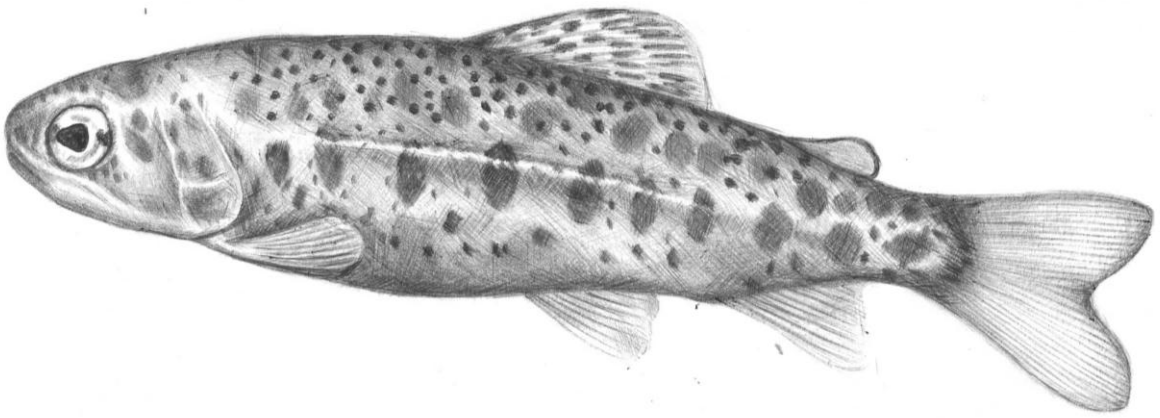
Impacts of non-native rainbow trout on stream food webs in the Cape Floristic Region, South Africa: integrating evidence from surveys and experiments

by

Jeremy Mark Shelton



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Declaration

This thesis reports original research carried out in the Department of Zoology, University of Cape Town, between 2009 and 2013. It has not been submitted in whole or in part for a degree at any other university. All data presented are original. Any assistance received is fully acknowledged.

Jeremy Mark Shelton

Date

This thesis is dedicated to my mother, Lynette Hampton – your love, friendship and support have inspired me beyond words

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Abstract

Impacts of invasive predators may be influenced by whether or not native predators which function in the same way as the invasive predator exist in the recipient system. Impacts are expected to be strong in isolated systems lacking functionally similar predators because native species will be naïve to the foraging behaviour of the introduced predator, and because the invasion is likely to change the role which the native predator assemblage performs. In this thesis I studied how the introduction of a functionally novel predatory fish, rainbow trout *Oncorhynchus mykiss*, has affected native fish, and how changes in the functioning of the predator assemblage have influenced lower trophic levels, in headwater streams in a catchment area within the Cape Floristic Region, South Africa.

Fish populations, benthic invertebrate assemblages, benthic algae and particulate organic matter were surveyed in each of 24 minimally-disturbed headwater streams in the upper Breede River catchment, and relevant environmental variables in each stream measured, over one summer. Twelve of the streams contained trout and 12 did not. It was hypothesized that native predatory fish would be vulnerable to predation by trout because they evolved in the absence of a functionally similar predator. The mean density and biomass of the native fish Breede River redfin *Pseudobarbus* sp., Cape kurper *Sandelia capensis* and Cape galaxias *Galaxias zebratus*, was 5-40 times higher in streams without trout than in streams with them, and although present at all 12 sites without trout, native fish were only recorded at five of the 12 sites with trout present. Multivariate analysis of variance revealed no consistent difference in environmental conditions between sites with and without trout, and distance-based linear models identified trout density as the best predictor of Breede river redfin and Cape kurper density. Cape galaxias density, on the other hand, was best predicted by other environmental variables including mean substrate size, site slope and riparian vegetation cover, but analyses for this species may have been compromised by the low frequency of Cape galaxias occurrence. A predation experiment conducted in mesocosms in one of the survey streams revealed that large trout selectively consumed small Breede River redfin (the dominant member of the native fish assemblage),

indicating that size-specific predation by trout reduces native fish abundance, and is also likely to change the size structure of native fish populations.

The taxonomic and functional composition of the invertebrate assemblage, as well as the biomass of benthic algae, in streams with trout differed consistently from that in streams without trout, implying that trout impacts extend beyond native fish to lower trophic levels. Herbivorous invertebrates were more abundant, and algal biomass lower, in streams with trout than in streams without them. Distance-based linear models identified trout presence as the best predictor of these patterns. It was concluded that by reducing native fish abundance, trout indirectly reduce the predation pressure on herbivorous invertebrates, resulting in increased grazing pressure on benthic algae. These results indicate that trout do not functionally compensate for the native fish that they have replaced, being weaker regulators of herbivorous invertebrates than are native fish.

Differences in the functional role performed by trout and redfin were examined by characterizing and contrasting their trophic niches in a subset of the survey streams (trout: *n* = 3, redfin: *n* = 3). Behavioural observations showed that while redfin fed mostly from the stream bed, trout fed primarily from the drift. Gut contents and stable isotope analysis revealed that herbivorous aquatic invertebrates contributed more to the diet of redfin than to that of trout, and that trout augment their diet of aquatic invertebrate prey by consuming terrestrial prey more than do redfin. Collectively these results support the conclusion that trout and redfin occupy different trophic niches, and that trout may exert weaker top-down control over lower trophic levels than do redfin.

An in-stream cage experiment was conducted to test the hypothesis that redfin suppress benthic invertebrate abundance more strongly than trout do, and that the differences in their effects on invertebrates would cascade down to the base of the food web. As predicted, redfin reduced benthic invertebrate abundance more than did trout, but significant cascading effects on benthic algae and particulate organic matter were not detected. Possible reasons for this are discussed. The results confirmed that differences in predation by trout and native fish are likely to be a key driver of the patterns in invertebrate abundance measured in the field, but indicate that these differences will not necessarily affect algal biomass.

Abstract

Through a combination of surveys and experiments, my thesis contributes to knowledge and understanding about impacts of novel predators on food webs in isolated systems which lack functionally similar native predators. My findings provide the first quantitative evidence that trout have a strong impact on native fish communities and invertebrate assemblages in the upper Breede River catchment. Since trout invasions pose arguably the greatest threat to remaining naturally-functioning food webs in Cape Floristic Region streams, it is recommended that the prevention of new trout invasions, and their selective eradication in key areas, should be management priorities.

Chapter 1

General introduction

1.1 Forces shaping the structure of biological communities

Understanding the factors regulating the structure of biological communities has long been a prominent focus in ecology. Traditionally, communities were seen as being controlled "from the bottom-up". Lindeman (1942) described communities as distinct sets of functionally similar organisms called "trophic levels", with each successive level dependent on the level below it as a source of energy. In this view, resource (e.g. the nutrients on which plants depend) availability is the major factor constraining the structure of communities. Nearly two decades later, Hairston *et al.* (1960) proposed the complementary hypothesis that the "top-down" influence of predators is the major force shaping biological communities. They reasoned that by suppressing herbivores, predators indirectly release plants from herbivory, allowing their abundance to increase (commonly referred to as the "green world" hypothesis). In this way, predators are capable of shaping the structure of entire communities through a combination of direct and indirect top-down effects. For several decades, ecologists debated the applicability of these two apparently opposing models. The debate inspired vigorous research into the factors driving community structure, and compelling examples of both bottom-up and top-down control surfaced in the literature (Hunter & Price 1992).

It is now widely accepted that these processes are not exclusive, but rather are complementary, opposing forces that act simultaneously to shape the structure of biological communities (Terborgh & Estes 2010). Bottom-up processes are inherent in every system and determine the flow of resources into a given community, while top-down forces act upon the stage set by bottom-up processes, and dictate the distribution of resources among different trophic levels (Terborgh & Estes 2010). The primary focus of community ecologists has now shifted towards trying to understand the factors and mechanisms responsible for variation in bottom-up and top-down effects among systems (Power 1990a), and in

particular, on how human-related perturbations modify the nature, and outcome, of bottom-up and top-down forces (Estes *et al.* 2011).

1.2 Modification of biological communities by humans

Human activities can alter the structure and function of biological communities by modifying bottom up and/or top-down forces in a system (Österblom *et al.* 2007). An example of the former is the addition of nutrients to aquatic systems. Nutrients are important in limiting primary production in aquatic systems such as lakes, and an increase in nutrients such as nitrogen and phosphorus through pollution (eutrophication) can lead to excessive plant growth (Carpenter *et al.* 2001). The decomposition of large amounts of plant material can then lead to oxygen depletion (hypoxia), which can in turn have serious consequences for consumers such as fish and invertebrates (Winder & Schindler 2004). Ultimately the community structure of polluted systems becomes quite different from that of similar, but unpolluted, systems (Pace *et al.* 1999, Carpenter *et al.* 2001).

Anthropogenic activities can also alter community structure by modifying the top-down effects of predators (Terborgh & Estes 2010), and it has been suggested that the modification of native predator assemblages may well be one of humankind's most pervasive influences on natural systems the world over (Estes *et al.* 2011). Predators are important because from their position at the top of the food web, they can regulate the structure and function of biological communities below them through a combination of direct and indirect effects. They directly affect their prey by reducing their abundance or by changing their foraging behaviour, and this can indirectly influence other components of the food to which the prey are linked as food or competitors (Allan & Castillo 2007). This phenomenon is known as a "trophic cascade", which Pace *et al.* (1999) have defined as a process in which there are "reciprocal predator–prey effects that alter the abundance, biomass or productivity of a population, community or trophic level across more than one link in a food web" (Figure 1.1). Consequently, the disruption of natural predator assemblages can lead to changes in the structure and function of entire communities, in addition to direct effects on prey assemblages (Estes *et al.* 2011).

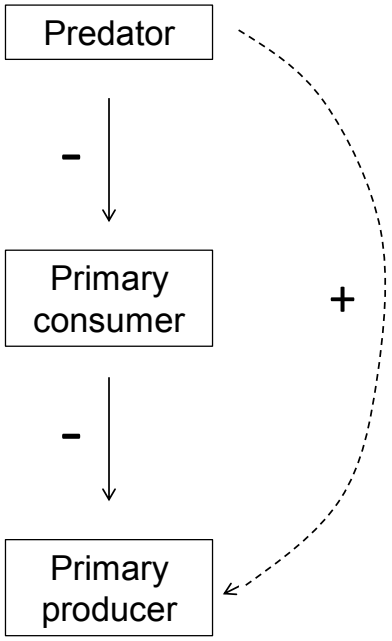

Figure 1.1 Conceptual diagram showing the mechanism of an abundance-mediated trophic cascade. Predators reduce primary consumer abundance through direct consumption. A decrease in primary consumer abundance decreases the quantity of primary producers consumed, allowing primary producer abundance to increase. Thus, by directly regulating primary consumer populations, predators indirectly promote primary producer abundance. Solid arrows indicate direct effects, dashed arrows indicate indirect effects. The + and – signs indicate whether the effect has a positive or negative outcome on abundance.

Humans have altered top predator assemblages in a variety of ways, and in all major ecosystem types (Terborgh & Estes 2010). In terrestrial systems, the range and abundance of large predators have been dramatically reduced by hunting, poisoning and trapping, because of the predatory threat that they pose to humans and livestock (Beschta & Ripple 2009). In marine systems, top predators have been targeted by recreational and commercial fishing (Pauly *et al.* 1998), or by hunting (Estes *et al.* 1978), and overexploitation has resulted in severe declines in their abundance on a global scale. In freshwater environments, fishing has contributed to predatory fish declines in lakes and streams the world over (Allan *et al.* 2005). However, perhaps the greatest threat to native top predators in freshwaters at present is that posed by introduced, non-native predatory fish (Moyle & Light 1996, Eby *et al.* 2006).

1.3 Non-native predatory fish in freshwater ecosystems

The human-assisted spread of non-native, predatory fishes for angling and aquaculture has led to a disproportionately large number of predator invasions in freshwater systems relative to their terrestrial and marine counterparts (Rahel 2000, Dudgeon *et al.* 2006, Leprieur *et al.* 2008) (Ricciardi & MacIsaac 2011). In many cases, introduced predatory fish have had strong effects on native biotas and ecosystem functioning (Eby *et al.* 2006, Cox & Lima 2006), but in other cases impacts appear to have been relatively minor (Mack *et al.* 2000, Ricciardi & Atkinson 2004). Unfortunately, there are few generalities about the factors dictating the outcome of predator invasions, and consequently our ability to predict their impact is limited (Moyle & Light 1996, Parker *et al.* 1999, Salo *et al.* 2007).

Introduced predatory fish can have impacts at multiple levels of ecological organization. At the individual level they can cause behavioural and morphological changes in native species; at the population level they can affect the abundance, size structure and genetic structure of a species; at the community level they can modify trophic interactions between species and alter patterns of richness and diversity through a combination of direct and indirect effects; and at the ecosystem level they can alter habitat structure and modify nutrient fluxes and energy pathways (Townsend 2003, Simon & Townsend 2003).

Traditionally, studies on the impacts of invasive predatory fish have focused mainly on direct impacts on native species at the individual or population level (Flecker & Townsend 1994, Townsend 2003). More recently, the realization that predator impacts can extend beyond direct effects on native species has led to an increase in studies at the community and ecosystem levels (e.g. Huryn 1998, Baxter *et al.* 2004, 2007, Cheever & Simon 2009, Herbst *et al.* 2009, Ho *et al.* 2011). Simon and Townsend, in their 2003 review of salmonid impacts in freshwater ecosystems, stressed that only by broadening our approach and incorporating multiple trophic levels and the links between them, we can hope to unravel the true extent of predatory fish impacts in freshwater systems, and manage fish invasions accordingly. The focus of my thesis is on understanding how an introduced predatory fish has influenced the abundance of native predatory fish species (population-level impact), and on what the consequences of this are for lower trophic levels (community-level impact).

The consequences of a predatory fish invasion for populations of native species are difficult to predict because the effects can be influenced by multiple biotic and abiotic aspects of the recipient system, resulting in large variation in impacts among systems (McIntosh 2000). One hypothesis that has been put forward to explain variation in non-native predator impacts is the "naïve prey" hypothesis (Cox & Lima 2006) which proposes that impact strength will be influenced by whether or not native species have prior experience with a predator which is functionally similar to the introduced one. Salo *et al.* (2007) surmise that impacts will be strongest in insular systems that lack functionally similar predators, because native species, having evolved in isolation, are probably not adapted to the hunting methods of the new predator. Freshwater systems function as insular systems within terrestrial systems, and often have long histories of evolutionary isolation (Cox & Lima 2006). The native species they support are therefore unlikely to have acquired traits that enable them to cope with the threat posed by a novel predator, and consequently may be highly vulnerable to the effects of introduced predators (Cox & Lima 2006).

Whether or not a predator invasion has consequences at the community-level may depend upon the degree to which the introduced predator changes the functional role performed by the native predator assemblage. Traditionally, models of trophic relationships within biological communities have been based on the premise that groups of species can be lumped into distinct functional units such as "trophic levels", or "guilds" (Oksanen *et al.* 1981, Menge & Sutherland 1987). This approach assumes that all species within a functional unit have the same role in a community and are therefore functionally equivalent (Chalcraft & Resetarits 2003). More recently, community ecologists have begun to realize that this assumption is, in many cases, an over-simplification (Schmitz & Suttle 2001, Schmitz 2007). For example, predator species within the same trophic level may have different hunting strategies, which can lead to different effects on prey assemblages, and ultimately on the structure of entire communities (Schmitz 2008). In freshwater systems, differences in the effects of predatory fish on communities have been attributed to, among other factors, differences in fish foraging modes. Specifically, Dahl & Greenberg (1996) postulated that drift-feeding fish should have a weaker effect on benthic communities than should benthic-feeding fish, because their diet is often augmented by terrestrial invertebrates.

1.4 Rainbow trout: a formidable freshwater invader

Rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) is a member of the family Salmonidae which includes species of salmon, trout and charr. Rainbow trout are medium- to large-sized fish (commonly 50–300 mm in length, but in some environments individuals can exceed 1000 mm) with elongate bodies that are covered in small scales (Adams *et al.* 2008, Montgomery & Bernstein 2008). Their mouths are large and terminal, and rows of sharp teeth occur on the palate, tongue and on both the upper and lower jaws. Color and markings vary with habitat, size and spawning condition. Adults tend to be silver to green with black spots on their backs and fins, and a band of pink along their sides, while juveniles have several dark, oval "par marks" along their sides (Montgomery & Bernstein 2008) (Figure 1.2). Their fins, except for the adipose fin which is lobate, are supported by branched soft rays (Montgomery & Bernstein 2008). They are primarily a freshwater species, but some populations migrate to the sea as juveniles and return to freshwater environments as adults to breed (i.e. they have an anadramous life cycle) (Raleigh *et al.* 1984). They prefer cool, well-oxygenated, unpolluted water, and their optimal temperature range is 12-18°C (Raleigh *et al.* 1984), with a critical thermal maximum of ~25°C (Adams *et al.* 2008). Rainbow trout reach sexual maturity at 2-3 years of age, and at a length of approximately 150-200 mm (Adams *et al.* 2008, Montgomery & Bernstein 2008). They can spawn in temperatures ranging from 2–20˚C, within an optimal range of 7–15˚C, and flowing water is essential for successful reproduction (Raleigh *et al.* 1984, Montgomery & Bernstein 2008). Rainbow trout are opportunistic predators that prey on a wide variety of animal foods including aquatic invertebrates, terrestrial invertebrates, crabs, frogs and fish (Raleigh *et al.* 1984, Adams *et al.* 2008, Montgomery & Bernstein 2008). In general, juveniles feed mostly on small invertebrates, and as they grow, larger prey items such as vertebrates, become increasingly common in their diet (Montgomery & Bernstein 2008).

