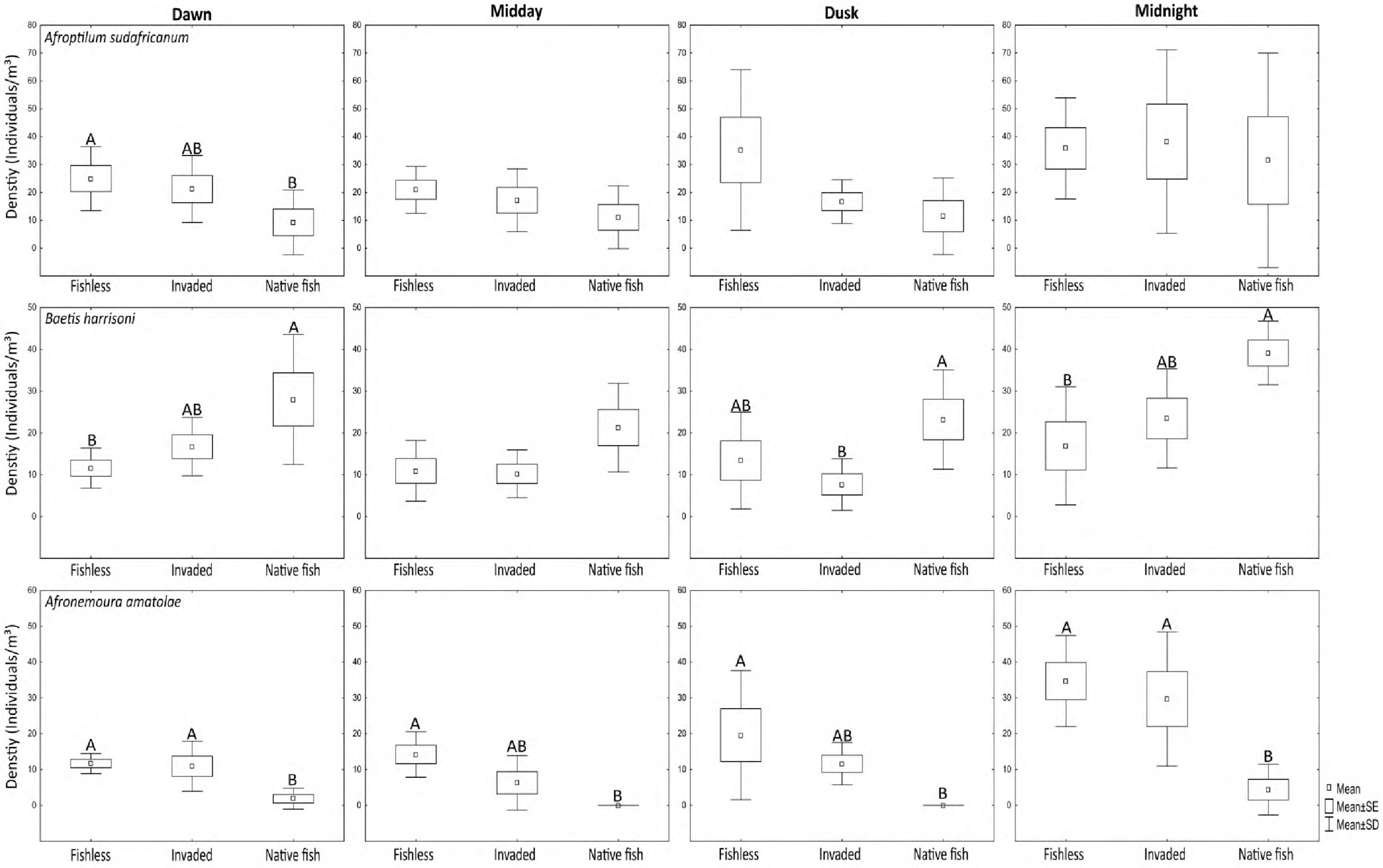

Figure 4.6: Square root transformation of drift density of individuals per m^3 of the species *Afroptilum sudafricanum*, *Baetis harrisoni* (Ephemeroptera: Baetidae) and *Afronemoura amatolae* (Plecoptera: Notonemouridae) from fishless, invaded and native fish reaches of headwater tributaries of the Keiskamma River system, Eastern Cape, South Africa. Differing letters above the drift density correspond to significant differences, where this was observed only.

4.4 Discussion

Macroinvertebrates play a pivotal role in freshwater ecosystems by facilitating and contributing to functional stream processes like decomposition, nutrient turnover and ultimately secondary production, as a link between nutrients in the stream and predatory fish (Rader 1997). Macroinvertebrate drift was sampled from fishless, invaded and native fish reaches of headwater tributaries of the Keiskamma River, to discern if patterns of influence by predatory fish were evident. The working hypothesis posed was that absolute drift density, measured as density of macroinvertebrates per volume of stream-flow, would not differ significantly between reach. However, a significant difference would be observed in the timing of drift density between fishless reaches and those containing predatory fishes.

An examination of cumulative drift for each insect order, including invertebrate rain, suggested that only one group varied significantly in drift density between reach. Therefore, the hypothesis that absolute density between reaches would not differ was tentatively accepted. Multivariate modelling analysis suggested that the predictor variables "season", "reach" and "time of day" accounted for significant amounts of variation each, in explaining observed drift patterns. This finding is corroborated for some species, and not others. In particular, the density of drifting *B. harrisoni*, *A. sudafricanum* (Ephemeroptera: Baetidae) and *A. amatolae* (Plecoptera: Notonemouridae) were suggested to differ significantly between reach. Therefore, the hypothesis that differences in drift timing would be observed was also tentatively accepted.

4.4.1 Invertebrate drift along fishless, invaded and native fish reaches of the Keiskamma River headwaters

Stream-dwelling salmonids, like *S. trutta* and *O. mykiss*, are known to feed extensively within the drift (Bachman 1984, Cada et al. 1987, Clapp & Clarck, 1990, Hill & Grossman 1993, Hayes & Jowett 1994), though feeding from the benthos is also reported (Tippets & Moyle 1978, Feltmate & Williams 1988, Bechara et al. 1993, Dahl & Greenberg 1996). Bachman (1984) reported that along a clear Pennsylvanian stream, over three years, *S. trutta* between the ages of one and eight years spent 86 % of their foraging time in a sit-and-wait search state, with the majority of their food taken from the drift and only 15% taken from the benthos. *Oncorhynchus mykiss* in an invaded Japanese streams received 49% of their annual total prey resource from terrestrial input along forested reaches, while a contribution of 53% was observed for grassland reaches, suggesting that drift-feeding is an important strategy for the salmonid predatory archetype (Kawaguchi & Nakano 2001). As the above studies suggest, studying the composition and timing of drift density may be useful in exploring for the effects of non-native predatory fish species.

Invertebrates are known to use drifting as an energetically low-cost way for locating unutilized food resources or to avoid predators (Huhta et al. 2000). An examination of the density of drift for each order of aquatic macroinvertebrate, including invertebrate rain defined as terrestrial in-fall, suggest significant differences in drift density between reach for the Plecoptera only. In this case, observed differences are almost certainly not attributable to the presence or absence of fish, but rather to an influence of the effect of sampling reach described in Chapter 2, and elucidated through multivariate analysis, suggesting "reach" as a significant predictor variable.

The most abundant stonefly, *A. amatolae* accounted for 66% of total plecopteran drift sampled, and is known to favour the uppermost headwater reaches in river of the Amatolo-Winterberg Highlands Ecoregion (Palmer et al. 1991). A similar example is the megalopteran *Taeniochauliodes attenuatus* Liu & Price, 2013 (Corydalidae), which was completely absent from the drift along the native fish reaches (Figure 4.4), corresponding to what is known from their favoured habitat (Liu et al. 2013). As discussed in Chapter 2 (Section 2.3), these taxa are characteristic of the upper reaches of the Cata, Mnyameni and Gwiligwili Rivers. Additionally, in certain a sense, the preferred instream habitat of these groups places them at greater risk of effects of non-native predatory fish, as they share very similar environmental requirements.

The abundance of terrestrial invertebrate rain may provide an important source of food for trout (Bachman 1984, Clapp & Clark 1990, Nakano et al. 1999, Kawaguchi & Nakano 2001), more so than for native cyprinids (Gaigher 1975, Cambray 1983, Jackson et al. 2016, Shelton et al. 2016). Invertebrate rain density was highest along the native fish reaches of the Cata and Mnyameni Rivers, which are not canopied like the invaded and fishless reaches (Chapter 2, Figure 2.9). This finding contrasts that of Kawaguchi & Nakano (2001) who found that invertebrate rain contributed 1.7 times more from a forested reach than form a grassland reach of the same stream. Therefore, this finding warrants further investigation. However, as no significant difference in density of invertebrate rain between reaches was observed, this suggests that while trout may utilize this resource, they do not do so at a rate that influences overall density compared to fishless reaches.

Plecopteran and ephemeropteran species both showed significant differences in drift density between sampling periods, and shifts from diurnal drift to predominantly nocturnal drift were observed for selected species *B. harrisoni* and *A. amatolae* (Figure 4.5). Shifts in drift periodicity as an antipredator response have been suggested for Ephemeroptera (Culp et al. 1991, Flecker 1992, Cowan & Peckarsky 1994, Douglas et al. 1994, Huhta 1999, 2000) and Plecoptera (Feltmate & Williams 1989, Wooster & Sih 1995), and the significant differences observed in diel drift density changes may be a predator avoidance mechanism for these taxa. However, while patterns are observable, these data represented small sample sizes, and only for specific species and groups. Further investigation using multiple nets placed across the width of the stream to increase sample size, and therefore statistical power, may facilitate stronger conclusions.

The paucity of Megaloptera drifting along the invaded reaches may also be attributable to the presence of trout; as large predatory invertebrates, they are susceptible to predation from fish (Rader 1997, Huhta 2000) while macroinvertebrate groups that are predominantly composed of smaller-sized individuals may drift aperiodically (Allan 1984, Kohler 1985). This is supported from studies by Meissner & Muotka (2006), who found significantly lower densities of larger predatory macroinvertebrates from forested streams containing trout, suggesting that *S. trutta* exert a strong size-selective effect. Similarly, Flecker & Allan (1984) suggested that fish predation was responsible for depressing species richness for the largest macroinvertebrate size classes (>8 mm), a size easily attainable for megalopteran larvae from these streams. Further study with increased sample sizes is required to verify, or refute, this observation.

Patterns of drift among the remaining groups of invertebrate (Hemiptera, Coleoptera, Trichoptera and Diptera) are attributable to their occurrence along the continuum of the stream. Most hemipteran fauna in the Keiskamma River favour slower-flowing foothill reaches, like those along the native fish reaches, which suggests why their drift densities were higher here. Palmer (1991; Palmer et al. 1994) found the macroinvertebrates of the neighbouring Buffalo River to be biotope-specific, and thus selective of where they occur along the course of a stream.

The classic study by Alan (1982) concluded that the presence or absence of trout was not a major influence on the invertebrate density or species composition of Cement Creek, USA. He speculated that there would be mixed effects for different taxa, based on their abundance, size and behaviour. Wooster & Sih (1995) reviewed the activity response that invertebrate and vertebrate predators have on prey, and deduced that vertebrate predators like trout had mixed effects on prey drift rates. The findings of this study tentatively support their findings in that changes in the timing of drift were demonstrable and significant for some species but not others. Therefore, specific groups appear to react to the presence of fish while others do not. More focussed work in the headwaters of the Keiskamma River is necessary to better account for observed patterns.

4.5 Conclusion

Studies the world over have shown significant behavioural responses by stream macroinvertebrates in the presence of salmonids (see review by Wooster & Sih 1995) and even as a response to the scent of salmonids (McIntosh & Peckarsky 2004). Drift patterns, and the temporal distribution of specific macroinvertebrate species within the drift, may be attributable to the presence of non-native trout in the headwaters of the Keiskamma River. The data presented here represents a snap-shot of drift patterns from these streams, and further study is warranted to provide more compelling evidence that the patterns observed are meaningful. The altered timing of drift density from fishless to invaded reaches by *B. harrisoni* and *A. amatolae* suggests a possible behavioural response amongst invertebrate faunas that may be due to the presence of vertebrate predators. The influence of fish needs to be teased out from the natural distribution of different species and groups along the length of the Keiskamma River, as conceptualised by the river continuum concept. Some taxa, like mayflies and stoneflies, appear to be affected by the presence of fish, while others are not. Given the data, both parts of the working hypothesis posed are tentatively accepted but further investigation is warranted. In the following chapter, I will examine food webs across each of the three treatment reaches using stable isotope data to test whether the presence of predatory fish may cause trophic interactions and diet shifts among invertebrate groups as a result of top-down pressure through predation.

Chapter 5: Trophic interactions within invaded and non-invaded reaches of the Keiskamma River headwaters, Eastern Cape, South Africa

5.1 Introduction

Freshwater and island environments are among the most damaged ecosystems on earth as a result of biological invasions (D' Antonio & Dudley 1995, Cambray 2003a, Cox & Lima 2006, Yiming et al. 2006, Strayer 2010, Sagouis et al. 2015). It has been proposed that because of their inherently insular nature, the impacts of invasions on these ecosystems are more severe (Elton 1958, Carlquist 1965, Richardson & Pyšek 2006, Cucherousset & Olden 2011). This also makes these systems good models for testing the impacts of non-native species on the recipient system (Jackson et al. 2017). Examining the trophic food-web dynamics along invaded and uninvaded reaches of stream can thus provide evidence of the impacts of non-native invasive species, and to do this, an understanding of ecological space is important.

When a novel predatory archetype is introduced into an ecosystem, its effects on the native faunas may take place through direct predator-prey links (Townsend & Crowl 1991), and indirectly through competition for resources or space (McIntosh et al. 1992). Documented cases exist of stream macroinvertebrates altering their behaviour in the presence of trout (Buria et al. 2007, Albariño & Buria 2011), which may have cascading food-web effects (Epanchin et al. 2010, Jackson et al. 2016). In the previous chapters that examined macroinvertebrate assemblages for the effects of fishes, flow and fish presence were found to be the most important correlates of macroinvertebrate assemblage structure, while time of day, season and reach of stream were significant correlates of macroinvertebrate drift. To better understand the role of fishes in structuring macroinvertebrate assemblages in streams requires an understanding of resource use of each species.

5.1.1 Stable isotope ecology

One way to represent the ecological space occupied by a species with respect to available resources, competitors and predators is the concept of a *niche*. The *n*-dimensional hypervolume, originally proposed by Hutchinson (1957) [*sensu* Wilson et al. (1987) and Blonder et al. (2014)], is used to describe an abstract niche constrained by *n* axes that represent biologically significant, independent variables within which a population of an organism may exist indefinitely. The species occupies a region within the hypervolume bounded by suitable values for each of the variables (Williams et al. 2010). These variables exist as continua within the environment, following from and providing a quantifiable "structure" to Charles Elton's definition of a species' "niche" as the sum of the interactions that link a particular species to all of the remaining species in its environment (Elton 1927, Layman et al. 2007).

Stable isotopes have taken a prominent role in ecology as the isotopic ratios within an organism's body are influenced by the trophic pathways leading to that individual (Layman et al. 2007). This means that the individual's position on the resource axes referred to in Hutchinson's (1957) niche concept and its trophic position relative to the rest of the organisms within an environment referred to in Elton's (1927) niche concept, can be estimated using stable isotope ratios (Bolnick et al. 2003, Bearhop et al. 2004). This has been formalized into the concept of an isotopic niche (Newsome et al. 2007, Jackson et al. 2011). Thus, stable isotope techniques have the ability to provide a continuous measure of trophic position that combines the assimilation of energy or mass flow through the trophic pathways leading to an organism (Post 2002, Hopkins & Ferguson 2012).

The fundamental "how" of isotopic ecology is based on the understanding that when organic molecules are used by organisms for biological process like respiration and tissue manufacture, the enzymatic pathways discriminate between isotopes (Ehleringer & Rundel 1989, Martínez del Rio & Wolf 2005), and because of the general rule that it is energetically easier to break or form bonds with molecules that are isotopically lighter, these molecules are more easily incorporated, resulting in the unreacted residue becoming relatively enriched in heavier isotopes (Martínez del Rio & Wolf 2005). A predictable change in the ratio of ^{15}N /^{14}N (expressed as $\delta^{15}N$) takes place with the assimilation of tissue in a consumer relative to its diet; typical values are a 3-4‰ enrichment (Minagawa & Wada 1984, Post 2002, Martínez del Rio & Wolf 2005), and for this reason $\delta^{15}N$ is often used as a time-integrated estimator of trophic position (Vander Zanden & Rasmussen 1999). This cannot however be done in an absolute manner without the estimation of a $\delta^{15}N$ baseline, as there is often between-ecosystem variation in organisms at the base of the food-web (Cabana & Rasmussen 1996, Anderson & Cabana 2007). The isotope enrichment of $\delta^{13}C$ and $\delta^{15}N$ are by far the most commonly used today to explore and describe food-web dynamics and trophic interactions in ecology (Bearhop et al. 2004, Martínez del Rio & Wolf 2005, Wolf et al. 2009). Carbon and nitrogen stable isotopes are thus extremely useful as they can be employed as time-integrated measures of an organism's habitat use (Ben-David et al. 1998, Hobson 1999, Harrod et al. 2005, Hobson et al. 2009), diet (Hobson et al. 1994, Grey 2006, Vinson & Budy 2008) and trophic position (Vander Zanden & Rasmussen 1999, Post 2002).

5.1.2 Stable isotopes and invasion biology

Isotopic ecology was reviewed by Newsome et al. (2007), and the use of isotopes in the field of invasion biology with respect to non-native fish invasions by Cucherousset et al. (2012). Examples of the use of stable isotopes in studying invasion biology include those of Jackson & Britton (2014), who found that three invasive species, the common carp *Cyprinus carpio* Linnaeus 1758, topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846) and signal crayfish *Pacifastacus leniusculus* (Dana 1852), occurring sympatrically in ponds in England showed niche conservatism. Though all three species are demonstrably dietary generalists (Britton et al. 2010, Jackson et al. 2012, Jackson & Britton 2013), they were revealed to avoid competition by utilizing distinct resources. Vander Zanden et al. (1999) used stable isotopes to show that the introduction of largemouth bass *Micropterus salmoides* (Lacepède 1802) into lakes in North America resulted in diet shifts of the native trout species, which moved to consuming pelagic invertebrates instead of littoral fish species that contributed bulk of their diet prior to the introduction of bass. Zengeya et al. (2011) studied the diet of three species of *Oreochromis* tilapiine cichlids through stomach content analysis and stable isotope analysis. Stomach content analysis suggested high dietary overlap, but stable isotope analysis revealed evidence of resource partitioning between the two native and one invasive species. Olsson et al. (2009) demonstrated that the invasive signal crayfish *P. leniusculus* had a greater niche width than the native noble crayfish *Astacus astacus* L. in Swedish streams, and concluded from this that niche width assessments using stable isotopes are a useful tool to explain and predict the effects of invaders at multiple scales. Kadye & Booth (2013) suggests that the African sharptooth catfish *Clarias gariepinus* Burchell, 1882, extralimital within the Amatole study area but invasive in the nearby Great Fish River system, will probably proliferate, posing a potential threat to native barb species found in the headwater tributaries. The proliferation and survival of *C. gariepinus* are attributed to a highly oportunistic diet, confirmed by stable isotope analysis.

The salmonids, in particular rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta*, are among the predatory fish most frequently introduced outside their native range, primarily for the purpose of recreational angling (Welcomme 1992, Cambray 2003b). Their introduction has resulted in impacts on the recipient environment from the genetic to the ecosystem level (Simon & Townsend 2003, Cucherousset & Olden 2011). Studies employing stable isotopes to describe their impacts on recipient eco-systems in the Yosemite National Park in the United States of America suggested that the introduction of non-native trout lead to declines in numbers of native herpetofauna (Knapp 2005). Similar studies suggested that non-native trout impinge upon native garter snake populations by usurping amphibian populations (Matthews et al. 2002); Epanchin et al. (2010) found that *O. mykiss* caused a five-fold decrease in the number of nesting passerine birds by altering a key lake-derived subsidy, compared to lakes without trout. In the Keiskamma River system, isotopes demonstrated resource competition between native riparian spiders and non-native salmonids from headwater streams (Jackson et al. 2016). Thus, the trophic subsidy component of the stream food-web of the system is well explored, but the within-stream dynamics are not.

5.1.3 Aims and hypotheses

European brown trout *S. trutta* and rainbow trout *O. mykiss* were introduced into the Keiskamma catchment more than a century ago (de Moor & Bruton 1988, McCafferty et al. 2012), and have become completely naturalized within the headwater reaches of the system and are effectively trapped as a result of impoundments constructed in the 1980s (Ellender et al. 2016). The native fish fauna is severely affected by a combination of the introduction of trout and anthropogenic driven habitat alteration; where trout persist, the two native cyprinid species *Enteromius anoplus* and *Pseudobarbus trevelyani* no longer occur (Ellender 2013).

The aims of this chapter were therefore to briefly describe and assess trophic niche structure and niche width of macroinvertebrate FFGs within the Keiskamma River headwaters to test whether these reflect top-down pressure from fish predators. To do this, stable isotope data of representative samples of all food resources and macroinvertebrates from all functional feeding groups (FFG), and vertebrate predators were used to compare food webs from fishless, invaded and native fish reaches, and to determine the trophic level occupied by each species where they occur. It must be made clear that, while the fishless reach is impermeable to non-native trout because of waterfall barriers, the native fish reach may be invaded via the surface-release outlets at the dam walls.

I hypothesized that predation by non-native trout would cause a shift in the trophic niche of macroinvertebrate species due to top-down pressure exerted through predator-prey links. I also hypothesized that both salmonid species would occupy the highest trophic level within the food-web where they occur, in keeping with their documented status as efficient invaders of headwater streams (Cambray et al. 2003b).

5.2 Materials and methods

5.2.1 Sampling procedure

Basal food resources including allochthonous vegetation, phytoplankton, detrital organic matter, algal bio-film, macroinvertebrates and fish were sampled during November 2013 from the same reaches sampled for macroinvertebrates described in Chapter 2 (Figure 5.1). Table 5.1 contains the details of resources, macroinvertebrate and fish taxa sampled for isotopic analysis. Vegetation consisted of young leaves sampled from three separate specimens of each of three plant species that were common along the stream bank of each reach. To obtain a sample of suspended phytoplankton/seston, three two-litre water samples were collected and 750 ml from each was filtered using pre-combusted GF-75 grade glass fibre filters and a hand-held vacuum pump (Post 2002). Three samples of detritus consisting of leaf litter and fine sediment were collected from depositional zones within the streambed, and individually filtered through a stacked gradation sieve system using the remaining stream water.

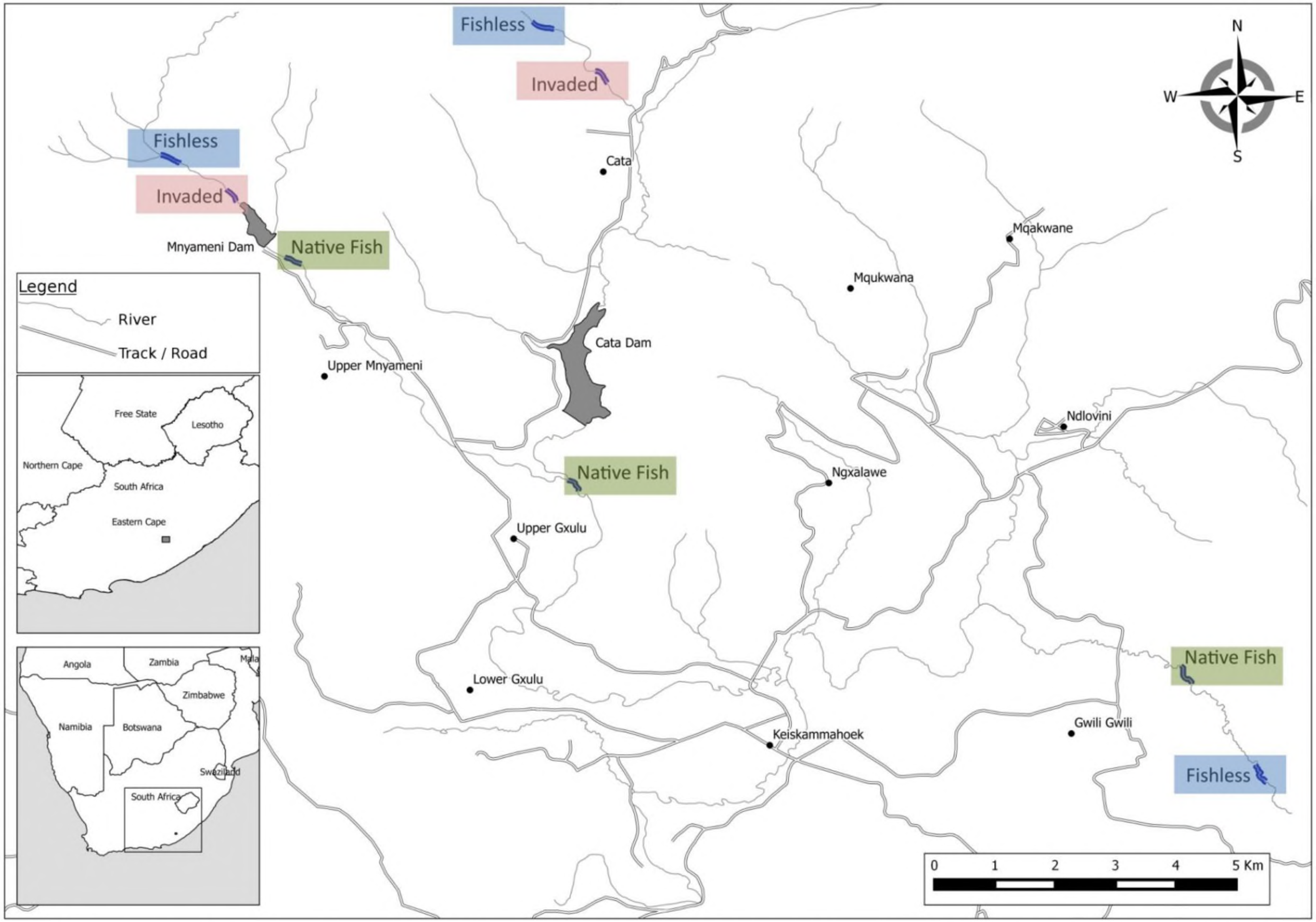

Figure 5.1: A map of the eastern tributary headwaters of the Keiskamma River system, Eastern Cape, South Africa, showing important landmarks and the position of fishless (blue), invaded (red) and native fish (green) sampling reaches of the Cata, Gwiligwili and Mnyameni Rivers.

Table 5.1: Organisms and resources collected during this study for which isotopic signatures were obtained, sorted by functional feeding group. Fish prey noted according to (Gaigher 1975, Cambray 1983, Skelton, 2001); macroinvertebrate food noted according to Palmer (1991), Appleton (2002a, 2002b), de Moor (2002, 2003), Harrison et al. (2002a, 2002b), van Hoven & Day (2002), Barber-James & Lugo-Ortiz (2003), Mansell (2003), Reavell (2003), Samways & Wilmot (2003), Stevens & Picker (2003), Biström (2007), Shepard & Lee (2007), Stals (2007). Superscripts denote sampling reach where 1 = Fishless, 2 = Invaded, 3 native fish, * = all reaches.

Family	Genera	Species	Functional feeding group	Prey/food
Cyprinidae, Pyxicephalidae	*Enteromius*^{3}, *Pseudobarbus*^{3}, *Amietia**	*E. anoplus*, *P. trevelyani*, *A. fuscigula*	Native predator	Aquatic invertebrates, terrestrial invertebrates
Salmonidae	*Salmo*^{2}, *Oncorhynchus*^{2,3}	*S. trutta*, *O. mykiss*	Non-native predator	Aquatic invertebrates, terrestrial invertebrates
Hydropsychidae, Simuliidae	*Cheumatopsyche**, *Simulium**	*C. afra*, *S. medusaeforme*, *S. rutherfoordi*, *S. vorax*	Filtering collector	Phytoplankton, drifting detritus
Pyxicephalidae, Leptophlebiidae, Baetidae, Caenidae, Chironomidae^{3}, Oligochaeta^{3}, Potamonautidae, Scirtidae^{1,2},	*Amietia*^{2,3}, *Aprionyx**, *Afroptilum*^{1,2,3}, *Baetis*^{3}, *Demoreptus*^{1,2}, *Caenis*^{1,2}, *Potamonautes**,	*A. fuscigula* (tadpoles), *A. tricuspidatus*, *A. sudafricanum*, *B. harrisoni*, *D. capensis*, *D. monticola*, *P. sidneyi*,	Gathering collector	Detritus
Aeshnidae, Athericidae, Coenagrionidae, Corydalidae, Dugesiidae, Dytiscidae, Gyrinidae, Naucoridae, Nepidae, Perlidae, Platycnemididae, Synlestidae, Tabanidae^{3}	*Aeshna**, *Atherix* sp 1*, *Pseudagrion* sp 1^{3}, *Taeniochauliodes*^{1,2}, *Dugesia**, *Berosus*^{3}, *Aulonogyrus*^{1,2}, *Orectogyrus*^{1,2}, *Dineutes*^{3}, *Laccocoris*^{3}, *Ranatra*^{3}, *Neoperla**, *Allocnemis*^{1,2}, *Chlorolestes**,	*Aeshna* sp 1, *Atherix* sp 1, *Pseudagrion* sp 1, *Taeniochauliodes attenuatus*, *Dugesia* sp 1, *Berosus* sp 1, *Aulonogyrus* sp 1, *Orectogyrus* sp 1, *Dineutes* sp 1, *Laccocoris* sp 1, *Ranatra* sp 1, *Neoperla spio*, *Allocnemis leucosticta*, *Chlorolestes tessellatus*,	Invertebrate predator	Aquatic invertebrates, terrestrial invertebrates
Blephariceridae, Ancylidae, Leptophlebiidae, Heptageniidae, Psephenidae, Teloganodidae, Tricorythidae	*Elporia**, *Burnupia*^{1,2}, *Euthraulus**, *Afronurus**, *Afrobrianax** Lestagella^{1,2}, *Tricorythus**	*Elporia* sp 1, *Burnupia* sp 1, *Euthraulus* sp 1, *Afronurus* sp 1, *Afrobrianax ferdyi*, *Lestagella penicillata*, *Tricorythus discolour*	Scraper	Algae and diatoms from substrate surfaces
Lepidostomatidae, Notonemouridae, Tipulidae	*Goerodes*^{1,2}, *Afronemoura*^{1,2}, *Tipula**	*Goerodes caffrariae*, *Afronemoura amatolae*, *Tipula* sp 1, *Tipula* sp 2	Shredder	Allochthonous vegetable material

Resources	
Resource name	Contents
Phytoplankton	Filtered river water*
FPOM	Fine particulate organic matter*
CPOM	Coarse particulate organic matter*
Algal biofilm	Film of algae scrubbed from surfaces of submerged stones*
Riparian plants	*Podocarpus falcatus**, *Polystichum pungens*[1,2], *Apodytes dimidiata*[2,3], *Pteridium aquilinum*[1,3]

Organic material collected in the sieve mesh sizes larger than 500 µm was allocated to coarse-particulate-organic-matter (CPOM) and material collected from the 75 µm sieve was allocated to fine-particulate-organic matter (FPOM). Three stones were scrubbed using a brush for one minute inside a plastic tub filled with 500 ml of water to collect algal bio-film. Care was taken to remove all clinging invertebrates before scrubbing. The three solutions were filtered in the same manner as the phytoplankton samples. The resource algal biofilm was unfortunately only represented from the Gwiligwili River, for the native fish reaches, as the vacuum pump malfunctioned before other reaches could be processed.