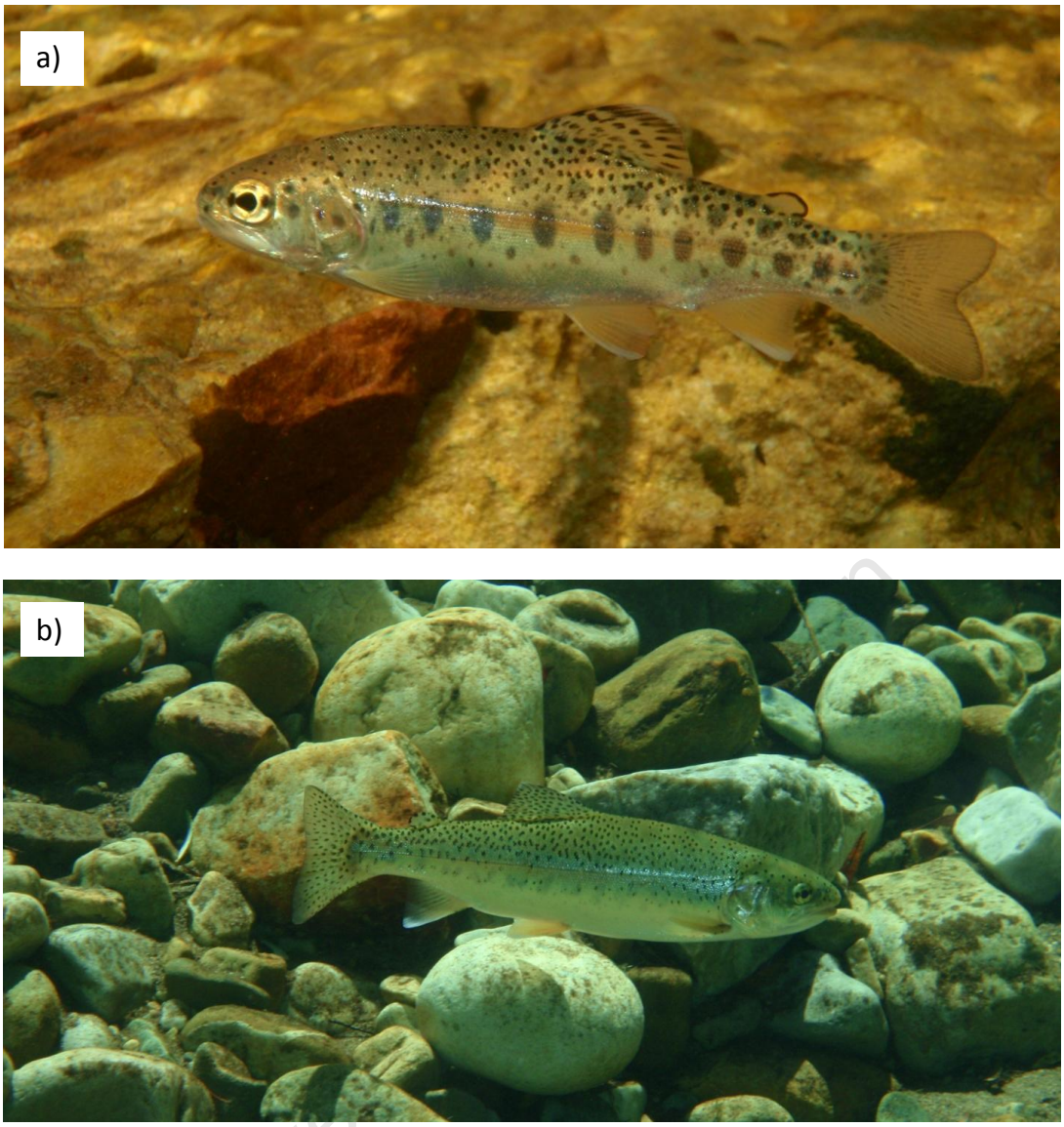

Figure 1.2 a) A juvenile (~70 mm) rainbow trout *O. mykiss* feeding in a riffle in Jan du Toit Stream, and b) an adult (~300 mm) rainbow trout in a deep pool in Morainekloof Stream.

Rainbow trout (henceforth "trout") is the third most widely introduced fish in the world (Fausch 2007), with only common carp *Cyprinus carpio* and Nile perch *Lates niloticus* being more widely introduced (Welcomme 1988). From its native range in Pacific North America and eastern Russia, it has been introduced to at least 97 countries, and to every continent except Antarctica (Crawford & Muir 2008, Figure 1.3). In many cases, introduced trout have established self-sustaining populations (Fausch 2007) and have had severe impacts on native biotas, ranging from impacts on a species' behaviour (Garcia de Leaniz *et al.* 2010) to the re-structuring of entire stream ecosystems (Baxter *et al.* 2004, 2007). Introduced trout

have had strong, negative impacts on populations of native vertebrates, including fish (e.g. Blinn *et al.* 1993, Lintermans 2000, Kitano 2004, Fausch *et al.* 2010, Young *et al.* 2010) and amphibians (e.g. Pilliod & Peterson 2001, Gillespie 2001, Vredenburg 2004), as well as on populations of aquatic invertebrates (e.g. Baxter *et al.* 2007, Molineri 2008, Albariño & Buria 2011). By altering the abundance, and/or behaviour, of native aquatic consumers such as herbivorous invertebrates, introduced trout have indirectly altered levels of algae (e.g. Herbst *et al.* 2009, Buria *et al.* 2010), and detritus (e.g. Buria *et al.* 2010), which are key resources at the base of aquatic food webs (Allan & Castillo 2007). The consequences of rainbow trout invasions have also been shown to extend beyond the boundaries of aquatic ecosystems. For example, trout-induced changes in aquatic invertebrate abundance, and the corresponding flux of aquatic invertebrates from aquatic to terrestrial systems has been shown to influence the abundance of riparian consumers (Nakano *et al.* 1999b, Baxter *et al.* 2004, 2007). Negative impacts of trout invasions have been quantified in New Zealand (McDowall 2003), Australia (Crowl *et al.* 1992, Lintermans 2000, McDowall 2006), South America (Young *et al.* 2010, Habit *et al.* 2010), Japan (Nakano *et al.* 1999b, Baxter *et al.* 2004, 2007, Kitano 2004) and in the U.S. (Dunham *et al.* 2004). Because of their widespread impact, rainbow trout have been listed by the World Conservation Union Global Invasive Species Programme (GISP) as one of the World's 100 Worst Alien Invasive Species (Lowe *et al.* 2000). Although the consequences of trout introductions have received detailed study in many areas, impacts in other areas, such as South Africa, remain largely unknown.

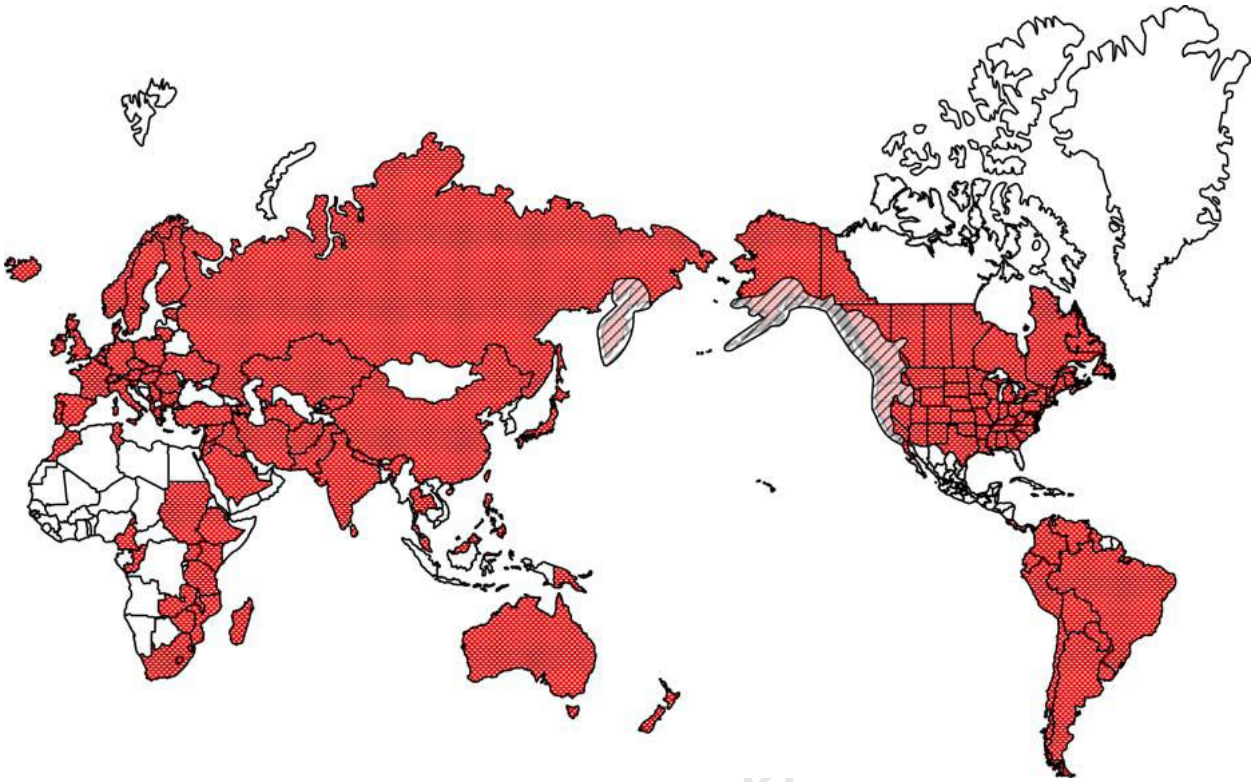

Figure 1.3 Distribution of native and introduced rainbow trout. Countries, states or provinces where rainbow trout are native are indicated in pink with black diagonals, and where trout have been introduced in red (from Crawford & Muir 2008).

1.5 Rainbow trout in South Africa

Early British colonists decided that South Africa's indigenous freshwater fish species were unsuitable for angling, and felt it necessary to import and stock South African rivers with (among other fishes) salmonids which were considered premier angling species in other parts of the world (de Moor & Bruton 1988, Skelton 2001). The first rainbow trout ova were successfully brought to South Africa from England in 1897, and were hatched and reared at the Jonkershoek Hatchery in Stellenbosch, close to Cape Town (de Moor & Bruton 1988). These fish were bred successfully and their ova were sent to several newly-established hatcheries in other parts of the country (de Moor & Bruton 1988). Widespread stocking of South African rivers followed (see Figure 1.4), and by the 1920s the cool, clear-flowing headwaters of many of the country's major river systems supported self-sustaining populations of rainbow trout (Hey 1926). For much of the 20th century, trout were bred, and trout populations were created, maintained and protected, by government-funded nature

conservation authorities (Skelton 2001). Beginning in the 1960s, conservation organizations gradually became aware that trout appeared to be having a negative impact on native aquatic species, particularly fishes (de Moor & Bruton 1988). This realization led to policy changes in the 1980s, and the focus of conservation organizations shifted away from the production and promotion of non-native fish species towards the conservation of threatened native aquatic species (de Moor & Bruton 1988). Figure 1.5 shows the present range of introduced rainbow trout in South Africa.

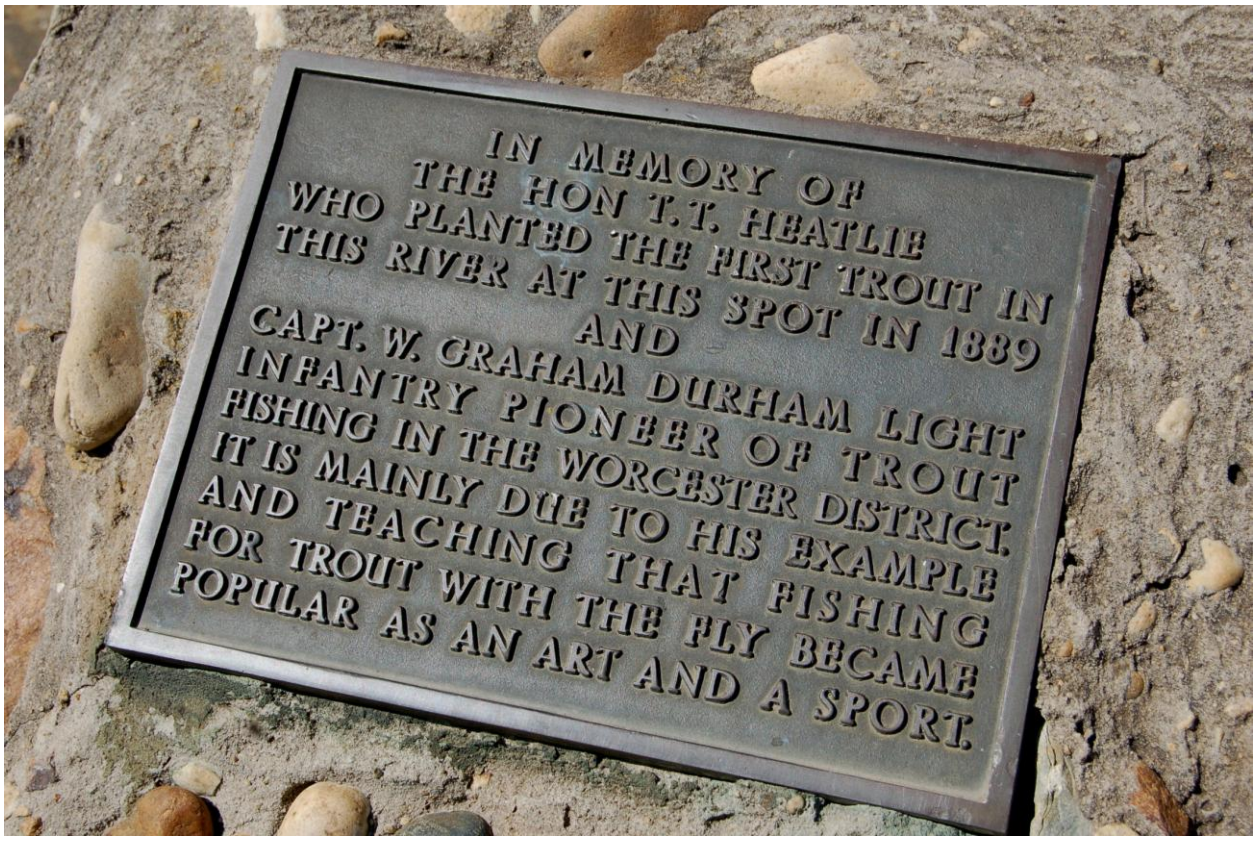

Figure 1.4 Plaque on the bank of the Hex River detailing when and where trout were first introduced to the river (1889), and the attitude towards the introduction of trout in South Africa at that time (photograph Sean Marr).

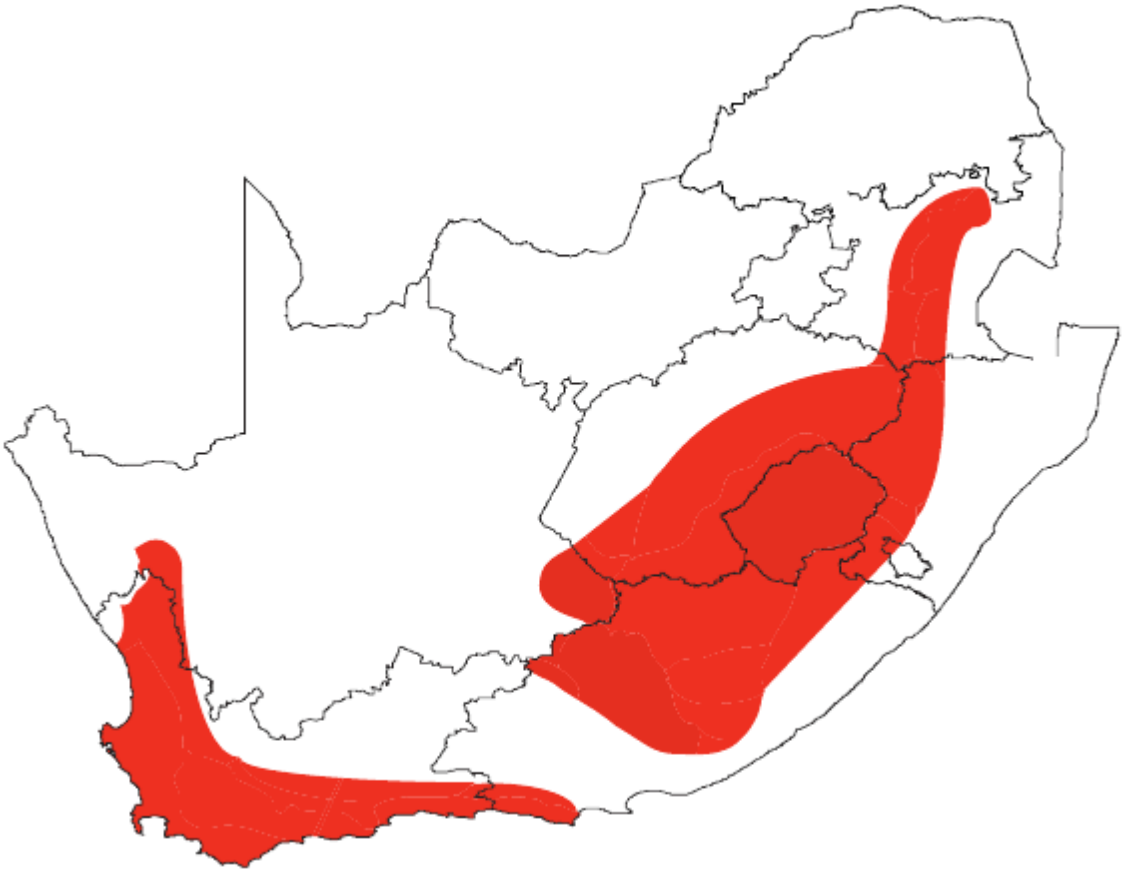

Figure 1.5 Range (shown in red) of introduced rainbow trout in South Africa (from Picker & Griffiths 2011).

Today, rainbow trout is a species of significant economic value in South Africa (Bainbridge *et al.* 2005). They are exploited commercially for food production, and recreationally as an angling species. Commercially, trout are farmed and sold locally, or exported as a high-value food source (Bainbridge *et al.* 2005). After abalone *Haliotis midae*, trout is the second most important aquaculture species in South Africa, and in 2008, the trout aquaculture sector consisted of 24 registered trout farms which were collectively valued at 27.9 million ZAR, and employed 346 permanent and 163 part time employees (Britz *et al.* 2009). The recreational trout industry sustains a considerable industry of tackle manufacturers and retailers, tourist operators, professional guides and accommodation establishments (Bainbridge *et al.* 2005, du Preez & Hosking 2010), and is an important source of income and job creation in some of the poorest parts of South Africa (du Preez & Hosking 2010). Clearly,

trout have an important place in the South African economy, but unfortunately our understanding of their impacts on native species and ecosystems is rudimentary.

Although it has long been suspected by ecologists that the environmental impacts of trout in South Africa have been predominantly negative, evidence for this is mostly circumstantial and anecdotal (de Moor & Bruton 1988, Cambray 2003). To my knowledge, just three scientific studies have attempted quantitative assessments of trout impacts on native biotas in southern Africa, two in South Africa and one in Malawi. Woodford & Impson (2004) studied sympatric populations of trout and three species of native fish (Berg River redfin *Pseudobarbus burgi*, Cape kurper *Sandelia capensis* and Cape galaxias *Galaxias zebratus*) in a single headwater stream in the south-western Cape and found some evidence that trout consume one of the species of native fish (*Galaxias zebratus*), and that native fish avoided areas occupied by trout. In KwaZulu-Natal, Karssing *et al.* (2012) studied populations of tadpoles of the native Natal cascade frog *Hadromophryne natalensis* above and below waterfalls which acted as physical barriers to trout invasions in two headwater streams (one containing rainbow trout, and the other brown trout *Salmo trutta*), and found trout presence to be the most likely cause of abrupt declines in amphibian abundance downstream of the waterfalls. In a survey conducted at 24 sites on streams on the Nyika Plateau, Malawi, Kadye & Magadza (2008) found that the feeding behaviour and habitat preferences of the native mountain catfish *Amphilius uranoscopus* appeared to be influenced by the presence of trout. Taken together, these studies suggest that trout may well be preying upon (and negatively impacting) native fish and amphibian populations, and altering native fish behaviour, in southern Africa. Considering that many species of South African native fish have evolved in isolation, and in the absence of large predatory fish such as trout, it seems likely that they will be especially vulnerable to predation by introduced trout, as predicted by the "naïve prey" hypothesis of Cox & Lima (2006).

In addition to concern over direct effects on charismatic species such as fish and amphibians, there has been mounting concern that trout may also affect other components of stream communities (de Moor & Bruton 1988, Cambray 2003). Information on the impacts of trout at the community-level in South Africa is scarce, and to my knowledge no quantitative assessments of trout effects on invertebrate assemblages and lower trophic levels have yet been attempted. The feeding biology of trout in some other parts of the

world has been well studied (see reviews in Raleigh *et al.* 1984, Adams *et al.* 2008, Montgomery & Bernstein 2008). Trout are mostly drift feeders that usually feed primarily on aquatic invertebrates, but frequently derive a significant proportion of their diet from terrestrial invertebrates that fall into the stream from the riparian zone (Kido *et al.* 1999, Nakano *et al.* 1999b, Nakano & Murakami 2001, Baxter *et al.* 2004, White & Harvey 2007). The feeding biology of native fish has been less well studied, but the available literature suggests that *Pseudobarbus* spp., the dominant component of native fish assemblages in many South African headwater streams (Skelton 2001), are primarily benthic feeders that consume mostly aquatic invertebrates (Cambray & Stewart 1985, Whitehead *et al.* 2007, Lowe *et al.* 2008), but often also notable quantities of algae and detritus (Esterhuizen 1978, de Wet 1990). This information suggests that trout and native *Pseudobarbus* spp. may differ in the functional role that they perform in CFR headwater streams, and thus may influence community structure and functioning differently. For example, according to the "foraging mode" hypothesis proposed by Dahl & Greenberg (1996), if terrestrial invertebrates feature more prominently in the diet of drift-feeding trout than in that of benthic-feeding redfin, trout should have a weaker effect on benthic communities in South African streams than should native redfin.

Nowhere is research on trout impacts in South Africa needed more urgently than in the Cape Floristic Region (CFR), because of its importance as a biodiversity hotspot.

1.6 The threat posed by trout in the CFR

The CFR is one of only 25 global biodiversity hotspots identified by Myers et al. (2000), and is best known for its exceptionally high plant diversity (~9000 species) and endemicity (~70% of species) (Rouget *et al.* 2003, de Moor & Day 2013). It is less well known that the region's freshwater fauna also exhibits unusually high levels of endemism, with ~64% of the ~700 currently recognized invertebrate species (Wishart & Day 2002), and ~89% of the 27 currently recognized fish taxa (Tweddle *et al.* 2009), being endemic. Freshwater ecosystems in the CFR are highly threatened by multiple human-related factors including habitat loss and fragmentation, hydrological alteration, climate change, overexploitation, pollution and invasions by non-native plants and animals (Tweddle *et al.* 2009, de Moor & Day 2013). In

general, the middle and lower sections of rivers have been most severely degraded because the surrounding terrain tends to be suitable for agriculture and urban developments (Swartz *et al.* 2004, Impson *et al.* 2007, Tweddle *et al.* 2009, Chakona & Swartz 2012, de Moor & Day 2013). The upper headwater sections of rivers in the region have generally been less severely impacted by human-related activities, because they are situated in mountainous areas that are inaccessible and unsuitable for agriculture, human settlements and reservoirs (Swartz *et al.* 2004, Tweddle *et al.* 2009, de Moor & Day 2013). Consequently, headwater streams function as ecological refugia within a relatively degraded landscape and are thus critical habitats for conserving freshwater biodiversity in the CFR.

Most of the non-native fish species introduced into the CFR (including bass *Micropterus* spp., bluegill sunfish *Lepomis macrochirus*, Mozambique tilapia *Oreochromis mossambicus*, banded tilapia *Tilapia sparrmanii*, common carp *Cyprinus carpio* and sharptooth catfish *Clarias gariepnus*) are not well-adapted to environmental conditions in headwater streams, and cannot tolerate the high flows and low temperatures that prevail in winter (de Moor & Bruton 1988, Cambray 2003). Trout, on the other hand, are well adapted to headwater stream environments, and may therefore pose the single greatest threat to the ecological integrity of headwater stream environments in the CFR. If we are to meet the dual goals of maintaining trout populations for economical and recreational purposes and conserving our native freshwater biodiversity and aquatic ecosystems, it is imperative that we develop a thorough understanding of the impact of introduced trout in headwater stream environments in the CFR.

1.7 Approaches to investigating non-native predators impacts

Quantifying impacts of non-native predators is a challenging task, and unless approached carefully and thoughtfully will not provide reliable and comprehensive information on which management decisions can be based. A variety of approaches can be used for assessing non-native predator impacts, including predictive studies based on information on similar invasions elsewhere; comparative and correlational studies that take advantage of natural variations in non-native predator presence/abundance; dietary studies and experimental manipulations (Park 2004). Each approach offers unique insights into the nature and extent

of predator impacts and has specific strengths and weaknesses which need to be recognized when choosing which to apply (Kats & Ferrer 2003, Park 2004).

Monitoring an invasion in action is an ideal way to study the effects of a non-native predator on a recipient system, but unfortunately most studies only begin long after the impacts have already occurred (Lintermans 2000). It is sometimes possible to create a "new" invasion by means of an experimental introduction (e.g. Fletcher 1979), but this approach is usually unacceptable for ethical reasons (McIntosh 2000, McDowall 2003, Meissner & Muotka 2006, Peterson & Fausch 2008). When pre-invasion data are not available, and studying an invasion in action is not an option, various alternative techniques can be employed to infer invader impacts.

One possibility is to conduct comparisons among systems with and without the introduced predator, and to use differences in native species abundance to infer impacts. The major weakness of this approach is that it does not take into account other factors that may influence the abundance of the native species, such as variation in habitat characteristics among the sites being compared (Townsend & Crowl 1991). Furthermore, there is usually a large degree of natural variation among freshwater systems making it difficult, in many cases, to separate the predator impact "signal" from the environmental "noise" (Meissner 2000). These problems can by partly overcome by incorporating variation in other environmental factors into analyses, and assessing the influence of environmental factors alongside that of the introduced predator (Townsend & Crowl 1991). However, an important limitation of the survey approach is that it can only be used to describe patterns in species abundance, and to relate these to other factors, not to identify mechanisms driving the patterns or to infer "cause-and-effect type" relationships (Park 2004). This is because several different processes could potentially have given rise to an observed pattern, and there is always a possibility that some unmeasured variable has obscured the relationship of interest (Cooper & Dudley 1988).

The identification of mechanisms behind patterns observed in the field requires experiments that enable the factor of interest (e.g. predatory fish presence) to be manipulated, while controlling for all other potential sources of variation (e.g. environmental conditions) (Meissner 2000, Park 2004). Unfortunately, to achieve control

over potentially confounding variables the experiments often have to be conducted on unrealistically small spatial scales (Miller 1986, Cooper & Dudley 1988). Surveys, on the other hand, can be conducted on large, realistic, spatial scales, and thus incorporate natural levels of environmental heterogeneity. Because surveys and experiments offer contrasting advantages and insights into non-native predator impacts, the use of these approaches in complementary roles has been strongly advocated by several authors (e.g. Cooper & Dudley 1988, McIntosh *et al.* 2002, Kats & Ferrer 2003). Concordance between experimental and survey results is generally considered strong evidence for an impact (Meissner 2000, Park 2004). In this study, I use a combination of comparative and experimental approaches to investigate the impact of introduced trout on native fish populations, and on the functional structure of benthic communities, in headwater streams in the CFR.

1.8 Thesis overview

Broadly, this thesis aims to improve knowledge and understanding about impacts of novel predators in insular systems where biological communities evolved in the absence of functionally similar native predators. Of particular interest is how populations of naïve native predators are affected by a novel predator, and how changes in predator functional role influence the structure and functioning of the "recipient" community. This work builds on our limited knowledge about trout impacts on South African fish populations, and is the first quantitative assessment of community-level consequences of trout invasions in South African streams.

The fact that trout have invaded some, but not all, headwater tributaries of the upper Breede River (one of the major river systems in the CFR) presents a unique opportunity to investigate trout impacts on native fish populations and on stream community structure by means of broad-scale comparative field surveys. Hypotheses about impacts formulated from patterns uncovered during these surveys are then tested using small-scale, controlled field experiments. In addition, investigation of fish foraging behaviours and diets are used to ascertain whether or not native and non-native fish are functionally equivalent predators, and whether any differences in community structure between streams with and without trout were linked to differences in fish trophic niche.

The individual chapters presented in this thesis have been written as "stand alone" bodies of work. There is therefore some overlap in the introduction and methods sections of the chapters. A list of references covering the literature cited in the individual chapters can be found following Chapter 6. The writing and analyses presented in all chapters is entirely my own work.

In Chapter 2, I study the impact of introduced trout on native fish populations in headwater streams in the upper Breede River catchment. I predict that trout should have a strong, negative impact on native fish abundance, since the native fish species in these streams evolved in the absence of top predators functionally similar to trout. Native fish abundance is estimated, and relevant environmental variables measured, in 24 minimally-disturbed headwater streams, 12 with trout and 12 without trout. The relative importance of trout abundance and other environmental variables in explaining variation in native fish abundance among streams is assessed. Native fish size distributions are compared between streams with and without trout, and a small-scale field experiment conducted to evaluate how size-selective predation by trout might affect the structure of native fish populations is described.

In Chapter 3, I survey benthic invertebrate assemblages, algal biomass and standing stocks of organic matter in all of the streams where fish populations and environmental variables were surveyed to ascertain whether benthic community structure differs between streams with and without trout. The relative importance of trout presence and other environmental variables in explaining variation among the streams in the taxonomic and functional structure of benthic invertebrate assemblages, as well as in algal biomass and the biomass of fine and coarse particulate organic matter, is assessed.

In Chapter 4, I characterize and compare the trophic niches of trout and redfin (the dominant member of the native fish assemblage) to ascertain whether these two species perform different roles in the stream community. Focal animal watching (FAW) is used to compare the foraging behaviours of trout and redfin, while gut content analysis (GCA) and stable isotope analysis (SIA) are employed to characterize and compare the diets of these two species in three of the streams sampled during the broad-scale surveys (Chapters 2 and 3) which contained only trout and three which contained only redfin. GCA provides detailed

information about recently ingested foods, while SIA offers a coarser-resolution, time-integrated measure of fish dietary habits.

In Chapter 5, I describe a small-scale, manipulative field experiment carried out in one of the headwater streams which was conducted to ascertain whether top-down fish effects are important in regulating community structure, and whether trout and redfin affect lower trophic levels differently. I predict that trout should have a weaker effect on benthic invertebrate assemblages than should redfin, because trout are drift feeders and their diet is likely to be augmented by terrestrial prey. I predict furthermore that if redfin suppress benthic invertebrate abundance more strongly than do trout, the grazing pressure exerted by benthic invertebrates on benthic algae should be relaxed, and algal biomass should be greater in the presence of redfin than in the presence of trout.

Finally, in Chapter 6, I summarize and synthesize the main findings from each chapter, and place my research within the context of current knowledge about the population- and community-level consequences of predator invasions. I then assess the implications of my findings with regard to the threat posed by trout to native fish populations and other components of stream communities, and discuss the relevance of my findings for the management of non-native trout in South Africa. I conclude by highlighting key avenues for future research on trout impacts in South Africa.

Chapter 2

Predatory impact of non-native rainbow trout on native fish populations in headwater streams of the upper Breede River catchment, South Africa

2.1 INTRODUCTION

Understanding the consequences of predation in biological systems has long been an important goal in ecology, and is becoming increasingly pertinent as the number of predators introduced outside of their natural ranges increases (Vitousek *et al.* 1997). Impacts of introduced predators are difficult to predict because they can be influenced by multiple biotic and abiotic features of the recipient system (Lodge 1993, McIntosh 2000, Sih *et al.* 2010). While some predator introductions result in severe consequences for native species, others appear largely inconsequential (Mack *et al.* 2000, Ricciardi & Atkinson 2004). An ability to distinguish introductions likely to have major impacts from those that are not would be useful for guiding management efforts (Ricciardi & Atkinson 2004, Salo *et al.* 2007, Sih *et al.* 2010). Unfortunately, few generalities exist about the factors dictating the outcome of predator invasions, and consequently our ability to predict impact strength is limited (Moyle & Light 1996, Parker *et al.* 1999, Korsu *et al.* 2007).

One hypothesis that has been put forward to explain variation in non-native predator impacts is the "naïve prey" hypothesis (Cox & Lima 2006) which proposes that impact strength should be influenced by whether or not native species have prior experience with a predator functionally similar to that which is introduced. Impacts are expected to be strongest in insular systems, such as oceanic islands, that lack functionally similar predators (Courchamp *et al.* 2003, Blackburn *et al.* 2004) because native species will have evolved in isolation and will thus be naïve to the hunting tactics of the novel predator (Ricciardi & Atkinson 2004, Salo *et al.* 2007). Well known examples of strong impacts of introduced predators on islands include the impact of the red foxes *Vulpes vulpes* on small marsupials in Australia (Jones *et al.* 2004), the impact of brown tree snakes *Boiga irregularis* on birds on

Guam (Savidge 1987) and impacts of introduced black rats *Rattus rattus* on sea birds in the Balearic and Canary Islands (Traveset *et al.* 2009).

Freshwater systems with a history of geographic isolation function like islands, and may be similarly sensitive to effects of introduced predators (Cox & Lima 2006). Predatory freshwater fish have been widely introduced for angling purposes (Eby *et al.* 2006), and may pose a serious threat to native species in systems that evolved without functionally similar predators (Blackburn *et al.* 2004, Salo *et al.* 2007, Sih *et al.* 2010). Indeed, one of the most destructive predator introductions ever documented is that of the Nile perch *Lates niloticus* into Lakes Victoria and Kyoga in Africa. These lakes were historically inhabited by over 300 endemic haplochromine cichlid species that evolved in the absence of large piscivorous fish (Witte *et al.* 1992). Predation by introduced Nile perch has resulted in the extinction of approximately two-thirds of the cichlid species inhabiting these lakes, and the remaining species may also be under threat (Ogutu-ohwayo 1993). In the Cape Floristic Region (CFR) of South Africa, native stream fish have also evolved in the absence of large predatory fish (Skelton 2001, Swartz *et al.* 2004, Tweddle *et al.* 2009), and may therefore be naïve and vulnerable to predation by the large, predatory game fish which have been introduced to the region for angling relatively recently (de Moor & Bruton 1988). The focus of this chapter is on quantifying the predatory impact of rainbow trout *Oncorhynchus mykiss*, one such introduced predatory fish, on native fish populations in CFR streams.