A long-handled water net with frame dimensions of 300 mm x 300 mm and a mesh size of 0.5 mm was used to collect macroinvertebrates from the three principle aquatic biotopes: stones-in-current (SIC), stones-out-of-current (SOC) and gravel-sand-and-mud (GSM). The GSM biotope included leaf packs found in current and leaf packs deposited in areas of slow stream flow. The invertebrates sampled were transferred into buckets and the water aerated using battery-operated compressors. The buckets, along with the water, bio-film, and detritus samples were taken to a "field laboratory" for processing. Macroinvertebrates were processed last, allowing them time to evacuate their guts. While shredders were collected from the native fish Cata and Gwiligwili reach, too few were collected to obtain a niche width measurement. Favourable habitat and resources to support shredding taxa along the native fish reaches were rare, making representatives of that FFG difficult to find (Chapter 3).

All invertebrate, fish and filter paper samples were processed and dried in the field in a portable oven at 60°C for a minimum of 48 h. Macroinvertebrate samples consisted of at least three whole individuals per sample. Carbonate-containing shells of molluscs were removed prior to drying. Samples taken from the crab *Potamonautes sidneyi* consisted of muscle tissue extracted from the legs and pincers. Where it was suspected that excess (non-dietary or environmental) carbonate may have accumulated on samples, for example leaves of vegetation collected alongside the streambed, the samples were rinsed in a weak 0.1mol HCl solution, followed by distilled water before desiccation (Bunn et al. 1995). Fish and amphibians were sampled using a backpack electrofisher (SAMUS-725GN) and euthanized by placing them in a polyurethane cool box containing water and ice until they were immobilized and were euthanized by pithing. Samples were taken from each specimen in the form of white muscle tissue from the legs or tails of amphibians, or from below the dorsal fin of fish (Pinnegar & Polunin 1999). Care was taken to ensure that no skin, slime or scales were included in the muscle sample. The fork length of each fish was recorded.

The carbon and nitrogen stable isotope analysis was conducted using a Europa Scientific 20-20 isotope ratio mass spectrometer (IRMS) interfaced to an ANCA SL elemental analyzer at IsoEnvironmental Laboratory, South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa. The data outputs were in the format of $\delta^{13}C$ and $\delta^{15}N$ isotope ratios expressed in parts per thousand, termed *per mille* (‰), which for each element is the difference between the ratio from the sample (R_{sample}) and the ratio from an international standard ($R_{standard}$), by the equation (1), where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$:

$$\delta^{13}\mathrm{C} \text{ or } \delta^{15}\mathrm{N} = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000 \tag{1}$$

5.2.2 Analyses

5.2.2.1 Functional feeding group allocations

To simplify the interpretation of the food-web results from fishless, invaded and native fish reaches, organisms were classified into functional feeding groups (FFG) prior to analysis (Table 5.1). This provides an intuitive understanding of how the food-web functions, where a taxonomic approach would require an explanation of why each taxon in the analysis is positioned where it is, relative to the other taxa and the $\delta^{13}C$ and $\delta^{15}N$ axes; a FFG approach incorporates this information and is simpler to understand (Tomanova et al. 2006, Herbst et al. 2009). FFG allocations were made using the methods described in Chapter 3, and based on an understanding of the ecology of the macroinvertebrates partly derived from Palmer (1991), who studied diets of macroinvertebrates from the neighbouring Buffalo River system.

Invertebrate predators such as the predacious diving beetles (Coleoptera: Dytiscidae), dragonfly and damselfly larvae (Odonata: Anisoptera, Zygoptera) and whirligig beetles (Coleoptera: Gyrinidae) were assigned to the group "invertebrate predators" to distinguish between secondary and primary consumers. Cape river frog, *Amietia fuscigula* (Duméril & Bibron 1841) (Anura: Pyxicephalidae) adults were classified as "native predators" and their tadpoles as gathering collectors (Sikutshwa 2015). The salmonids, *S. trutta* and *O. mykiss*, were assigned to the FFG "non-native predators". Native fish species *E. anoplus* and *P. trevelyani* were assigned to the group "native predators".

5.2.2.2 Data analysis

$\delta^{13}C$ and $\delta^{15}N$ values were plotted using Sigmaplot v12.5 (Systat Software, Inc. San Jose, USA), and convex hulls were drawn around the members of each FFG, which included filtering collectors, gathering collectors, scrapers, shredders, invertebrate predators, native predators and non-native predators using Inkscape v0.91 (Free Software Foundation, Inc. Boston, U.S.A.). This was done accurately using Inkscape's "handle-to-object-midpoint" function.

Isotope data ($\delta^{15}N$ and $\delta^{13}C$ values) for macroinvertebrate FFGs only were collated across headwater tributaries to compare the fishless, invaded and native fish reaches of the Cata, Mnyameni and Gwiligwili rivers, in the same way that Jackson et al. (2016) compared fishless and invaded reaches from the Cata, Lotheni, Mnyameni and Mooi Rivers. Differences in the size of the food-web of each reach was determined by comparing the Layman metrics (Layman et al. 2007) produced for each of the FFGs using the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011). These metrics provide a measure of niche space occupied by each FFG. The total area of the convex hull (TA) estimates the total isotopic niche space occupied by all members of the focal FFG. The TA consequently provides a meaningful estimate of the total extent of the trophic diversity of organisms, in this case guilds of species (Layman et al. 2007). To examine the average degree of trophic diversity exhibited within each assemblage, the average Euclidean distance (CD) of each FFG to the assemblage food web centroid is measured, where the centroid is described as the mean δ^{15}N and δ^{13}C value of each food web (Layman et al. 2007, Jackson et al. 2012).

The mean nearest neighbour distance (MNND) is the mean Euclidean distance from representative points in each functional group to its nearest neighbour in the isotopic bi-plot. The MNND therefore represents the overall packing density of functional groups within the food web (i.e. species packing; Layman et al. 2007). For example, a food web that exhibits a small MNND value suggests that a large proportion of the observed functional groups share similar trophic spaces, while a larger MNND value will suggest that functional groups are more divergent in their isotopic niche space (Layman et al. 2007, Martínez del Rio et al. 2009).

Lastly, the standard deviation of nearest neighbour distance (SDNND) provides a measure of the evenness of the packing of functional groups within the isoscape, whereby a low SDNND value represents a more even distribution of trophic niches and *vice versa* (Layman et al. 2007). To discern differences in the overall δ^{15}N and δ^{13}C composition of each invertebrate assemblage from different reaches (i.e. fishless vs. invaded vs. native fish), a multivariate analysis of variance (MANOVA) was employed. The MANOVA was used to test for differences in the position of each invertebrate assemblage's centroid (±SD) to each other and provides a representation of spatial niche shifts due to the distribution of fish, along the different reaches of these tributaries.

The trophic position of each individual of the introduced and native fish species was calculated from the sampling site where each of the respective species occurred, using δ^{15}N values, and equation (2), from Anderson & Cabana (2007):

$$\text{Trophic position}_{\text{consumer}} = \left[\left(\frac{\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}}{f}\right)\right] + 2 \qquad (2)$$

where $\delta^{15}\text{N}_{\text{consumer}}$ is the δ^{15}N value for the individual fish for which the trophic position is being estimated; $\delta^{15}\text{N}_{\text{baseline}}$ is the δ^{15}N of the baseline organism (discussed below); 2 represents the expected trophic level of the organism used to estimate baseline δ^{15}N (representing a primary consumer); and f is the fractionation factor that is expected between a consumer and its prey, which equates to approximately one trophic level. This factor varies considerably, though a value between 2.6‰ and 3.4‰ is commonly used (Minagawa & Wada 1984, Peterson & Fry 1987, McCutchan et al. 2003, Jackson et al. 2012). As each system is inherently different and natural variation exists, using a published value may not be accurate. Therefore, following Taylor & Soucek (2010), the correction value for $\delta^{15}N$ for each site where fish were present was calculated based on the median of all site-specific differences. This was done by calculating the average $\delta^{15}N$ between the secondary consumers (native fish at each site) and primary consumers (scrapers), and primary producers (algal biofilm, FPOM). A median (±SE) f value of 2.27 ± 0.42 was obtained. This value, while below the aforementioned range of 2.6‰ and 3.4‰, was comparable to published findings (McCutchan et al. 2003). A possible reason for this lower value is the degree of depletion of $\delta^{15}N$, which varies spatially at the bases of food-chains (Anderson & Cabana 2007).

Organisms used for baseline $\delta^{15}N$ values are usually long-lived, primary consumers, for example unionid bivalves (Vander Zanden et al. 1999, Post et al. 2000, Post 2002), but the headwaters of the Keiskamma are devoid of large, long-lived, filter-feeding molluscs and so an alternative was required. Anderson & Cabana (2007) studied the $\delta^{15}N$ fractionation of lotic food-webs to determine which organism, and its respective FFG, would provide the best $\delta^{15}N_{\text{baseline}}$ value. They found that the specialist scrapers, water penny beetles (Coleoptera: Psephenidae), had the lowest $\delta^{15}N$ values consistently as primary consumers displaying minimal omnivory and therefore best suited to use as $\delta^{15}N_{\text{baseline}}$ organisms. Where the trophic levels of each of the fish species was to be examined, average $\delta^{15}N$ for scrapers was used as this group displayed the lowest $\delta^{15}N$ values of all of the FFGs common to all sites, directly congruent with the findings of Anderson & Cabana (2007). Three statistical comparisons of trophic position were made: *S. trutta* and *O. mykiss* were compared between invaded and native fish reaches using a one-way ANOVA; data suited parametric analysis (Shapiro-Wilk's W, $P > 0.05$; Brown-Forsythe test $P = 0.187$); *O. mykiss* was compared to native fish species *E. anoplus* and *P. trevelyani* where they occurred sympatrically along the Mnyameni River native fish reach using a Kruskal-Wallis test as the test data residuals were not normally distributed (Shapiro-Wilk's W, $P < 0.05$); *E. anoplus* and *P. trevelyani* were compared when they co-occurred with *O. mykiss* and when *O. mykiss* were absent using a Kruskal-Wallis test as the test data did not meet the assumptions of ANOVA (Shapiro-Wilk's W, $P < 0.05$). Tests were carried out in Sigmaplot v12.5 (Systat Software, Inc. San Jose, USA).

5.3 Results

5.3.1 Food-web analyses

The standard $\delta^{13}C$ and $\delta^{15}N$ stable isotope bi-plot (average ± SE) and the scatter bi-plot displaying the convex hulls of each FFG (Figure 5.2) portray slightly different perspectives and emphasize different aspects of the trophic ecology of a system. Structuring of basal resources with respect to $\delta^{15}N$ and $\delta^{13}C$ axes was most similar along the fishless and invaded reaches, beginning with the riparian vegetation at the base of the food-web, followed by CPOM and above that FPOM. Relative to reaches upstream of impoundments, the native fish reaches displayed depleted $\delta^{13}C$ values for the algal biofilm, and enriched $\delta^{13}C$ values for riparian vegetation. The CPOM, FPOM and phytoplankton $\delta^{13}C$ and $\delta^{15}N$ values were comparable across all reaches (Figure 5.2).

The fishless reaches showed very little variation in available resources as the convex hulls for all of the FFGs overlapped considerably. The gathering collectors and scrapers displayed the broadest niches, suggesting a generalized diet for the macroinvertebrates of this FFG from these reaches. The shredder and filtering collector macroinvertebrates displayed narrower niches suggesting a more specialized diet. The invertebrate predator FFG was enriched in $\delta^{15}N$ compared to the remaining FFGs, including the native predators (adult *A. fuscigula*), though these were slightly more enriched in $\delta^{13}C$ (Figure 5.2).

The invaded reaches displayed almost exactly the same overall trend in trophic structuring of the food-web as the fishless reaches. The niche space occupied by the gathering collector and scraper FFGs contracted markedly along the $\delta^{13}C$ axis, as did the filtering collectors, though not as strongly. The highest position in the isospace was occupied by the non-native predator FFG, relative to the $\delta^{15}N$ axis. Similar to the fishless reaches, the native predators are the most enriched in $\delta^{13}C$, suggesting an alternative dietary source to the non-native predators along the invaded reaches (Figure 5.2). All macroinvertebrate FFGs along the native fish reaches display similar levels of $\delta^{13}C$ resource use, compared to the fishless reaches, the only exception being the shredding taxa. The native predators, *E. anoplus* and *P. trevelyani* occupy a position within the isotopic niche space above all of the remaining FFGs, with the exception of the non-native predator group. This point represents four *O. mykiss* individuals sampled from below the Mnyameni impoundment, from among the native fishes. Similar to the invaded reaches, the non-native fish occupied the highest position within the isotopic niche space.

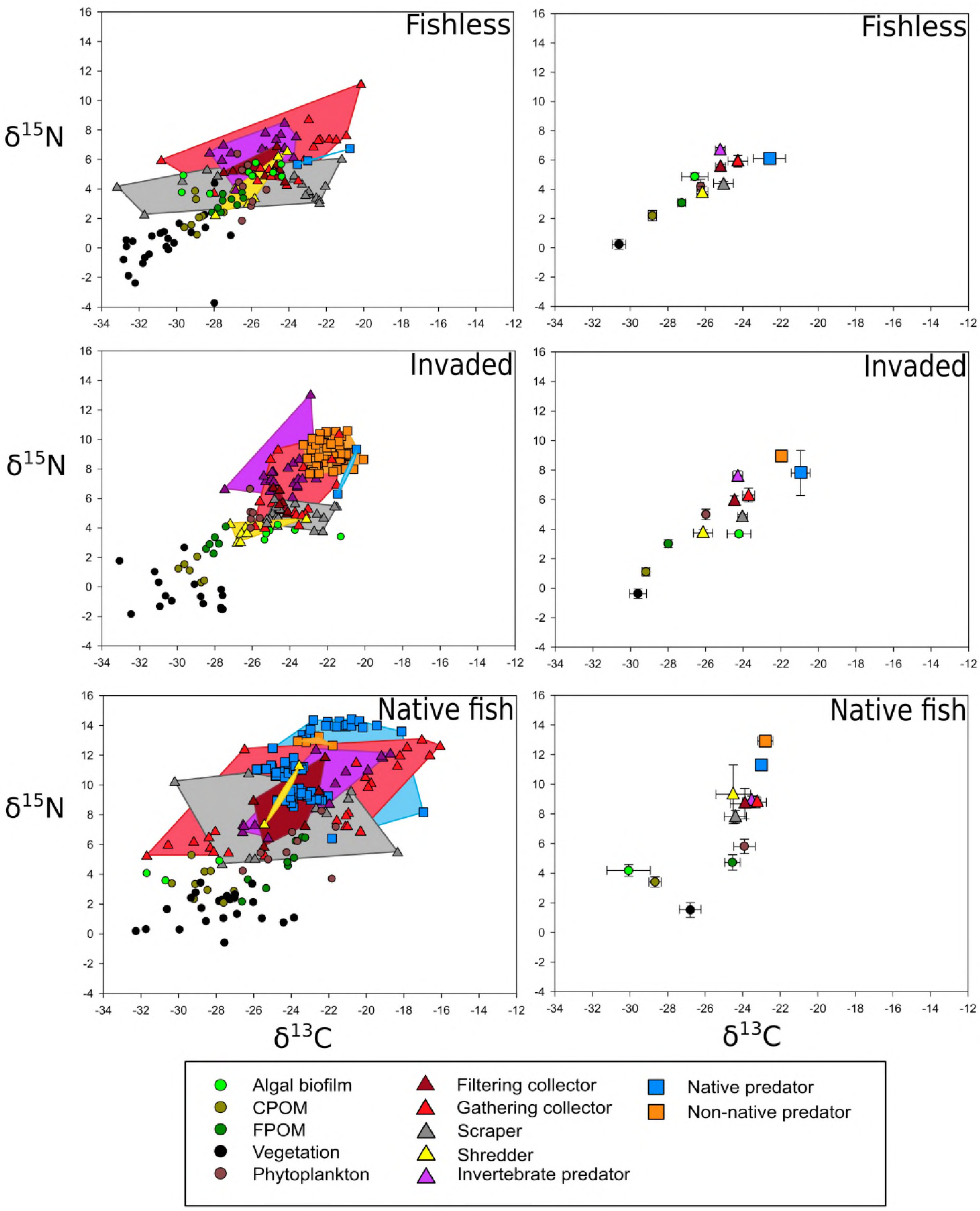

Figure 5.2: Stable isotope $\delta^{13}C$ and $\delta^{15}N$ bi-plots displaying food resources and consumers from fishless, invaded and native fish reaches of the Cata, Mnyameni and Gwiligwili rivers, Eastern Cape, South Africa. Food resources, including algal bio-film, CPOM, FPOM, vegetation and phytoplankton were plotted as dots, primary consumers as triangles and secondary consumers as squares. The convex hulls for each of the FFGs are displayed on the left, and the average isotopic value (± SE) is displayed on the right for resources and FFGs.

The SIBER ellipses (Jackson et al. 2011) generated from the macroinvertebrate FFGs for each reach are presented in Figure 5.3. As suggested from the isotopic niche plots (Figure 5.2), in particular gathering collector and scraper FFGs, a contraction in overall functional niche area is observed when comparing the fishless reaches to the invaded reaches. Macroinvertebrate FFGs of the native fish reaches display the largest functional niche area (Figure 5.3).

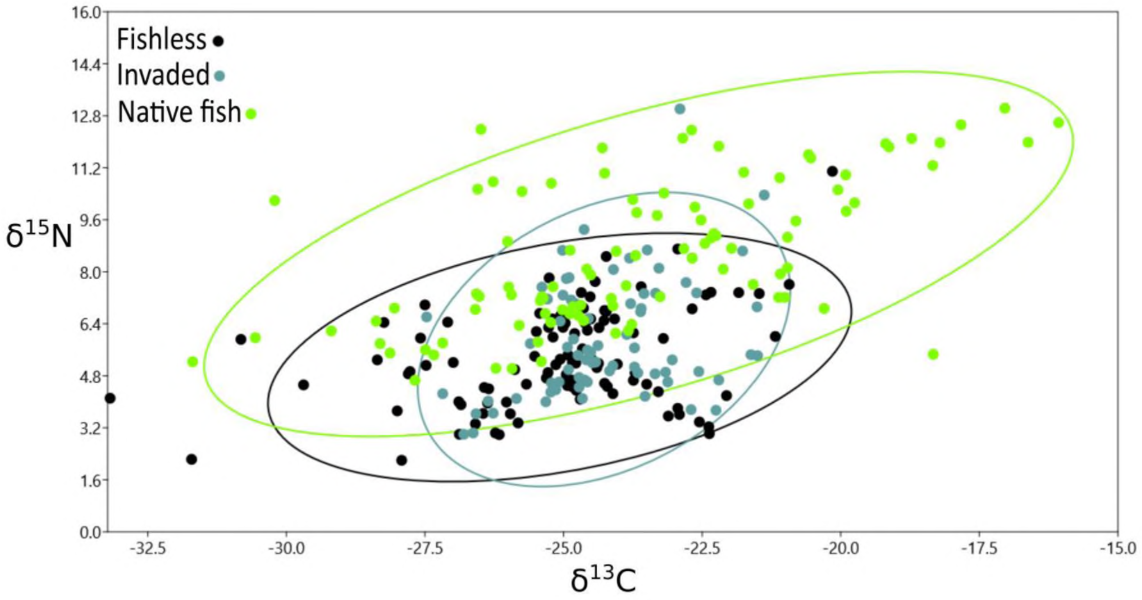

Figure 5.3: SIBER Ellipses of macroinvertebrate functional feeding groups from fishless, invaded and native fish reaches along headwater tributaries of the Keiskamma River, Eastern Cape, South Africa.

The Layman's metrics (Layman et al. 2007) calculated for each reach are presented in Table 5.1. The native fish reaches displayed the largest Total Area, and Standard Ellipse Area, followed by the fishless and invaded reaches. The Native fish reaches also displayed the lowest Average Euclidean Distance to Centroid, a measure of average trophic diversity (Layman et al. 2007), the invaded reaches the highest. The same trend followed for the remaining two measures of food-web characteristics, the Mean Nearest Neighbour Distance, a measure of overall packing density of the FFGs and the Standard Deviation of Nearest Neighbour, a measure of the evenness of the packing of the FFGs.

Table 5.1: Layman's metric (Layman et al. 2007) outputs for macroinvertebrate functional feeding groups from fishless, invaded and native fish reaches of headwater tributaries of the Keiskamma River, Eastern Cape, South Africa.

Reach	Fishless	Invaded	Native fish
Total Area	61.83	44.54	98.34
Standard Ellipse Area	9.39	7.77	17.34
Average Euclidean Distance to Centroid	1.08	1.34	0.58
Mean Nearest Neighbour Distance	1.11	1.34	0.42
Standard Deviation of Nearest Neighbour Distance	0.11	0.63	0.23

The MANOVA comparing the overall δ^{15}N and δ^{13}C of the macroinvertebrate FFGs of the fishless, invaded and native fish reaches (Table 5.2) suggests that significant differences existed between the reaches ($F = 37.07$; $P < 0.05$). The p-value obtained from univariate F-tests suggests that significant differences existed in overall niche size between the native fish reaches and the two upstream reaches, but that the difference between the fishless and invaded reaches was not significant.

Table 5.2: Results from a multivariate analysis of variance (MANOVA) comparing the centroid position for each macroinvertebrate functional feeding group between fishless, invaded and native fish reaches of headwater tributaries of the Keiskamma River, Eastern Cape, South Africa.

MANOVA	Wilks' λ	df1	df2	*F*	*P*-value
	0.61	4	538	37.07	< 0.05
Univariate *F*-tests					
Fishless reach vs. Invaded reach					> 0.05
Fishless reach vs. Native fish reach					< 0.05
Invaded reach vs. Native fish reach					< 0.05

5.3.2 Trophic level assessment of native and non-native fishes

The Mnyameni River native fish reach produced the unexpected addition of non-native trout, facilitating the testing of trophic position of native and non-native fish species under differing scenarios. The trophic levels (± 95% CI) for each fish species are provided in Figure 5.6 as three comparisons: (1) salmonids from invaded reaches along the Cata and Mnyameni Rivers, and the native fish reach along the Mnyameni River; (2) native fishes *E. anoplus* and *P. trevelyani* and non-native *O. mykiss* from the native fish reach along the Mnyameni River; (3) *E. anoplus* and *P. trevelyani* from the native fish reach along the Mnyameni River where *O. mykiss* was present and the native fish reach along the Gwiligwili River where *O. mykiss* was absent.

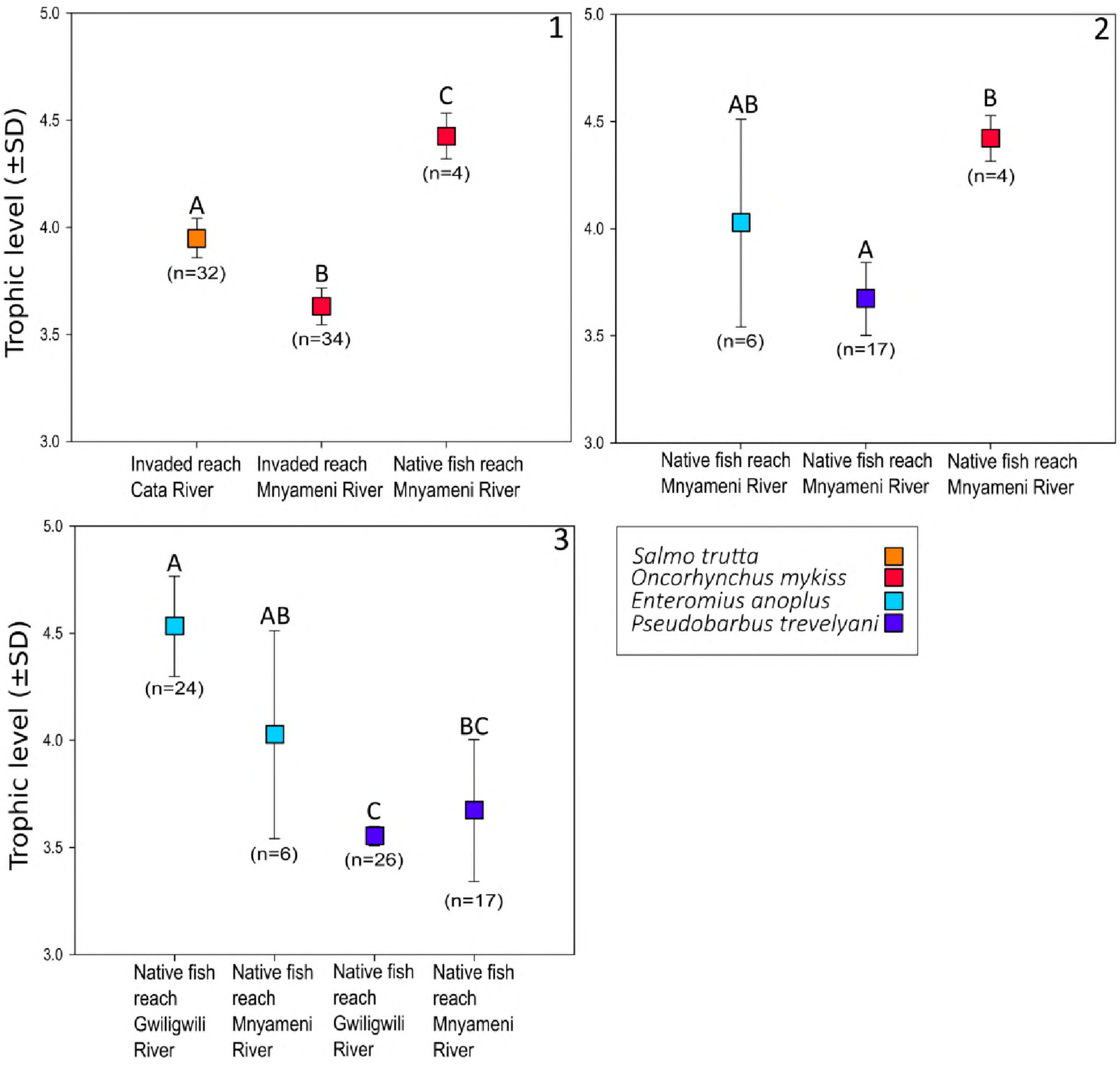

Figure 5.6: Scatter plot (±SD) showing the trophic positions of the two native fish species, chubbyhead barb *Enteromius anoplus* and Border barb *Pseudobarbus trevelyani*, and the two non-native salmonids, brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*, sampled from headwater tributary reaches of the Keiskamma River, Eastern Cape, South Africa. Plot 1: trophic position of the salmonids only; Plot 2: trophic position of *O. mykiss* and native fishes when they co-occur; Plot 3: trophic positions of the native fishes from reaches where *O. mykiss* were present and absent. Letters above the trophic correspond to significant differences in trophic level within each plot. The number of fish (n) per calculation of trophic position is given in parentheses.

Salmo trutta sampled from the invaded reach of the Cata River occupied a significantly higher trophic level than *O. mykiss* from the invaded reach of the Mnyameni River; *O. mykiss* sampled along the native fish Mnyameni reach occupied a significantly higher trophic position compared both to conspecifics from the upstream invaded reach, above the impoundment, and *S. trutta* from the invaded reach along the Cata River (Figure 5.6, (1); Table 5.3).

Table 5.3: Summarised results from an ANOVA comparing trophic position of *Salmo trutta* from the invaded reach along the Cata River and *Oncorhynchus mykiss* from the invaded and native fish reaches along the Mnyameni River, Eastern Cape, South Africa. Comparisons are illustrated in plot 1 of Figure 5.6; significant differences are highlighted in bold.

Group Name	n	Missing	Mean	Std Dev	SEM
S. trutta Cata invaded reach	32	0	3.95	0.23	0.05
O. mykiss Mnyameni River invaded reach	34	0	3.63	0.25	0.04
O. mykiss Mnyameni River native fish reach	4	0	4.42	0.11	0.05

Source of Variation	df	SS	MS	*F*	*P*
Between Groups	2	3.19	1.59	26.46	**<0.01**
Residual	67	4.05	0.06		
Total	69	7.24			

Comparisons for factor:	Diff of Means	t	*P*
O. mykiss Mnyameni Native fish vs. *O. mykiss* Mnyameni invaded	0.79	6.1	**<0.01**
S. trutta Cata invaded vs. *O. mykiss* Mnyameni invaded	0.32	5.24	**<0.01**
O. mykiss Mnyameni native fish vs. *S. trutta* Cata invaded	0.48	3.65	**<0.01**

When *O. mykiss* occurred sympatrically with native fishes along the Mnyameni River native fish reach, it occupied a significantly higher trophic level than *P. trevelyani* but not *E. anoplus* (Figure 5.6, (2); Table 5.4).