Geologic conditions in the CFR have remained relatively stable since the beginning of the Cenozoic era 65 million years ago (Deacon *et al.* 1992), and the majority of the regions river catchments have probably been confined to their present catchments since that time (de Moor & Day 2013). Biogeographic studies indicate that many of the CFR freshwater fish lineages have probably existed and evolved in relative isolation for more than 20 million years (Skelton 1980, 2001, Swartz *et al.* 2004, 2009, Wishart *et al.* 2006). The region's freshwater fish fauna is characterized by low species diversity and a high level of endemism (Linder *et al.* 2010). Only four families of primary freshwater fish (species that spend their entire lives in freshwater) occur in the region, namely Cyprinidae, Galaxiidae, Anabantidae and Austroglanididae, and only the genera *Pseudobarbus*, *Galaxias* and *Sandelia* are widespread (Swartz *et al.* 2004). Historically, 19 species of primary freshwater fish have been recognized in the region (Skelton 2001, Impson *et al.* 2002), but this number has

recently been elevated to 27 following taxonomic work on the genus *Pseudobarbus* (Tweddle *et al.* 2009, Swartz *et al.* 2009). Further ongoing taxonomic work on the genera *Galaxias* and *Sandelia* indicate that what were once thought to be single widespread species' are in fact species complexes, and over 40 distinct genetic lineages have been identified in the region (Linder *et al.* 2010). Most species are relatively small-bodied, rarely exceeding 150 mm as adults (Skelton 2001), and none of these native species is primarily piscivorous (Swartz *et al.* 2004), although larger individuals of some species, such as *Sandelia capensis* and *Labeobarbus capensis*, may become partly piscivorous as adults (Skelton 2001). Of the 27 currently recognized taxa, 24 (~89%) are endemic to the region (Impson *et al.* 2002, Tweddle *et al.* 2009), and 19 (~70%, all endemics) are listed as threatened in the IUCN Red List of Threatened Species (Impson *et al.* 2007, Tweddle *et al.* 2009).

Threats to CFR freshwater fishes include habitat loss and fragmentation, hydrological alteration, climate change, overexploitation and pollution, but perhaps the greatest threat at present is that posed by introduced predatory fish (Tweddle *et al.* 2009). To date, 16 species of non-native freshwater fish have established self-sustaining populations in the CFR (Marr 2011), and the majority of these species have been linked to declines in native fish populations (de Moor & Bruton 1988). In particular, the smallmouth bass *Micropterus dolomieu* introduced from North America has led to dramatic declines in native fish populations. For example, Woodford *et al.* (2005) found that four of the five species of native fish that inhabit the Rondegat River (Olifants River system, CFR) were entirely absent from a section of the river invaded by smallmouth bass, and only one native species persisted, although in severely reduced numbers. Interactions with smallmouth bass and other non-native fish, in combination with the effects of habitat degradation, have led to the disappearance of native fish from the middle and lower sections of many rivers in the CFR, and as a result the region's native fish populations are highly fragmented, with many species now largely restricted to headwater tributaries (Swartz *et al.* 2004, Tweddle *et al.* 2009, Chakona & Swartz 2012). Headwaters have generally been less severely impacted by human-related activities than lower-lying reaches because they are situated in mountainous areas that are difficult to access and unsuitable for agriculture, human settlements and reservoirs (Swartz *et al.* 2004, Tweddle *et al.* 2009, de Moor & Day 2013). Furthermore,

most of the non-native fish species introduced into the CFR are not well-adapted to environmental conditions in headwater streams, and cannot tolerate the high flows and low temperatures that prevail in winter (de Moor & Bruton 1988, Cambray 2003, Tweddle *et al.* 2009). Trout, on the other hand, are well adapted to headwater stream environments (Skelton 2001), and consequently may pose a serious threat to remaining native fish populations in the CFR.

Rainbow trout is the third most widely introduced fish in the world (Fausch 2007), having been introduced to at least 97 countries from its native range in Pacific North America and eastern Russia (Crawford & Muir 2008). In many cases, strong, negative impacts on native biotas have been reported following introductions, resulting in rainbow trout being listed by the World Conservation Union Global Invasive Species Programme as one of the World's 100 Worst Alien Invasive Species (Lowe *et al.* 2000). Introduced trout have had impacts at the individual, population, community and ecosystem levels of ecological organization (Townsend 2003, Simon & Townsend 2003). Effects on native fish populations have been the focus of many studies, and impacts range from severe declines in native fish abundance (e.g. Townsend & Crowl 1991) to subtle impacts on native fish population dynamics (e.g. McIntosh 2000). Mechanisms for impact include competition for space or food, transfer of parasites and hybridization, but perhaps the most commonly reported mechanism is predation (Townsend 1996). Predation by non-native trout can alter the distribution, density and size structure of native fish populations (McIntosh 2000, Simon & Townsend 2003). Trout are gape-limited, visual predators, and as a result, certain size-classes of native fish are more vulnerable to predation than are others (McIntosh 2000).

Rainbow trout were introduced to the CFR in 1897 for angling purposes and self-sustaining populations have established in many headwater streams (de Moor & Bruton 1988, Scott *et al.* 2006). Trout represent an economically and recreationally valuable species (Bainbridge *et al.* 2005) so there is pressure from interest groups to retain existing populations, and potentially to stock additional rivers (Impson 2001a), but our understanding of the impact of trout in rivers of the CFR is limited. It has long been claimed that trout eliminate native fish from South African streams through predation, but this is based mostly on circumstantial evidence and anecdotal observations (see De Moor & Bruton 1988, Cambray 2003 for comprehensive reviews of information on trout impacts in South Africa). If we are to meet

the dual goals of maintaining trout populations for economic and recreational purposes, and conserving our native fish species, it is imperative that we develop a good understanding of the predatory threat posed by non-native trout to native fish populations in CFR headwater streams.

In some cases the impact of an introduced predatory fish can be inferred by a subsequent decline in the population of native fish abundance (e.g. Habit *et al.* 2010), but such inferences are not possible in the CFR because pre-invasion information about native fish distributions is scarce. An alternative approach is to compare otherwise similar systems with and without the introduced predator (Meissner 2000). If native fish density differs between these two types of systems, and if these differences cannot be attributed to differences in environmental conditions among systems, then the introduced predator is implicated as a contributing factor (White & Harvey 2001). Controlled experiments can then be used to investigate specific processes suspected to be driving patterns in native fish populations (Park 2004). I used this approach to investigate the predatory impact of introduced trout on native fish populations in the CFR headwater streams. The upper Breede River catchment in the CFR is partially invaded by non-native rainbow trout, providing a valuable opportunity to conduct a comparative study of the type mentioned above. A small-scale field experiment was then used to examine the role of predation in structuring native fish populations.

The broad aim of this study was to test the hypothesis that trout have strongly reduced the density and distribution of native fish populations in the CFR, because native fish lack evolutionary experience with a predator that is functionally similar to trout. Specifically five questions were addressed:

1) Does the density and biomass of the native fish species differ between streams with and without trout?
2) Do environmental conditions differ between sites with and without trout?
3) What is the relative importance of trout density and other environmental variables in explaining variation in the density of the native fish species among streams?
4) Does the native fish size distribution differ between sites with and without trout?
5) Is size-selective predation by trout a mechanism that could account for differences in native fish density and size distribution between streams with and without trout?

2.2 METHODS

2.2.1 Study area

The Breede River catchment is the fourth largest in the CFR, and drains an area of approximately 12 600 km^2. From its source in the Skurweberg Mountains near the town of Ceres, it flows in a south-westerly direction for 322 km before opening into the Indian Ocean near the town of Witsand (Steynor *et al.* 2009). The present study was conducted in the mountainous upper catchment which includes the tributary systems that join the main river upstream of its confluence with the Doring River near Brandvlei Reservoir, as well as the tributaries of the upper Sonderend River (Figure 2.1). This area experiences a Mediterranean climate, with warm, dry summers and cool, wet winters (Cowling & Holmes 1992). Mean annual rainfall is ~800 mm per annum, of which 80% falls between the months of April and September (Steynor *et al.* 2009). Natural vegetation is predominantly Sandstone Fynbos (Rebelo *et al.* 2006), a diverse assemblage of low-growing, fine-leafed, sclerophylous shrubs. Riparian vegetation is largely composed of broad-leaved woody species including perennial shrubs and small trees, but also characteristic fynbos elements such as species of Restionaceae and Ericaceae (Cowling & Holmes 1992). The mountains generally comprise hard, quartzitic sandstones of the Table Mountain group (Tankard *et al.* 1982), and the streams flowing over this stratum are acidic, oligotrophic and low in dissolved solids (de Moor & Day 2013).

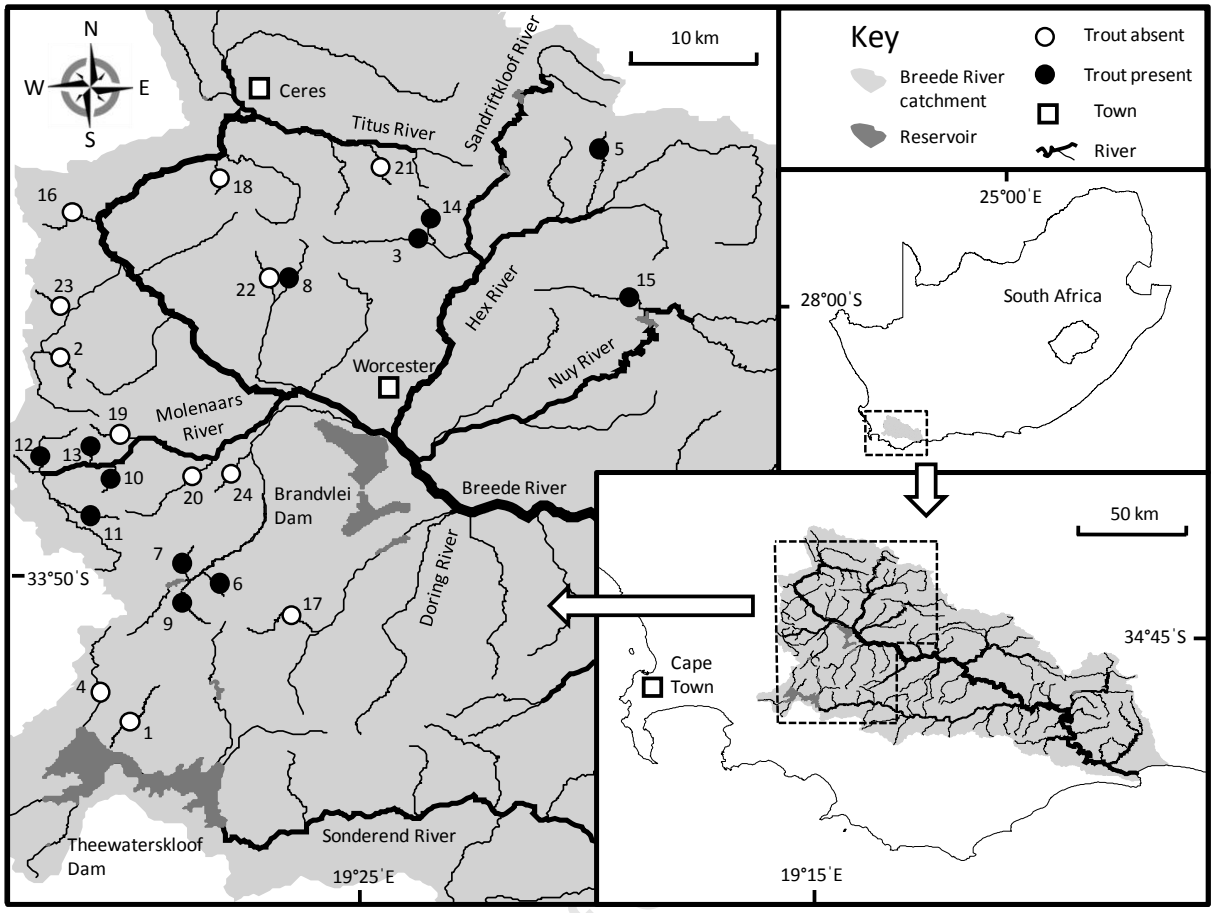

Figure 2.1 Location of sampling sites in the upper Breede River catchment in the CFR of South Africa. White circles represent sampling sites without trout, and black circles represent sites with trout. The numbers of the sampling sites correspond to the numbers in Table 2.1. Names of towns, as well as major rivers and reservoirs, are shown.

The middle and lower reaches of many streams in the upper catchment have been degraded by human-related activities. Agriculture, in particular vineyards and citrus orchards, has resulted in large-scale displacement of natural vegetation, stream channel modification, water abstraction and water quality deterioration (Swartz *et al.* 2004, Impson *et al.* 2007, Tweddle *et al.* 2009, RHP 2011, Chakona & Swartz 2012, de Moor & Day 2013). Reservoirs such as Brandvlei and Theewaterskloof modify flows and sediment loads transported by rivers (Davies & Day 1998). Infestations of non-native plants, especially those in the genera *Acacia*, *Pinus* and *Hakea*, are common (Brown *et al.* 2004). In general, the headwater reaches of streams are less severely impacted by human-related activities than lower-lying reaches (RHP 2011), although water is abstracted from many headwater streams for

agriculture, and in some cases long stretches of perennial streams run dry, or near dry, during summer (Tharme & King 1998). Additionally, non-native plants such as the silky hakea *Hakea sericea* and black wattle *Acacia mearnsii* have invaded several headwater catchments (Brown *et al.* 2004, RHP 2011).

Four native species of primary freshwater fish, namely the Breede River redfin *Pseudobarbus* sp. "Burchelli Breede" (henceforth "redfin"), the Cape Kurper *Sandelia capensis* Cuvier 1831 (henceforth "kurper"), the Cape galaxias *Galaxias zebratus* Castelnau 1861 (henceforth "galaxias") and the Berg-Breede River whitefish *Barbus andrewi* Barnard 1937, inhabit streams in the upper Breede River catchment. Whitefish, which prefer deep, slow-flowing pools found in middle and lower reaches of streams have disappeared from much of their native range, and at present very few riverine populations exist (Impson 2001b). This species can survive in reservoirs and large populations of whitefish occur in the Brandvlei and Roode-Els Berg Reservoirs, although co-inhabiting populations of predatory alien fish pose a serious threat to these populations (Impson 2001b). Whitefish is listed as Endangered on the IUCN Red List (Tweddle *et al.* 2009). Compared to whitefish, the other three species (Figure 2.2) are relatively widespread in the upper Breede River catchment, however populations are largely confined to headwater habitats (RHP 2011). The Breede River redfin is listed as Near Threatened, and the Cape kurper and Cape galaxias as data-deficient in the IUCN Red List (Tweddle *et al.* 2009).

Rainbow trout were stocked into several of the larger tributaries of the upper Breede River, including the Molenaars, Holsloot, Dwars, Hex and Elandspad Rivers, in the first half of the 20th century (Harrison 1949), and were more recently stocked into the Jan du Toits and Witels Rivers (de Moor & Bruton 1988). Additional inadvertent stockings have likely occurred as a result of trout aquaculture operations found at various localities throughout the upper catchment (RHP 2011). Trout have subsequently spread from these larger tributaries into many of the smaller headwater streams that feed into them (Tweddle *et al.* 2009, RHP 2011). A considerable number of headwater streams, however, have not been invaded by trout.

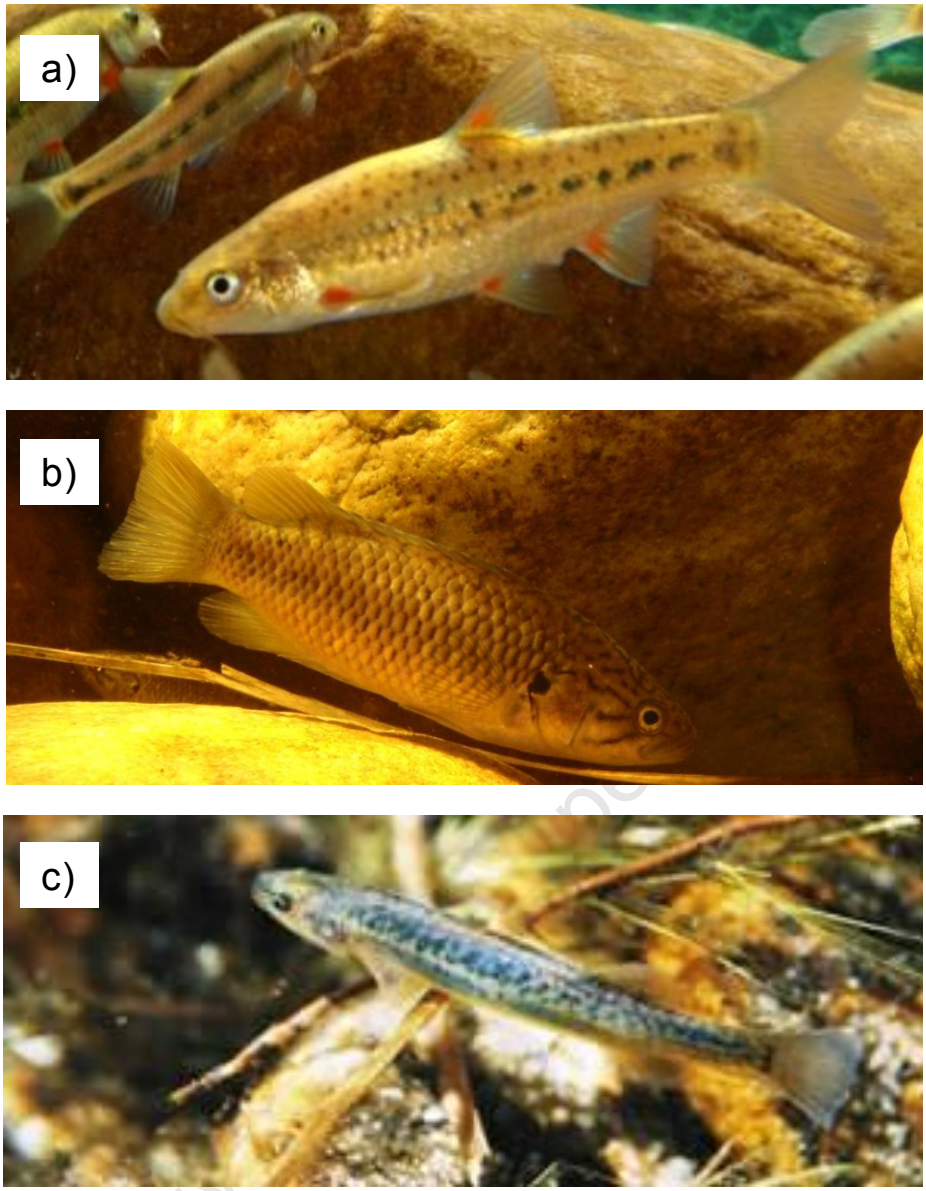

Figure 2.2 Native fish species commonly present in headwater streams in the upper Breede River catchment. Breede River redfin *Pseudobarbus* sp. "Burchelli Breede" (a), Cape kurper *Sandelia capensis* (b) and Cape galaxias *Galaxias zebratus* (c) (photograph Sean Marr).

2.2.2 Site selection

One of the greatest challenges when making inferences about an impact from a comparative study is accounting for factors other than trout that may influence native fish abundance. I attempted to account for such factors in two ways. Firstly, an attempt was made to select sites with and without trout that were as similar as possible in terms of environmental conditions, and secondly, a range physical and chemical factors that

potentially influence stream fish abundances was measured and their influence on fish abundance assessed. The present study was restricted to streams in the upper Breede River catchment that fell within the Western Folded Mountains Aquatic Ecoregion (Kleynhans *et al.* 2005) to avoid cross-Ecoregion, and cross-catchment, comparisons. The literature suggests that, longitudinal zone, canopy cover, amount of bedrock and human-related disturbance are key drivers of variability among stream communities (Davies & Day 1998, Rowntree & Wadeson 1999, King & Schael 2001, Allan & Castillo 2007). This study was therefore restricted to headwater streams with an open canopy, minimal bedrock and minimal human-related disturbance (Figure 2.3). To avoid the confounding influence of other introduced fish species, sites containing non-native species other than trout were avoided, and sites with no fish present were also avoided. Sixty-four potential sites were identified based on the opinion of local freshwater conservation biologists (including Dean Impson and Dr Martine Jordaan (Cape Nature), Dr Sean Marr and Dr Helen Dallas (University of Cape Town), and Dr Ernst Swartz and Dr Steven Lowe (South African Institute for Aquatic Biodiversity)), Google Earth, and topographic maps.

Figure 2.3 Jan du Toit Stream (site 8), a typical headwater tributary in the upper Breede River catchment.

2.2.3 Pilot study

A pilot survey was conducted during summer 2009 (January-March) to establish which of the 64 headwater stream sites met the site selection criteria. Single-pass snorkel-surveys (Thurow 1994) of one 100 m reach were conducted at each site to ascertain which species of fish were present. Sketch-maps were drawn at each site to estimate the percentage cover of bedrock and riparian canopy, and sites where canopy or bedrock cover exceeded 50% were dismissed because these factors are key drivers of variability in stream communities (King and Schael 2001). On-site observations, discussions with farmers and topographic maps were used to ascertain whether water abstraction and/or water pollution occurred upstream of any of the sites, and all sites downstream of an abstraction/pollution point were excluded. The riparian zone at each site was searched for non-native plant species, and all sites where non-native plants were present were excluded. The GPS coordinates at each site were recorded. In total, 24 of the 64 sites met the selection criteria; 12 with trout present and 12 without trout (Figure 2.1, Appendices 1a and b).

2.2.4 Fish surveys

One 50 m long site was selected on each of the 24 headwater streams that met the site selection criteria. This site length was chosen based on the recommendation of Bovee (1982) that a stream segment of 7-10 times the stream width is generally sufficient to capture the physical heterogeneity of that stream reach; the study sites were usually about 3-4 m wide. A range of techniques including fyke netting, seine netting, electrofishing, mark-recapture and underwater observation is available for estimating stream fish abundance (Thurow 1994, Wildman & Neumann 2003, Hardie *et al.* 2006). In small streams, multi-pass electrofishing is a widely used technique that can provide accurate estimates of fish density and biomass. Headwater streams in the CFR are difficult to access, and the conductivity of the water is low, which make electrofishing logistically difficult, potentially dangerous, and less effective than in streams where conductivity is higher (O'Neal 2007). Additionally, electrofishing may be undesirable when surveying fish populations under threat, due to unavoidable mortality associated with this method (Snyder 2003). An alternative is to use snorkelling surveys to estimate fish abundance. Snorkel sampling requires minimal

equipment, does not harm fish, is effective in clear streams with low conductivity and may therefore be a more appropriate technique than electrofishing for sampling fish populations in headwater streams in the CFR (Hankin & Reeves 1988). It has the additional advantages of being a relatively cheap and rapid sampling method (Hankin & Reeves 1988, Dolloff *et al.* 1993).

Like all sampling methods, snorkel sampling has certain biases that need to be taken into account. Several factors including the behaviour of the fish, and attributes of the physical stream environment, can bias underwater estimates of fish populations (Thurow 1994). Problems with snorkel counts include failure to detect fish, counting a fish more than once, incorrectly estimating fish size and miss-identifying fish species'. In general, the accuracy of snorkel counts decreases in large, turbid streams, with high flows, low temperatures and extensive cover for fish to hide in (Thurow 1994). The small size and high water clarity of the headwater streams in my study area allowed fish to be easily seen, identified, enumerated and sized, and thus snorkel sampling was considered to be an appropriate technique for estimating differences in relative fish abundance among streams.

Surveys were conducted during summer (16 February – 19 March 2010) when water clarity and temperature was high, and flows low, and between 11h30 and 13h30, when the sun was directly overhead, which maximized the accuracy of the estimates (Thurow 1994). One site was sampled per day and sites were sampled in a random order. The multiple-pass snorkel survey method described by Thurow (1994) was used to estimate mean abundance of each fish species at each site. Since the study streams were small and clear, a single diver (J. M. Shelton) did the surveys because the entire channel width could be seen. The same diver conducted all snorkel censuses so that sampling effort among sites was consistent (Hankin & Reeves 1988). The diver begun at the downstream end of the 50 m site and proceeded upstream in a zigzag pattern (Hankin & Reeves 1988, Mullner *et al.* 1998) recording the species, number and length of all fish seen. Fish length (total length, TL) was estimated to the nearest 10 mm with an underwater ruler (Thurow & Schill 1996), and fish data were recorded underwater on a perspex slate. Three passes were made, in order to estimate the mean and variance of fish numbers per size class (Thurow 1994), and passes were conducted 10-15 min apart to allow fish to recover from the disturbance caused by the snorkeler during the previous pass. Mean fish density was calculated for each site using the

mean number of fish of each species recorded and surface area of water over the site (estimated from 10 width transects measured at each site, see Section 2.2.5).

Trout and native fish were collected in the field using a 3 m seine net and measurements of weight (g) and length (mm) were taken so that length-weight regressions could be constructed. Indigenous fish were collected from sites 19, 20 and 22, and trout were collected from sites 5, 10 and 11, and I aimed to collect 30-40 individuals of each species at each site (although this was not always possible). Fish were kept temporarily in aerated plastic buckets on the stream bank, measured (TL) using a measure board, weighed using an Ohaus Scout Pro 400 g scale to the nearest 0.01 g and released back into the stream.

2.2.5 Habitat surveys

Stream habitat was surveyed following completion of fish surveys at each study site. Nineteen physico-chemical variables known to influence stream biota (Allan & Castillo 2007) were measured. Temperature (°C), pH, conductivity (µS/cm), dissolved oxygen (% saturation) and turbidity (NTU) were measured at three random points within each site. Temperature and dissolved oxygen were measured with a Crison OXI45 oxygen meter, pH with a Crison pH25 meter, conductivity with a Crison CM35 conductivity meter, and turbidity with a Hach 2100P turbidimeter. Three 500 ml water samples were collected at randomly-selected locations at each site, thoroughly homogenized, and a 200 ml sub-sample was taken for analysis of nutrient levels in the laboratory. Nutrient samples were held on ice in the field and frozen in the dark within 12 h of collection. Measurements of width (cm), depth (cm), substrate (mm), flow (m/s), canopy cover, submerged macrophytes and woody debris were made at three equidistant points along 10 transects laid across stream, perpendicular to the direction of current flow, at 5 m intervals. The presence or absence of riparian vegetation and undercut banks was noted on either end of each habitat transect. Width was measured using a tape measure stretched across the stream. Depth was recorded using a calibrated depth rod placed vertically on the streambed at each point. The substrate particle on which the depth rod was placed was then measured by recording maximum particle diameter using plastic callipers or a tape measure. The average flow of the water column at each point was measured with a Global FP101 Digital Water Velocity

Meter. Canopy cover was estimated by recording whether canopy was present or absent directly above each point along each transect. This design resulted in ten data points for stream width, 20 data points for the occurrence of riparian vegetation and undercut banks and 30 data points where depth, substrate particle length, canopy cover, submerged macrophytes and woody debris were assessed. The spatial coordinates of each site was recorded with a Garmin eTrex Vista ® HCx GPS, and elevation and site slope were measured with 1:250 000 topographic maps.

2.2.6 Laboratory methods

NO_3^-+NO_2^-–N, PO_4^{3+}–P and NH_4^+–N concentrations were estimated using a Lachat Flow Injection Analyser, as follows: NH_4^+-N was measured using Lachat's QuikChem® Method 31-107-06-1, based on the Berthelot reaction in which indophenol blue is generated; NO_3^- and NO_2^- were estimated using Lachat's QuikChem® Method 31-107-04-1-E, in which NO_3^- is converted to NO_2^- and diazotized with sulfanilamide to form an azo dye; PO_4^{3+} was measured by forming an antimony-phospho-molybdate complex using QuikChem® Method 31-115-01-1. Approximate detection limits are: for PO_4^{3+} 15µg.L^{-1} P; for NO_3^- and NO_2^- 2.5 µg.L^{-1} N; and for NH_4^+5 µg.L^{-1} N. These variables are herein referred to in the text as "phosphates", "nitrates + nitrites" and "ammonium" respectively.

2.2.7 Predation experiment

Historical records from South Africa (e.g. Cambray & Meyer 1988) suggest that trout deplete small-bodied native stream fish populations through predation, and results from the field surveys in the present study indicate that predation may be size-selective in that trout selectively consume small size classes of native fish. A predation experiment was therefore conducted to measure consumption of three sizes of native redfin by two sizes of trout. The redfin was chosen for this experiment because it was the numerically dominant native fish species at most of the study sites. Ideally, a separate experiment would have been conducted for each native fish species, but this was not possible for logistical reasons. The experiment was conducted on March 2011 in Morainekloof Stream (site 14, Figure 2.1), one

of the field-survey sites. This site was chosen because it was one of the few sites where trout and native fish co-occur, allowing both species to be manipulated within the same stream, and because it was relatively accessible.

The fish were held in 12 rectangular, plastic tanks. Tanks were 90 cm long, 45 cm wide and 40 cm deep, and had a total volume of 162 L (Figure 2.4a). Windows were cut from the front and back ends of tanks (30 x 20 cm) and from the top (60 x 20 cm), and lined with 2 mm plastic mesh. Windows at the front and back ends allowed some degree of flow through the tanks, and windows on the tops of tanks allowed sunlight to penetrate. Tanks were lined with 6 small cobbles (80-120 mm) and 6 large cobbles (180-220 mm) collected from the stream using a 30 x 30 cm diameter hand net with 250 μm mesh. Cobbles were lifted from the streambed into the net and then placed in a tank, so that the invertebrates on the cobble, as well as those dislodged when the cobble was lifted, were transferred to the tank. Cobbles were used to anchor tanks to the streambed and to provide shelter for the fish, and associated invertebrates provided potential food source for trout and redfin.

Redfin for the tanks were captured with seine and hand nets from Morainekloof Stream the day before the experiment. Since no small-sized individuals were caught, additional redfin were collected at a downstream site on the Amandel River (into which the Morainekloof Stream flows) where small-sized redfin were known to occur. Three size classes of redfin were used, <30 mm (small), 30-60 mm (medium) and >60 mm (large), and three individuals in each size class were stocked in each tank. Trout were caught using fyke nets set at the study site the night before the experiment begun. All fish were held in aerated buckets containing stream water, cobbles and invertebrates for food, for a maximum of 24 h before being placed into the experimental tanks.

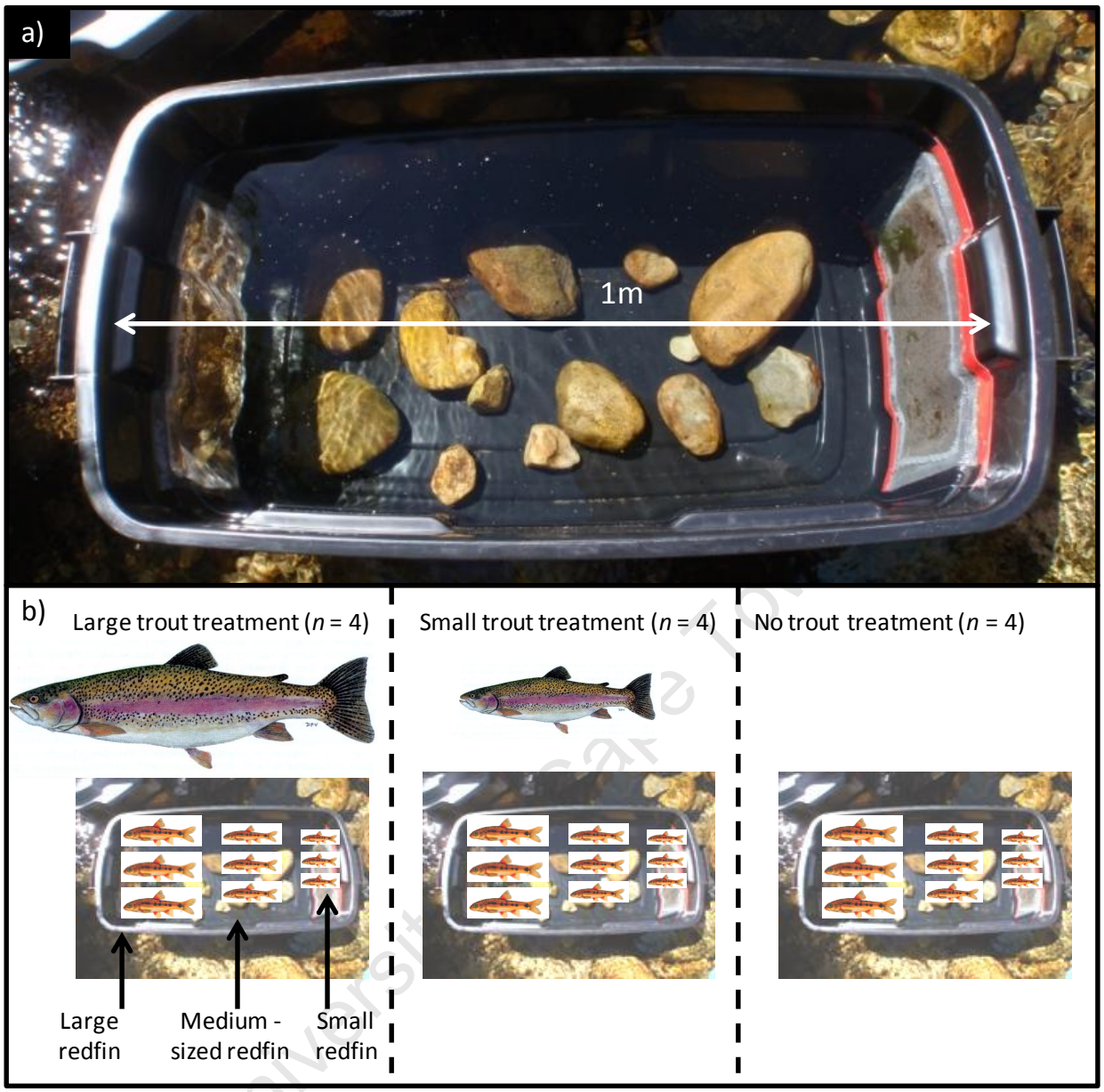

Figure 2.4 a) Experimental mesocosm in Morainekloof Stream lined with mesh windows, and containing cobbles and invertebrates collected from the surrounding stream, b) the three experimental treatments, including large trout, small trout and no trout, each of which received nine redfin; three large, three medium-sized and three small.

Three predator treatments were established, namely no trout, small trout (one individual <150 mm) and large trout (one individual >150 mm, Figure 2.4b), and each treatment was replicated four times in a randomized complete block design. Blocks were placed in runs, and each block was separated from the other blocks by at least 10 m of pool habitat. Redfin were stocked into the tanks between 11h00 and 12h00 on March 11, and trout were

stocked approximately 1 h later to allow redfin to acclimatize to conditions in the tanks. The experiment ran for 48 h and was terminated at 13h00 on March 13. At the end of the experiment cobbles were removed from tanks and the remaining redfin were measured and counted. Physico-chemical conditions in the tanks, including flow (m/s), pH, temperature (°C), oxygen (% saturation), conductivity (µS/cm) and turbidity (NTU) were measured once in each tank on the second day of the experiment between 12h00 and 14h00.

2.2.8 Statistical analyses

Comparing fish density and biomass between sites with and without trout

I estimated the mean density of each fish species at each site by dividing the average of the three snorkel passes by an estimate of the stream area sampled. Stream area was estimated by multiplying site length by the mean of the ten width measurements taken at a site. Length-weight regressions were constructed from data pooled among the three sites where length and weight measurements were taken for each species to maximize sample sizes. Both length (mm) and weight (mg) were ln(x+1) transformed and regression construction and biomass estimation followed the method of (Anderson & Neumann 1996) (see Appendix 2 for sample sizes, regression equations and regression plots). The density and biomass of each native fish species was compared between sites with and without trout using Mann-Whitney *U* tests, since data did not meet assumptions of parametric tests, even after transformation (Zar 1999). The mean total density and biomass of fish (i.e. native plus non-native species) was also estimated for sites with and without trout, and compared using independent sample *t* tests since the data met the assumptions of this test after ln(x+1) transformation was performed.