Table 5.4: Summarised results from a Kruskal Wallis test comparing trophic position of *Oncorhynchus mykiss*, *Enteromius anoplus* and *Pseudobarbus trevelyani* sampled occurring sympatrically along the native fish reach of the Mnyameni River, Eastern Cape, South Africa. Comparisons are illustrated in plot 2 of Figure 5.6.

Group	n	Missing	Median	25%	75%
O. mykiss Mnyameni River native fish reach	4	0	4.42	4.33	4.53
P. trevelyani Mnyameni River native fish reach	17	0	3.60	3.52	3.69
E. anoplus Mnyameni River native fish reach	6	0	3.83	3.66	4.54

H = 10.91 with 2 degrees of freedom, ($P < 0.01$)

Pairwise multiple comparisons:	Diff of Ranks	Q	*P* < 0.05
O. mykiss vs. *P. trevelyani*	12.51	2.837	**Yes**
O. mykiss vs. *E. anoplus*	3.92	0.764	No
E. anoplus vs. *P. trevelyani*	8.59	2.281	No

Enteromius anoplus occupied a significantly higher trophic level than *P. trevelyani* both in the absence and presence of *O. mykiss* (Figure 5.6, plot 3; Table 5.5), but no significant difference was observed in trophic level between *E. anoplus* in the presence and absence of *O. mykiss*, suggesting that predation by *O. mykiss* is not resulting in a diet shift by *E. anoplus*.

Table 5.5: Summarised results from a Kruskal Wallis test comparing trophic position of *Enteromius anoplus* and *Pseudobarbus trevelyani* sampled occurring sympatrically with *O. mykiss* along the native fish reach of the Mnyameni River, and when *O. mykiss* was absent along the Gwiligwili River, Eastern Cape, South Africa. Comparisons are illustrated in plot 3 of Figure 5.6.

Group	N	Missing	Median	25%	75%
E. anoplus Gwiligwili native fish reach	24	0	4.69	3.94	4.99
P. trevelyani Gwiligwili native fish reach	26	0	3.54	3.48	3.63
E. anoplus Mnyameni River native fish reach	6	0	3.83	3.66	4.54
P. trevelyani Mnyameni River native fish reach	17	0	3.61	3.51	3.69

H = 39.15 with 3 degrees of freedom. (*P* = <0.01)

Pairwise multiple comparisons:	Diff of Ranks	Q	*P* < 0.05
E. anoplus Gwiligwili vs. *P. trevelyani* Gwiligwili	35.5	5.91	**Yes**
E. anoplus Gwiligwili vs. *P. trevelyani* Mnyameni	28.18	4.19	**Yes**
E. anoplus Gwiligwili vs. *E. anoplus* Mnyameni	9.67	0.99	No
E. anoplus Mnyameni vs. *P. trevelyani* Gwiligwili	25.83	2.69	**Yes**
E. anoplus Mnyameni vs. *P. trevelyani* Mnyameni	18.51	1.84	No
P. trevelyani Mnyameni vs. *P. trevelyani* Gwiligwili	7.32	1.11	No

5.4 Discussion

The data presented here support only one of the proposed hypotheses, in part. The contraction of the isotopic niche space of particular FFG suggests that niche specialization has taken place along the invaded reach, where salmonids are abundant (Chapter 2), compared to upstream fishless reaches. However, this apparent contraction was not demonstrably significant. Along the invaded reaches, trout occupied the highest trophic level within the food web, supporting the understanding of them assuming the role of apex predators within systems they invade. This partly supports the second hypothesis, as *O. mykiss* sampled from the native fish reach along the Mnyameni River occupied a significantly higher trophic position than *P. trevelyani*, suggesting predation by the trout, but shared a similar trophic level to *E. anoplus*, suggesting competition between these species.

5.4.1 Food-web structure and niche width variation of macroinvertebrate functional feeding groups

The isotopic niche plots describe the food-webs of three headwater streams in the Keiskamma River system, from reaches of stream that are fishless, reaches invaded by trout, and reaches below impoundments where native fishes occurred. Among the primary consumers at these sites, the shredder and filtering collector FFGs appear to be more trophically specialised, having comparatively small isotopic niche spaces. The scrapers were more generalist in their food acquisition, though they are restricted to feeding on primary sources, based on how they fed (Palmer et al. 1993a, 1993b). The gathering collector FFG is difficult to categorize because they are selective, but not restricted in their resource use. This is evident from their large $\delta^{13}C$ and $\delta^{15}N$ ranges, suggesting omnivory in some of the taxa (Palmer et al. 1993a), particularly the river crabs, where feeding spans multiple trophic levels and a wide variety of carbon sources (Hill & O'Keeffe 1992). It should be remembered though, that specialisation or generalism in this context relates only to resource use and for the most part, not to the behaviour or functionality of the study organisms.

The contraction of isotopic niche space by the scraper and gathering collector FFGs sampled from the invaded reaches, compared to the fishless reaches, suggests niche specialization. A bottom-up approach as proposed by the river continuum concept (Vannote et al. 1980) suggests that this could be as a result of a limitation in the variety of available $\delta^{13}C$ resources, but no such variation appears to exist in the primary resources sampled from the invaded reach. The native reach showed higher variation in $\delta^{13}C$ isotopic signature, which would in turn account for larger variation at higher trophic levels. The shift from a shaded, canopied stream, as found in the fishless and invaded reaches, to a more open stream in the native fish reaches may also have contributed to the observed shift in diet by macroinvertebrates from the functional groups "gathering collectors" and "scrapers" because light is no longer limited and autochthonous carbon is more abundant as opposed to allochthonous carbon from riparian vegetation (Finlay & Kendall 2007, Evangelista et al. 2014). The effects of this shift are reflected in the lower abundance of "shredder" taxa observed along the native fish reaches in the Keiskamma system (see Chapter 3, Section 3.3.2). This does, however, not explain why the isotopic niche spaces of these two groups are as large as they are in the fishless reach.

When comparing the macroinvertebrate FFG overall trophic niche characteristics, Layman's Niche Metrics (Layman et al. 2007) support this finding, where the total niche area and standard ellipse area are largest for the native fish reaches, followed by the fishless reaches and smallest along the invaded reaches. This suggests a contraction of niche spaces of FFGs in the presence of salmonids, suggesting a top-down effect on macroinvertebrate assemblage resource use by these fishes. Interestingly, the average degree of trophic diversity of macroinvertebrate FFGs, derived from the Average Euclidean Distance to Centroid metric, is highest along the invaded reaches, which also suggests that there is a greater degree of trophic spacing between the FFGs along this reach (Layman et al. 2007). The small

Mean Nearest Neighbour Distance metric derived for the native fish reaches suggests that the macroinvertebrate FFGs share similar trophic ecology, while the largest value derived for the invaded reaches suggests that FFGs deviate more in their trophic niches, indicating a lesser degree of "species packing", and a less even distribution of FFG trophic niches, as suggested by the highest Standard Deviation of Nearest Neighbour metric (See Table 5.1; Layman et al. 2007). While these differences between invaded reaches and fishless reaches are also evident from the SIBER ellipses constructed from macroinvertebrate FFGs, and from Layman's metrics, the results of the MANOVA suggested that the differences observed were only significant between the native fish reaches and the remaining upstream reaches, and that fishless reaches and invaded reaches were not significantly different.

5.4.2 Trophic level differences between fish species

The $\delta^{15}N$ fractionation value of 2.27 was obtained following the exact procedure described in Taylor & Soucek (2010). While values within the range of 2.6-3.4 are widely used, McCutchan et al. (2003) suggest that spatial and temporal variation in $\delta^{15}N$ enrichment/depletion at the base of the food-web may either increase or decrease the fraction factor within food-webs (see also Vander Zanden & Rasmussen 1999). Using baseline-corrected $\delta^{15}N$ to calculate trophic position specific to reaches when the salmonids *S. trutta* and *O. mykiss* were present, they occupied the highest trophic level, with *S. trutta* occupying a significantly higher position than *O. mykiss*, a finding supported by literature comparing the diet and isotopic ecology of these species (Vinson & Budy 2011). While resource partitioning has been shown to occur amongst aquatic consumers (Jackson et al. 2013, Jackson & Britton 2014, Hill et al. 2015), the Keiskamma headwaters do not appear to present a case of this, where trout occurring sympatrically may have excluded native fish predators through competition for resources. Lake Trout *Salvelinus namaycush* (Walbaum, 1972) were demonstrated to occupy the highest trophic position available in the food chain in reference lakes, but when additional non-native species were invaded, they occupied lower trophic levels by shifting their diets to pelagic primary consumers (Vander Zanden et al. 1999). The data presented by Jensen et al. (2008) suggest that trout usurp native fish predators through predation rather than competitive exclusion. The evidence presented here to substantiate this suggestion is that trout collected below the impoundment in the native fish reach fed at a higher trophic level, and displayed distinct $\delta^{13}C$ and $\delta^{15}N$ signatures compared to the trout upstream in the invaded reach where the only difference in reaches was the presence and absence, respectively, of native fish species. Further exploration of this would be interesting, using mixing models, but this lies outside of the scope of this work.

5.5 Conclusion

The analyses conducted in this chapter do not suggest significant differences in the isotopic niche space of macroinvertebrate groups between fishless and invaded river reaches, the hypothesis that salmonids would cause a shift in functional isotopic niches of macroinvertebrates is rejected. The hypothesis that salmonids occupied the highest trophic level in all reaches of stream where they occur is tentatively accepted. The scenario of trout "overtopping" downstream of the impoundments and co-occurring with native barbs is an example of what may have occurred when trout where first introduced into the Keiskamma River more than a century ago (de Moor & Bruton, 1988). Barbs would have been replaced as the top predator, in the case of *E. anoplus* being the top native predator within this study, by *O. mykiss* and *S. trutta* and then driven to extinction locally through predation and competition. In the long term, *O. mykiss* and *S. trutta* cannot co-exist with the native fishes of the Keiskamma River headwaters due to the differing thermal tolerances of these species (Ellender et al. 2016). During winter when water temperatures are low enough along the native fish reaches downstream of impoundments, trout may persist and as suggested by the data, there may be competitive impacts on *E. anoplus*, but this requires further study through the use of mixing models. These impacts on native fishes have resulted in projects to exclude them from streams where they pose a threat to imperilled native fishes (Lintermans & Raadik 2003, Pham et al. 2013).

The conservation management of non-native fishes towards their removal from rivers in South Africa is still in its infancy, but where data has been collected that demonstrated that non-native fishes pose a threat to native fish populations (Woodford et al. 2005, Marr et al. 2012), national policy exists that mandates their removal if feasible (National Environmental Management: Biodiversity Act, Act No. 10 of 2004). The process is expensive, time-consuming and requires a considerable degree of manpower, but if done correctly the results are rewarding (Allendorf et al. 1997, Dunham et al. 2002, Simberloff 2003). A valid concern is the collateral effects of this process, as these measures may affect non-target native faunas (Vinson et al. 2010, Pham et al. 2013). In the following chapter, the impacts on the macroinvertebrate assemblage of removing a non-native fish predator are described, where the goal was to better facilitate the survival of an imperilled, endemic, fish assemblage.

Chapter 6: The impacts of rotenone on the macroinvertebrate assemblage along the lower reaches of the Rondegat River, Western Cape, South Africa

6.1 Introduction

Invasive predatory fish have been responsible for disproportionately high losses of aquatic diversity worldwide (Cox & Lima 2006). As a result of the negative effects of such invasions, conservation management of non-native species is seen as a priority where their continued presence and geographical expansion threaten native ecosystems (Britton et al. 2006, Vander Zanden & Olden 2008, Weyl et al. 2014). A solution to this problem is through eradication or population reduction of the introduced predator from the invaded ecosystem by mechanical or chemical means (Ling 2003, Lintermans & Raadik 2003, Rayner & Creese 2006, Blackburn et al. 2011, Pham et al. 2013).

The use of rotenone in natural systems poses a challenge to conservation managers in that it may have detrimental impacts on non-target organisms such as amphibians (Billman et al. 2011) and invertebrates (Vinson et al. 2010 Kjærstad & Arnekleiv 2011). The impacts on aquatic macroinvertebrates tend to be highly diverse and taxon-specific, making the outcomes of proposed rotenone applications difficult to predict (Finlayson et al. 2010; Vinson et al. 2010). For example, Ephemeroptera (mayflies) have been shown to be highly susceptible to rotenone (Arnekleiv et al. 2001, Booth et al. 2014, Dalu et al. 2015). For this reason, the use of rotenone as a conservation tool in natural systems needs to be weighed up against the negative effects of the target organism, so that non-target effects may be justified as "the lesser of two evils" (Marr et al. 2012, Woodford et al. 2013).

The opportunity was presented to assess the impacts of rotenone on non-target invertebrate faunas, as the local nature conservation authority in the Western Cape, CapeNature, implemented the removal of non-native smallmouth bass *Micropterus dolomieu* (Lacepède, 1802) from the Rondegat River (Marr et al. 2012, Impson et al. 2013). *Micropterus dolomieu* were considered to be invasive in the Rondegat River, with only a physical waterfall barrier preventing their further spread upstream (Woodford et al. 2005, Weyl et al. 2013). They were found to be responsible for the complete extirpation of several native fish species along the invaded reach, occurring sympatrically with large adults of only one of the five native fish species (Woodford et al. 2005; Weyl et al. 2013). Additionally, Lowe et al. (2008) demonstrated an impact by *M. dolomieu* on the invertebrate assemblage, where shifts in the invertebrate abundance were observed from specific groups.

Following the management recommendations within the unified framework of Blackburn et al. (2011), eradication of the *M. dolomieu* population was deemed feasible (Marr et al. 2012). Therefore, in accordance with standard operating procedures (Finlayson et al. 2000), rotenone treatments were carried out by CapeNature on the Rondegat River in February 2012 and a year later in early March 2013 (Slabbert et al. 2014; Weyl et al. 2014).

Rotenone is a naturally occurring isoflavone found in the seeds, stems and roots of specific plants, almost exclusively within the family Fabaceae (Finlayson et al. 2000). Rotenone affects cellular aerobic respiration, particularly within gill-breathing organisms, by preventing mitochondrial electron transport and inhibiting NADH-ubiquinone reductase (Kjærstad et al. 2016 *sensu* Singer & Ramsey 1994). It has been used as a piscicide for centuries; its earliest use as a means for fishermen to capture food fish in parts of South America where the plants that produce it occur (Finlayson et al. 2000). The compound has been used in North America as a fisheries management tool from as early as the 1930s (Haley 1978). Many examples of its use as a conservation management tool followed (Haley 1978, Vinson et al. 2010); more recent examples of its use include operations in the United States (Demong 2001), the United Kingdom (Britton & Brazier 2006), Australia (Lintermans 2000), Norway (Gladsø & Raddum 2002) and New Zealand (Pham 2013), all with the objective of improving native fish conservation status in the respective treated river and lake systems.

6.1.1 The Rondegat River

The Rondegat River is a small, perennial, second-order tributary of the Olifants River (Figures 6.1, 6.2), that drains from the Cedarberg mountains in a north-westerly direction for approximately 20 km, into the Clanwilliam Dam, a water storage reservoir on the main stem of the Olifants River (Figure 6.2). The Cedarberg forms part of the western edge of the Cape Fold Mountains, running parallel to the south-western coastline of South Africa. The topography of the mountain range is rugged, and is largely composed of depositional origin sequences characteristic of the Table Mountain Group.

The source of the Rondegat River arises in the Ou-Uitkyk Pass from three spring-fed first-order tributaries, approximately 4 km upstream of the small settlement of Algeria (Woodford 2005; Figure 6.2). The catchment of the Rondegat River is quite small considering the perennial nature of the river, spanning 111 km^2 (February 2002). The upper catchment and riparian zone of approximately the first 10 km of the river's length fall within the Cedarberg Wilderness Area, managed by CapeNature as part of the Greater Cedarberg Biodiversity Corridor, and consist almost entirely of near pristine mountain fynbos (February 2002). The remaining half of the Rondegat River traverses agricultural land, moving from the sclerophylous Fynbos biome to the succulent Karoo biome, and was characterized by dense stands of alien vegetation such as Australian black wattle *Acacia mearnsii*, blackwood *Acacia melanoxylon* and Port Jackson wattle *Acacia saligna* in places; bramble *Rubus* sp.

and gum trees *Eucalyptus* spp. were also present (Figure 6.1). By comparison, overall bank-side conditions of the treatment monitoring sites were more transformed than the control sites.

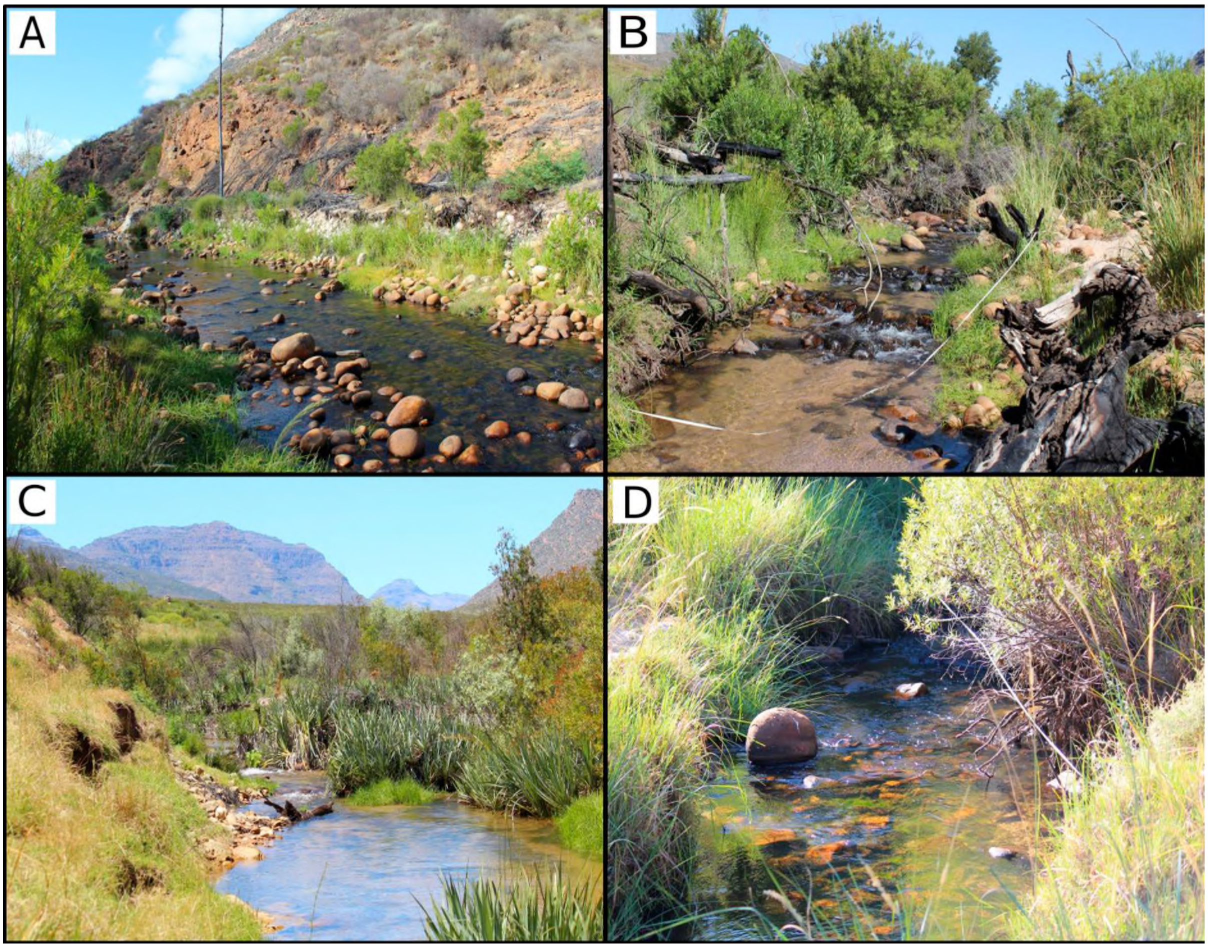

Figure 6.1: Photographs illustrating flow and riparian habitat of a selection of sampling sites along the Rondegat River, Western Cape, South Africa. (Control reach: A = C3; B = C2; Treatment reach: C = T2; D = T1; Reach codes correspond to Figure 6.2).

6.1.2 Aims and hypotheses

The aims of this study were to assess short- and long-term impact of rotenone operations on non-target invertebrate taxa. Based on preliminary observations published in Woodford et al. (2013) and from observations elsewhere (e.g. Vinson et al. 2010), it was hypothesized that there would be an immediate, catastrophic, impact on invertebrate assemblage integrity, followed by a complete recovery of the assemblage to a state comparable to the pre-treatment state.

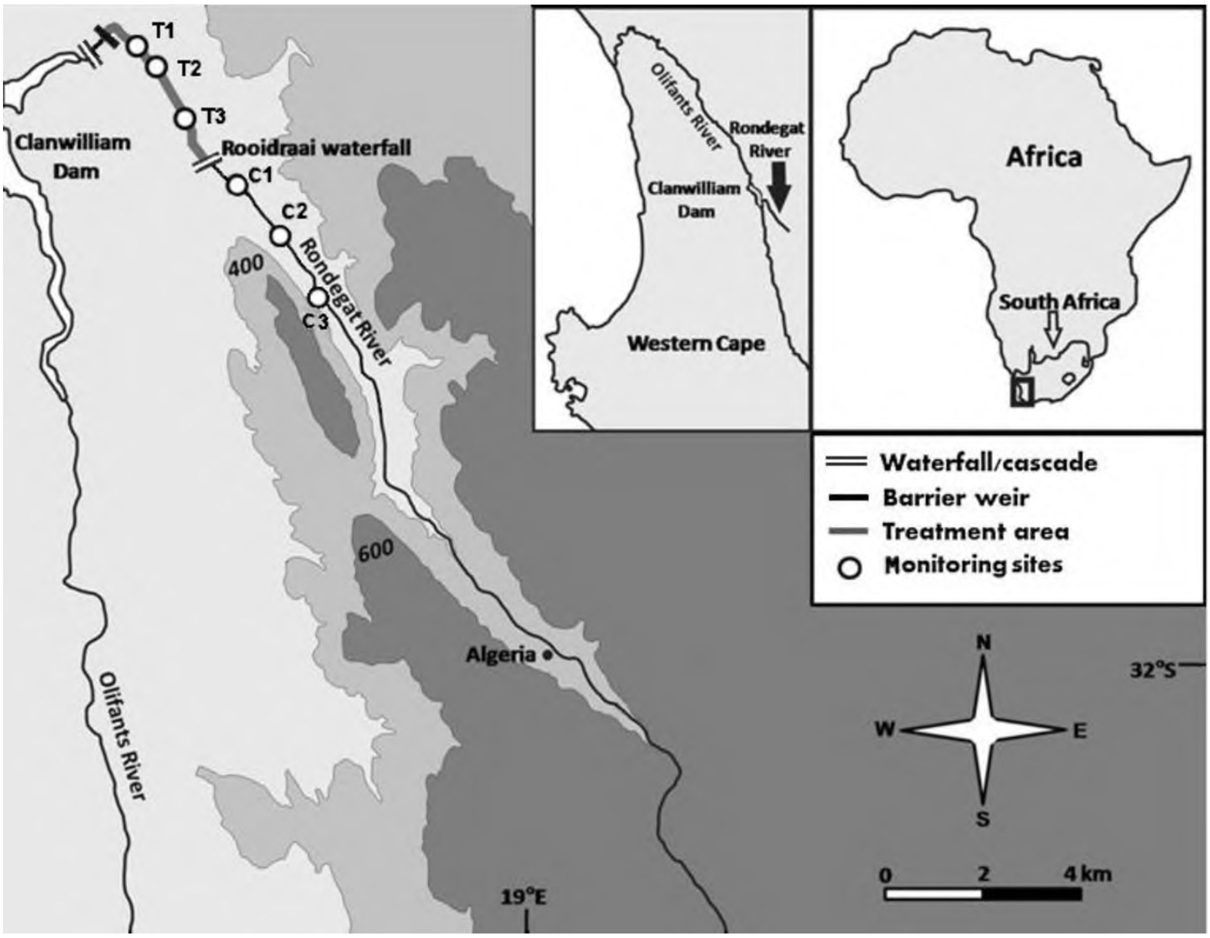

Figure 6.2: Map of the Rondegat River, Western Cape Province, South Africa, showing important landmarks altitudinal relief and the location of monitoring sites in the control (C1-C3) and treatment (T1-T3) reaches (Modified from Woodford et al. 2013).

6.2 Materials and methods

6.2.1 Monitoring site selection

After a visual assessment of the proposed reach earmarked by CapeNature for treatment with rotenone, three monitoring sites were selected within this reach ensuring that similar proportions of the necessary habitat were available for sampling of macroinvertebrate within a 20 m stretch of river (Figure 6.1). Three matching sites were selected upstream of the Rooidraai waterfall (invasion barrier), to serve as control monitoring sites (Figure 6.2). Control monitoring sites were chosen that had proportions of instream habitat similar to those of the treatment monitoring sites, to facilitate comparisons of invertebrate assemblages before, during and after rotenone treatment time period (Underwood 1994, Underwood & Chapman, 2003). Monitoring sites were located in areas that were easily accessible and, where applicable, lay at least 200 m downstream of the nearest rotenone application point, to ensure that the rotenone passing through each site was as evenly and well mixed with the river water as possible.

Table 6.1 summarises the details of monitoring and the rotenone application events. Two sampling events were aborted: heavy rains in May of 2010 prevented the sampling of the control reach due to flooding; post-treatment sampling of the control monitoring sites was not carried out in March of 2012 due to logistical and time constraints. Thus, for 2012 before and after rotenone comparisons, the control data collected in February are used.

Table 6.1: Details of monitoring events carried out during this study. The double lines represent the timing of the two applications of rotenone to the Rondegat River, Western Cape.

Year	Date	Monitoring event	Control		Treatment	
			Stones	Kick	Stones	Kick
2010	May	2 Years pre-treatment	✗	✗	✓	✓
2010	October	2 Years pre-treatment	✓	✓	✓	✓
2011	February	1 Year pre-treatment	✓	✓	✓	✓
2012	February	Pre-treatment	✓	✓	✓	✓
2012	March	Post-treatment	✗	✗	✓	✓
2012	May	3 Months post-treatment	✓	✓	✓	✓
2012	October	8 Month post-treatment	✓	✓	✓	✓
2013	February	Pre-treatment	✓	✓	✓	✓
2013	March	Post-treatment	✓	✓	✓	✓
2013	October	8 Month post-treatment	✓	✓	✓	✓
2014	March	1 Year post-treatment	✓	✓	✓	✓
2014	October	1 Year post-treatment	✓	✓	✓	✓
2015	February	2 Years post-treatment	✓	✓	✓	✓

6.2.2 Sampling procedure and techniques

The details of the rotenone treatment carried out by CapeNature are described in Jordaan & Weyl (2013) and Slabbert et al. (2014). The rotenone treatment followed standard operating procedures (Finlayson et al. 2010). Briefly, in each year rotenone was applied to the river from drip stations at a consistent rate for six hours, allowing for a steady concentration of rotenone to build up over the whole length of the treatment reach. To prevent rotenone from reaching the Clanwilliam Dam, which is an important *M. dolomieu* angling venue, rotenone was neutralized using potassium permanganate below the water abstraction weir (Figure 6.2).

Post-treatment sampling (Table 6.1) took place within two days of each treatment in both years to avoid any confounding stochastic factors attributable to seasonal variation or weather events. Monitoring of the macroinvertebrate assemblage for the effects of rotenone was performed in three ways. Quantitative sampling of invertebrates from stones was carried out to determine if a change in the number of invertebrates per surface area of substrate before and after rotenone treatment could be detected and semi-quantitative kick sampling following the SASS5 (South African Scoring System, version 5) methodology (Dickens & Graham 2002) was employed to detect responses from the aquatic macroinvertebrate assemblage to changing water quality because of rotenone.

6.2.2.1 Stone sampling

Stone sampling is a quantitative method designed to collect macroinvertebrates from the surface of stones (Wrona et al. 1986, Woodford et al. 2013). The sampling procedure undertaken followed that of Woodford et al. (2013). Briefly, four stones were sampled from each of the three sampling sites, per control and treatment reach. Invertebrates on each stone were carefully removed on site by visual inspection of the stone, and thereafter scrubbing the entire stone surface; macroinvertebrates were preserved on site using 95% ethanol and returned to the laboratory enumeration and identification.

Stones were measured across their three longest orthogonal axes to the nearest millimetre, to estimate surface area, using the equation:

$$\text{Surface area} = 1.15*(X*Y + Y*Z + X*Z) \qquad (1)$$

where X, Y and Z refer to the three longest orthogonal axes of length, width and height of stones (Graham et al. 1988). Once the surface area was obtained from equation (1), the number of invertebrates was divided by the surface area of each stone to obtain a density that could be compared across all monitoring sites.

6.2.2.2 Kick sampling

Following the SASS5 methodology prescribed by Dickens & Graham (2002) closely, three kick samples were collected at each monitoring site. Instead of returning the sample to the stream, as is standard protocol when performing SASS5 assessments, samples were preserved in 95% ethanol at a ratio of one part sample to three parts ethanol and brought back to the laboratory for detailed taxonomic analysis. Sample processing and identifications followed the same procedure described in Section 3.2.3 of Chapter 3. The SASS5 method assesses invertebrate diversity at the familial or ordinal rank and assigns sensitivity scores to each family based on an understanding of each group's sensitivity to perturbations in habitat or water quality. The river is then assessed by using its overall SASS5 score and its average score per taxon sampled (ASPT), which provide complementary indicators of the richness and sensitivity of the invertebrate community (Dickens & Graham 2002). To ensure consistent and accurate generation of SASS5 water quality scores, the SASS5 kick sampling and scoring was carried out by qualified SASS5 practitioners for each sampling event.

6.2.3 Habitat characteristics

For each monitoring event at each monitoring site, standard water quality parameters including pH, electrical conductivity, temperature and dissolved oxygen were made measured using an Aqualytic AL15 hand-held water quality meter. Depth measurements were taken along transects constructed in the same fashion, at the top, middle and bottom end of each site. Five readings were taken spaced roughly evenly apart, while the width at each point was recorded. From these data, the surface area, average width, average depth and volume of each site was quantified.

6.2.4 Analyses

The invertebrate density data sampled from stones was graphed using Microsoft Excel to illustrate fluctuations in densities of only the Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa across all sampling periods. This was done because there is evidence suggesting that different taxa respond differently to rotenone, and EPT taxa are reported to be more sensitive (Vinson et al. 2010, Woodford et al. 2013, Booth et al. 2015, Dalu et al. 2015). This allowed a visual representation to be made of the response from taxa known to be sensitive to rotenone. The number of taxa collected from kick sampling was graphed in the same way, across all sampling events.

Student’s t-test for paired samples was used to test for significant differences between EPT densities before and after rotenone treatment within the same year. To test for long-term differences between control and treatment reaches for EPT density from stones, and taxonomic richness from kick samples, two-way repeated measures ANOVA were employed using site as the subject, with reach and sampling event as grouping factors. To avoid incorporating the observed natural seasonal variation that may confound rotenone effects, data from February 2011, 2012, 2013, March 2014 and February 2015 were used to approximate a Before-After-Control-Impact (BACI) design with a one-year sampling interval (Underwood 1994, Underwood & Chapman, 2003). Only samples collected before annual rotenone applications in 2012 and 2013 were used such that the measurements were one year after potential impact. EPT density data were square-root transformed to conform to the requirements of parametric analysis (Zar 1999). Repeated measures ANOVA tests were carried out Using Sigmaplot v12.5 (Systat Software, Inc. San Jose, USA). In both instances were repeated measures ANOVA were employed, data residuals met the assumptions of parametric analysis (normality: Shapiro Wilk's Test, $P > 0.05$; equal variance: Brown-Forsythe test $P > 0.05$). Where significant differences were observed, pair-wise multiple comparisons were made using Tukey's HSD tests.

SASS5 and ASPT scores from the treatment reach were graphed using Sigmaplot v12.5 (Systat Software, Inc. San Jose, USA). Similarly, due to large amounts of seasonal variation observed for each index, SASS and ASPT scores calculated per river reach were compared within years using paired t-tests, pre- and post treatment in 2012 and 2013. F-tests for homogeneity of variance were carried out, and the variances of the scores were found to not differ significantly. The statistical package Statistica® (Version 12.0; Statsoft Ltd.) was used for these analyses.

6.3 Results

6.3.1 Habitat characteristics

The habitat and physicochemical characteristics of the control and treatment reaches of the Rondegat River are provided in Table 6.2. The two uppermost control monitoring sites were the largest on average by surface area and volume, while the lower C1 site was the smallest of all of the monitoring sites. The T3 and T2 sites were very similar in surface area and volume, while T1 was the smallest of the treatment reach monitoring sites. Readings of pH remained stable across monitoring sites, along with dissolved oxygen recordings. Electrical conductivity readings where on average lowest at the higher altitude control sites and increased progressively downstream. This is to be expected along the continuum of the river, with higher increasing inputs of solutes down its length. However, the environmental characteristics remained comparable between monitoring sites throughout the duration of the study (Table 6.2).

6.3.2 Invertebrate diversity from the Rondegat River

During 13 monitoring events across 5 years, 212 kick samples were collected from the treatment and control monitoring sites, producing 144 species of invertebrate (46 032 individuals) from 122 genera, 60 families and 14 orders (Appendix 3, Table 1). A total of 288 stone samples were collected from the treatment and control monitoring sites producing 84 species of invertebrate (32 602 individuals) from 72 genera, from 41 families and 15 Orders (Appendix 3, Table 2). Only 6 taxa were unique to the stone samples, meaning that the two sampling methods shared 79 taxa in common, and that the kick sampling method produced 68 more taxa. A combination of the sampling methods produced 152 taxa in total (Appendix 3, Tables 1,2).

A total of 127 taxa were sampled from the treatment reach prior to the rotenone dosing, and 110 taxa were sampled thereafter. From the control reach, 110 taxa were sampled before the application of rotenone, and 101 thereafter. The taxa that were not resampled from the control and treatment reaches, following the rotenone dosing, are provided in Table 6.3. These total 28 taxa from the treatment reach and 23 from the control reach respectively. Appendix 3, Table 3 contains the full list of taxa from both reaches, summed across both sampling methods, and summed across sampling event as pre- and post- treatments, to provide a detailed reference for this summary.

Table 6.2: Summary of the habitat and physico-chemical characteristics (mean ± SD) for seven sites sampled along the Rondegat River between May of 2010 and February of 2015. Parameters marked with an asterisk (*) indicate qualitative estimations.

	Control			Treatment		
Parameter	C3	C2	C1	T3	T2	T1
Altitude (meters above sea level)	234	223	220	178	170	162
	32.29389 S	32.28878 S	32.28472 S	32.27592 S	32.26623 S	32.26164 S
GPS waypoint (Decimal degrees)	18.99550 E	18.99097 E	18.98759 E	18.97453 E	18.96977 E	18.96649 E
Width (m) (n = 11)	6.84 ± 0.90	6.15 ± 0.47	3.19 ± 0.45	4.03 ± 0.76	4.40 ± 0.60	3.08 ± 0.45
Depth (m) (n = 11)	0.25 ± 0.13	0.23 ± 0.06	0.18 ± 0.07	0.35 ± 0.09	0.33 ± 0.09	0.31 ± 0.13
Length (m) (n = 11)	20	20	20	20	20	20
Surface Area (m^2) (n = 11)	136.91 ± 19.80	123.09 ± 9.42	63.76 ± 9.00	80.65 ± 15.38	87.86 ± 12.16	61.51 ± 9.11
Site volume (m^3) (n = 11)	36.44 ± 24.58	28.30 ± 8.70	11.61 ± 5.21	29.31 ± 13.02	28.83 ± 8.62	19.87 ± 10.64
Cobbles* (%)	50	45	20	17	22	10
Leaf litter* (%)	5	13	5	13	9	15
Bedrock* (%)	7	22	4	32	2	15
Sand/Gravel* (%)	35	18	65	30	64	45
Mud* (%)	3	2	6	8	3	15
Pool* (%)	15	15	70	55	20	35
Riffle/Run* (%)	85	85	30	45	80	65
pH (n = 9)	6.32 ± 0.54	6.22 ± 0.48	6.02 ± 0.37	7.77 ± 4.04	6.38 ± 1.56	6.06 ± 1.51
Temperature (°C) (n = 11)	19.23 ± 3.32	19.79 ± 3.47	23.88 ± 4.36	19.78 ± 3.04	20.60 ± 2.93	22.15 ± 3.63
Oxygen concentration (mg l^{-1}) (n = 11)	10.89 ± 3.69	10.31 ± 3.31	12.73 ± 8.30	10.11 ± 3.61	11.66 ± 7.83	9.62 ± 3.37
Conductivity (µS cm^{-1}) (n = 11)	44.3 ± 12.67	47.15 ± 11.83	43.97 ± 12.01	48.76 ± 13.11	45.56 ± 21.10	55.76 ± 16.42

Table 6.3 provides that names of taxa that were sampled from the control and treatment reaches prior to the application of rotenone and thereafter were not resampled, i.e. taxa whose absence may be attributable to the application of rotenone.

Table 6.3: Taxa identified from stone and kick samples that were collected from the control and treatment reaches of the Rondegat River, Western Cape, South Africa. These taxa were sampled from each reach prior to the application of rotenone, whereafter they were not detected again through continued sampling. Morphospecies allocations correspond to those provided in Appendix 3, Tables 1 and 2, accompanied by the higher rank classification of each taxon. The number of specimens of each taxon is provided in parentheses.

Control reach		Treatment reach	
Burnupia sp 1(1)	*Nychia limpida* (1)	*Burnupia* sp 1(7)	*Sharphydrus* sp 1 (1)
Daphnia sp 1 (16)	Larval dytiscid sp 1 (8)	*Demolinia crassi* (10)	*Helminthopsis* sp 1 (4)
Bugelisia sp 1 (3)	*Laccophilus* sp 1 (3)	*Glossidion* sp 1 (1)	*Microdinodes* sp 1 (2)
Nadinetella sp 1 (2)	*Helminthopsis* sp 1 (9)	*Lestagella penicillata* (1)	*Dineutes* sp 1 (2)
Lithogloea sp 1(1)	*Dineutes* sp 1 (1)	*Platycypha* sp 1 (2)	*Haliplidus* sp 1 (1)
Ictinogomphus sp 1 (1)	*Parhydraena* sp 1 (1)	*Ellatoneura* sp 1 (1)	*Discozantaena* sp 1 (3)
Aphanicercopsis sp 1 (16)	*Discozantaena* sp 1 (1)	*Mesovelia* sp 1 (1)	Aulacochthebius sp 1 (1)
Limnogonus sp 1(2)	*Berosus* sp 1 (8)	*Anisops* sp 1 (2)	*Parhydraena* sp 1 (3)
Trepobates sp 1 (1)	*Simulium bequaerti* (15)	*Barbarochthon* sp 1 (1)	*Prosthetops* sp 1 (2)
Hydrometra sp 1 (1)	*Gonomyia* sp 1 (4)	Pyralid sp 1 (1)	*Elporia* sp1 (3)
Microvelia sp 1 (1)		*Strina* sp 1 (1)	*Simulium bequaerti* (8)
Laccotrephes sp 1 (1)		*Laccophilus* sp 1(1)	*Simulium ruficorne* (2)
Borborophilus afzelii (1)		*Uvarus* sp 1 (1)	*Tipula* sp 1 (1)
Anisops sp 1(4)		*Yola* sp 1 (1)	

Figure 6.3 shows the mean number of taxa collected through kick sampling during each monitoring event across the five-year sampling period, for each reach. The average number of taxa collected varied considerably between 2010 and 2015. Both the treatment and control reaches follow similar fluctuations, suggesting comparable seasonal effects along the length of both reaches (Figure 6.3).

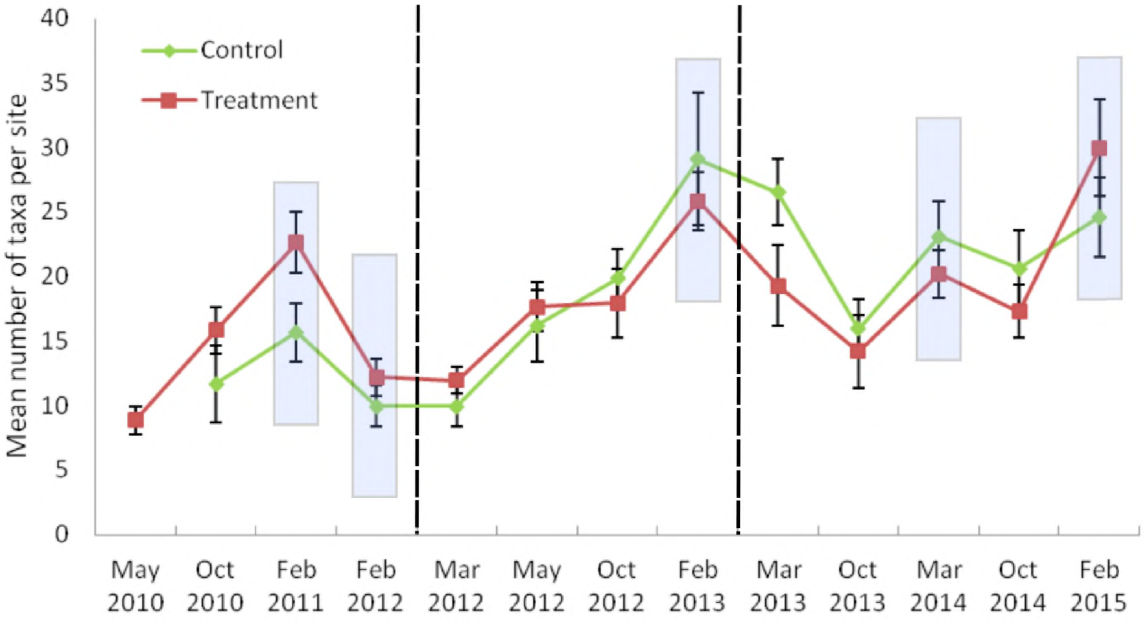

Figure 6.3: Mean number of taxa (±SD) collected from the kick samples per monitoring event for the control and treatment monitoring reaches of the Rondegat River, Western Cape, South Africa. The dashed lines represent rotenone doses in 2012 and 2013; the shaded boxes overlaying specific treatment events represent the comparisons made using a two-way repeated measures ANOVA. For both reaches n = 3 per monitoring event.

Table 6.4 contains the summary statistics from the two-way repeated measures ANOVA carried out comparing the number of taxa in each year across the five-year sampling period. This comparison is illustrated by the shaded boxes in Figure 6.3 above. No significant difference was observed between reach after allowing for the effects of differences in Sampling event (Repeated measures ANOVA; $P > 0.05$), similarly, no significant difference in interaction was observed between sampling event and reach; however, significant differences were observed between sampling events.

Table 6.4: Two-way repeated-measures ANOVA results comparing the number of taxa sampled from semi-quantitative kick samples collected from the control and treatment reaches of the Rondegat River, Western Cape, South Africa. The comparison made corresponds to the blocks in Figure 6.3. Significant differences are highlighted in bold.

Source of variation	df	SS	MS	*F*	*P*
Reach	1	50.7	50.7	1.54	> 0.05
Site(Reach)	4	131.6	32.9		
Sampling event	4	3390.2	847.55	18.35	**<0.01**
Reach x Sampling event	4	345.13	86.28	1.87	> 0.05
Residual	16	739.07	46.19		
Total	29	4656.7	160.58		

Tukey's post-hoc tests suggested significant differences between several comparison of sampling event, but the only meaningful comparison found to be significantly different, i.e. following the sequence of rotenone dosing, was between February 2012 and February 2013. A significant increase in taxon richness was observed, following the 2012 rotenone treatment.

Table 6.5: Multiple comparison tests generated using Tukey's post-hoc tests following a repeated measures ANOVA comparing taxon richness between treatment and control reaches of the Rondegat River, Western Cape, South Africa. The comparisons are represented by boxes around the sampling events, spaced one year apart, in Figure 6.3. Significant differences are highlighted in bold.

Comparison	Diff of means	p	q	*P*
Feb 2011 vs. Feb 2012	11.5	5	4.15	> 0.05
Feb 2012 vs. Feb 2013	27.17	5	9.79	**< 0.01**
Feb 2013 vs. March 2014	7.5	5	2.70	> 0.05
Feb 2014 vs. March 2015	9.0	5	3.24	> 0.05
Feb 2015 vs. Feb 2012	28.67	5	10.33	**< 0.01**
Feb 2015 vs. Feb 2011	17.17	5	6.19	**< 0.01**
Feb 2015 vs. Feb 2013	1.5	5	0.54	> 0.05
Feb 2013 vs. Feb 2011	15.67	5	5.65	**< 0.01**
Mar 2014 vs. Feb 2012	19.67	5	7.09	**< 0.01**
Mar 2014 vs. Feb 2011	8.17	5	2.94	> 0.05

6.3.3 Quantitative measurements of the response to rotenone

A short-term immediate response from the EPT taxon density to rotenone treatment is represented in Figure 6.4. Rotenone treatment significantly altered the EPT densities in 2012 (Paired t-test, $t = 4.11$; $df = 11$; $P < 0.05$) and 2013 (Paired t-test, $t = 6.85$; $df = 11$; $P < 0.05$). No significant difference was observed between 2012 and 2013 pre-treatment samples, or between 2012 and 2013 post-treatment samples (Figure 6.4).

Measurements made of the densities of EPT taxa from stones from both reaches over the entire monitoring period are presented in Figure 6.5 below. As was observed from the plots of the average number of taxa sampled from kick samples (Figure 6.3), there was considerable variation in the densities of EPT taxa collected from stones over the monitoring period.

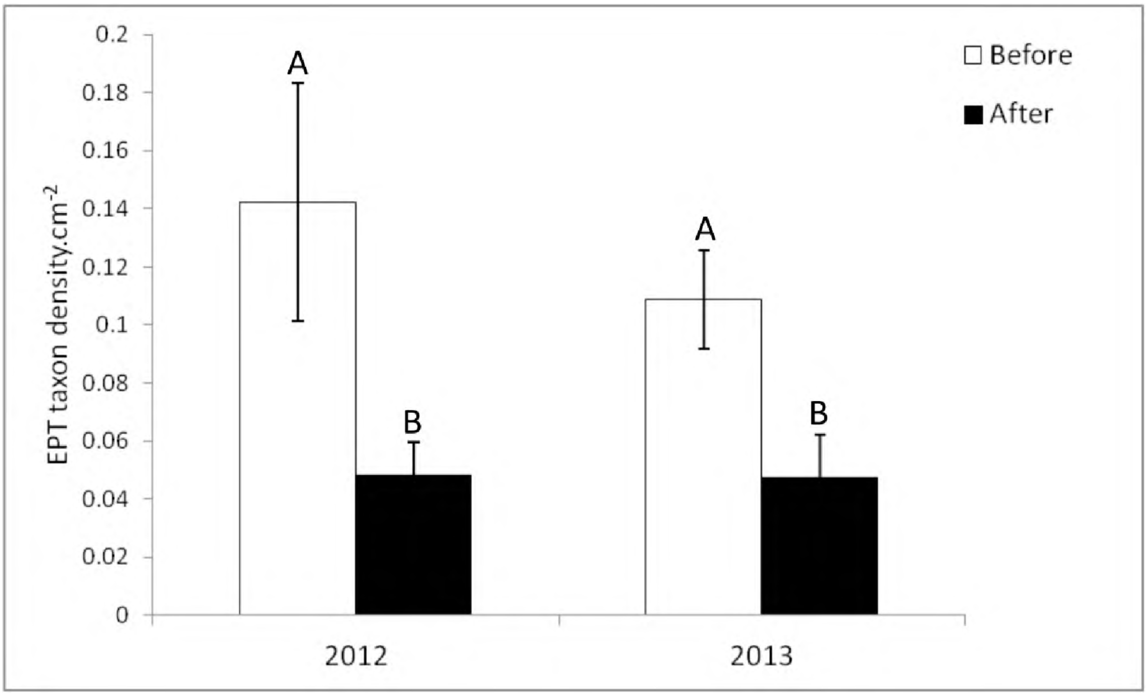

Figure 6.4: Mean (±SE) densities of taxa belonging to the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) on stones collected 1 week before, and 2 days after rotenone treatment in 2012 and 2013. Differing letters above bars indicate significant difference in EPT taxa density obtained from t-tests ($P < 0.05$), attributable to the application of rotenone. Differing letters above EPT density correspond to significant differences.

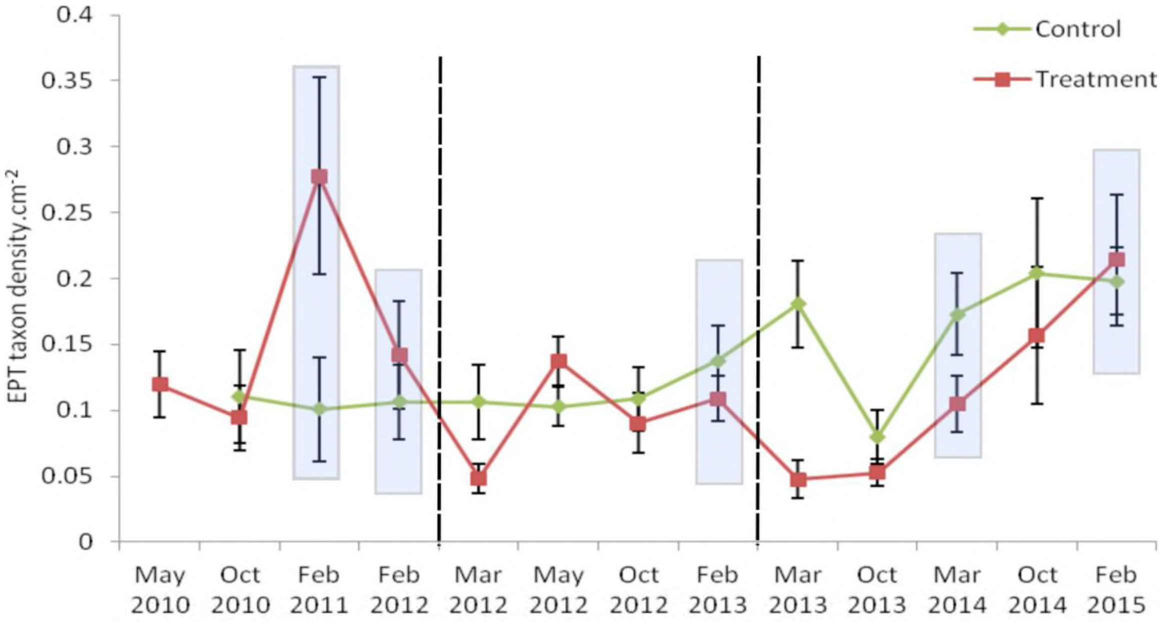

Figure 6.5: Mean (±SE) density of ephemeropteran, plecopteran and trichopteran (EPT) taxa sampled per cm^2 of stone surface from the control and treatment reaches over 13 monitoring events along the Rondegat River, Western Cape. The dotted lines indicate the applications of rotenone in 2012 and 2013.

Table 6.5 contains the summary statistics from the two-way repeated measures ANOVA comparing the number of taxa in each year across the five-year sampling period. This comparison is illustrated by the shaded boxes in Figure 6.3 above. The mean values among levels of site and event suggest that no significant difference was observed for comparisons between treatment and control reaches (Repeated measures ANOVA, $P < 0.05$; Table 6.5); differences observed may be due to random sampling variability after allowing for the effects of differences in sampling event and sampling reach. Thus, in consecutive years from the same time period, the density of EPT taxa from stones was not significantly different between the control and treatment reaches.

Table 6.5: Table of the results of a two-way repeated-measures ANOVA comparing density of ephemeropteran, plecopteran and trichopteran (EPT) taxa sampled per cm^2 of stone surface between the control and treatment reaches of the Rondegat River, Western Cape, South Africa. The comparison was made for five sampling events, and corresponds to the shaded blocks in Figure 6.3

Source of Variation	df	SS	MS	*F*	*P*
Reach	1	0.01	0.01	0.29	> 0.05
Site (Reach)	4	0.07	0.01		
Sampling event	4	0.04	0.01	1.29	> 0.05
Reach x Sampling event	4	0.05	0.01	1.90	> 0.05
Residual	16	0.11	0.01		
Total	29	0.27	0.01		

6.3.4 Semi-quantitative measurements of the response to rotenone

Both the control and treatment reaches were characterized by large amounts variation in SASS5 and ASPT scores over the five year monitoring period, attributable to seasonal variation similar to what was observed for the kick-sample taxon richness and stone-sample EPT densities (Figures 6.3& 6.5). Therefore, only comparisons within year for each treatment year (2012, 2013) are presented, to assess short term effects of rotenone. Tests for significant difference before and after rotenone treatment in 2012 for SASS5 scores (Paired t-test, $t = 23.00$; df = 2; n = 3; $P < 0.05$) and ASPT scores ($t = 11.06$; df = 2; n = 3; $P < 0.05$) suggested the scores were significantly higher before rotenone treatment. However, no significant difference was observed when comparing water quality indices before and after rotenone treatment in 2013 (Figure 6.6).

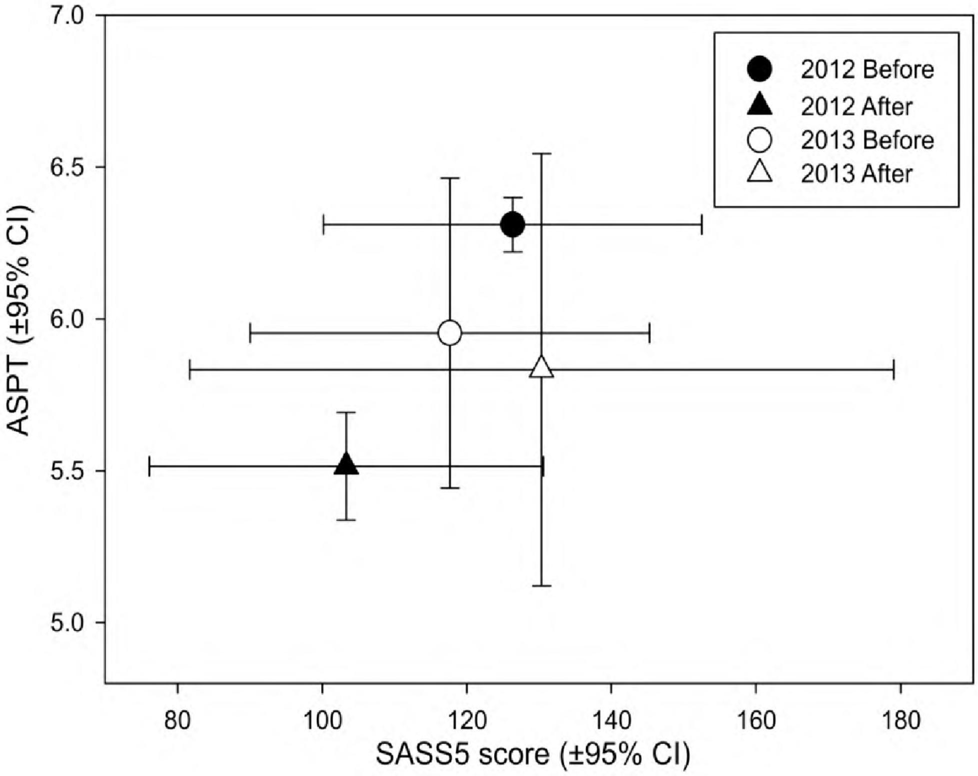

Figure 6.6: Mean (±95% confidence interval) total SASS5 score and average score per taxon (ASPT) for the treatment reach before and after rotenone dosing in 2012 and 2013.

6.4 Discussion

The main findings of this study were that macroinvertebrate densities sampled from stones fluctuated considerably throughout the five year monitoring period; similarly, the mean number of taxa collected using the semi-quantitative kick sampling method also varied widely, from both reaches sampled. Within the overall trends of seasonal fluctuation, comparisons between the control and treatment reaches facilitated the assessment of the effects of the rotenone treatment. Were significant declines in density, or diversity, observed from the treatment reach, without a corresponding decline from the control reach, it could be argued that the application of rotenone was responsible. Therefore, the hypothesis that rotenone would have a short-term negative effect on the density and diversity of the macroinvertebrate assemblage was supported by the short-term data only. Significant declines in densities of macroinvertebrates from stones following both treatments were observed, accompanied by significant declines in SASS5 and ASPT water quality index values after the 2012 treatment only. This suggests that the working hypothesis proposed may be accepted. However, the only significant difference was an increase in the number of taxa between February of 2012 and February of the following year. Therefore, no significant long-term decline of the macroinvertebrate assemblage was observed, the observed increase having occurred in both reaches (Figure 6.3) is suggested to represent seasonal fluctuation within the data. However, a short term impact of the rotenone treatment was detected using the methods employed, within these fluctuations.

6.4.1 Quantitative assessment for responses to rotenone

Because of their ubiquitous nature and sensitivity to environmental perturbations, the use of macroinvertebrates to detect changes in water quality is internationally accepted practice (Wright et al. 1984; Reynoldson & Metcalfe-Smith 1992; Resh & Jackson 1993; Chessman 1995; Alba-Tercedor 2002; Ollis et al. 2006). Within the diverse array of invertebrates found in lotic environments, certain taxa are more susceptible to water quality changes than others, particularly where rotenone is concerned (Arnekleiv et al. 2001; Lintermans & Raadik 2003, Booth et al. 2014, Dalu et al. 2015). Ephemeroptera are widely used as indicator taxa due to their consistently high sensitivity to water quality, and they are known to be highly susceptible to rotenone (Vinson et al. 2010), while the Plecoptera and Trichoptera carry among the highest sensitivities scores in the SASS5 scoring system because of their responsiveness to poor water quality (Dickens & Graham 2002).

Having analysed data from monitoring events examining the macroinvertebrate assemblage for five years which included sampling for two years prior to, for a year during and two years following the treatment, provides an important sense of scale as to how the density and species richness of invertebrates fluctuates seasonally and inter-annually. The significant decline in the density of EPT taxa for both rotenone treatments indicated that both doses had an immediate and detectable effect on these groups. However, no significant difference in taxon richness and EPT density were observed over the long term by paired comparisons between treated and untreated reaches. This suggests that no long term detrimental effect of rotenone was detectable using these methods. Furthermore, the data suggest that the natural variation within the system varies in amplitude to a greater degree than the observed effect of rotenone. In other words, changes in the densities of invertebrates occurred naturally and seasonally, and to a greater degree than the effect of the rotenone application. In addition, the macroinvertebrate assemblage, through measurements of density and taxon richness, returned to a state comparable to what was observed in the years prior to the application of rotenone along the treatment reach. This was noted to have taken place within months of both treatments.

6.4.2 Semi-quantitative assessment for responses to rotenone

Given the high levels of diversity and endemicity within the CFE (Wishart & Day 2002, de Moor & Day 2013), the use of the piscicide rotenone as a conservation tool was accompanied by valid concerns of the loss of sensitive taxa from the Rondegat River. However, given the extremely low abundance and frequency of occurrence (often only as singletons), the species that were "lost" from the treatment reach, after each rotenone application, may all be considered either rare or incidental within the lower reaches of the Rondegat River (Table 6.3). Furthermore, a similar number of species were "lost" from the control reach during the same period of time, where no rotenone was applied.

This suggests that these "losses” may be an artefact of sampling effort, given that the numbers of individuals of each species were less than 20 in all instances, out of over 70 000 insect specimens.

The significant decline in SASS5 and ASPT scores following the 2012 dose was accompanied by significant declines in density of EPT taxa in both years. This suggests that rotenone had a significant short-term effect on the macroinvertebrate assemblage of the treatment reach. However, with no significant difference observed by comparison of EPT density between the control and treatment reaches, no long-term negative effect was observed. These results do suggest that both SASS5 and stone sampling may be useful in detecting water quality perturbations associated with piscicide treatments.