Comparing environmental conditions between sites with and without trout

A combination of univariate and multivariate analyses were used to compare environmental conditions between sites with and without trout. Independent sample *t* tests were used to compare each variable separately between the two groups of sites. Percentage oxygen

saturation, % riparian vegetation and % canopy cover, were arcsin square root transformed, while turbidity, flow velocity and elevation were ln(x+1) transformed to meet the assumptions of the analysis. A varimax-rotated principal components analysis (PCA) was used to visualize differences among sites. Principal components (PCs) with eigenvalues >1 were retained and variables with loadings >0.7 were considered important (Quinn & Keough 2002) and used to interpret individual components. Non-parametric, one-way, permutational multivariate analysis of variance (PERMANOVA, Anderson *et al.* 2008) using Euclidean distance with 9999 permutations was used to test the null hypothesis of no difference in environmental conditions between sites with and without trout. Variables were normalized prior to analysis, and % oxygen saturation, % riparian vegetation and % canopy cover were arcsin square root transformed, and turbidity, flow velocity and elevation were ln(x+1) transformed to even out their skewed distributions. The assumption of no significant in dispersion between the groups being compared was tested prior to the analysis using a PERMDISP test (Anderson *et al.* 2008).

Assessing the role of environmental factors and trout density in explaining variation in native fish density among sites

Relationships between native fish density and a set of predictor variables including environmental factors, as well as the density of trout, were investigated using distance-based linear models (DISTLM, Anderson *et al.* 2008). DISTLM is a non-parametric multivariate multiple regression technique for analyzing and modeling the relationship between one or more response variables (as described by a resemblance matrix) and a set of biotic and/or abiotic predictor variables (Anderson *et al.* 2008). Since DISTLM is a permutation-based technique performed on a resemblance matrix, it avoids the assumption of normality associated with standard linear modeling approaches, and is thus an appropriate option for analyzing community datasets which often fail to meet this assumption. The varimax-rotated PCA described above was used to reduce the 19 environmental variables to a limited number of independent, uncorrelated factors (Quinn & Keough 2002) which could then be used, along with trout density, as predictors in DISTLM models (Budaev 2010). Based on the recommendation of (Quinn & Keough 2002), all PCA

factors with eigenvalues >1 were retained and used as predictors that represent major axes of variation in environmental conditions. Predictor variables were checked for multicollinearity, but the correlation coefficient *r* never exceeded 0.7 so no variables were dropped from the analysis (Anderson *et al.* 2008, Budaev 2010). Resemblance matrices were calculated using Euclidian distance, which is appropriate for models with a single response variable (Anderson *et al.* 2008). The density of each species was ln(x+1) transformed prior to analysis to even out their skewed distributions.

The DISTLM routine was used achieve two primary objectives. Firstly, the proportion of the variation in the density of each native fish species explained by each predictor was assessed individually in marginal tests. Second, a step-wise procedure with the "adjusted R^2" selection criterion was used to identify the combination of predictors that produced the most parsimonious model explaining the variation in native fish density among sites. (In this context, parsimony refers to the trade-off between explaining the largest possible proportion of variation in the response variable, but at the same time minimizing the number of predictors included in the model.) First, the predictor explaining the greatest proportion of the variability is fitted, and then predictors are sequentially added to, and subtracted from the model, in an attempt to improve the selection criterion (adjusted R^2). The procedure is complete when no further improvement to the selection criterion can be made by adding or deleting a term from the model. Simple linear regression was used to investigate relationships between response variables and any predictor found to explain a significant proportion of variation in the final model. DISTLM models were also run using the fish biomass dataset, but since the results were similar to those produced using density data, only results from the density-based analyses are presented here.

Comparing native fish size distributions between sites with and without trout

Kolmogorov-Smirnov goodness-of-fit tests for discrete data (Zar 1999) were used to compare the size distributions of each native fish species between sites with and without trout. This test was chosen because it is more powerful than a Chi-square test when the sample size is small, or when the number of observations in certain categories is small.

Predation experiment

Mixed model ANOVA with block as a random factor and treatment as a fixed factor was used to test for differences in redfin survival and physico-chemical conditions among treatments (Quinn & Keough 2002). Temperature was log-transformed and % oxygen arcsin square root transformed prior to analysis to improve normality and meet the assumptions of the test.

Software used

All univariate analyses were carried out with SPSS 20.0 (SPSS 2011), and multivariate analyses were performed using PRIMER-E (Clarke & Gorley 2006) with the add-on package PERMANOVA+ (Anderson *et al.* 2008).

2.3 RESULTS

2.3.1 Fish density at sites with and without trout

Native fish were generally abundant at sites without trout, but absent or present only at relatively low density at sites where trout occurred (Figure 2.5, Table 2.1). Native fish were absent from sites where trout density was relatively high (>3 fish/100 m^2), but persisted at a relatively low density at some of the sites where trout density was relatively low (<3 fish/100 m^2). The redfin was the most abundant native fish species at the majority of sites where trout were absent, and on average made up 64.37 and 76.23% of the native fish assemblage by number at sites with and without trout, respectively. Redfin was recorded at all 12 sites lacking trout, but only at four of the twelve sites containing trout. The mean density of redfin was more than 30 fold higher, and biomass more than 40 fold higher, at sites lacking trout than at sites containing trout (Figure 2.6a, d), and Mann-Whitney *U* tests indicated that differences in both redfin density (Mann-Whitney *U* test, $U_{1, 22} = 8.00$, $p < 0.001$) and biomass (Mann-Whitney *U* test, $U_{1, 22} = 8.50$, $p < 0.001$) were highly significant.

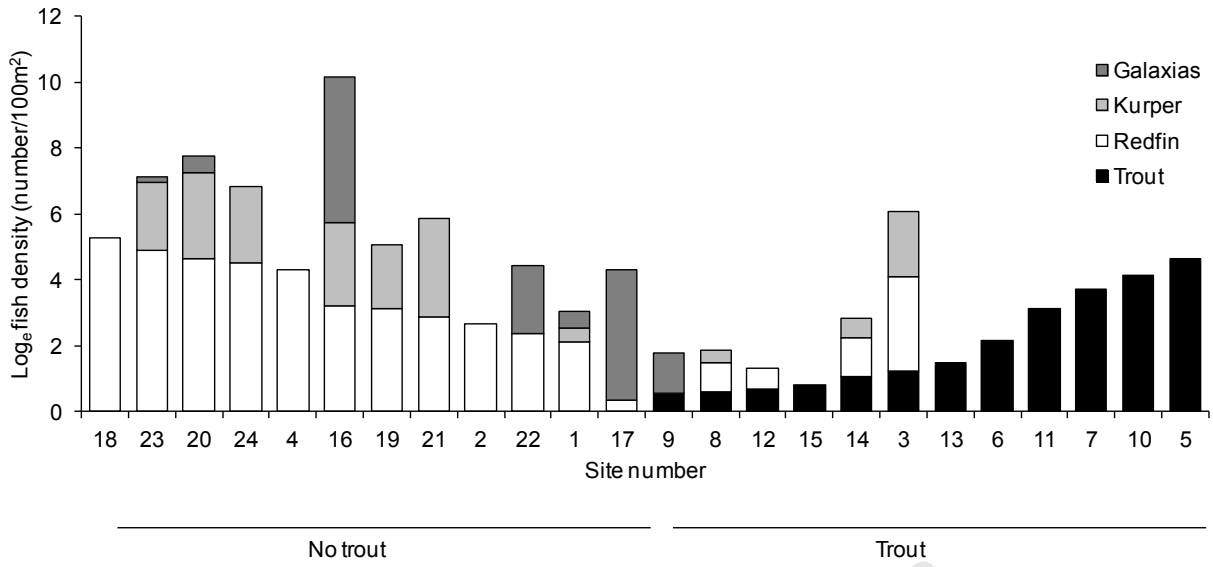

Figure 2.5 Density estimates for each fish species at each of the 24 sampling sites. Density estimates were $\log_e$ transformed in order to accommodate all four species on the same set of axes.

Kurper occurred at seven of the 12 sites without trout and only three of the 12 sites with trout. The mean density of kurper was more than 10 times greater (Figure 2.6b), and biomass more than five times greater (Figure 2.6e), at sites without trout than at sites with trout. Differences in both density (Mann-Whitney *U* test, $U_{1,\ 22}$ = 41.50, p = 0.031), and biomass (Mann-Whitney *U* test, $U_{1,\ 22}$ = 41.55, p = 0.048) between sites containing and lacking trout were statistically significant.

Galaxias was only present at seven of the 24 sites, six without trout and one with trout. The mean density of galaxias was more than 30 times greater (Figure 2.6c), and biomass more than 15 times greater (Figure 2.6f), at sites without trout than at sites with trout, although neither differences in density (Mann-Whitney *U* test, $U_{1,\ 22}$ = 42.00, p = 0.172) nor in biomass (Mann-Whitney *U* test, $U_{1,\ 22}$ = 41.20, p = 0.088) were statistically significant. When all fish species were combined (including trout), the mean total density of fish at sites lacking trout was significantly greater than that at sites containing trout (*t* test, $t_{1,22}$ = 3.23, $p < 0.001$, Figure 2.7a), while no significant difference in mean total biomass was detected (*t* test, $t_{1,22}$ = -0.37, p = 0.712, Figure 2.7b).

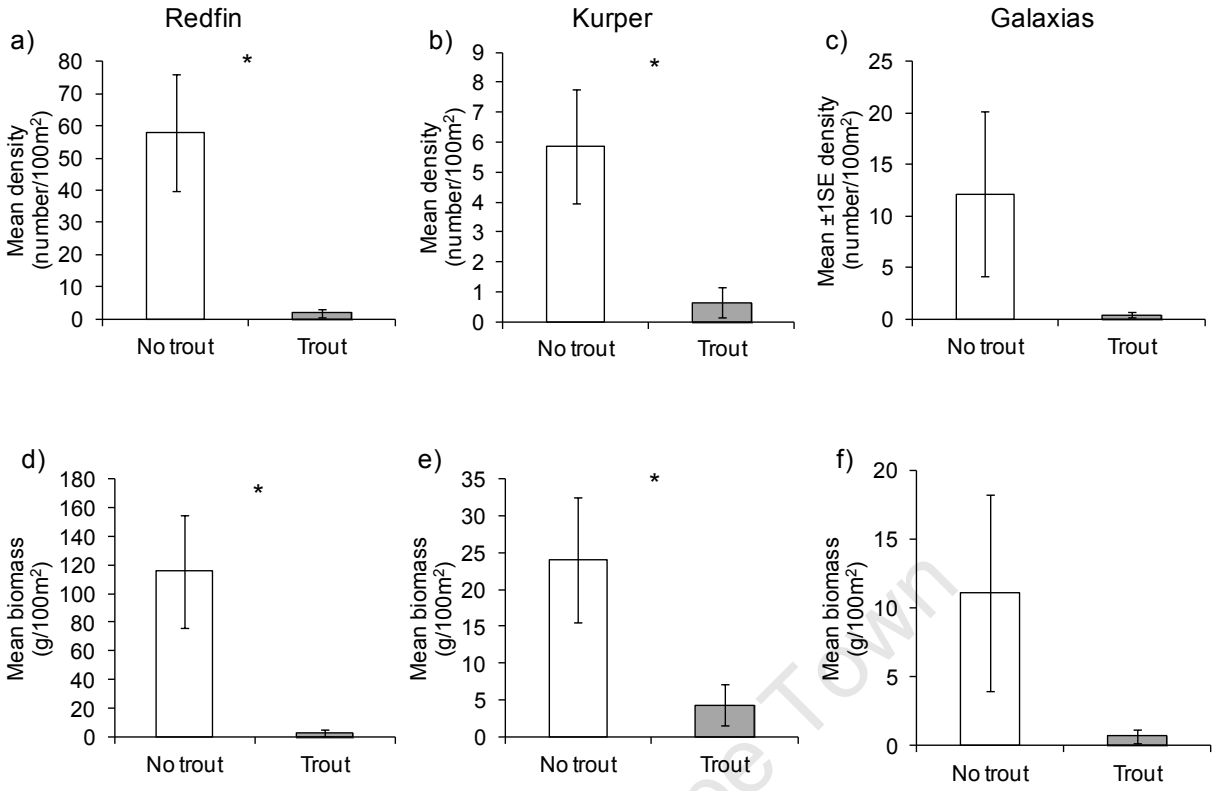

Figure 2.6 Mean ± standard error (SE) density (a-c) and biomass (d-f) of redfin, kurper and galaxias at sites with and without trout. An asterisk indicates a significant difference based on the results of Mann-Whitney *U* tests (α = 0.05).

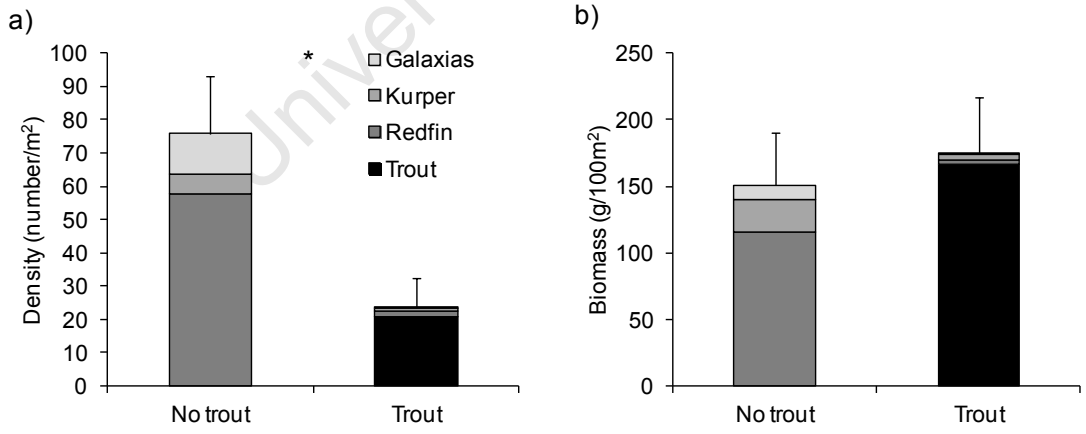

Figure 2.7 Mean ± SE total density (a) and total biomass (b) of fish at sites with and without trout. An asterisk indicates a significant difference based on the results of Mann-Whitney *U* tests (α = 0.05).

Table 2.1 Mean ± SE fish density (number/100m^2) estimates from snorkel 3-pass surveys conducted at the 24 sampling sites. "Site no." indicates site number which corresponds to the numbers in Figure 2.1.

Site name	Site no.	Redfin		Kurper		Galaxias		Trout	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Amandel	1	7.43	0.61	0.51	-	0.68	0.34	-	-
Bobbejaans	2	13.30	0.28	-	-	-	-	-	-
Buffelshoek	3	16.51	2.43	6.11	2.95	-	-	2.45	0.40
Du Toits	4	72.29	24.52	-	-	-	-	-	-
Groothoek	5	-	-	-	-	-	-	102.32	7.36
Hartmanskloof	6	-	-	-	-	-	-	7.85	3.72
Houtboskloof	7	-	-	-	-	-	-	40.79	5.90
Jan du Toit	8	1.36	0.34	0.51	0.29	-	-	0.85	0.17
Kaaimansgat	9	-	-	-	-	2.46	0.63	0.72	0.14
Klip	10	-	-	-	-	-	-	61.71	5.13
Kraalstroom	11	-	-	-	-	-	-	21.51	1.62
Krom	12	0.84	0.17	-	-	-	-	1.01	0.00
Mol trib.	13	-	-	-	-	-	-	3.51	0.40
Morainekloof	14	2.25	0.16	0.80	0.16	-	-	1.93	0.50
Raaswater	15	-	-	-	-	-	-	1.30	0.86
Sandspruit	16	23.79	5.15	11.32	2.77	82.69	20.85	-	-
Stettyn	17	0.41	0.20	-	-	51.93	3.49	-	-
Tierhok	18	196.71	29.10	-	-	-	-	-	-
Tierkloof	19	21.51	2.03	6.09	3.06	-	-	-	-
Tierstel	20	103.00	6.87	12.63	0.83	0.66	0.17	-	-
Titus trib.	21	16.49	2.36	19.59	2.29	-	-	-	-
Waaihoek	22	9.65	0.79	-	-	6.83	1.04	-	-
Wolwekloof	23	136.08	18.88	6.69	0.30	0.17	0.17	-	-
Wolwenberg	24	92.36	6.94	8.77	1.55	-	-	-	-

2.3.2 Environmental conditions at sites with and without trout

Environmental conditions, with respect to the variables measured in this study, were similar at sites with and without trout. Independent-sample *t* tests conducted separately on each environmental variable indicated that none of the measured variables differed significantly between the two groups of sites (Table 2.2). The PCA ordination (Figure 2.8) shows that the two groups of sites do not separate clearly in the multivariate habitat space, indicating a lack of consistent difference in environmental conditions between sites with and without trout. The PCA produced seven principal components that had eigenvalues >1, which together accounted for 74.10% of the variation in environmental conditions among sites

(Table 2.3). These seven components were therefore used as predictors in DISTLM models (see Section 2.3.3). The percentage variation explained by the first two axes combined was only 31.70%. Although similar patterns were observed when principal component (PC) numbers 3, 4, 5, 6 and 7 were plotted (results not presented).

PERMANOVA results were consistent with the pattern shown by the PCA ordination, and revealed that overall, environmental conditions did not differ significantly between sites with and without trout (one-way PERMANOVA, $F_{1,\ 22} = 0.96$, $p_{perm} = 0.497$), and that there was no significant dispersion effect among site groups (one-way PERMDISP, $F_{1,\ 22} = 2.80$, $p_{perm} = 0.125$).

Table 2.2 Mean ± SE of each environmental variable at sites with and without trout. Results for independent-sample *t* tests for each variable are shown. Variable transformations are indicated by the symbols, † = ln(x+1) transformed, ‡ = arcsin square root transformed.

Variable	No trout		Trout		*t* test	
	Mean	SE	Mean	SE	$t_{1,22}$	*p*
Nitrates + nitrites (mg/l)†	6.21	1.88	9.52	2.06	-0.99	0.335
Ammonium (mg/l)†	24.97	1.94	33.09	7.02	-0.68	0.504
Phosphates (mg/l)	17.69	3.13	16.68	3.40	0.22	0.831
pH	4.90	0.16	5.30	0.15	-1.88	0.074
Temperature (°C)	22.39	0.69	21.07	0.53	1.52	0.144
Conductivity (µS/cm)	16.30	1.46	15.38	1.64	0.42	0.677
Oxygen saturation (%)‡	92.13	1.97	90.51	1.92	0.79	0.441
Turbidity (NTU)†	0.67	0.08	0.58	0.11	0.84	0.411
Width (cm)	389.75	13.76	384.58	17.51	0.23	0.819
Depth (cm)	25.16	1.03	24.09	1.23	0.66	0.515
Substrate length (mm)	295.30	16.68	291.26	16.25	0.17	0.864
Flow velocity (m/s)†	0.20	0.02	0.18	0.02	0.49	0.628
Riparian vegetation (%)‡	65.83	5.14	62.08	6.56	0.55	0.586
Canopy cover (%)‡	19.44	1.92	28.33	4.09	-1.08	0.102
Elevation (m)†	419.17	35.94	473.83	25.05	-1.62	0.121
Site slope (%)	6.71	0.52	6.72	0.86	0.46	0.651
Submerged macrophytes (%)‡	16.39	5.82	4.44	1.38	1.96	0.063
Undercut bank (%)‡	3.33	1.36	3.06	1.04	0.19	0.848
Woody debris (%)‡	5.28	0.96	8.06	3.03	-0.18	0.859

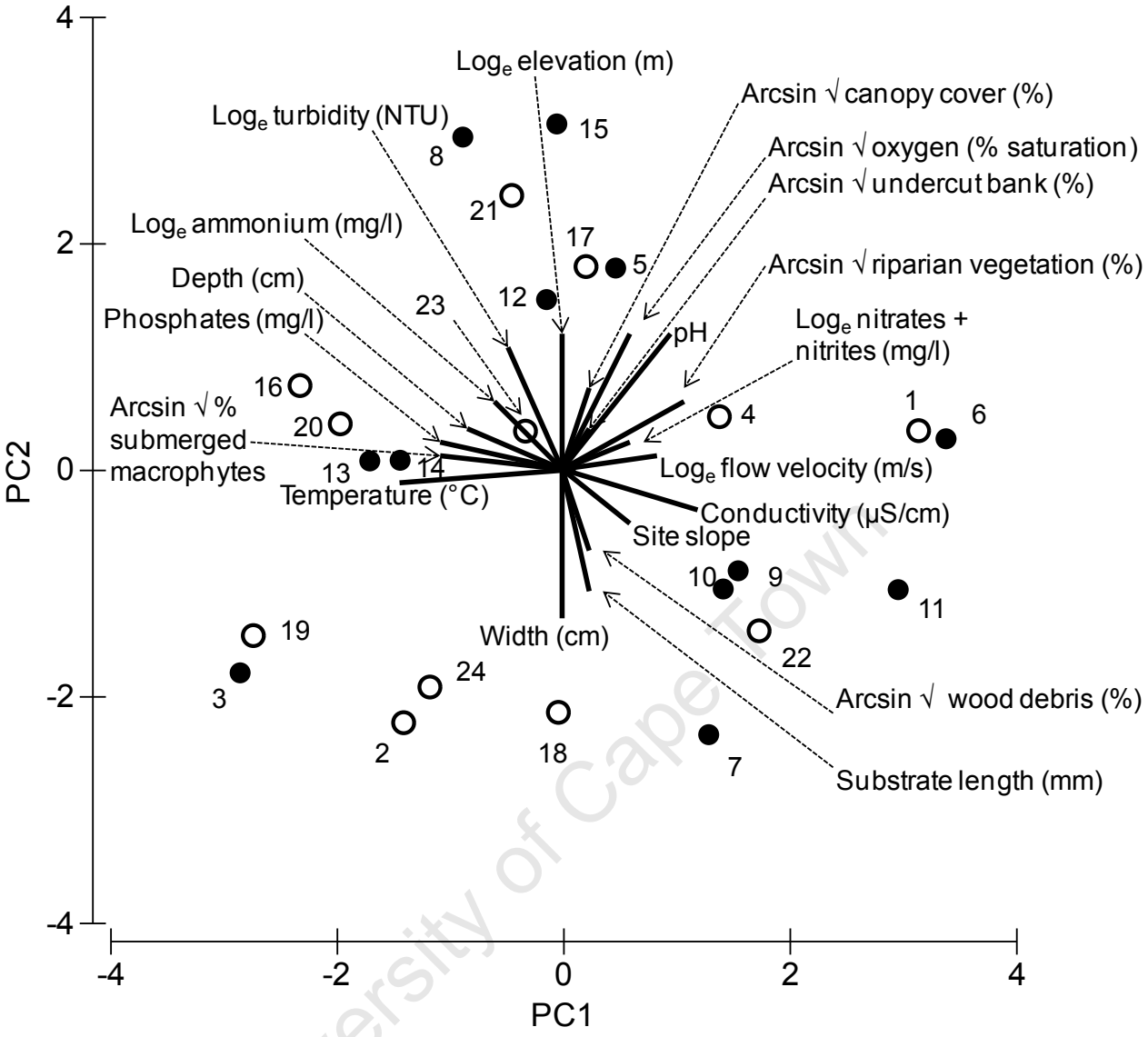

Figure 2.8 Principle components analysis plot summarizing environmental conditions at sites with (black circles) and without (white circles) trout. PC 1 represents 17.30%, and PC 2 represents 14.40%, of the total variation in environmental conditions among sites. The length and direction of vectors (solid black lines) in the vector overlay indicate the direction and strength of the influence of each environmental variable on the variation in overall environmental conditions among sampling sites.

Table 2.3 Component loadings produced by principal components analysis on the environmental variables measured at the 24 sampling sites. Variables with loadings >0.7 are considered important and are indicated in bold. The percentage variation, and cumulative percentage variation, explained by principal component axes with eigenvalues >1 is shown.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
NO3 + NO2 (mg/l)	-0.33	-0.16	-0.04	-0.27	0.33	0.44	-0.33
NH4 (mg/l)	-0.07	**0.78**	-0.07	0.18	0.22	-0.07	-0.11
PO4 (mg/l)	**0.83**	0.05	-0.05	0.01	0.06	-0.01	-0.13
pH	-0.23	-0.24	**0.77**	0.38	0.13	-0.04	0.10
Temperature (°C)	**0.77**	0.31	-0.06	-0.12	-0.21	0.10	0.00
Conductivity (µS/cm)	-0.12	-0.68	-0.21	0.13	0.41	-0.34	0.01
Oxygen saturation (%)	-0.17	0.00	-0.05	**0.91**	0.09	0.15	-0.12
Turbidity (NTU)	0.48	-0.03	0.06	0.42	-0.03	0.53	-0.25
Width (cm)	-0.12	-0.15	-0.34	-0.65	-0.10	0.07	0.08
Depth (cm)	0.26	0.62	0.24	-0.23	0.22	0.09	0.09
Substrate length (mm)	-0.10	0.16	-0.24	-0.21	0.12	-0.09	**0.80**
Flow velocity (m/s)	-0.34	-0.18	-0.20	0.33	0.05	0.56	0.30
Riparian vegetation (%)	-0.35	-0.04	0.08	0.22	**0.72**	0.08	0.09
Canopy cover (%)	0.12	0.12	0.09	0.01	**0.88**	0.06	-0.04
Elevation (m)	0.07	0.10	**0.89**	-0.06	0.08	0.14	-0.14
Site slope	-0.09	-0.20	0.11	-0.03	-0.07	0.13	**0.81**
Submerged macrophytes (%)	0.28	0.64	-0.23	0.18	-0.17	-0.14	-0.02
Undercut bank (%)	-0.33	0.17	0.50	0.21	-0.38	-0.52	-0.04
Woody debris (%)	-0.09	-0.15	-0.17	-0.04	-0.03	**-0.75**	-0.05
Eigenvalues	3.29	2.74	2.15	1.74	1.55	1.52	1.10
Variation (%)	17.30	14.40	11.30	9.20	8.10	8.00	5.80
Cumulative variation (%)	17.30	31.70	43.10	52.20	60.30	68.30	74.10

2.3.3 The influence of trout density and other environmental factors on native fish density

Redfin

Table 2.4 summarizes the results of the step-wise DISTLM used to investigate the relative influence of trout density and other environmental factors on variation in redfin density among the 24 sampling sites. The marginal tests show that the proportion of variation explained by each environmental predictor alone was low (≤10%), and that none of the environmental predictors explained a significant proportion of the variation in redfin density

among sites. Trout density, on the other hand, explained 41.45% of the overall variation which was found, by permutation, to be highly significant ($F_{1, 22} = 15.58$, $p_{perm} < 0.001$).

Table 2.4 Test statistics for distance-based linear model (DISTLM) analysis investigating relationships between redfin density and a set of predictor variables including seven principal components that represent major axes in variation in environmental conditions, as well as trout density. Marginal tests indicate the proportion of variation in redfin density explained by each variable alone, while the sequential tests indicate the cumulative variation explained by each variable fitted to the final model in the order specified, and taking previously-fitted variables into account. Sequential tests were based on a step-wise selection procedure, and the selection criterion used was adjusted R^2. "Var. (%)" = percentage of variation explained and "Cum. var. (%)" = the cumulative percentage of variation. Asterisks indicate predictors explaining a significant proportion of variation in the response variable ($\alpha = 0.05$).

Variable	Adjusted R^2	SS	*F*	p_{perm}	Var. (%)	Cum. var. (%)	Residual *df*
Marginal tests							
PC 1	-	7.76	2.32	0.142	9.55	-	-
PC 2	-	0.36	0.10	0.754	0.44	-	-
PC 3	-	7.12	2.11	0.162	8.76	-	-
PC 4	-	3.87	1.10	0.321	4.76	-	-
PC 5	-	3.15	0.89	0.350	3.88	-	-
PC 6	-	0.04	0.01	0.929	0.04	-	-
PC 7	-	0.19	0.05	0.835	0.23	-	-
Trout density	-	33.69	15.58	0.001*	41.45	-	-
Sequential tests							
+Trout density	0.39	33.69	15.58	0.001*	41.45	41.45	22
+PC 5	0.46	7.54	3.96	0.061	9.28	50.73	21

The sequential tests produced a final model that contained two predictors, namely trout density and PC 5, and the overall proportion of variation explained by this model was 50.73%. Trout density was fitted first, and was the only predictor that explained a significant proportion of the overall variation in the final model (41.45%). PC 5 represents gradients in % canopy cover and % riparian vegetation, but this predictor explained only 9.28% of the variation (see Table 2.4) over and above the 41.45% already explained by trout density. Taken together, these results indicate that trout density is clearly the single best predictor

of redfin density in the study streams, but that % canopy cover and % riparian vegetation were also correlated with variation in redfin density detected among the study sites.

Kurper

Table 2.5 summarizes the results of the step-wise DISTLM used to investigate the relative influence of trout density and other environmental factors on variation in kurper density among the 24 sampling sites. The marginal tests show that trout density was the only predictor that explained a significant proportion (21.91% explained, $F_{1,\ 22}$ = 6.17, p_{perm} = 0.024) of the variation in kurper density. The predictor explaining the next highest proportion of variation was PC 1 (16.46% explained), which represents gradients in phosphate concentration and water temperature. The proportion of variation explained by the remaining predictors on their own was relatively low (<10%), and found not to be statistically significant. The sequential tests produced a final model that contained four predictors, namely trout density, PC 7, PC 1 and PC 2, and the overall proportion of variation explained by this model was 52.71%. Trout presence was fitted first, and explained approximately half of the overall variation captured by the model. The density of trout was therefore considered to be the single best predictor of variation in kurper density among the study sites. The next predictor selected by the model was PC 7 which explained a further 16.61% of the variation, over and above the 21.91% already accounted for by trout, and the proportion of variation explained was statistically significant ($F_{1,22}$ = 5.67, p_{perm} = 0.025). PC 7 represents gradients in site slope and substrate length, and Figure 2.9 shows that kurper density was higher at sites with a relatively gentle gradient and a fine mean substrate length, but regression analysis revealed that the relationship between Log_e kurper density and PC 7 was not statistically significant. As mentioned above, PC 1 represents gradients in phosphate concentration and water temperature, while PC 2 largely represents gradients in ammonium concentration, and although not significant, these two predictors explained 7.38% and 6.81% respectively of the variation captured by the final model. Taken together, these results indicate that although trout density is the single best predictor of kurper density in the study streams, environmental variables such as site slope and substrate

length, were also important predictors of the variation in kurper density among the study sites.

Table 2.5 Test statistics for distance-based linear model (DISTLM) analysis investigating relationships between kurper density and a set of predictor variables including seven principal components that represent major axes in variation in environmental conditions, as well as trout density. Marginal tests indicate the proportion of variation in kurper density explained by each variable alone, while the sequential tests indicate the cumulative variation explained by each variable fitted to the final model in the order specified, and taking previously-fitted variables into account. Sequential tests were based on a step-wise selection procedure, and the selection criterion used was adjusted R^2. "Var. (%)" = percentage of variation explained and "Cum. var. (%)" = the cumulative percentage of variation. Asterisks indicate predictors explaining a significant proportion of variation in the response variable (α = 0.05).

Variable	Adjusted R^2	SS	*F*	p_{perm}	Var. (%)	Cum. var. (%)	Residual *df*
Marginal tests							
PC 1	-	4.41	4.33	0.058	16.46	-	-
PC 2	-	1.27	1.09	0.326	4.74	-	-
PC 3	-	0.06	0.05	0.837	0.22	-	-
PC 4	-	1.08	0.93	0.357	4.04	-	-
PC 5	-	0.44	0.37	0.556	1.64	-	-
PC 6	-	0.04	0.03	0.873	0.14	-	-
PC 7	-	2.16	1.93	0.190	8.07	-	-
Trout density	-	5.87	6.17	0.024*	21.91	-	-
Sequential tests							
+Trout density	0.18	5.87	6.17	0.035*	21.91	21.91	22
+PC 7	0.33	4.45	5.67	0.025*	16.61	38.52	21
+PC 1	0.38	1.98	2.73	0.120	7.38	45.90	20
+PC 2	0.43	1.83	2.74	0.129	6.81	52.71	19

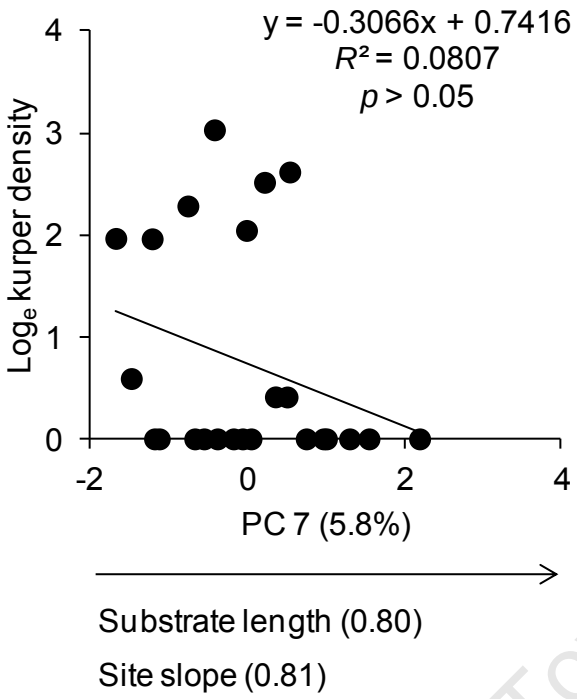

Figure 2.9 Relationship between log transformed kurper density (number/100 m^2) and scores along principal component axis 7 (PC 7). The percentage of variation explained, and variables with loadings >0.7 are shown for PC 7 (non-significant regression line is shown).

Galaxias

Table 2.6 summarizes the results of the step-wise DISTLM used to investigate the relative influence of trout density and other environmental factors on variation in galaxias density among the 24 sampling sites. The marginal tests show that when analysed separately, none of the predictors explained a significant proportion of the variation in galaxias density. The sequential tests produced a final model that contained three predictors, namely PC 7, PC 3 and PC 5, and the overall proportion of variation explained by the model was 43.23%. PC 5 and PC 7 both explained significant proportions of the overall variation. PC 7 represents gradients in site slope and substrate length, and Figure 2.10a shows that galaxias density was generally higher at sites where site slope and mean substrate length were greater, and regression analysis revealed that the relationship between Log_e galaxias density and PC 7 was statistically significant at the $p < 0.05$ level. Galaxias density was also positively correlated with PC 5, indicating that galaxias density was greater at sites with high levels of canopy cover and riparian vegetation (Figure 2.10b), and regression analysis revealed that the relationship between Log_e galaxias density and PC 5 was statistically significant at the *p*

< 0.05 level. Taken together, these results indicate that the variation in galaxias density among the study sites was best explained by variation in environmental conditions, rather than trout density, and that galaxias density was generally highest at sites with the steepest gradient, where the mean substrate size was high, and the cover of riparian vegetation and canopy were high.

Table 2.6 Test statistics for distance-based linear model (DISTLM) analysis investigating relationships between galaxias density and a set of predictor variables including seven principal components that represent major axes in variation in environmental conditions, as well as trout density. Marginal tests indicate the proportion of variation in galaxias density explained by each variable alone, while the sequential tests indicate the cumulative variation explained by each variable fitted to the final model in the order specified, and taking previously-fitted variables into account. Sequential tests were based on a step-wise selection procedure, and the selection criterion used was adjusted R^2. "Var. (%)" = percentage of variation explained and "Cum. var. (%)" = the cumulative percentage of variation. Asterisks indicate predictors explaining a significant proportion of variation in the response variable (α = 0.05).