6.5 Conclusion

At least 624 species of fish have been introduced into water bodies outside of their native distributional ranges (Gozlan 2008). In a relatively small number of instances, these introductions lead to damaging ecological consequences for the recipient systems, and management of the non-native fish is necessary (Britton et al. 2011). Where management interventions using rotenone for conservation purposes are implemented, impacts on non-target taxa are a concern due to those that rotenone can have (Mangum & Madrigal 1999, Woodford et al. 2013). Despite this, there are few field assessments of the long-term responses of invertebrate communities to rotenone treatments (Vinson et al. 2010).

The monitoring of invertebrate densities on stones to quantitatively assess the impacts of rotenone applications was successful in demonstrating an immediate short term impact on the biologically sensitive members of the assemblage. As per standard operating procedure (Finlayson et al. 2000), the first treatment carried out was suggested to be detrimental through SASS5 rapid bioassessment only. While SASS5 is useful as a cost-effective technique for assessing the integrity of riverine ecological health (Bonada et al. 2006), it is suggested that detailed biological surveys should be carried out when assessing the efficacy and collateral conservation impacts of river rehabilitation using a piscicide like rotenone. That these changes were detectable using SASS5 suggests that it is a good generalised method for long-term monitoring of river restoration programmes, though the monitoring of species-specific changes in assemblage structure is still needed to confidently assess impacts of these activities (McGeoch 1998, Samways et al. 2011, Woodford et al. 2013, Bellingan et al. 2015).

Chapter 7: General discussion and conclusion

7.1 Introduction

This thesis had two explicit objectives. First, it tests if macroinvertebrate assemblages from invaded and native fish reaches responded to the presence of predatory fish, using fishless reaches as a basis for comparison. This was undertaken by making a detailed taxonomic and functional assessment of the diversity and density of macroinvertebrates along these reaches. Measurements of behavioural aspects and food-web interactions under the differing fish predation regimes were also made, while testing specific hypotheses relating to each, based on an understanding of streams elsewhere around the world. Secondly, through a focussed assessment, the opportunity to study the impacts of rotenone on the macroinvertebrate assemblage of the Rondegat River was taken. This was done to further understanding of non-target effects of a commonly used conservation measure in remediating invasions by non-native fishes.

In this chapter I provide an overview of the main findings of the previous chapters and discuss these in a conservation context. In this regard, recommendations are made for the benefit of the macroinvertebrate assemblages from these respective rivers, particular with regard to future non-native fish removal operations. Recommendations for future research are also made.

7.1.1 Overview of non-native fish and aquatic macroinvertebrate impacts

In a southern African context, non-native predatory fishes like the centrarchids smallmouth bass *Micropterus dolomieu* and largemouth bass *Micropterus salmoides*, and the salmonids brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*, have been suggested to negatively affect native fish species were they have been introduced locally (Hamman & Jordaan 1988, Cambray 2003a, 2003b, Woodford et al. 2005, Lowe et al. 2008, Shelton et al. 2008, Clark et al. 2009, Weyl et al. 2010, Ellender et al. 2011, Ellender & Weyl 2014, Magoro et al. 2015, Shelton et al. 2015a, 2015b, 2016) and elsewhere in the world (Vitousek et al. 1997, Simon & Townsend 2003, Gurevitch & Padilla 2004). It is, therefore, genuinely ironic that the first publications emanating from South Africa pertaining to freshwater conservation were regarding trout, and were aimed at the protection of these non-native fishes (*sensu* O'Keeffe 1989). Day (1932) noted the threat of consumption of trout ova by native cyprinid species, while Bush (1933) suggested that streams containing trout should be afforded conservation at the catchment level to maintain suitable habitat for the invaders, going so far as to suggest insect species from Europe should be stocked to provide more suitable fodder for trout (O'Keeffe 1989).

Few studies have examined the impacts of these fishes from the perspective of lotic macroinvertebrates. To my knowledge, only eight have emanated from the southern African region to date: one addressing the impacts of *M. salmoides* (Weyl et al. 2010); another on the impacts of *M. dolomieu* (Lowe et al. 2008); a study examining the effects of *O. mykiss* from Afro-montane streams in eastern Zimbabwe (Kadye et al. 2013); a study by Rivers-Moore et al. (2013) examining the effects of *O. mykiss* and *S. trutta* on macroinvertebrates of the Maloti-Drakensberg Ecoregion (MDE); three recent papers by Shelton et al. (2015a, 2015b, 2016) examining the influence of *O. mykiss* on macroinvertebrates in rivers of the Cape Fold Ecoregion (CFE) and a large scale study by Jackson et al. (2016) on the impacts of *S. trutta* on ecosystem subsidies in the Amatole-Winterberg Highlands (AWHE) and Maloti-Drakensberg Ecoregions. In addition, while not testing for the effects of trout explicitly, Samways (1995, 1996, 1999) suggested trout limit the distribution of a regionally endemic damselfly *Ecchlorolestes peringueyi*, through predation of nymphs, restricting its distribution to uninvaded reaches of stream upstream of waterfall barriers. The studies by Kadye & Booth (2012a, 2012b) examining the effects on invertebrates by native, but extralimital and invasive within the study system, African sharptooth catfish *Clarias gariepinus* should be included here too. Most of these studies suggest that non-native invasive fishes have measureable, negative, effects on the macroinvertebrate communities within the recipient ecosystem.

To better understand the impacts of non-native fishes on headwater ecosystems, I assessed the drivers of macroinvertebrate assemblage structure in the Keiskamma River headwaters (Chapters 3 and 4). I demonstrated that the headwaters of the Keiskamma River are home to a notably diverse suite of macroinvertebrate faunas. The abiotic factors of season and flow were concluded to be the strongest drivers describing the variation within macroinvertebrate assemblages of the three reaches. While the presence of predatory fish was found to be a significant driver of macroinvertebrate assemblage structure, this was not as important as the abiotic drivers. Similarly, the presence of predatory fish could not be conclusively shown to account for significant differences in the density and functional feeding group composition of macroinvertebrates from invaded and uninvaded headwater tributaries. Other than the difference in fish predation regime, factors like temperature played a role in delineating the native reaches from the invaded and fishless reaches, along with differences in riparian vegetation, flow rate and sediment load. This was similar to observations elsewhere (Mhangara & Kakembo 2012, Mhangara et al. 2012, Ellender 2013). The Keiskamma river has been suggested to be impacted through poor land use practice, resulting in increased sediment loads (Mhangara & Kakembo 2012, Mhangara et al. 2012) that, coupled with invasion by trout, have negatively affected the distribution of the endemic, endangered Border barb, *Pseudobarbus trevelyani* (Ellender 2013). However, detecting impacts of trout on macroinvertebrates was not straightforward, as the combinations of abiotic factors appear to override the effect of predatory fishes, compared to upstream fishless reaches. Functional feeding guilds for example, responded to changing river gradient as predicted by the river continuum concept, where, for example, the vast majority of shredders were found in the upper forested reaches that are characterized by high inputs of leaf litter and were nearly entirely absent from the lower reaches.

The significant differences that were observed in the timing of drift density of specific macroinvertebrates, within the Ephemeroptera and Plecoptera, between fishless, invaded and native fish reaches, warrant further in depth investigation to validate these findings. Tentatively, patterns of shift towards predominantly nocturnal drift for these species are attributed to the presence of predatory fish, both native and non-native. This phenomenon is a well documented and expected response to fish predation when behavioural drift densities are highest during times of low light, demonstrated elsewhere in the world (Culp et al. 1991, Flecker 1992, Douglas et al. 1994, McIntosh et al. 2002) and now for the first time to my knowledge in southern Africa.

An examination of isotopic niche space of macroinvertebrate functional feeding groups concluded that, while contraction of niche space was observed from fishless to invaded reaches, followed by an expansion again along native fish reaches, this was not demonstrably significant. The testing of trophic position between native and non-native fish species however suggested that trout usurp native barb species. What was found, using baseline corrected δ^{15}N values, was a significant difference in the trophic level between *O. mykiss* co-occurring with native fish species, and those that occurred where no native fish remain, which suggests that *O. mykiss* feed preferentially on native fish species, elevating their trophic level to one situated above the co-occurring native fish, through predation. This adds further weight to the evidence that the strongest predictor of native fish distribution in the Keiskamma River is the presence of non-native predatory fishes (Ellender 2013).

The use of piscicides in managing fisheries, for enhancement and conservation purposes, is an internationally accepted practice (Haley 1978, Finlayson et al. 2000, Cailteux et al. 2001, Ling 2003). Impacts on non-target faunas have been reported (Binns 1967, Dudgeon 1990, Arnekleiv et al. 2001, Finlayson et al. 2010), though there are contradictory findings (Vinson et al. 2010). The removal of *M. dolomieu* from the lower stretch of the Rondegat River by local conservation authority CapeNature represented the first example of the use of a piscicide for conservation purposes in South Africa. The operation also represented the first application of regulations set forth in the National Environmental Management: Biodiversity Act (NEM:BA: Republic of South Africa 2004) which seeks to remove non-native, invasive, species from priority areas, by mandating action through conservation bodies (Marr et al. 2012, McCafferty et al. 2012). South Africa is severely impacted by non-native fish invasions (Ellender & Weyl 2014), and while it is recognised that these species are the most important targets of the recreational angling industry (Ellender et al. 2014), areas where invasive fish pose a quantifiable threat to native fish species are targeted to alleviate the threat to native biodiversity (Marr et al. 2012). Rotenone was selected as the method of choice for remediating the *M. dolomieu* invasion following a comprehensive Environmental Impact Assessment (Marr et al. 2012) and strategic coordination between a wide variety of stakeholders (Impson et al. 2013).

Non-target effects observed from the macroinvertebrate assemblage included significant declines in the density of sensitive taxa from the surfaces of stones following both rotenone treatments; significant declines in SASS5 water quality scores, based on macroinvertebrate presence/absence, followed the first treatment only. However, it is confidently concluded that the macroinvertebrate assemblage of the lower reaches of the Rondegat River was resilient to the rotenone application, and no long-term detrimental effects were observed.

7.2 Management recommendations and future research avenues

7.2.1 Invasions in headwater tributaries

While a significant behavioural response from specific taxa within the macroinvertebrate assemblage to the presence of predatory fish was observed (Chapter 4), the assemblage as a whole, in terms of overall diversity and density (Chapter 3) and trophic ecology (Chapter 5), does not appear to be to the detriment for the presence of trout, or native cyprinids. Dahl & Greenberg (1996) concluded from a meta-analysis of published studies on the effects of benthivorous vs. drift-feeding fish that the density of benthic invertebrates is more strongly affected by fish that feed from the benthos, rather than the drift-feeding fish. This suggests that the native cyprinids may have a stronger influence on the macroinvertebrate assemblage than non-native salmonids do, as was concluded by the study comparing predatory impacts of native barbs to non-native trout in a CFE stream (Shelton et al. 2016).

Stomach contents analysis of *P. trevelyani* suggested that their diet was composed predominantly of aquatic invertebrates, particularly Ephemeroptera (Gaigher 1975). Thus, the next step in this work is a detailed assessment of the stomach contents of both trout species and native cyprinids, that were preserved after collection of isotope tissue samples during Chapter 4, to determine the contribution of various prey resources to their respective diets. Having quantified both terrestrial invertebrate rain and allochthonous invertebrates as drift and densities of benthic invertebrates, estimates of the specificity of their diet and of prey availability for each predatory archetype can be made. As both benthic and drift samples were collected simultaneously from the same streams, instantaneous drift probabilities can be calculated for species that occurred in common between the two sample types (Huhta et al. 2000 *sensu* Elliot 1967) to determine if trout in the headwaters of the Keiskamma are selecting specific prey or feeding on whatever happens to appear before them, possibly within the benthos, as has been documented for trout elsewhere (Tippets & Moyle 1978, Inoue et al. 2009). Gaigher (1975) report "[Pseudo]*Barbus trevelyani*, ...aquatic insects, crabs, a frog and some terrestrial insects" from the stomachs of large *O. mykiss* collected near an impoundment on the neighbouring Buffalo River, suggesting a wide range of feeding preference, as salmonids are known to possess (Elliot 1967, 1970, 1973, Cada et al. 1987, White & Harvey 2007, Albariño & Buria 2011).

Better resolution in detecting impacts that predatory fish may have on invertebrate assemblages can be achieved by adding the factor of "size" and "mass" in testing for the effects of native and non-native fish (Culp & Scrimgeour 1993, Rader 1997, Buria et al. 2007). This may be done by determining a length-mass regression for invertebrate groups. Using the Ephemeroptera as an example, it is possible to elucidate the strength of the effect that native and non-native predatory fish have (Buria et al. 2007). By calculating the biomass and size of drift components, size selective responses to fish can be tested, to determine if different responses are evident within different larval instars of the same species, or between absolute size classes across the board (Nakano et al. 1999, Albariño & Buria 2011). This may also help to shape a better understanding of the responses of Megaloptera larvae in the invaded reaches of the Cata and Mnyameni Rivers, as they attain a relatively large size and are predatory and therefore forage actively, making them one of the most conspicuous invertebrates within the river from a predatory fish's point of view (Douglas et al. 1994, Buria et al. 2007, Herbst et al. 2009, Weyl et al. 2010).

These advances in this line of research are worth taking, not only for shedding light on the interactions between fish and macroinvertebrates, and for adding to the body of research into the effects that non-native invasive salmonids have on recipient ecosystems, but also for establishing a baseline on which future research can be structured. Extending this study to the reaches of stream that still represent an invasion debt within the headwater tributaries of the Keiskamma River (Ellender et al. 2016), for example the Rabula River (Figure 1.2, Chapter 1), may also provide insight into macroinvertebrate assemblages not yet infiltrated by salmonids.

With respect to macroinvertebrate conservation, there is difficulty with managing non-native invasive fish, like the two salmonid species in the headwaters of the Keiskamma, as its tricky to detect what long-term effect they may have had on the invertebrate assemblage over the last century of inhabiting these streams. Conventionally, upstream reaches are repopulated by adults from downstream, following Müller's (1954) "colonisation cycle" (Townsend & Hildrew 1976, Campbell & McIntosh 2013), which in the case of the Cata and Mnyameni Rivers means immature stages potentially occupying invaded reaches of stream by entering the drift from fishless reaches downward. In this way, predation pressure is exerted across the macroinvertebrate community and a thinning effect may occur over time, depending on the selectivity of predation by non-native trout.

Historical accounts exist for fish species native to the system relatively early on (Visser 1968, Gaigher 1975) and references to the Keiskamma in Hey (2008), but until now, the closest estimate of macroinvertebrate diversity has been from the neighbouring Buffalo River catchment (Palmer 1991), nearly one hundred years after trout were introduced into these catchments (de Moor & Bruton 1988). There is, as with almost all fields of entomology except possibly Odonatology and Lepidopterology, a severe paucity of research into freshwater macroinvertebrate biodiversity and thus a need for a better grasp of the diversity of insects and other invertebrate groups in the face of human-mediated environmental decay (McAllister et al. 1997, Lenat & Resh 2001, Abell 2002, Wishart & Davies 2003). Freshwater biologists in South Africa, let alone the rest of Africa, barely grasp the extent of freshwater biodiversity, and therefore there is no way of knowing if it is lost, if we never knew that existed to begin with (Ricciardi & Rasmussen 1999, Abell 2002, Wishart & Davies 2003, Linke et al. 2011). Ultimately, the best that can be achieved is an *a priori* comparison of macroinvertebrate assemblages from reaches of streams that have not been impacted by impoundments or invasion by non-native fishes, which is what has been attempted here. It is for these reasons that studies like those of Ellender (2013) and Shelton (2013), are so important in paving the way for studies like this one; were it not for the surveys carried out by Ellender (2013), the "natural experiment" (Diamond, 1983) used to conduct this work would have been impossible.

While presence of predatory fish was found to be a significant driver of macroinvertebrate assemblage structure, the fishless and invaded reaches did not differ significantly in either density of macroinvertebrates or functional feeding group composition (Chapter 3). By studying fishless reaches, it was demonstrated that enough habitat exists as refugia upstream of waterfall barriers where macroinvertebrates that may be sensitive to non-native salmonid affects could continue to exist, as has been documented elsewhere in the world where trout have invaded previously fishless headwater reaches (Buria et al. 2007, Herbst et al. 2009) and South Africa (Samways 1995, 1996, 1999). Furthermore, having a self-sustaining population of wild trout in these streams represents a functional recreational fishery, and indirectly provides an opportunity for research, both avenues bringing much-needed revenue into a rural catchment (Ellender et al. 2014, Kinghorn 2014). The existence of the non-consumptive catch-and-release recreational fishery attracts fly-fishermen, who pay for daily fishing permits and accommodation, and as a product of buy-in from the local community there is an incentive to maintain the stream and forest habitat in as pristine a condition as possible (Kinghorn 2014). Removal of the non-native trout, by whatever means, would therefore be more likely to cause longer-term damage to the catchment and therefore streams, through development of the rural community and consumption of natural resources further into the catchment.

Therefore, the only management recommendation that can be made, with specific reference to minimizing the impacts of non-native salmonids, would be to encourage fishermen to release trout in the same reaches where they are caught, and discourage stocking upstream of waterfalls or chutes, to keep fishless reaches intact. In light of the socio-economic circumstances within the catchment, the removal of trout is not recommend, as they are probably currently the best reason for these particular streams remaining pristine. Following the framework of Blackburn et al. (2011) the populations of non-native fishes within the Keiskamma and Rondegat Rivers represented naturalized/established units, with both species of trout and the *M. dolomieu* species occupying the full extent of the reaches of river that contained them, limited only by natural physical barriers which prevented further spread, with all stages prior to spread outlined within Blackburn et al. (2011) having been overcome. No significant conflicts of interest existed to the eradication of *M. dolomieu* population along the Rondegat River and, following extensive research, the initiative was deemed feasible and carried out successfully (Marr et al. 2012, Ellender et al. 2014, Weyl et al. 2014).

7.2.2 Non-native fish eradication operations

Monitoring of the macroinvertebrate assemblage of the Rondegat River ceased two years after the second rotenone treatment, but monitoring of the recovery of the native fish population along the reach below the waterfall barrier previously invaded by *M. dolomieu* still continues (Weyl et al. 2016). The recovery of the native fish community along a reach of stream where they were almost entirely absent (Weyl et al. 2013) represents an opportunity to gauge the response of the invertebrate community to the recolonization process of the natural fish community. A large amount of data exists describing the macroinvertebrate assemblage of the lower Rondegat River in the presence of *M. dolomieu* (Lowe et al. 2008) and during the first two years of monitoring conducted here. It would be interesting to examine macroinvertebrate assemblage structure once native fishes have established fully, given that native cyprinids might be stronger regulators of the benthic invertebrate assemblage compared to non-native *M. dolomieu*, similar to the findings of Shelton et al. (2015b, 2016) comparing *O. mykiss* to native barbs.

Continued assessment of the Rondegat River for the presence of *M. dolomieu* should be carried out, as the river drains into one the best angling destinations for *M. dolomieu* on the African continent; the wolves are literally at the door. For this reason, I strongly recommend the use of social media, where possible national media, and other forms of easily digestible literature to tell the story of the recovery of the Rondegat River in a positive light, for it is only through widespread stakeholder buy-in, driven by a genuine concern for the plight of imperilled native CFE fishes, that is going to prevent the re-introduction of *M. dolomieu* into the Rondegat River. Attention should be drawn, through social media and popular articles to the unique and poorly described macroinvertebrate fauna of the Rondegat River (Woodford et al. 2013). Many previously unknown taxa have been discovered, and as part of the conservation program highlighting the plight of imperilled native minnows, emphasis can be drawn to the impressive collateral diversity of endemic stream insects.

While being able to describe the macroinvertebrate faunas of the Rondegat River provides interesting findings of itself, it is recommended that future non-native fish eradication operations using rotenone focus on quantitative aspects of measuring impacts. Species- and morphospecies-level identifications take an extraordinarily large amount of time to do, when the entire suite of macroinvertebrates is examined, and this may be a hindrance to the reporting of important results. The measurement of density responses of sensitive taxa as demonstrated here is relatively simple and quick to carry out, both in the field and in the laboratory. If it is possible, and depending on in-stream conditions of course, it is recommended that more stones should be sampled to improve the statistical power of tests, and therefore the reliability of the results. I also recommend carrying out the SASS5 protocol, as was employed for this study, but increasing in the number of sites, for the same reasons. This may also limit the impact that one biotope-poor site may have in masking the effects of rotenone on macroinvertebrate assemblages.

7.3 Conclusions

A sound understanding of native biota is imperative for conserving them and the ecosystem services they provide (Lenat & Resh 2001). During the course of this work I have made an attempt to adequately represent aspects of macroinvertebrate assemblages using measurements of density, diversity, assemblage structure, diel behaviour and niche utilization to quantify the effects that non-native and native fishes may have on macroinvertebrate assemblages. In itself, there is a general lack of baseline descriptions of these aspects of invertebrate communities from the regions worked in, and across South Africa as a whole (Wishart & Day 2002, de Moor & day 2013). For the Keiskamma and Rondegat Rivers, good baseline data now exist describing assemblage structure and function.

The river continuum concept predicts changes in stream community composition down the length of a river, particularly well for forested headwater reaches, and impacts of predatory headwater species like salmonids are thus likely to have unequal effects, as their direct influence on an assemblage may only be exerted where they can maintain self-sustaining populations. In the Keiskamma these reaches were reported to be occupied by native predatory cyprinids (Gaigher 1975) that are now permanently excluded by trout (Ellender 2013). The effects of trout on the macroinvertebrate assemblage of the Keiskamma headwaters, in terms of the ecological parameters measured, appear to be weak and nearly undetectable, with the exception of behavioural responses from specific groups. These responses suggest that macroinvertebrates may be resilient to the presence of trout, in that they have the means to avoid predation in reaches where trout occur, while natural states above these reaches may continue to exist immediately unaffected. As described by Rivers-Moore (2012), macroinvertebrate communities within South African Rivers exhibit upland and lowland assemblages, in keeping with what is predicted by the river continuum concept. Because of the habitat requirements of trout, direct effects on the macroinvertebrate assemblages in the systems they have been introduced into and have invaded are likely to be unequal, thus affecting only upland headwater invertebrate assemblages.

The use of rotenone was demonstrated to have a detectable immediate effect on densities of sensitive taxa, and rapid bioassessment indices following the first treatment (Woodford et al. 2013, Bellingan et al. 2015). However, no long-term, detrimental impact on the macroinvertebrate community of the Rondegat River was observed, while the native fish community of the previously-invaded reaches recovered towards observed upstream states where no *M. dolomieu* were present (Weyl et al. 2014). The two rotenone applications represented pulse disturbances and, as predicted by Lake (2000), species richness and overall macroinvertebrate diversity is strongly regulated at the local habitat scale, and this regulation operates post-disturbance affording rapid colonization even after repeated disturbance. This was confirmed within the macroinvertebrate assemblage of the treatment reach from the Rondegat River.

This thesis has contributed a better understanding of the impacts of non-native fishes on groups of taxa that are not well studied in the region. In this regard, data generated during its course have already contributed to several papers on the topic (Woodford et al. 2013, Bellingan et al. 2015, Ellender et al. 2016, Jackson et al. 2016). It is therefore hoped that the management recommendations made here may better aid conservation practices in South Africa, and that this thesis has furthered our understanding of headwater ecosystems from the perspective of macroinvertebrates. This thesis also represents the first species-level checklist from the Keiskamma River system, upon which further work may be built. While this may be considered basic information, it is ultimately the point of departure of any study, and thus this thesis is concluded with the following thought attributed to Linnaeus *sensu* Lenat & Resh (2001): ‘‘Who knoweth not the name, knoweth not the subject.’’

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Appendix 1

Table 1: Functional feeding group (FFG) allocations made for each species and the corresponding species name abbreviation used for the multivariate analyses, arranged alphabetically by FFG followed by name. Species authorities are also provided where a positive determination to species level has been made.

Species name	Functional feeding group
Anopheles sp 1	Filtering collector
Cheumatopsyche afra Moseley, 1935	Filtering collector
Chimarra sp 1	Filtering collector
Hydropsyche longifurca Kimmins, 1957	Filtering collector
Leptonema natalense Mosely, 1933	Filtering collector
Oligoneuriopsis lawrencei Angew, 1973	Filtering collector
Pisidium ovampicum Ancey, 1890	Filtering Collector
Simulium damnosum Theobald, 1903	Filtering collector
Simulium dentulosum Roubaud, 1915	Filtering collector
Simulium impukane de Meillon, 1936	Filtering collector
Simulium medusaeforme Pomeroy, 1920	Filtering collector
Simulium nigritarse Coquillet, 1901	Filtering collector
Simulium rutherfoordi Meillon, 1937	Filtering collector
Simulium sp 1	Filtering collector
Simulium unicornutum Pomeroy, 1920	Filtering collector
Simulium vorax Pomeroy, 1920	Filtering collector
Aclosma sp 1	Gathering Collector
Afroptilum sudafricanum (Lestage, 1924)	Gathering collector
Aprionyx tricuspidatus Crass, 1947	Gathering Collector
Bezzia sp 1	Gathering Collector
Caenis sp 1	Gathering collector
Caenis sp 2	Gathering collector
Cheleocloeon excisum (Barnard, 1932)	Gathering collector
Chironiminae	Gathering Collector
Choroterpes nigrescens Barnard, 1932	Gathering Collector
Cloeodes inzingae (Crass, 1947)	Gathering collector
Dabulamanzia sp 1	Gathering collector
Dixa sp 1	Gathering Collector
Dolichopodid sp 1	Gathering Collector
Elmid sp 1	Gathering Collector
Elmid sp 2	Gathering Collector
Empidid sp 1	Gathering Collector
Helminthocaris sp 1	Gathering Collector
Helminthopsis sp 1	Gathering Collector
Limnophora sp 1	Gathering Collector

Species name	Functional feeding group
Nematode sp 1	Gathering collector
Pachyelmis sp 1	Gathering Collector
Nigrobaetis bethunae Lugo-Ortiz & de Moor, 2000	Gathering collector
Oligochete sp 1	Gathering collector
Paduniella sp 1	Gathering Collector
Peloriolus sp 1	Gathering Collector
Pericoma sp 1	Gathering Collector
Potamonautes sidneyi (Rathbun, 1904)	Gathering collector
Pseudocloeon maculosum Crass, 1947	Gathering collector
Ptylodactylid sp 1	Gathering Collector
Rhoizema sp 1	Gathering Collector
Scirtid sp 1	Gathering Collector
Aeshna sp 1	Predator
Allocnemis leucosticta Sélys, 1836	Predator
Arrenura sp 1	Predator
Atherix sp 1	Predator
Aulonogyrus sp 1	Predator
Berosus sp 1	Predator
Centroptiloides bifasciata (Esben-Petersen, 1913)	Predator
Cheumatopsyche thomasseti (Ulmer, 1931)	Predator
Chlorolestes tessellates (Burmeister, 1839)	Predator
Copelatus sp 1	Predator
Dugesia sp 1	Predator
Eurymetra natalensis (Distant, 1903)	Predator
Hydrodroma sp 1	Predator
Hydrophilid larva	Predator
Laccocoris sp 1	Predator
Libellulid sp 1	Predator
Micronecta sp 1	Predator
Neoperla spio (Newman, 1839)	Predator
Oecetis sp 1	Predator
Orectogyrus sp 1	Predator
Paragomphus cognatus (Rambur, 1842)	Predator
Pseudagrion sp 1	Predator
Rhagovelia sp 1	Predator
Tabanid sp 1	Predator
Taeniochauliodes attenuates Liu, 2013	Predator
Teratothyasa sp 1	Predator
Acanthiops erepens Gillies, 1990	Scraper
Adenophlebia auriculata (Eaton, 1871)	Scraper
Afrobrianax ferdyi Lee 2003	Scraper
Afronurus harrisoni Barnard, 1932	Scraper
Athripsodes sp 1	Scraper

Baetis harrisoni Barnard, 1932	Scraper
Biomphalaria pfeifferi (Krauss, 1948)	Scraper
Burnupia sp 1	Scraper
Castanophlebia calida Barnard, 1932	Scraper
Catoxyethira sp 1	Scraper
Demoreptus capensis (Barnard, 1932)	Scraper
Demoreptus monticola (Crass, 1947)	Scraper
Elporia Sp 1	Scraper
Euthraulus elegans Barnard, 1932	Scraper
Hydraena sp 1	Scraper
Hydroptila cruciata Ulmer, 1912	Scraper
Hydroptila sp 2	Scraper
Lestagella penicillata (Barnard, 1932)	Scraper
Orthotrichia sp 1	Scraper
Parasthetops sp 1	Scraper
Pyralid sp 1	Scraper
Tricorythus discolor (Burmeister, 1939)	Scraper
Afronemoura amatolae (Balinsky, 1956)	Shredder
Antocha sp 1	Shredder
Aphanicercella sp 1	Shredder
Goerodes caffrariae (Barnard, 1943)	Shredder
Limnophila sp 1	Shredder
Limonia sp 1	Shredder
Pisulia austrina Morse, 1974	Shredder
Tipula sp 1	Shredder
Tipula sp 2	Shredder

Table 2: Species used in multivariate analyses in Chapter 2 including their FFG assignations.

Species name	Abbreviation	Functional feeding group (FFG)
Oligochete sp 1	Sp2	Gathering collector
Dugesia sp 1	Sp3	Predator
Burnupia sp 1	Sp4	Scraper
Pisidium ovampicum	Sp6	Filtering
Potamonautes sp 1	Sp10	Gathering collector
Acanthiops erepens	Sp11	Scraper
Afroptilum sudafricanum	Sp12	Gathering collector
Baetis harrisoni	Sp13	Scraper
Cheleocloeon excisum	Sp15	Gathering collector
Cloeodes sp 1	Sp16	Gathering collector
Demoreptus monticola	Sp17	Scraper
Demoreptus capensis	Sp18	Scraper
Afronurus harrisoni	Sp22	Scraper
Tricorythus discolor	Sp24	Scraper
Lestagella penicillata	Sp25	Scraper
Caenis sp 1	Sp26	Gathering collector
Caenis sp 2	Sp27	Gathering collector
Adenophlebia sp 1	Sp28	Scraper
Castanophlebia sp 1	Sp30	Scraper
Euthraulus sp 1	Sp32	Scraper
Aeshna sp 1	Sp37	Predator
Afronemoura amatolae	Sp39	Shredders
Neoperla sp 1	Sp41	Predator
Orthotrichia sp 1	Sp50	Scraper
Cheumatopsyche afra	Sp52	Filtering collector
Cheumatopsyche thomasseti	Sp53	Predator
Goerodes caffrariae	Sp57	Shredder
Pisulia austrina	Sp58	Shredder
Oecetis sp 1	Sp60	Predator
Scirtid sp 1	Sp71	Gathering collector
Elmid sp 1	Sp72	Gathering collector
Afrobrianax ferdyi	Sp78	Scraper
Limnophila sp 1	Sp81	Shredder
Tipula sp 1	Sp83	Shredder
Elporia sp 1	Sp85	Scraper
Pericoma sp 1	Sp86	Gathering collector
Bezzia sp 1	Sp87	Gathering collector
Simulium medusaeforme	Sp88	Filtering collector
Simulium impukane	Sp89	Filtering collector
Simulium dentulosum	Sp91	Filtering collector
Simulium rutherfoordi	Sp92	Filtering collector
Simulium vorax	Sp96	Filtering collector
Atherix sp 1	Sp99	Predator
Empidid sp 1	Sp102	Gathering collector

Table 3: Species identified from Surber sampled from each of the fishless, invaded and native fish reaches of stream sampled in the headwaters of the Keiskamma River, Eastern Cape, South Africa. Each row represents ten replicate samples that have been summed per season, in order to fit to page margins. Site codes correspond to the following: MnTop - Fishless Mnyameni reach; MnMid - Invaded Mnyameni reach; MnLow - Native fish Mnyameni reach; CaTop - Fishless Cata reach; CaMid - Invaded Cata reach; CaLow - Native fish Cata reach; GwTop - Fishless Gwiligwili reach; GwMid - Native fish Gwiligwili reach.

Site Code	Season	**Nematoda**	Nematode sp 1	**Annelida**	Oligochete sp 1	**Platyhelminthes**	Tricladida	**Dugesiidae**	Dugesia sp 1	**Mollusca**	**Ancylidae**	Burnupia sp 1	**Planoboridae**	Biomphalaria pfeifferi	**Sphaeriidae**	Pisidium ovampicum	**Arachnida**	Acarina	**Hydrodromidae**	Hydrodroma sp 1	**Teratothyasidae**	Teratothyasa sp 1	**Arrenuridae**	Arrenura sp 1	**Malocostraca**	Decapoda	**Potamonautidae**	Potamonautes sidneyi
MnTop	**Autumn**				13				26			25				6												1
MnTop	**Spring**				31				30			75				118												
MnTop	**Summer**		4		30				20			6				7												6
MnTop	**Winter**				49				9			24				22												
MnMid	**Autumn**				9				32			84				14												1
MnMid	**Spring**				64				29			23				1												1
MnMid	**Summer**				18				78			24				22				2								1
MnMid	**Winter**				35				17			28				3												
MnLow	**Autumn**				3				4			3		1										1				4
MnLow	**Spring**				7				9			1				7												2
MnLow	**Summer**				3				4			2				2												4
MnLow	**Winter**				15				11			3		2		17												1
CaTop	**Autumn**				7				11			13				1												2
CaTop	**Spring**				31				17			7				8												1
CaTop	**Summer**		8		26				47			2		3		6												
CaTop	**Winter**				2				37			8				26												2
CaMid	**Autumn**				3				22			89				1												1
CaMid	**Spring**				6				9			9																
CaMid	**Summer**				3				13			23								1								
CaMid	**Winter**				2				3			27				3												1
CaLow	**Autumn**				1				6																			
CaLow	**Spring**				7				7			3				1												
CaLow	**Summer**								3																			5
CaLow	**Winter**				1				5			7																3
GwTop	**Autumn**				2				5			4																1
GwTop	**Spring**				1				11			5				6												2
GwTop	**Summer**				3				3			8				2						3						3
GwTop	**Winter**				4				9			1				1				1		1						2
GwMid	**Autumn**								5			4		1														3
GwMid	**Spring**				38				38			2				1												4
GwMid	**Summer**		1		20				18			13										1						5
GwMid	**Winter**				1				57			8																

Table 3 contd.

Site Code	Season	**Insecta**	Ephemeroptera	**Baetidae**	Acanthiops erepens	Afroptilum sudafricanum	Baetis harrisonii	Centroptiloides bifasciata	Cheleocloeon excisum	Cloeodes sp 1	Demoreptus monticola	Demoreptus capensis	Dabulamanizia sp 1	Nigrobaetis bethunae	Pseudocloeon sp 1	**Heptageniidae**	Afronurus sp 1	**Oligoneuriidae**	Oligoneuriopsis sp 1	**Tricorythidae**	Trichorythus sp 1	**Teloganodidae**	Lestagella penicillata	**Caenidae**	Caenis sp 1	Caenis sp 2	**Leptophlebiidae**	Adenophlebia sp 1	Aprionyx sp 1	Castanophlebia sp 1	Choroterpes sp 1	Euthraulus sp 1
MnTop	**Autumn**					30	1				11	6					48				26		29		13	4		141		102		
MnTop	**Spring**					74	19			1	11	12					6				11		26		781	415		147	1	103		6
MnTop	**Summer**				1	544	47			5	16	2					3				14		13		3	1		20	3	1		
MnTop	**Winter**					228	3			1	15						20				7		64		445	140		246	2	126		
MnMid	**Autumn**					15		1			9	3					20				61		3		11	1		40		43		14
MnMid	**Spring**					99	29				3	1					15				68		8		20	58		2		161		45
MnMid	**Summer**				1	151	38		12			2	7		5		16				24				9	5		7		5		10
MnMid	**Winter**					102	11		1		4				2						13				4	39		26		191		22
MnLow	**Autumn**					14	49		7		12	7	6	3	1		40				13					4		17		18		4
MnLow	**Spring**					11	51		15	3		38		3			24				9				9	31				1		45
MnLow	**Summer**					65	55		2		18	16	11				9		2		120					7		28				1
MnLow	**Winter**					33	93		33	30	1						74								90	5		25		38	2	81
CaTop	**Autumn**				1	8					3	6					26				33		9		2	2		21	1	27		
CaTop	**Spring**					274					76	6					15				1		17		25	51		100	1	87		1
CaTop	**Summer**					161					46	7					4						12					12		6		
CaTop	**Winter**					66					10	1					23						32		62	97		50	3	70		
CaMid	**Autumn**					8	6				44	7					10				171		2		7			17		54		1
CaMid	**Spring**				2	29	19				167	17					7						1		15	67		17		52		
CaMid	**Summer**					43	11				77	6					1				25							6		3		
CaMid	**Winter**					118					51	18									18		2		68	26		7		50		10
CaLow	**Autumn**					13	46					5	3	6			66				25		1		1	2		8				2
CaLow	**Spring**					4	14		20			13		12			39				17				8	3						34
CaLow	**Summer**					13	258	2	1			20	20	17	2		31				73				2	3		25				5
CaLow	**Winter**					29	116		4	9		3					48				18				11			9		3		9
GwTop	**Autumn**					21					1	2					4				9							65		10		
GwTop	**Spring**				8	174				4	3	6					12									1		24	6	2		
GwTop	**Summer**				8	234	1			1	66	1			1		4				19				1	3		63		1		
GwTop	**Winter**				3	102				1		8					14				5		1			1		105	3	64		
GwMid	**Autumn**						6				1	1			2						31				9			8		96		
GwMid	**Spring**				2	88	22		5	5				4	13											42		60		14		6
GwMid	**Summer**				1	119	33		28			8			44		4				45				37	1		22		26		
GwMid	**Winter**				8	23	31		42	10		3									11				17	10		21		518		

Table 3 contd.

Site Code	Season	Odonata	**Synlestidae**	Chlorolestes tessellatus	**Platycnemididae**	Allocnemis leucosticta	**Coenagrionidae**	Pseudagrion sp 1	**Gomphidae**	Paragomphus sp 1	**Aeshnidae**	Aeshna sp 1	**Libellulidae**	Libellulid sp 1	Plecoptera	**Notonemouridae**	Afronemoura sp 1	Aphanicercella sp 1	**Perlidae**	Neoperla sp 1	Hemiptera	**Gerridae**	Eurymetra natalensis	**Veliidae**	Rhagovelia sp 1	**Corixidae**	Micronecta sp 1	**Naucoridae**	Laccocoris sp 1	Megaloptera	**Corydalidae**	Taeniochauliodes attenuatus
MnTop	**Autumn**																12			2												1
MnTop	**Spring**			1													17			2												
MnTop	**Summer**			3																6					1							
MnTop	**Winter**											1					9	1		3												1
MnMid	**Autumn**																1			2												1
MnMid	**Spring**					4						1					8			5												
MnMid	**Summer**											1								1												
MnMid	**Winter**																5	1		1												
MnLow	**Autumn**																	5		2					1							
MnLow	**Spring**									1		1								5									1			
MnLow	**Summer**											1								3					1		1					
MnLow	**Winter**											2						3														
CaTop	**Autumn**											2					7			1												3
CaTop	**Spring**											1					20															
CaTop	**Summer**											1					3			4												1
CaTop	**Winter**			1										1			16	1		2												1
CaMid	**Autumn**																3			5												
CaMid	**Spring**																															
CaMid	**Summer**											3					2			4												
CaMid	**Winter**																2															
CaLow	**Autumn**							1				1								3												
CaLow	**Spring**					1				4		1																	6			
CaLow	**Summer**									1		1					1			6												
CaLow	**Winter**											2																				
GwTop	**Autumn**			3		1						1					3	1														
GwTop	**Spring**					8											5															
GwTop	**Summer**			6		11						3					4															
GwTop	**Winter**			7		2											12			1												
GwMid	**Autumn**									1		8					1	1														
GwMid	**Spring**			1						3		3																				
GwMid	**Summer**			5				8		3		8					1						1		3		1					
GwMid	**Winter**									3		1					6	4							1							

Table 3 contd.

Site Code	Season	Trichoptera	**Hydroptilidae**	Catoxyethira sp 1	Hydroptila cruciata	Hydroptila sp 2	Orthotrichia sp 1	**Philopotamidae**	Chimarra sp 1	**Hydropsychidae**	Cheumatopsyche afra	Cheumatopsyche thomasseti	Hydropsyche longifurca	Leptonema natalense	**Psychomyiidae**	Paduniella sp 1	**Lepidostomatidae**	Goerodes caffrariae	**Pisuliidae**	Pisulia austrina	**Leptoceridae**	Athripsodes sp 1	Oecetis sp 1	**Sericostomatidae**	Aclosma sp 1	Rhoizema sp 1	Lepidoptera	**Pyralidae**	Pyralid sp 1
MnTop	**Autumn**										19							10		3		1							
MnTop	**Spring**										8	6						80		1		4			1				4
MnTop	**Summer**						1				16							21		2		3	6						
MnTop	**Winter**										4	2						22				1	3			2			
MnMid	**Autumn**										6	15	1			4		13		1		1			1				
MnMid	**Spring**								2		11	23				39		6		4			2						
MnMid	**Summer**								1		8	24				1		71		3		1	10						
MnMid	**Winter**				1						4	12	1			1		57		1			2						
MnLow	**Autumn**					1					5	9	6			1									1				
MnLow	**Spring**										1	25	1					1											
MnLow	**Summer**										8	20	3			2													
MnLow	**Winter**					1						4																	
CaTop	**Autumn**										23	7						10					1						2
CaTop	**Spring**						2		1		1							14				2			2				
CaTop	**Summer**										11							162		2		1			2				
CaTop	**Winter**																	69		2		2			2				
CaMid	**Autumn**										25	12						23											1
CaMid	**Spring**											2						28					2						
CaMid	**Summer**						2				8	1						102		2									
CaMid	**Winter**								5		8	7						6											
CaLow	**Autumn**										3	4	1									1							
CaLow	**Spring**										3	2										1	1						
CaLow	**Summer**										8	13	3																
CaLow	**Winter**						1				1	9																	
GwTop	**Autumn**						1					6		2				3											
GwTop	**Spring**				1													12		1			2						
GwTop	**Summer**			1			4					20		4				23		2			1						1
GwTop	**Winter**				1		1		1		1	3						21		3									
GwMid	**Autumn**						1					11																	1
GwMid	**Spring**						4					6						24					8						
GwMid	**Summer**						4		7		2	11						7					1						3
GwMid	**Winter**				1		1		15		3	4	1					34					1						

Table 3 contd.

Site Code	Season	Coleoptera	**Gyrinidae**	Aulonogyrus sp 1	Orectogyrus sp 1	**Dytiscidae**	Berosus sp 1	Copelatus sp 1	**Hydrophilidae**	Hydrophilid larva	**Hydraenidae**	Hydraena sp 1	Parasthetops sp 1	**Scirtidae**	Scirtid sp 1	**Elmidae**	Elmid sp 1	Elmid sp 2	Helminthocaris sp 1	Helminthopsis sp 1	Pachyelmis sp 1	Peloriolus sp 1	**Psephenidae**	Afrobrianax ferdyi	**Ptilodactylidae**	Ptylodactylid sp 1
MnTop	**Autumn**												1		3		2									
MnTop	**Spring**														18		4	1								
MnTop	**Summer**																2							6		
MnTop	**Winter**														1		4									
MnMid	**Autumn**														1		3							3		
MnMid	**Spring**														15		2							1		
MnMid	**Summer**														2		5							1		
MnMid	**Winter**														2		1									
MnLow	**Autumn**						1						1				4				1					
MnLow	**Spring**																							4		
MnLow	**Summer**												1					3		1		1		7		1
MnLow	**Winter**																							1		
CaTop	**Autumn**																2									
CaTop	**Spring**														28		2									
CaTop	**Summer**														1		6									
CaTop	**Winter**														5		2									
CaMid	**Autumn**									4														2		
CaMid	**Spring**														7		1							4		
CaMid	**Summer**														1		1							2		
CaMid	**Winter**												2		4									2		
CaLow	**Autumn**																									
CaLow	**Spring**			1														1			1			1		
CaLow	**Summer**																									
CaLow	**Winter**																									
GwTop	**Autumn**												2		6											
GwTop	**Spring**														45			1								
GwTop	**Summer**														10		2							4		
GwTop	**Winter**														39							1		3		
GwMid	**Autumn**												1		1									1		
GwMid	**Spring**			1									3		1			1						1		
GwMid	**Summer**			4	3			1				2			3				1		2			2		
GwMid	**Winter**			1											15											

Table 3 contd.

Site Code	Season	Diptera	**Tipulidae**	Antocha sp 1	Limnophila sp 1	Limonia sp 1	Tipula sp 1	Tipula sp 2	**Blephariceridae**	Elporia Sp 1	**Psychodidae**	Pericoma sp 1	**Ceratopogonidae**	Bezzia sp 1	**Chironomidae**	Chironiminae sp 1	**Simuliidae**	Simulium medusaeforme	Simulium impukane	Simulium damnosum	Simulium dentulosum	Simulium rutherfoordi	Simulium nigritarse	Simulium sp 1	Simulium unicornutum	Simulium vorax	**Dixidae**	Dixa sp 1	**Culicidae**	Anopheles sp 1	**Athericidae**	Atherix sp 1	**Tabanidae**	Tabanid sp 1	**Dolichopodidae**	Dolichopodid sp 1	**Empididae**	Empidid sp 1	**Muscidae**	Limnophora sp 1
MnTop	**Autumn**						2							4		13		2	5			12				10						9						3		
MnTop	**Spring**				5		2					7		43		64		4			1	14				10						13						1		
MnTop	**Summer**						4			1						33			1		1					5						16						2		
MnTop	**Winter**				2									20		43					2	3				3						12				1		1		
MnMid	**Autumn**						1			1						9			2			6				16						7								
MnMid	**Spring**				3									6		20					1	2				3						17		1				3		
MnMid	**Summer**					1								3		134			12			1										13						1		
MnMid	**Winter**											1		1		30		6			1	6				13						15								
MnLow	**Autumn**				2											16																						4		1
MnLow	**Spring**			1												28		6		16						41						4		1						
MnLow	**Summer**				2			2				1		1		35			1	1						3						1						1		
MnLow	**Winter**													1		37		1				2				1						5								
CaTop	**Autumn**				1					2				1		12		6	1	2	5	13		3		26						9								
CaTop	**Spring**				11		1			3		4		3		57					1	3				4						18		4						
CaTop	**Summer**						1			2						50		2			1	4		4		7						10						1		
CaTop	**Winter**				12		1	1		2		4		15		80					9	1				5						22								
CaMid	**Autumn**			2			1			9				5		24		2	2			4				80						7						2		
CaMid	**Spring**				4					8						7			2		7	21				65						6								
CaMid	**Summer**						1			3		1		1		41			1			2										7						1		
CaMid	**Winter**				1					24		1		10		45			1			56				13						6						2		
CaLow	**Autumn**									1						22																								
CaLow	**Spring**			2	2											30		271								1						3								
CaLow	**Summer**				2											45		48		49						9														
CaLow	**Winter**			5										3		62		1			1	1				1						2								
GwTop	**Autumn**															7						61		1		3						9								
GwTop	**Spring**											1		2		32		5	4									1				5								
GwTop	**Summer**				1	1	2			1		6		1		78		3	6		2	35				25						4						3		
GwTop	**Winter**				2							1		4		42						12						1				10								
GwMid	**Autumn**				3											2		17	1		1	52				11						8								
GwMid	**Spring**						2									223						4								3		20								
GwMid	**Summer**				2		1									91		38	50	10		12	1		3	8		1		7		1						1		
GwMid	**Winter**						1									195					1	43	3									9								

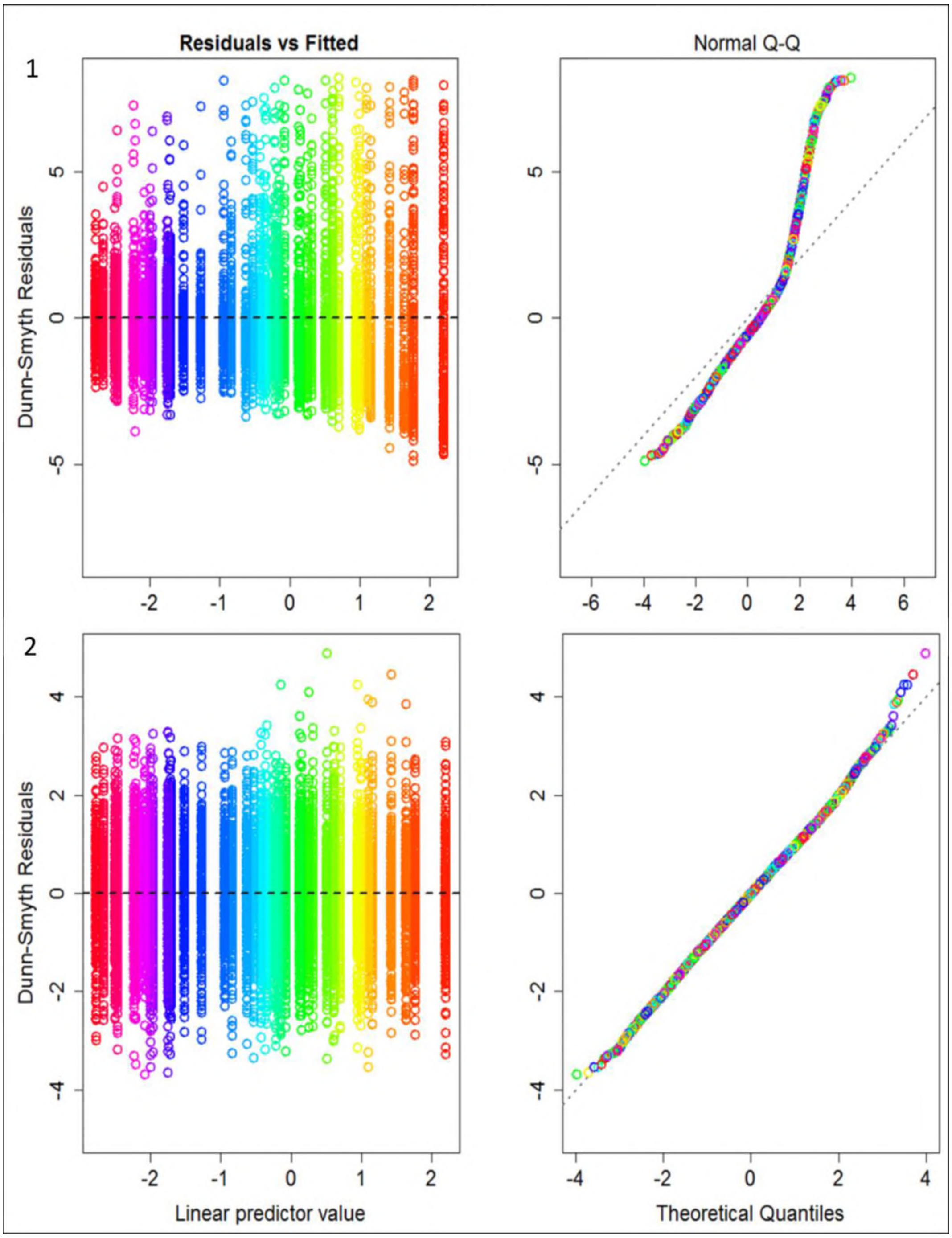

Figure 1: Diagnostic plots from when the model was run under distribution family 1) Poisson and 2) Negatively binomial. The negative binomial family fit was superior, demonstrated from the lower two plots. The model built using macroinvertebrate count data consisted of 44 species and 320 samples in the species matrix as response variables, using the categorical predictors flow rate, biotope, fish presence and season, and continuous predictors pH and conductivity (Chapter 3, Table 3.1).

Table 4: Generalized Variance Inflation Factors (GVIF) produced from tests for collinearity between predictor variables used in the multivariate analysis of macroinvertebrate species data and environmental variables, collected from headwater streams of the Keiskamma River system, Eastern Cape, South Africa.

Predictor	GVIF	DF	$GVIF^{1/(2.df)}$
as.factor(Flow rate)	1.283138	3	1.042427
pH	2.233633	1	1.494534
Conductivity	3.405246	1	1.845331
as.factor(Biotope)	1.327603	2	1.073414
as.factor(Fish presence)	2.805971	2	1.294258
as.factor(Season)	1.08209	3	1.013236

Appendix 2:

Table 1: Species identified from drift samples from headwater tributaries of the Keiskamma River, Eastern Cape, South Africa. Site codes correspond to the following: MnTop - Fishless Mnyameni reach; MnMid - Invaded Mnyameni reach; MnLow - Native fish Mnyameni reach; CaTop - Fishless Cata reach; CaMid - Invaded Cata reach; CaLow - Native fish Cata reach. Samples are summed across season, per drift period, in order to fit to page margins

Site code	Time of day	**Invertebrate rain**	**Mollusca**	**Isopoda**	**Arachnida**	**Pseudoscorpiones**	**Acari**	**Chilopoda**	**Collembola**	**Ephemeroptera**	**Thysanura**	**Hemiptera**	**Orthoptera**	**Mantodea**	**Termitoidea**	**Neuroptera**	**Plecoptera**	**Psocoptera**	**Trichoptera**	**Lepidoptera**	**Diptera**	**Coleoptera**	**Hymenoptera**
CaLow	Dawn				5	1	3		3	40	4	70						4	1	3	96	12	24
CaLow	Dusk				1		1			34	9	25						2	1	2	64	6	61
CaLow	Midday				2	1	4			3	30	32			1			3	1	4	107	13	75
CaLow	Midnight				2		3				9	31						4	3	3	44	8	28
CaMid	Dawn		1		1		7		1	5	10	6				1		3	2	2	55	7	15
CaMid	Dusk				3		7		6	20	3	12	1		1			1	1	4	49	13	11
CaMid	Midday				2	1	42		6	21	43	25						3		6	117	16	79
CaMid	Midnight				3		6	1	4	3	6	15				3	1	2		2	48	6	12
CaTop	Dawn		1		4		51	1	1			5								2	23	3	2
CaTop	Dusk			1	2		9		2			4						1	2	3	32		8
CaTop	Midday				5		9		2	6	2	6						1	1	9	48	2	7
CaTop	Midnight			2	6		11		4		8	8						3		5	28	12	13
MnLow	Dawn		1		4		1		1	2	5	24				1		30			69	8	19
MnLow	Dusk				5		3		4	45	13	76						7	1	3	58	20	54
MnLow	Midday				11		5		3	17	47	132				2		38	2	3	158	25	129
MnLow	Midnight		1	1	1		3		8	6	1	48						25		5	60	13	36
MnMid	Dawn		1				1		6	5		3					2	2		9	76	6	4
MnMid	Dusk		1			1	6		3	32	9	4		1				1	5	5	92	18	21
MnMid	Midday		1		3		8		3	28	8	6						2	2	5	104	7	25
MnMid	Midnight		3	1	7	1	7		14	10	6	11						1		3	68	24	20
MnTop	Dawn		2		2	1	4		5	53	1	12							1	4	74	15	9
MnTop	Dusk				11		12		1	38	59	19				1	2	5	5	12	360	27	36
MnTop	Midday		2		8		7		3	162	6	25				1	1	1	3	7	241	25	26
MnTop	Midnight		6	1	18	1	3		14	2	6	19			1	1				12	168	19	21

Site code	Time of day	**Nematoda**	Nematode sp 1	**Annelida**	Oligochete sp 1	**Platyhelminthes**	Tricladida	**Dugesiidae**	Dugesia sp 1	**Mollusca**	**Ancylidae**	Burnupia sp 1	**Sphaeriidae**	Pisidium ovampicum	**Cladocera**	Daphnia spp	**Copepoda**	Cyclopoida	**Ostracoda**	Cyprididae	**Arachnida**	Acarina	**Malocostraca**	Decapoda	**Potamonautidae**	Potamonautes sidneyi
CaLow	Dawn		1		18									1		5		1		3		3				
CaLow	Dusk		10		19									1		1						9				
CaLow	Midday		3		15							1				31				1		10				
CaLow	Midnight		10		35				1							41		18		7		17				1
CaMid	Dawn		1		3							2								2		14				
CaMid	Dusk		1									1										13				
CaMid	Midday		6		6							7								1		25				
CaMid	Midnight				2							6										10				
CaTop	Dawn				1							1								1		14				
CaTop	Dusk				3							1						1		3		20				
CaTop	Midday				1															4		19				
CaTop	Midnight		1		2							1								1		11				
MnLow	Dawn		1													724		5				6				
MnLow	Dusk				2											1422		64		1		5				
MnLow	Midday		1													2020		13		1		10				
MnLow	Midnight				2											18850		28		2		13				1
MnMid	Dawn		3		3									2						2		9				
MnMid	Dusk		4		7							2		2				2		8		19				
MnMid	Midday		1		5									2						10		12				
MnMid	Midnight		4		9				1			13		2						18		21				1
MnTop	Dawn				1							2		1				1		4		7				
MnTop	Dusk				1							2						1				22				1
MnTop	Midday		1		5				1											4		25				
MnTop	Midnight		1						6			2		1				2		1		18				

Table 1 contd.

Site code	Time of day	**Insecta**	Ephemeroptera	**Baetidae**	Tiny Baetidae	Acanthiops erepens	Afroptilum sudafricanum	Baetis harrisonii	Centroptiloides bifasciata	Cheleocloeon excisum	Cloeodes sp 1	Demoreptus monticola	Demoreptus capensis	Dabulamanizia sp 1	Nigrobaetis bethunae	Pseudocloeon sp 1	Securiops sp 1	**Heptageniidae**	Afronurus sp 1	**Oligoneuriidae**	Oligoneuriopsis sp 1	**Tricorythidae**	Tricorythus discolor	**Teloganodidae**	Lestagella penicillata	**Caenidae**	Caenis sp 1	Caenis sp 2	**Leptophlebiidae**	Adenophlebia sp 1	Aprionyx sp 1	Castanophlebia sp 1	Euthraulus sp 1	Odonata	**Tiny Odonata**	**Synlestidae**	Chlorolestes tessellatus	**Coenagrionidae**	Pseudagrion sp 1	**Gomphidae**	Paragomphus sp 1	**Aeshnidae**	Aeshna sp 1
CaLow	Dawn				123		4	176		9		2	3	18	3	5							1				2	2				4	1		1								1
CaLow	Dusk				148		7	81		2		1	5	9	6	1			1									1				2			2								
CaLow	Midday				54		2	54	1	11	5	2		4	4	5	1		3				1				7	3				4	2										
CaLow	Midnight				674		15	144		5			8	1	14	5			1				3				14					2	13		3				1		1		
CaMid	Dawn				26	1	29	19				37	8										3				6	2		1		12			14								
CaMid	Dusk				14	4	16	7				15	8										9				3	2				5											
CaMid	Midday				23	5	23	8				28	22										3				14	4			1	13											1
CaMid	Midnight				51		19	19				126	23								1		6				1	15				4			1								
CaTop	Dawn				54		58	9				69	12						1				3		1			1			1	15			3								
CaTop	Dusk				38	1	151	7				86	55						1				2		2		1	6				34			4		1						
CaTop	Midday				40	3	26	12				45	32						1								2	2				5			2								
CaTop	Midnight				64	1	67	5				53	47						1				1		1			1		2		35			1								
MnLow	Dawn				74	1	30	27		1		13	5			1											1					1			3				1				1
MnLow	Dusk				103		31	39	1			18	14			3			2				5							1		2							2		1		1
MnLow	Midday				51		36	48				4	12			6			1				1									4											
MnLow	Midnight				259	2	247	92	2	1		73	53		2	6							5				2	2		2		13							3				
MnMid	Dawn				49		128	55				9	7						1				4				11	10		1		6	5								1		
MnMid	Dusk				114	1	52	15			1	4	4						2				4				24	5				16			1		2						
MnMid	Midday				55	1	54	18		1	2	17	7						3				3				18	7				7											
MnMid	Midnight				307	1	581	110	1			43	37			1			2				16				21	29		7		50	5										
MnTop	Dawn				167	4	182	50				16	16			2			2				16		1		7	16				28			1		1						
MnTop	Dusk				907	7	648	156	1			132	98			1			10				154		13		51	68		16		395											
MnTop	Midday				222	25	198	47			1	20	16			1			2				31		1		42	14		6		41			1		1						
MnTop	Midnight				469	6	489	167				66	110			1			1				86		2		14	12		15	3	167					1						

Table1 contd.

Site code	Time of day	Plecoptera	**Notonemouridae**	Afronemoura amatolae	Aphanicercella sp 1	**Perlidae**	Neoperla sp 1	Hemiptera	**Gerridae**	Eurymetra natalensis	Limnogonus sp 1	**Veliidae**	Microvelia sp 1	Rhagovelia sp 1	**Corixidae**	Micronecta sp 1	**Notonectidae**	Anispos sp 1	**Pleidae**	Plea sp 1	**Naucoridae**	Laccocoris sp 1	Megaloptera	**Corydalidae**	Taeniochauliodes attenuatus	Trichoptera	**Hydroptilidae**	Hydroptila cruciata	Hydroptila sp 2	Orthotrichia sp 1	**Philopotamidae**	Chimarra sp 1	**Hydropsychidae**	Cheumatopsyche afra	Cheumatopsyche thomasseti	Hydropsyche longifurca	**Psychomyiidae**	Paduniella sp 1	**Lepidostomatidae**	Goerodes caffrariae	**Pisuliidae**	Pisulia austrina	**Leptoceridae**	Athripsodes sp 1	Athripsodes harrisoni	Oecetis sp 1	Triaenodes sp 1
CaLow	Dawn			1	1								3			3				2														1											3		
CaLow	Dusk													3		11												1																			1
CaLow	Midday												1			6				3														1	2	1									1		1
CaLow	Midnight															1				2															1										2		
CaMid	Dawn			26			5																		1					4				1						28						1	
CaMid	Dusk			17	1								1			1																1		3						11							
CaMid	Midday			9	1		1						3			1														3		1		4		1				13							
CaMid	Midnight			36			1						6																	2		1		2		2				43							1
CaTop	Dawn			12	1																				2					3				3						15						1	
CaTop	Dusk			45			2																		2					4				2	3					12							
CaTop	Midday			22			2						1												1					3		1		3	1	1				9							
CaTop	Midnight			94			1																		1					8				3	4					36				3		1	
MnLow	Dawn			1	1								3							2									1	5				7	4			1							1		1
MnLow	Dusk						1													1														3	2					1					1		
MnLow	Midday												1							2		1												2	1			4									
MnLow	Midnight			8	1								3					1		4										7				9	13	1		3		1				1		1	
MnMid	Dawn			11			1																							1		1		2	1	3		13		20		2		1		2	
MnMid	Dusk			16			1						2															1						2	3	3		12		9						3	
MnMid	Midday			5						1	1		2												1					2		1		6	2	1		24		12							
MnMid	Midnight			128	4		7																		1					6		2		3	26	3		8		73		1		1		4	
MnTop	Dawn			45	2		1						5																	6				9	1			2		17		1		1			
MnTop	Dusk			244	1		9						8																	5				14	15			2		48		1		1		2	
MnTop	Midday			55			1						7																1					18				7		5						1	
MnTop	Midnight			322	3		16						4												10					13		2		31	3					53		3		3			

Table 1. contd.

Site code	Time of day	Coleoptera	**Gyrinidae**	Aulonogyrus sp 1	Orectogyrus sp 1	**Noteridae**	Synchortus sp 1	**Dytiscidae**	Bidessus sp 1	Primospes sp 1	Laccophilus sp 1	Larval dytiscidae	**Haliplidae**	Peltodytes sp 1	**Helophoridae**	Helophorus aethiops	**Hydrophilidae**	Berosus sp 1	Laccobius sp 1	Laccobius sp 2	**Hydraenidae**	Discozantena sp 1	Discozantena sp 2	Hydraena sp 1	Mesoceration sp 1	Parasthetops sp 1	**Scirtidae**	Scirtid spp	**Elmidae**	Elmid sp 1	Elmid sp 2	Helminthocaris sp 1	Helminthopsis sp 1	Peloriolus sp 1	Protelmis chutteri	**Psephenidae**	Afrobrianax ferdyi
CaLow	Dawn			1			1					1													1	1		2			6		3				
CaLow	Dusk			2	1				1															1	1			1					3				
CaLow	Midday								2			1												1		1					1						
CaLow	Midnight			6	1				1			1												11	8	1		1					1				
CaMid	Dawn								2																5			5			1						
CaMid	Dusk																								1	1		1							2		
CaMid	Midday								2															2		1		6						1			1
CaMid	Midnight																							1	13					1			4	1	1		1
CaTop	Dawn																								3	1		16					1	1	1		
CaTop	Dusk																											22		2							
CaTop	Midday																											3									
CaTop	Midnight																								6	1		31						3	3		
MnLow	Dawn			1										1																							
MnLow	Dusk								1		1																										
MnLow	Midday			1	3				2							1			1	1				5	5	3							5				3
MnLow	Midnight			4	6				4			1						3						4	23	1		7		2			6		1		
MnMid	Dawn																								1			5							1		
MnMid	Dusk								2		1														7	3		1					1				
MnMid	Midday								1																1	1		3									
MnMid	Midnight									1															24	1		18		3	1			2	1		
MnTop	Dawn																						1		4	1		16									
MnTop	Dusk			1	1				1													1			17	5		30					2	1	1		1
MnTop	Midday								1										1				1		9	4		19						2			1
MnTop	Midnight																							1	34	6		77		1		1	1	2	1		

Table 1. contd.

Site code	Time of day	Diptera	**Tipulidae**	Antocha sp 1	Conosia sp 1	Tipula sp 1	Tipula sp 2	**Blephariceridae**	Elporia Sp 1	**Psychodidae**	Pericoma sp 1	**Ceratopogonidae**	Atrichopogon sp 1	Bezzia sp 1	Forcipomyia sp 1	**Chaoboridae**	Chaoborus microstictus	**Chironomidae**	Chironiminae spp	**Simuliidae**	Tiny Simuliidae	Simulium albivirgulatum	Simulium bequaerti	Simulium damnosum	Simulium dentulosum	Simulium hirsutum	Simulium impukane	Simulium medusaeforme	Simulium rutherfoordi	Simulium uniconutum	Simulium vorax	**Stratiomyidae**	Stratiomyia sp 1	**Syrphidae**	Eristalis sp 1	**Dixidae**	Dixa sp 1	**Athericidae**	Atherix sp 1	**Tabanidae**	Tabanid sp 1	**Empididae**	Empidid sp 1	**Muscidae**	Limnophora sp 1
CaLow	Dawn								1					2					122		235			2	1		1	15		1	2														
CaLow	Dusk			1	1									1					102		518			7			1	45	2		2														
CaLow	Midday								5										130		188		2	10			2	24	2	1															2
CaLow	Midnight			1					1										134		1226			5			2	22		1	1														1
CaMid	Dawn								14		3			2					166		47				2		7		27		46						2						2		
CaMid	Dusk			1					8		3								103		48				2		9		8		50												2		
CaMid	Midday			1		2			28		2				2				148		39				2		6		16		25				1		6		1						
CaMid	Midnight					2			16		1				1				112		36	1					8		15		39						2		5						1
CaTop	Dawn					1			2		1				1				62		37	5			3		2		9		23		1				2				1				
CaTop	Dusk								4		3								121		15	25			6		1		17		42						1		3				2		
CaTop	Midday								4		1				3				105		31	20			7				13		18						1						1		
CaTop	Midnight								11		2		1		1				93		58	20			7		2		12		50		1				5				1				1
MnLow	Dawn			1															23		13		7				8	19	2		1						1								
MnLow	Dusk																1		36		79				1		19	14	3		21														1
MnLow	Midday			1							1								36		73		2		1	1	16	18	10		12						3		1						
MnLow	Midnight			1											1		8		44		48				1	1	76	41	8		19												2		
MnMid	Dawn			1							4				2				45		31						18		8		9						1						1		2
MnMid	Dusk			3					9		4								149		101	1			2		27		23		75								2						1
MnMid	Midday								1		4			1	1				170		43				1		23		9		17												2		
MnMid	Midnight			1		6	2		2		1			1	2				202		132					1	96		25		57						3		4						3
MnTop	Dawn					2			3		4			1					107		38				1		9		6		27						3								
MnTop	Dusk					2			5		5								102		317	3			1		35		43		265						4		7						
MnTop	Midday					1			1		10								116		52	2			5		17		16		34		1				2		4						
MnTop	Midnight					12			1		1								125		83	3			1		50		46		110						1		1						

Table 2: Macroinvertebrate species incorporated into the multivariate analysis of the drift data under a general linear model (GLM). The species abbreviation for the analysis is provided, the species names correspond to those above in Table 1 of Appendix2. For ease of reference, the order of each macroinvertebrate is provided as well, corresponding to Figure 4.4 of Chapter 4.

Species name	Abbreviation	Order
Nematode sp 1	SP1	Nematoda
Oligochete sp 1	SP2	Oligochaeta
Dugesia sp 1	SP3	Mollusca
Burnupia sp 1	SP4	Mollusca
Pisidium ovampicum	SP5	Mollusca
Hydracarina sp 1	SP6	Acarina
Potamonautes sp 1	SP7	Malacostraca
Tiny Baetidae	SP8	Ephemeroptera
Acanthiops erepens	SP9	Ephemeroptera
Afroptilum sudafricanum	SP10	Ephemeroptera
Baetis harrisoni	SP11	Ephemeroptera
Centroptiloides bifasciata	SP12	Ephemeroptera
Cheleocloeon excisum	SP13	Ephemeroptera
Cloeodes sp 1	SP14	Ephemeroptera
Demoreptus monticola	SP15	Ephemeroptera
Demoreptus capensis	SP16	Ephemeroptera
Dabulamanizia sp 1	SP17	Ephemeroptera
Nigrobaetis bethunae	SP18	Ephemeroptera
Pseudocloeon sp 1	SP19	Ephemeroptera
Afronurus sp 1	SP21	Ephemeroptera
Tricorythus discolor	SP23	Ephemeroptera
Lestagella penicillata	SP24	Ephemeroptera
Caenis sp 1	SP25	Ephemeroptera
Caenis sp 2	SP26	Ephemeroptera
Adenophlebia sp 1	SP27	Ephemeroptera
Aprionyx sp 1	SP28	Ephemeroptera
Castanophlebia sp 1	SP29	Ephemeroptera
Euthraulus sp 1	SP30	Ephemeroptera
Tiny Odonata	SP31	Odonata
Chlorolestes tessellatus	SP32	Odonata
Pseudagrion sp 1	SP33	Odonata
Aeshna sp 1	SP35	Odonata
Afronemoura amatolae	SP36	Plecoptera
Aphanicercella sp 1	SP37	Plecoptera
Neoperla sp 1	SP38	Plecoptera
Microvelia sp 1	SP41	Hemiptera
Micronecta sp 1	SP43	Hemiptera
Plea sp 1	SP45	Hemiptera
Taeniochauliodes attenuatus	SP47	Megaloptera
Orthotrichia sp 1	SP50	Trichoptera

Chimarra sp 1	SP51	Trichoptera
Cheumatopsyche afra	SP52	Trichoptera
Cheumatopsyche thomasseti	SP53	Trichoptera
Hydropsyche longifurca	SP54	Trichoptera
Paduniella sp 1	SP55	Trichoptera
Goerodes caffrariae	SP56	Trichoptera
Pisulia austrina	SP57	Trichoptera
Athripsodes sp 1	SP58	Trichoptera
Athripsodes harrisoni sp 1	SP59	Trichoptera
Oecetis sp 1	SP60	Trichoptera
Triaenodes sp 1	SP61	Trichoptera
Aulonogyrus sp 1	SP62	Coleoptera
Orectogyrus sp 1	SP63	Coleoptera
Bidessus sp 1	SP65	Coleoptera
Larval dytiscid sp 1	SP68	Coleoptera
Hydraena sp 1	SP76	Coleoptera
Mesoceration sp 1	SP77	Coleoptera
Parasthetops sp 1	SP78	Coleoptera
Scirtid sp 1	SP79	Coleoptera
Elmid sp 1 elongate morph	SP80	Coleoptera
Elmid sp 2 chubby morph	SP81	Coleoptera
Helminthopsis sp 1	SP83	Coleoptera
Peloriolus sp 1	SP84	Coleoptera
Protelmis chutteri	SP85	Coleoptera
Afrobrianax ferdyi	SP86	Coleoptera
Antocha sp 1	SP87	Diptera
Tipula sp 1	SP89	Diptera
Elporia Sp 1	SP91	Diptera
Pericoma sp 1	SP92	Diptera
Bezzia sp 1	SP94	Diptera
Forcipomyia sp1	SP95	Diptera
Simulium albivirgulatum	SP97	Diptera
Simulium medusaeforme	SP98	Diptera
Simulium impukane	SP99	Diptera
Simulium damnosum	SP100	Diptera
Simulium dentulosum	SP101	Diptera
Simulium rutherfoordi	SP103	Diptera
Simulium vorax	SP106	Diptera
Dixa sp 1	SP109	Diptera
Atherix sp 1	SP110	Diptera
Empidid sp 1	SP112	Diptera
Limnophora sp 1	SP113	Diptera

Appendix 3:

Table 1: Species identified from kick samples collected over 5 years from the Rondegat River, Western Cape Province, South Africa. Kick samples have been summed by biotope, and across site, to fit page margins. Below treatment reach samples, a site sampled downstream of the barrier weir, represented in Figure 6.2, are presented here with additional species occurred along this reach which are noteworthy, particularly from the orders Ephemeroptera and Coleoptera. This reach is however not included in Chapter 6 analyses.

Date	Stream reach	**Annelida**	Oligochaeta	Oligochaete sp 1	**Platyhelminthes**	Tricladida	**Dugesiidae**	Dugesia sp 1	**Mollusca**	Gastropoda	**Ancylidae**	Burnupia sp 1	Ferrissia sp 1	**Arachnida**	Acarina	**Hydrodromidae**	Hydracarina sp	**Malacostraca**	Cladocera	Daphnia spp	Decapoda	**Potamonautidae**	Potamonautes sidneyi
May 2010	Treatment																						5
May 2010	Below treatment																						
Oct 2010	Control			1																			
Oct 2010	Treatment			3																			
Oct 2010	Below treatment			1																			
Feb 2011	Control			1																			
Feb 2011	Treatment			3									1				5						9
Feb 2011	Below treatment																						
Feb 2012	Control			7																			3
Feb 2012	Treatment			2																			6
Feb 2012	Below treatment																4						
Mar 2012	Treatment											1					1						6
Mar 2012	Below treatment																						
May 2012	Control			28													5						8
May 2012	Treatment			28								6					2						4
May 2012	Below treatment			9																			
Oct 2012	Control			4													193			1			4
Oct 2012	Treatment			8													42			2			1
Oct 2012	Below treatment			2													24						
Feb 2013	Control			352								1					294			1			1
Feb 2013	Treatment			56									1				38			3			8
Feb 2013	Below treatment			65									1				139			2			2
Mar 2013	Control			11													149						1
Mar 2013	Treatment			12													36						9
Mar 2013	Below treatment			2									1				48						1
Oct 2013	Control			4													3						
Oct 2013	Treatment			5																			1
Oct 2013	Below treatment			6																			1
Mar 2014	Control			105									1				3						1
Mar 2014	Treatment			34																			7
Mar 2014	Below treatment			1																			2
Oct 2014	Control			3													17						3
Oct 2014	Treatment			12				1									6						3
Oct 2014	Below treatment			9													3						
Feb 2015	Control			112													1						2
Feb 2015	Treatment			44									1				5						10
Feb 2015	Below treatment			1													3						3

Table 1 contd.

Date	Stream reach	**Insecta**	Ephemeroptera	**Baetidae**	Tiny baetids	Afroptilum sudafricanum	Baetis harrisoni	Bugelisia sp 1	Cheleocloeon excisum	Cloeon sp 1	Dabulamanzia media	Demoreptis monticola	Demoulinia crassi	Glossidion sp 1	Nigrobaetis sp 1	Peuhlella sp 1	Pseudopannota maculosa	Pseudocloeon glaucum	Pseudocloeon piscis	Pseudocloeon vinosum	Securiops macafertiorum	**Caenidae**	Afrocaenis sp 1	Caenis sp 1	**Heptageniidae**	Afronurus sp 1	**Leptophlebiidae**	Euthraulus elegans	Adenophlebia peringueyella	**Teloganodidae**	Nadinetella sp 1	Lithogloea sp 1
May 2010	Treatment						113		7		2					23			280	8						9		6				
May 2010	Below treatment				5																											
Oct 2010	Control					1	73		3			2				1	4		18	10			13	37		12		2				1
Oct 2010	Treatment					26	224		6			23				9	13		133	46			25	37		11		35			1	
Oct 2010	Below treatment						21										1			1								6				
Feb 2011	Control						5	3	45	8						2	14	1	26	6			172	132				22				
Feb 2011	Treatment					21	93		40	10	1		2	1		39	36	11	169	13	1		78	109		11		23				
Feb 2011	Below treatment						21		1						1		2		3				76	38		5		3				
Feb 2012	Control				2		4		29	3						6	9		12	9			18	40		2		3				
Feb 2012	Treatment				3	1	31		15							6	20		30	9			47	10		1		13				
Feb 2012	Below treatment						3		1							2	4		2				7	3								
Mar 2012	Treatment								26								1		12				90	26				13				
Mar 2012	Below treatment						7									3	1		2				5	4		1						
May 2012	Control				21		123		38							31	10		130	13			49	2		2		16				
May 2012	Treatment				4		63		11							3	15		113	14			58	1				5				
May 2012	Below treatment				4		5		1							1			2	1	3		14	1								
Oct 2012	Control				142	19	304		100			25				14	37		360	62			8	29		10		25			2	
Oct 2012	Treatment				8	4	422		45			29	8			4	6		549	45			70	36		1		21			1	1
Oct 2012	Below treatment				36	5	80		82			31				2	21	2	72	9	1		14	5				1				
Feb 2013	Control				378	10	228		375							135	98	1	750	91			1008	139		26		53				
Feb 2013	Treatment				34	2	58		323	1	6					25	26	9	1384	220			854	181		9		50				
Feb 2013	Below treatment				4		57		147		16					15	6		78		14		196	61				4				
Mar 2013	Control				18		265		348	1	7	1	3			239	134	9	883	177			505	61		20		21				
Mar 2013	Treatment						1		129	7						6			134	7			203	62		4		6				
Mar 2013	Below treatment																						5	7								
Oct 2013	Control					39	86		5			25				31	16	1	81	34			82	3		2		12				
Oct 2013	Treatment				1	35	51		4			52				14	25	18	142	35			110	8		7		10			2	
Oct 2013	Below treatment					9	50					21				3	1	10	1	27			12								1	
Mar 2014	Control				5		75		56							78	48		92	80			117	14		36		32				
Mar 2014	Treatment						49		101		3					6	18		543	55	1		224	17		8		15				
Mar 2014	Below treatment						12		14		3					7	5		8				12					2				
Oct 2014	Control				7	44	169		21			14				18	6	1	48	25			52	8		14		14				
Oct 2014	Treatment				2	9	140		55			5				9	1	13	136	135			103	2		4		6				1
Oct 2014	Below treatment				9	2	7		2			6					1	1	4	1			14									
Feb 2015	Control				5	7	66		115	3	6					37	31	1	127	175			57	18		20		26				
Feb 2015	Treatment				5	6	40		77	2	9					25	103	3	519	73	5		109	9		9		8	1			
Feb 2015	Below treatment				2		7		6		1						1	1	3	2			2	2		3		7				

Table 1 contd.

Date	Stream reach	Odonata	**Aeshnidae**	Aeshna sp 1	Anax sp 1	**Chlorocyphidae**	Platycypha sp 1	**Coenagrionidae**	Ceriagrion sp 1	Pseudagrion spp	**Gomphidae**	Ceratogomphus sp 1	Ictinogomphus sp 1	Paragomphus sp 1	**Libellulidae**	Tiny libellulids	Orthetrum sp 1	Sympetrum sp 1	Trithemis sp 1	Zyxomma sp 1	Zygonyx sp 1	**Protoneuridae**	Ellatoneura sp 1	Plecoptera	**Notonemouridae**	Tiny notonemourids	Aphanicercella sp 1
May 2010	Treatment									137		4		32							3						19
May 2010	Below treatment													2													
Oct 2010	Control				1					5				6							9						
Oct 2010	Treatment									18		4		1					1	1	3						
Oct 2010	Below treatment													2													
Feb 2011	Control			3	1					3		6		13			7		9							4	
Feb 2011	Treatment			10			1			22				54				1	29				1			2	
Feb 2011	Below treatment			1								2							1								
Feb 2012	Control									25				17					7								
Feb 2012	Treatment						1			46			1	36				1	5		1						
Feb 2012	Below treatment													12													
Mar 2012	Treatment			1	1					48				53					1		1					1	
Mar 2012	Below treatment													30							2						1
May 2012	Control			4	1					24				21					3								6
May 2012	Treatment			4	2				1	41				21					2								4
May 2012	Below treatment			1										5													
Oct 2012	Control									3				4												1	
Oct 2012	Treatment									6				2				1			2						
Oct 2012	Below treatment																										
Feb 2013	Control			7	6					20		3	1	93		90	1	3			5					4	
Feb 2013	Treatment			2	14					134		1		214		184			5		9					3	
Feb 2013	Below treatment											5		141		54										2	
Mar 2013	Control			3	5				4	45				36		58	4	15	2		2					3	1
Mar 2013	Treatment			9	25				12	48				63		16	14	5	7		6						
Mar 2013	Below treatment											1		30		26					3						
Oct 2013	Control									4				1							2					4	
Oct 2013	Treatment									2				8		1					1					1	
Oct 2013	Below treatment									2																	
Mar 2014	Control			11	1					9				20		16	3	8	16		4					20	
Mar 2014	Treatment			11	5					53		1		76		11	3	4	2	1	17						
Mar 2014	Below treatment			2								2		37		7			1		3						
Oct 2014	Control				2					7				2			1	1	1		4					8	
Oct 2014	Treatment				1					19				10				1			4					14	
Oct 2014	Below treatment									4							3				4						
Feb 2015	Control			5	2				2	16		10		15		40	1	3	6		8					1	
Feb 2015	Treatment			8	20				1	35		13	1	149		6	4	15	23		12					18	
Feb 2015	Below treatment			1										37		1					3						

Table 1 contd.

Date	Stream reach	Hemiptera	**Gerridae**	Limnogonus sp 1	Trepobates sp 1	**Hydrometridae**	Hydrometra sp 1	**Veliidae**	Microvelia sp 1	Rhagovelia sp 1	**Mesoveliidae**	Mesovelia sp 1	**Naucoridae**	Neomacrocoris sp 1	Ctenipocoris africana	**Nepidae**	Laccotrephes sp 1	Borborophilus afzelii	**Notonectidae**	Anisops sp 1	Notonecta lactitans	Enithares sp 1	Nychia limpida	**Corixidae**	Mirconecta sp 1	Megaloptera	**Corydalidae**	Platychauliodes sp 1
May 2010	Treatment									55																		
May 2010	Below treatment																											
Oct 2010	Control									11							1											
Oct 2010	Treatment									11		1		1														
Oct 2010	Below treatment																											
Feb 2011	Control			2	1		1			4										4			1					
Feb 2011	Treatment									76				1				2		2								
Feb 2011	Below treatment									4																		2
Feb 2012	Control									3				1														
Feb 2012	Treatment									4				5											1			
Feb 2012	Below treatment																											1
Mar 2012	Treatment									27				1								1						
Mar 2012	Below treatment																											
May 2012	Control									36											2							
May 2012	Treatment								4	147											1				2			
May 2012	Below treatment																								1			
Oct 2012	Control									2															6			
Oct 2012	Treatment									4											1				16			
Oct 2012	Below treatment																											2
Feb 2013	Control								1	63				2				1			1				174			
Feb 2013	Treatment								1	13				2							6				109			
Feb 2013	Below treatment									1															36			2
Mar 2013	Control									71				1	1						4				46			
Mar 2013	Treatment									35				2							5				11			
Mar 2013	Below treatment																								1			2
Oct 2013	Control									1																		
Oct 2013	Treatment								1	1																		
Oct 2013	Below treatment									3																		
Mar 2014	Control									89				4							2							1
Mar 2014	Treatment								2	24											1	1			3			
Mar 2014	Below treatment																											1
Oct 2014	Control									8																		
Oct 2014	Treatment									2															1			
Oct 2014	Below treatment																								1			1
Feb 2015	Control									39															1			1
Feb 2015	Treatment						1		2	88				1				1			1							
Feb 2015	Below treatment									1															1			1

Table 1 contd.

Date	Stream reach	Trichoptera	**Barbarochthonidae**	Barbarochthon sp 1	**Ecnomidae**	Ecnomus sp 1	**Hydropsychidae**	Tinty hydropsychids	Cheumatopsyche afra	Cheumatopsyche thomasseti	Macrostemum capense	**Hydroptilidae**	Oxyethira velocipes	Hydroptila cruciata	Hydroptila sp 1	Orthotrichia sp 1	**Leptoceridae**	Athripsodes harrisoni	Athripsodes prionii	Athripsodes spp	Leptecho sp 1	Unknown genus sp 1	Leptecho helicotheca	Oecetis sp 1	Oecetis modesta	**Philopotamidae**	Chimarra sp 1	**Polycentropodidae**	Paranyctiophylax sp 1	**Sericostomatidae**	Petroplax sp 1
May 2010	Treatment								8									35		1											
May 2010	Below treatment																														
Oct 2010	Control					1			8									42							1		1				
Oct 2010	Treatment								7		2							118							1						2
Oct 2010	Below treatment								3									15													
Feb 2011	Control					3			19	1			1	2				7		1	11				5				1		1
Feb 2011	Treatment					5			105		8		6	94				12	12		3		1	2	13						
Feb 2011	Below treatment																	1							1		1				
Feb 2012	Control							3	46				1	4				13		1					1						
Feb 2012	Treatment					4			35					1				17											2		
Feb 2012	Below treatment								4																						
Mar 2012	Treatment					4		1	13		5							32		1			1								
Mar 2012	Below treatment								7																		1				
May 2012	Control					4		9	82	1			6	2				101		2	1		1		1						
May 2012	Treatment					4		3	28		3							160			7				2						1
May 2012	Below treatment								1									14									1				
Oct 2012	Control					8			13	1	1					2		469			5	1	2		5						
Oct 2012	Treatment							1	7		1		1					102			3	1			2						
Oct 2012	Below treatment							3	1	2								73									1				
Feb 2013	Control					3			179	6			5	9		8		84					5	5	17						1
Feb 2013	Treatment			1				3	15	1			11	40		3		68			1	2	1	1	11						6
Feb 2013	Below treatment								15	3								11									7				
Mar 2013	Control					4		2	104	5			4	9				42		1	9		1		5		2				
Mar 2013	Treatment					3		1	25		6		1	3				20		9	8				4				3		
Mar 2013	Below treatment								5		1					1		1			2				1						
Oct 2013	Control					1			5						1	1		48	2	3	11	1			2						
Oct 2013	Treatment								3		1							6		4	1										
Oct 2013	Below treatment								1									15			1										
Mar 2014	Control								109	7	1		9	31	1	7		41		4	12		1		12		2				1
Mar 2014	Treatment					6			28				19	2		2		114		6	9		1		2						
Mar 2014	Below treatment								18											1	2										
Oct 2014	Control					4		1	38	3					1			38		1	9		1		2		4				
Oct 2014	Treatment								10				6	1				108			12				3						
Oct 2014	Below treatment								2									66			4						2				
Feb 2015	Control					4			63	3			12	26	2	10		24		2	5		1	5	18		32				
Feb 2015	Treatment							2	34	1	4		35	8		12		102	1		23		2	3	11		17		2		2
Feb 2015	Below treatment								3							1		2									4				

Table 1 contd.

Date	Stream reach	Lepidoptera	**Pyralidae**	Pyralid sp 1	Coleoptera	**Dryopidae**	Strina sp 1	**Dytiscidae**	Larval Dytiscid	Philaccolus lineatoguttatus	Primospes suturalis	Canthyporus sp 1	Hydrovatus sp 1	Laccophilus sp 1	Uvarus sp 1	Yola sp 1	Sharphydrus sp 1	**Elmidae**	Elpidelmis capensis	Tropidelmis hintoni	Ctenelmis sp 1	Helminthopsis sp 1	Microdinodes sp 1	Elmid giant morph	Elmid oval morph	Elmid semi-oval morph	Elmid elongate morph	Protelmis chutteri	Pelorioluls spp
May 2010	Treatment																								4	14	36		
May 2010	Below treatment																												
Oct 2010	Control																		6						10	1			
Oct 2010	Treatment								5							1			15	1					4				
Oct 2010	Below treatment																								1	1			
Feb 2011	Control								1																4				
Feb 2011	Treatment								5										3				2		37				5
Feb 2011	Below treatment																						1		1				
Feb 2012	Control								5																		1		
Feb 2012	Treatment								2						1												5		
Feb 2012	Below treatment																								4				1
Mar 2012	Treatment								2								1		2						3	2			
Mar 2012	Below treatment																		1										
May 2012	Control													3					3			1			5	15	10		
May 2012	Treatment						1				1			1					5			2			6	9	55		
May 2012	Below treatment																								1		2		
Oct 2012	Control								1										18			8				13	12		29
Oct 2012	Treatment								3		3								15	2						8	10	2	26
Oct 2012	Below treatment			1									1						1							6	2		6
Feb 2013	Control								1										14	1					2	42	78	6	22
Feb 2013	Treatment								34										3						7	17	53		63
Feb 2013	Below treatment								7										21						11	44	54	2	136
Mar 2013	Control																		9						4	13	37		29
Mar 2013	Treatment									1									5						3	8	16	3	44
Mar 2013	Below treatment																		11						8	26	41		68
Oct 2013	Control																		2	3						3	5		
Oct 2013	Treatment										1									1						1	2		
Oct 2013	Below treatment																		4							4			
Mar 2014	Control																		2	1						15	65		2
Mar 2014	Treatment								5			1														7	13		
Mar 2014	Below treatment																									3			
Oct 2014	Control																		20	12					2	27	18	2	1
Oct 2014	Treatment								1			1							6	17	1					8	11		
Oct 2014	Below treatment										1								9	1						2	1		1
Feb 2015	Control																		7					1		14	36	1	
Feb 2015	Treatment											1							12	4				3	2	16	65	2	5
Feb 2015	Below treatment																		7						4	6	3		

Table 1 contd.

Date	Stream reach	Coleoptera	**Gyrinidae**	Aulonogyrus spp	Dineutes sp 1	Orectogyrus sp 1	**Halipidae**	Haliplidus sp 1	**Hydrochidae**	Hydrochus sp 1	**Hydraenidae**	Mesoceration spp	Hydraena sp 1	Discozantaena sp 1	Parhydraena sp1	Parasthetops sp 1	Prosthetops sp 1	**Hydrophilidae**	Hydrophilid larvae	Laccobius sp 1	**Nitidulidae**	Nitidulida sp 1	**Psephenidae**	Afropsephenoides sp 1	**Ptylodactylidae**	Ptylodactylid sp 1	**Scirtidae**	Scirtid spp
May 2010	Treatment																									8		14
May 2010	Below treatment																											
Oct 2010	Control				1							1			1					1						2		2
Oct 2010	Treatment			3	2	2		1																		2		1
Oct 2010	Below treatment											2																
Feb 2011	Control			1								3														1		
Feb 2011	Treatment														2		2		1							6		10
Feb 2011	Below treatment																											1
Feb 2012	Control											1														2		3
Feb 2012	Treatment											2																
Feb 2012	Below treatment											3														1		
Mar 2012	Treatment			2								5														10		9
Mar 2012	Below treatment																											
May 2012	Control			3		1						8		1												3		34
May 2012	Treatment			4		2						2														5		3
May 2012	Below treatment											1																1
Oct 2012	Control			5		3						23				1	1					4				8		16
Oct 2012	Treatment			12		1				1		4												6		1		1
Oct 2012	Below treatment			6								5																2
Feb 2013	Control					4						122				5			4	1						17		102
Feb 2013	Treatment			1		2						5														5		75
Feb 2013	Below treatment			4								69					3			1								7
Mar 2013	Control			4		10						25							1	1						4		41
Mar 2013	Treatment					1						34							1							6		10
Mar 2013	Below treatment											33					1											4
Oct 2013	Control			18		2						4				1						10				9		5
Oct 2013	Treatment			15		1						1							1			4		3		1		1
Oct 2013	Below treatment			11		1																						
Mar 2014	Control					5				1		6				1	1									6		19
Mar 2014	Treatment			2		8				2		1														1		5
Mar 2014	Below treatment			3																								1
Oct 2014	Control			12		7						15	1			5	1					2				4		12
Oct 2014	Treatment			13		10						9				1												
Oct 2014	Below treatment			20		3																						2
Feb 2015	Control											3														11		7
Feb 2015	Treatment			17		2						6				2										7		24
Feb 2015	Below treatment			2								42														1		

Table 1 contd.

Date	Stream reach	Diptera	**Athericidae**	Atherix sp 1	**Blephariceridae**	Elporia sp	**Ceratopogonidae**	Atrichopogon sp 1	Bezzia sp 1	Forcipomyia sp 1	**Chironimidae**	Larval chironimid	**Culicidae**	Anopheles sp 1	Culex sp 1	**Dixidae**	Dixa sp 1	**Empididae**	Larval empidid	**Muscidae**	Larval muscid	**Psychodidae**	Pericoma sp 1	**Simuliidae**	Tiny simuliids	Simulium adersi	Simulium bequaerti	Simulium harrisoni	Simulium impukane	Simulium medusaeforme	Simulium nigritarse	Simulium pom sp 1	Simulium ruficorne	Simulium unicornutum	Simulium vorax	**Stratiomyidae**	Larval stratiomyiid	**Tabanidae**	Larval tabanid	**Tipulidae**	Antocha sp 1	Gonomyia sp 1	Rhabdiomastix sp 1	Limnophila sp 1
May 2010	Treatment											7																	2	8				7										
May 2010	Below treatment																																											
Oct 2010	Control											67					1										5		1	23									3					
Oct 2010	Treatment			1								115					23										3		16	140					2				3		1			
Oct 2010	Below treatment											47															5			83					9									
Feb 2011	Control			1					3			519		8	2														3	4	1								2		7			
Feb 2011	Treatment					1			8			421		24	199														20	139				10							5			2
Feb 2011	Below treatment											7																							18									
Feb 2012	Control							1				95		7															1	1									2		1	2	1	
Feb 2012	Treatment							1				48		11															1	45									1		4			
Feb 2012	Below treatment											4																																
Mar 2012	Treatment							1	1			150		7																10			1								2			
Mar 2012	Below treatment											1																		3														
May 2012	Control							7	1			199		11			17																	1					1		10	1	1	
May 2012	Treatment								1			140		3			30				1																1		2				2	
May 2012	Below treatment											10																																
Oct 2012	Control								2			978					21		6				2		92	1		28	36	214				28			8				2	1	1	1
Oct 2012	Treatment					2						431			2		35		4						123	2		19	12	356				18										
Oct 2012	Below treatment								1			115							2						40				3	2				8			1					2		
Feb 2013	Control			1				19	5			603		7					7				1		47	16		11	8	32				39							5			3
Feb 2013	Treatment							5	3			971		1	1		5		4						14	1		2	1	14				5			2				1	2		1
Feb 2013	Below treatment							3	1			183							4						3					17														
Mar 2013	Control							6		2		513		79			2								34			7	11	30				154							3			
Mar 2013	Treatment											278		8	9		2		2						1					31				1			1				1			1
Mar 2013	Below treatment							1				33		1																1														
Oct 2013	Control							2				991					2		21						36				1	114				37			1							2
Oct 2013	Treatment											260					12		6						12				1	346	2			10			1				1		1	5
Oct 2013	Below treatment											143					3		2						1					10														
Mar 2014	Control							6	3			456		2			2		3						8			3		9				12	1		1				3			3
Mar 2014	Treatment			2				1				303		2					2		1				3				31	67				52	3				3			1		
Mar 2014	Below treatment											9													3				1	4							1							
Oct 2014	Control							3	1	1		381					8		24		4		1		16			14	53	44	3			3							1		2	
Oct 2014	Treatment								1			236			1		12		28		1				9			1	4	29	1	1					1						1	1
Oct 2014	Below treatment							1	1			74							5						8				1	16				1									1	1
Feb 2015	Control			3				6	1			642		3	1				5						2			1	4	24				9					3		5			1
Feb 2015	Treatment							4				463		1			1		5						6			4	22	21				12	3							1		1
Feb 2015	Below treatment							1	1			8													2				2	10	1			1										

Table 2: : Species identified from stones surfaces sampled over 5 years from the Rondegat River, Western Cape Province, South Africa. Each of the four stones sampled has been summed, along with each sampling site, per river reach, in order to fit to page margins, similarly to Table 1 above.

Date	Stream reach	Stone area (mm2)	**Annelida**	Oligochaeta	Oligochaete sp 1	**Platyhelminthes**	Tricladida	**Dugesiidae**	Dugesia sp 1	**Arachnida**	Acarina	**Hydrodromidae**	**Acari**	**Hydrodromidae**	Hydracarina sp	**Malacostraca**	**Cladocera**	Daphnia spp	Decapoda	**Potamonautidae**	Potamonautes sidneyi	**Insecta**	**Ephemeroptera**	**Baetidae**	Tiny baetids	Afroptilum sudafricanum	Baetis harrisoni	Cheleocloeon excisum	Cloeodes sp 1	Dabulamanzia media	Peuhlella sp 1	Demoreptus monticola	Pseudocloeon piscis	Pseudocloeon vinosum	Pseudopannota maculosa	**Caenidae**	Afrocaenis sp 1	Caenis sp 1	**Heptageniidae**	Afronurus sp 1	**Leptophlebiidae**	Euthraulus elegans	**Teloganodidae**	Lestegella pennicillata
May 2010	Treatment	3207.70			10										22										285	4	15	1			1				2					5		33		
May 2010	Below treatment	907.56			1										9										12		4													5		13		
Oct 2010	Control	2910.72			7										69										223	2	28	1			1	2					1			4		6		
Oct 2010	Treatment	2688.01			6										21										178		30	1		1	5	1	1		1					5		12		
Oct 2010	Below treatment	1174.16													6										50	1	9				1		2									2		
Feb 2011	Control	2884.91			491										83			6							121			16			1		4		1			22		3		44		
Feb 2011	Treatment	2996.97			66										27			6			1				329	1	29	16			56		3		7		19	61		1		76		1
Feb 2011	Below treatment	1027.28			2										16										77		1		11						1							19		
Feb 2012	Control	2687.40			21										26										79			26			8		5		15		2	1		1		44		
Feb 2012	Treatment	2865.06			3										11						1				179		12	5			34				12		2	21		4		20		
Feb 2012	Below treatment	987.24													3			1							70		6	1			9							3		1				
Mar 2012	Treatment	2605.72			2										10						3				9			2									15					28		
Mar 2012	Below treatment	1120.87													25			1			1				34		1	1									9							
May 2012	Control	2885.93			13										17										135		45	7			17			1	11		1					18		
May 2012	Treatment	2723.90			3										3										260		59	1			4				9		1					16		
May 2012	Below treatment	1215.08			1										9			1							102		24				2			1								1		
Oct 2012	Control	3773.51													129										306		28	1			1		2	1						2		27		
Oct 2012	Treatment	3067.98			3										43										225		16					1	1	1								9		
Oct 2012	Below treatment	1173.87			6										54										329		5				1	1						1						
Feb 2013	Control	3375.08			193										87			2							226	1	4	24			32		3		6		14	13		10		40		
Feb 2013	Treatment	3290.37			17										63			3							137		9	29		1	5		2				13	22		6		26		
Feb 2013	Below treatment	1200.91			10										28			2							55		3	1		1	2		4				6	18						
Mar 2013	Control	2996.90			53										188										321	3	6	8			36			2	2		6	7		4		52		
Mar 2013	Treatment	2995.24			13										25										20		1	11									4	12		4		23		
Mar 2013	Below treatment	1001.87			1										10																							3						
Oct 2013	Control	3604.66			3										68										187	2	34				3		8				2			7		14		
Oct 2013	Treatment	3366.97			6										29										81	2	36				1		6	4			1			3		11		
Oct 2013	Below treatment	1269.35			1										11										45		27				1									1				
Mar 2014	Control	2905.19			222										105										260		2	8			28				2			1		13		49		
Mar 2014	Treatment	3053.03			9										60			2							99	1	36	48		2	32		1	3	8		5	1		7		13		
Mar 2014	Below treatment	1410.69			6										108										71		9	8		8	16		21		6		4	1		2				
Oct 2014	Control	3133.84			4										123										459		43				32									7		42		
Oct 2014	Treatment	3111.98			8				1						146										262		83	1			9		1	4						7		11		
Oct 2014	Below treatment	1080.40			3										105										208		20			1	7											2		
Feb 2015	Control	3027.80			49										89										324		24	7		1	38				18			1		15		37		
Feb 2015	Treatment	3506.37			24				1						115			1							353		32	35		8	66		2	1	17			9		19		55		
Feb 2015	Below treatment	924.67			1										11										37		8	2		80			11		1			2		5		6		

Table 2 contd.

Date	Stream reach	Stone area (mm2)	Odonata	**Aeshnidae**	Aeshna sp 1	Anax sp 1	**Gomphidae**	Paragomphus sp 1	**Libellulidae**	Tiny libellulids	Trithemis sp 1	Zygonyx sp 1	Plecoptera	**Notonemouridae**	Tiny nontonemourids	Aphanicercopsis sp 1	Aphanicercella sp 1	Hemiptera	**Corixidae**	Mirconecta sp 1	Trichoptera	**Ecnomidae**	Ecnomus sp 1	**Hydropsychidae**	Tiny hydropsychids	Cheumatopsyche afra	Cheumatopsyche thomasseti	Macrostemum capense	**Hydroptilidae**	Oxyethira velocipes	Catoxyethira sp 1	Hydroptila sp 1	Hydroptila cruciata	Othotrichia sp 1	**Leptoceridae**	Athripsodes sp 1	Leptecho helicotheca	Leptecho sp 1	Oecetis modesta	**Philopotamidae**	Chimarra sp 1	**Polycentropodidae**	Paranyctiophylax sp 1	**Sericostomatidae**	Petroplax sp 1
May 2010	Treatment	3207.70										1				1							13		4			1								3			1				11		
May 2010	Below treatment	907.56																																1											
Oct 2010	Control	2910.72													9	8							2													3									
Oct 2010	Treatment	2688.01													1											2																			2
Oct 2010	Below treatment	1174.16													1																					4			1						
Feb 2011	Control	2884.91						2							2								11										17					8	9						
Feb 2011	Treatment	2996.97			2			5			2	1			3		3						10			31	1						44			6		3	18				10		2
Feb 2011	Below treatment	1027.28			1										1								1			4													9						
Feb 2012	Control	2687.40						1							3					1			9		16	28							3			2			4				1		
Feb 2012	Treatment	2865.06													8					1			12		9	28	1	1								1			4				3		
Feb 2012	Below treatment	987.24						2																	1		1									1									
Mar 2012	Treatment	2605.72			2	1						2			19					1			9		22	9		2								7		6							
Mar 2012	Below treatment	1120.87						1			1												1		2											2									
May 2012	Control	2885.93			1																		15		16	20	2											3							
May 2012	Treatment	2723.90										1					1			1			3		12	8	2	2										2					1		
May 2012	Below treatment	1215.08																							8	1	1																		
Oct 2012	Control	3773.51													4								6		6	4										22	4		1						
Oct 2012	Treatment	3067.98										1			6								1		9	1										13									1
Oct 2012	Below treatment	1173.87													3										18											1									
Feb 2013	Control	3375.08								3					2					1			12		19	18	4					1	9	4		19	2		9						
Feb 2013	Treatment	3290.37				1		3		4					6					1			4		1	4						1	17	1		46			11						
Feb 2013	Below treatment	1200.91				1				1					1								2		2	3								1		8			1						
Mar 2013	Control	2996.90										1			4								17		50	13	2			1			7	1		3			1				1		
Mar 2013	Treatment	2995.24						3				1											3		3	2							3	1		38			4						1
Mar 2013	Below treatment	1001.87								2																										2									
Oct 2013	Control	3604.66													15								2		1											19									
Oct 2013	Treatment	3366.97													6										2	1										10									
Oct 2013	Below treatment	1269.35													2										1											3									
Mar 2014	Control	2905.19													3								55		5	7						3	12	10		18			15						
Mar 2014	Treatment	3053.03			1	3		1		2					6										8	9	1					2				12			5				4		
Mar 2014	Below treatment	1410.69				1				2													2		2	1								3		7			3						
Oct 2014	Control	3133.84								1					2								8		22	3										8	1		2						
Oct 2014	Treatment	3111.98										1			10								1		34	1										20			4						
Oct 2014	Below treatment	1080.40								1		1			2								1		9	4				1						3	1				1				
Feb 2015	Control	3027.80				1				1		1			9								11		33	26					1	10	4	6		11			2		12				
Feb 2015	Treatment	3506.37			1	1		6		4		2			4								3		38	16	1			1		1	1	3		30		1	26		1				
Feb 2015	Below treatment	924.67																					1			2						2							3				1		

Table 2 contd.

Date	Stream reach	Stone area (mm2)	Lepidoptera	**Pyralidae**	Pyralid sp 1	Coleoptera	**Dytiscidae**	Larval Dytiscid	**Elmidae**	Tropidelmis hintoni	Helminthopsis sp 1	Elpidelmis capensis	Elmid oval morph	Elmid semi-oval morph	Elmid elongate morph	Peloriolus spp	Protelmis chutteri	**Gyrinidae**	Orectogyrus sp 1	Aulonogyrus spp	**Hydraenidae**	Mesoceration spp	Discozantaena sp	Aulacochthebius sp 1	Parhydraena sp 1	**Hydrophilidae**	Berosus sp 1	Hydrophilid larvae	**Nitidulidae**	Nitidulida sp 1	**Psephenidae**	Afropsephenoides sp 1	**Ptylodactylidae**	Ptylodactylid sp 1	**Scirtidae**	Scirtid spp
May 2010	Treatment	3207.70										2	37	13	28	2						5	3	1	1									2		5
May 2010	Below treatment	907.56											9	5	9																					
Oct 2010	Control	2910.72										4	4		1	8	1					10			1		4			1				3		13
Oct 2010	Treatment	2688.01						1				6		1	15	13			1											3						7
Oct 2010	Below treatment	1174.16												1	1	1						1														2
Feb 2011	Control	2884.91											7	2	4	5																				7
Feb 2011	Treatment	2996.97											74	13	64	22	1					1												6		15
Feb 2011	Below treatment	1027.28										1	9	7	28	2			1			4												1		10
Feb 2012	Control	2687.40										3	20	6	7	16						11												2		10
Feb 2012	Treatment	2865.06								1	1	2	40	32	29	19			2			1												4		5
Feb 2012	Below treatment	987.24											19	6	11	2						1														
Mar 2012	Treatment	2605.72						1			1	7	22	6	14	21			1			12												4		14
Mar 2012	Below treatment	1120.87											29	2	12	4						11			1									1		
May 2012	Control	2885.93										1	18	46	30	11			2			10						1						3		7
May 2012	Treatment	2723.90											21	17	51	3				1		5												1		
May 2012	Below treatment	1215.08											13	8	8	6						11												1		
Oct 2012	Control	3773.51										1		6	3	17						3			1					6						24
Oct 2012	Treatment	3067.98			1							9	7	26	11	17			2	2		2										2				2
Oct 2012	Below treatment	1173.87										6	6	6	11	18				5		5														3
Feb 2013	Control	3375.08										2	12	73	14	16			1			6												3		4
Feb 2013	Treatment	3290.37										1	9	79	25	14						1												2		1
Feb 2013	Below treatment	1200.91										3	10	67	12	10						2														
Mar 2013	Control	2996.90										7	64	24	16	18			2	1		10												1		4
Mar 2013	Treatment	2995.24										3	57	91	57	36						12												2		2
Mar 2013	Below treatment	1001.87										1	42	173	12							11														
Oct 2013	Control	3604.66										3	4	3	17	20			1	4		21								4				3		31
Oct 2013	Treatment	3366.97										1	3	4	7	4			1	5		4								8						1
Oct 2013	Below treatment	1269.35											3	2	7	6				2		2														
Mar 2014	Control	2905.19											10	31	18	7			1			2												2		8
Mar 2014	Treatment	3053.03											12	96	19	2			1																	6
Mar 2014	Below treatment	1410.69										3	40	312	39	21						45			1											1
Oct 2014	Control	3133.84											4	10	4	17			2			4								1						31
Oct 2014	Treatment	3111.98										3	7	20	14	29			3	1		4														35
Oct 2014	Below treatment	1080.40										6	4	55	4	24			1																	1
Feb 2015	Control	3027.80										1	19	37	10	7			1	1		3			1											11
Feb 2015	Treatment	3506.37											82	82	48	12			1			3														1
Feb 2015	Below treatment	924.67										2	11	17	13	18	1					13			1											

Table 2 contd.

Date	Stream reach	Stone area (mm2)	Diptera	**Athericidae**	Athericid sp 1	**Ceratopogonidae**	Bezzia sp	Atrichopogon sp 1	Forcipomyia sp 1	**Chironimidae**	Larval chironimid	**Culicidae**	Anopheles sp 1	Culex sp 1	**Dixidae**	Dixid sp 1	**Empididae**	Empidid sp 1	**Simuliidae**	Tiny simuliids	Simulium adersi	Simulium bequaerti	Simulium impukane	Simulium medusaeforme	Simulium ruficorne	Simulium unicornutum	**Tabanidae**	Tabanid sp 1	**Tipulidae**	Antocha sp 1	Tipula sp 1	Limnophila sp 1
May 2010	Treatment	3207.70									348					1					1	2	4	10		3				7	1	
May 2010	Below treatment	907.56									18																					
Oct 2010	Control	2910.72					3				386									51		5	2	23						2		1
Oct 2010	Treatment	2688.01					1				413		1									2	3							3		
Oct 2010	Below treatment	1174.16									136													6								
Feb 2011	Control	2884.91					4	1			373		2	2						6										80		
Feb 2011	Treatment	2996.97					1				173									22		1	1	5		5				46		
Feb 2011	Below treatment	1027.28					1				26									2										1		
Feb 2012	Control	2687.40									296									3										44		
Feb 2012	Treatment	2865.06									61									10			2	4		1				30		
Feb 2012	Below treatment	987.24									19																			1		
Mar 2012	Treatment	2605.72									63									2					1					12		
Mar 2012	Below treatment	1120.87									12																					
May 2012	Control	2885.93									199		1							25								1		40		
May 2012	Treatment	2723.90			1						153									237						1				3		
May 2012	Below treatment	1215.08									38									9												
Oct 2012	Control	3773.51									3003							3		154				3		3				4		
Oct 2012	Treatment	3067.98					1				809			1				5		567				22								1
Oct 2012	Below treatment	1173.87									264									35												
Feb 2013	Control	3375.08									775							2		5								1		107		1
Feb 2013	Treatment	3290.37					2	1			162			1				1		3						1				4		1
Feb 2013	Below treatment	1200.91									43							1														
Mar 2013	Control	2996.90					1				1069							2		6										11		2
Mar 2013	Treatment	2995.24					4		1		220			1																4		1
Mar 2013	Below treatment	1001.87									9										1											
Oct 2013	Control	3604.66					2				768							9		51												2
Oct 2013	Treatment	3366.97									242							3		34				6		2				1		1
Oct 2013	Below treatment	1269.35									124							3		8												
Mar 2014	Control	2905.19						1			1206							2		3										24		
Mar 2014	Treatment	3053.03					2				337							3		24	1		1			1				2		
Mar 2014	Below treatment	1410.69						2			50							6		2	1					2						
Oct 2014	Control	3133.84							1		1736							11		7				1		5				6		1
Oct 2014	Treatment	3111.98									185							8		40	1					2						
Oct 2014	Below treatment	1080.40									196							10		221	1		6	1								
Feb 2015	Control	3027.80			1		1	7			1843							10		12	1							1		31		1
Feb 2015	Treatment	3506.37					1	2			1006							2		120	4		3			4				11		
Feb 2015	Below treatment	924.67									50							1		7				2								

Table 3: Taxa from stone samples and kick samples with the number of specimens for each taxon, summed across 13 sampling events to display trends of changing taxon richness pre- and post-rotenone applications, along the Rondegat River, Western Cape, South Africa.

Reach	Treatment				Control			
Event	Pre 2012 treat	Post 2012 treat	Pre 2013 Treat	Post 2013 Treat	Pre 2012 treat	Post 2012 treat	Pre 2013 Treat	Post 2013 Treat
Oligochaete sp 1	93	38	79	167	528	551	558	566
Dugesia sp 1				3				
Burnupia sp 1		7					1	
Ferrissia sp 1	1		1	1				1
Hydracarina sp	86	55	147	422	178	376	527	746
Daphnia spp	6	2	6	3	6	7	3	
Potamonautes sydneyi	22	14	8	30	3	12	1	7
Afroptilum sudafricanum	53	4	2	53	3	21	11	95
Baetis harrisoni	547	485	142	469	110	455	305	770
Bugelisia sp 1					3			
Cheleocloeon excisum	91	84	353	461	120	181	407	568
Cloeon sp 1	10		1	9	11			4
Dabulamanzia media	4		7	22				14
Demoreptus monticola	24	29	1	57	4	27		40
Demoulinia crassi	2	8						3
Glossidion sp 1	1							
Peuhlella sp 1	173	7	34	168	19	55	185	540
Pseudopannota maculosa	91	22	35	172	43	63	115	257
Pseudocloeon glaucum	11		9	34	1		1	12
Pseudocloeon piscis	616	674	1387	1484	65	499	755	1239
Pseudocloeon vinosum	76	59	221	317	25	75	93	493
Securiops macafertiorum	1			6				
Afrocaenis sp 1	171	233	868	759	206	60	1023	821
Caenis sp 1	238	63	203	120	232	54	152	113
Afronurus sp 1	47	1	15	72	22	20	38	138
Euthraulus elegans	218	67	101	158	121	135	138	299
Lestegella pennicillata	1							
Adenophlebia peringueyella				1				
Nadinetella sp 1	1	1		2		2		

Lithogloea sp 1		1		1	1			
Aeshna sp 1	12	7	2	30	3	4	8	19
Anax sp 1		4	15	55	2	1	6	11
Platycypha sp 1	2							
Ceriagrion sp 1		1		13				6
Pseudagrion spp	223	95	134	157	33	27	20	81
Ceratogomphus sp 1	8		1	14	6		3	10
Ictinogomphus sp?	1			1			1	
Paragomphus sp 1	128	76	217	316	39	28	93	74
Orthetrum sp 1				21	7		1	9
Sympetrum sp1	2	1		25			3	27
Trithemis sp 1	37	3	5	32	16	3		25
Zyxomma sp 1	1			1				
Zygonyx sp 1	9	5	11	44	9		5	22
Ellatoneura sp 1	1							
Aphanicercella sp 1	22	4	1			6		1
Aphanicercopsis sp 1	1				8	8		
Limnogonus sp 1					2			
Trepobates sp 1					1			
Hydrometra sp 1				1	1			
Microvelia sp 1		4	1	5			1	
Rhagovelia sp 1	146	178	13	150	18	38	63	208
Mesovelia sp 1	1							
Neomacrocoris sp	7	1	2	3	1		2	5
Ctenipocoris africana								1
Laccotrephes sp 1					1			
Borborophilus afzelii	2			1			1	
Anisops sp 1	2				4			
Notonecta lactitans		2	6	7		2	1	6
Enithares sp 1		1		1				
Nychia limpida					1			
Mirconecta sp 1	2	19	111	15	1	7	175	47
Platychauliodes sp 1								2
Barbarochthon sp 1			1					
Ecnomus sp 1	44	17	8	16	26	34	36	106
Cheumatopsyche afra	216	57	28	129	101	123	221	368
Cheumatopsyche thomasseti	2		3	3	1	2	12	20
Macrostemum capense	12	11	2	11		1		1
Oxyethira velocipes	6	1	11	62	2	6	5	26
Catoxyethira sp 1								1
Hydroptila cruciata	139		57	18	26	22	18	89
Hydroptila sp 1			1	3			1	18

Orthotrichia sp 1			4	18		2	12	35
Athripsodes harrisoni	182	294	68	350	62	570	84	193
Athripsodes prionii	12			1				2
Athripsodes spp	11	8	59	129	7	7	41	70
Leptecho sp 1	6	16	3	54	19	14	3	46
Unknown genus sp 1		1	2			1		1
Leptecho helicotheca	1	1	1	3		3	11	5
Oecetis sp 1	2		1	3			5	5
Oecetis modesta	37	4	22	59	20	19	27	59
Chimarra sp 1				18	1			52
Paranyctiophylax sp 1	26		1	9	2	1		1
Petroplax sp 1	6	1	7	3	1		1	1
Pyralid sp 1			1					
Strina sp 1		1						
Larval *Dytiscid*	13	6	34	6	6	1	1	
Philaccolus lineatoguttatus				1				
Primospes suturalis		4		1				
Canthyporus sp 1				3				
Laccophilus sp 1		1				3		
Uvarus sp 1	1							
Yola sp 1	1							
Sharphydrus sp 1		1						
Elpidelmis capensis	28	29	13	30	13	28	18	51
Tropidelmis hintoni	2	2		22			1	16
Ctenelmis sp 1				1				
Helminthopsis sp 1	1	3				9		
Microdinodes sp 1	2							
Elmid giant morph				3				1
Elmid oval morph	196	31	44	166	45	36	32	107
Elmid semi-oval morph	73	25	139	333	9	36	167	177
Elmid elongate morph	177	79	140	252	13	34	125	226
Protelmis chutteri	1	2		5	1	1	6	3
Peloriolus spp	61	47	97	132	29	58	66	101
Aulonogyrus spp	3	18	4	53	1	8		40
Dineutes sp 1	2				1			
Orectogyrus sp 1	5	4	4	28		4	7	31
Halipidus sp 1	1							
Hydrochus sp 1		1		2				1
Mesoceration spp	9	23	13	74	26	52	141	93
Hydraena sp 1								1
Discozantaena sp 1	3					1		

Aulacochthebius sp 1	1							
Parhydraena sp1	3				2	1	1	1
Parasthetops sp 1				3		1	5	7
Prosthetops sp 1	2					1		2
Hydrophilid larvae	1			2			5	1
Berosus sp 1					4	4		
Laccobius sp 1					1		1	1
Nitidulida sp 1	3			12	1	5	6	17
Afropsephenoides sp 1		6	2	3				
Ptylodactylid sp 1	28	20	8	17	10	16	23	40
Scirtid spp	57	27	78	85	35	80	137	169
Athericid sp 1	1		1	2	1		1	4
Elporia sp	1	2						
Atrichopogon sp 1	1	1	6	7	2	8	19	31
Bezzia sp 1	10	2	6	8	10	10	5	9
Forcipomyia s1				1				4
Larval chironimid	1586	784	2095	3530	1736	2232	4580	9605
Anopheles sp 1	36	10	1	11	17	13	8	84
Culex sp 1	199	2	3	11	4	2		1
Dixa sp 1	24	65	5	27	1	38		14
Larval empidid		4	10	59		6	12	87
Larval muscid		1		2				4
Pericoma sp 1						2	1	1
Simulium adersi	1	2	1	6		1	16	1
Simulium bequaerti	8				10	5		
Simulium impukane	49	12	1	62	7	38	8	69
Simulium harrisoni		19	2	5		28	11	25
Simulium medusaeforme	351	366	36	500	51	237	35	222
Simulium nigritarse				3	1			3
Simulium pomeroyellum sp 1				1				
Simulium ruficorne		2						
Simulium unicornutum	26	18	7	84		29	42	220
Simulium vorax	2			6				1
Larval stratiomyiid		1	2	3		8		2
Larval tabanid	4	2		3	7	1	2	4
Antocha sp 1	96	14	8	20	134	138	156	84
Gonomyia sp 1			2	2	2	2		
Rhabdiomastix sp 1		2		2	1	2		2
Limnophila sp 1	2		3	10	1	2	4	12
Tipula sp 1	1							