Variable	Adjusted R^2	SS	F	p_{perm}	Var. (%)	Cum. var. (%)	Residual *df*
Marginal tests							
PC 1	-	0.69	0.45	0.487	2.00	-	-
PC 2	-	0.43	0.27	0.590	1.22	-	-
PC 3	-	4.84	3.56	0.064	13.93	-	-
PC 4	-	0.01	0.01	0.926	0.03	-	-
PC 5	-	5.28	3.94	0.059	15.19	-	-
PC 6	-	0.46	0.29	0.603	1.31	-	-
PC 7	-	4.91	3.61	0.064	14.11	-	-
Trout density	-	3.11	2.16	0.103	8.95	-	-
Sequential tests							
+PC 5	0.11	5.28	3.94	0.046*	15.19	15.19	22
+PC 7	0.23	4.91	4.19	0.048*	14.11	29.29	21
+PC 3	0.35	4.84	4.91	0.057	13.93	43.23	20

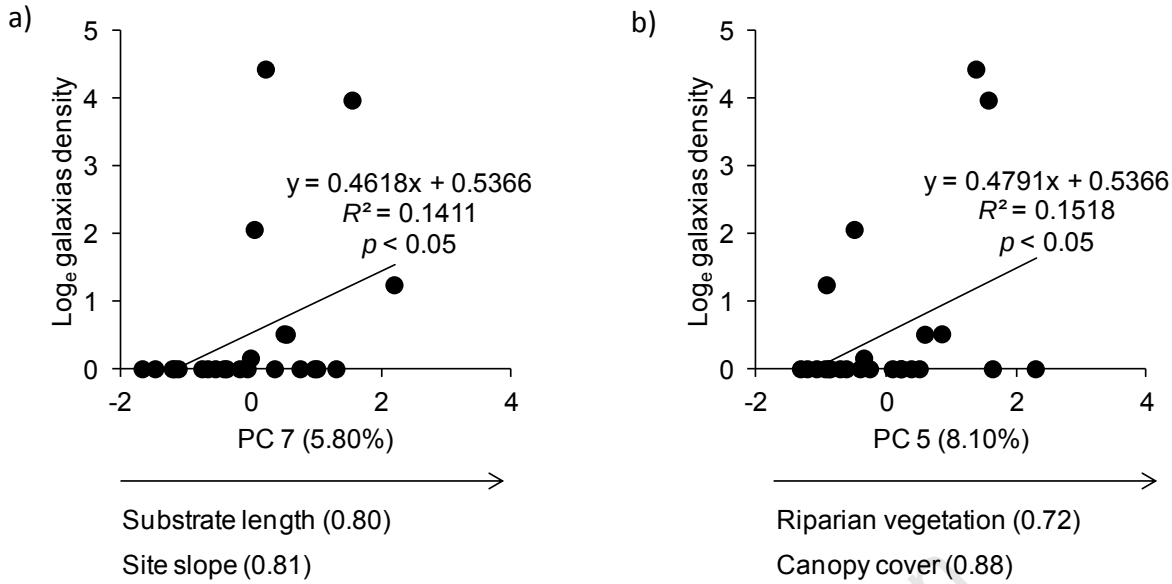

Figure 2.10 Relationship between log-transformed galaxias density (number/100 m^2) and scores along (a) PC 7 and (b) PC 5. The percentage of variation explained, and variables with loadings >0.7 are shown for PC 7 and PC 5, and significant regression lines are shown.

2.3.4 Native fish size distributions at sites with and without trout

The size distribution of each native species at sites without trout differed significantly from its distribution at sites with trout (Kolmogorov-Smirnov goodness-of-fit tests, redfin: d_{max} = 36.67, p < 0.001; kurper: d_{max} = 41.21, p < 0.001; Galaxias: d_{max} = 72.56, p < 0.001). For all three species, small individuals (≤40 mm) were relatively abundant at sites without trout, but all but absent at sites where trout were present (Figure 2.11).

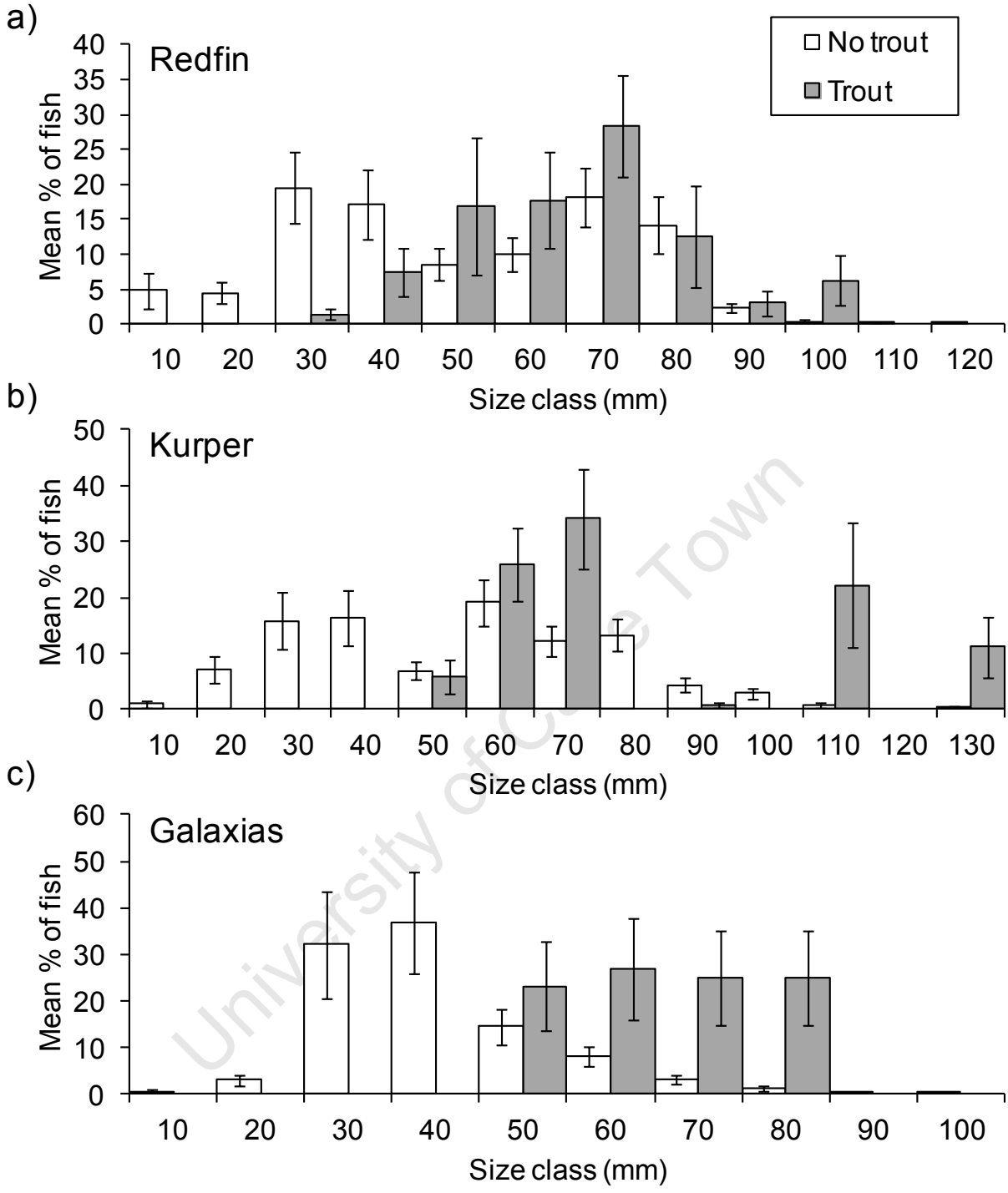

Figure 2.11 Size distributions of (a) redfin, (b) kurper and (c) galaxias at sites with and without trout. Bars show the mean ± SE of fish in each size class.

2.3.5 Predation experiment

All medium- and large-sized redfin survived in all tanks, indicating that predation by trout on these size-classes did not occur during the experiment (Figure 2.12). In total, two of the 12 small-sized redfin did not survive in the tanks with small trout, and seven of the 12 small-sized redfin did not survive in the tanks with large trout. Mixed model ANOVA (block = random factor, treatment = fixed factor) detected a significant difference in small redfin survival among treatments ($F_{2, 9} = 13.00$, $p = 0.007$), with survival in treatments with large trout being significantly less than in treatments with small trout (Tukey post-hoc test, $p = 0.028$) or no trout (Tukey post-hoc test, $p = 0.006$). Mixed model ANOVAs detected no significant treatment effect for any of the physico-chemical variables measured, however, a significant block effect was detected for flow velocity (Table 2.7).

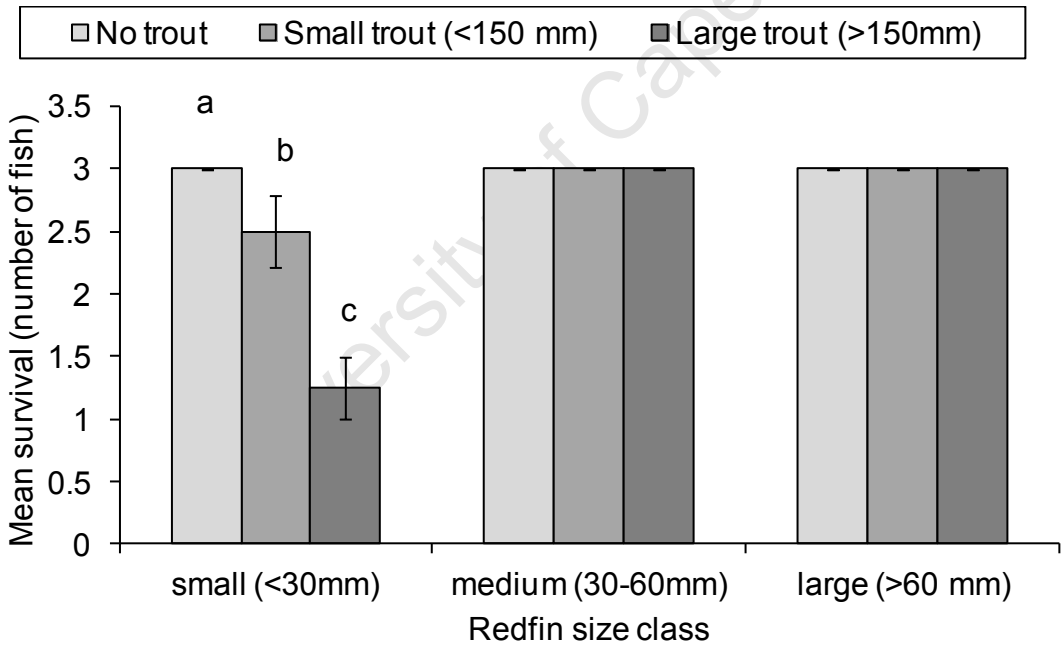

Figure 2.12 Mean ± SE number of small, medium and large-sized redfin surviving in tanks with no trout ($n = 4$), small trout ($n = 4$) and large trout ($n = 4$). Different letters indicate a significant difference in redfin survival as detected by mixed model ANOVA and Tukey post-hoc tests ($\alpha = 0.05$).

Table 2.7 Mean ± SE values of environmental variables in tanks with no trout, small trout and large trout. Significant effects detected by mixed model ANOVAs are indicated with an asterisk (α = 0.05). Variable transformations are indicated by the symbols, † = ln(x+1) transformed, ‡ = arcsin square root transformed.

Variable	No trout		Small trout		Large trout		*p* value	
	Mean	SE	Mean	SE	Mean	SE	Block	Treatment
Flow velocity (m/s)	0.07	0.04	0.04	0.02	0.05	0.03	0.010*	0.160
pH	4.23	0.07	4.14	0.03	4.19	0.02	0.189	0.290
Temperature (°C)†	20.70	0.33	20.35	0.06	20.40	0.07	0.217	0.369
Oxygen saturation (%)‡	84.05	0.85	86.04	1.02	84.73	0.37	0.961	0.383
Conductivity (µs/cm)	11.77	0.01	11.77	0.01	11.79	0.01	0.497	0.288
Turbidity (NTU)	0.61	0.07	0.70	0.06	0.70	0.08	0.072	0.392

2.4 DISCUSSION

In the following section, I first discuss survey-based evidence for trout impacts on each of the three native fish species, and then examine experimental evidence for size-selective predation by trout on redfin. I also discuss factors that could potentially influence trout density in CFR streams, and touch on some methodological issues associated with the snorkel sampling technique. Finally I summarize the major findings from this chapter and comment on the conservation implications of these findings.

2.4.1 Patterns in native fish density and biomass in relation to trout and other environmental factors

Redfin

Results from this study show that the average density and biomass of redfin at sites containing trout were significantly lower than at similar sites lacking trout. Additionally, while redfin were present at all 12 sites lacking trout, they were only detected at four of the 12 sites containing trout. The fact that no consistent differences in environmental conditions were found between sites with and without trout suggests that the presence of trout is the main factor driving these patterns. Further evidence in support of these conclusions comes from historical records of the abundance of small native cyprinids in relation to trout in South African streams. In a survey of freshwater systems in South Africa,

Hey (1926) noted that once-abundant redfin minnows (*Pseudobarbus* spp.) appeared to have disappeared from many streams where trout had been introduced. Harrison (1950a) reported a decline in numbers of the Berg River redfin *P. burgi* in the Eerste and Lourens rivers following the introduction of trout, and *P. burgi* has since gone extinct in the Eerste River (de Moor & Bruton 1988). Trout appear to have contributed to the disappearance of *Barbus trevelyani* in the upper reaches of the Buffalo and Tyume rivers in the Eastern Cape (de Moor & Bruton 1988), and to the disappearance of small-bodied *Barbus* and *Pseudobarbus* species that historically occurred in the Heks and Krom rivers in the Olifants River system in the CFR (Tweddle *et al.* 2009). In their survey of fish populations in pools in the upper Berg River, Woodford & Impson (2004) found large numbers of redfin co-inhabiting pools with trout, suggesting that the predatory impact is not always severe, and probably varies among systems. In the present study, redfin were often seen schooling in open water, even at sites where they co-occurred with trout, and this behaviour, and apparent naivety, is likely to make them vulnerable to large introduced predators like trout.

Analysis of the factors contributing to the variation in redfin density among sites adds further support to the conclusion that introduced trout, rather than some other aspect of the stream environment, is primarily responsible for the reduced density (or absence) of redfin at sites with trout. Moreover, the DISTLM results indicate that the impact of trout on redfin is density-dependent. Redfin were able to persist at some of the sites where trout density was low, but not at any of the sites where trout density was high. This finding is consistent with the results of other studies investigating trout impacts on populations of small-bodied, stream-dwelling fish elsewhere. In the Waimakariri River in New Zealand, the native *G. paucispondylus* only co-occurred with non-native trout where trout density was low, or where large trout were absent (McIntosh *et al.* 2010). Similarly, in northern Colorado (USA), Peterson *et al.* (2004) found that survival of juvenile native cutthroat trout in large fenced off stream reaches was inversely related to non-native brook trout density.

Kurper

As in the case of redfin, the mean density and biomass of kurper was significantly lower at sites with trout than at sites where trout were absent, and while present at seven of the sites without trout was only found at three of the sites where trout occurred. The fact that no consistent differences in environmental conditions were detected between sites with and without trout suggests that the presence of trout is the main factor driving these patterns. Reports from the literature suggest that introduced trout may similarly impact kurper populations elsewhere in South Africa. Hey (1926) reported that while kurper were abundant in many South African streams, they were absent or rare in streams where trout were present. Skelton (1987) listed predation by trout as an important threat to *Sandelia bainsii*, an ecologically similar anabantid fish. However, in a survey of pools in the Upper Berg River, Woodford & Impson (2004) found large numbers of kurper co-inhabiting pools with trout, suggesting that the predatory impact is not always severe. In the present study, kurper were generally observed on their own, or in small groups, among cobbles and boulders, although they were inquisitive towards the snorkeler, often leaving the shelter of the streambed to swim out into open water to investigate the snorkeler. This type of behaviour is likely to make them especially vulnerable to a large, predatory fish like trout.

Analysis of the factors potentially influencing kurper density added further support to the conclusion that introduced trout are largely responsible for the reduced kurper density at sites with trout, although other environmental factors, including site slope and mean substrate length, were also important. Kurper density was higher at sites with finer mean substrate particle size and lower gradient, which is consistent with their known preference for relatively slow-flowing, quiet stream habitats (Skelton 2001). As was the case for redfin, the impact of trout on kurper populations appears to be density-dependent, in that kurper were able to persist at some of the sites where trout density was low, but not at any of the sites where trout density was high.

Galaxias

The fact that mean galaxias density was approximately thirty times lower at sites with trout than at sites without trout, suggests that trout have a strong, negative impact on galaxias density in the study streams, yet a Mann-Whitney *U* test comparing galaxias density between the two groups of sites, did not detect a significant difference. Galaxias only occurred at seven of the 24 sites, thus the large number of zero data points likely reduced the statistical power of the test. Since this species was present at six of the 12 sites without trout, but only one of the 12 sites with trout, and was only found in high abundance where trout were absent, it seems likely that trout may indeed have had a considerable influence on galaxias distribution in the study streams, despite my lack of statistical evidence for this.

Records of trout impacts on galaxias populations in South Africa are scarce. In the Keurbooms River in the CFR, a once-abundant galaxias population appears to have disappeared following the introduction of brown trout (*Salmo trutta*) (Cambray 2003), and high-density stocking for angling may have exacerbated the impact of trout in this case. Woodford & Impson (2004) found that although galaxias inhabit the upper Berg River (CFR), they were not found in pools containing rainbow trout. The authors identified competitive displacement and predation by trout as potential drivers of this distribution pattern. McVeigh's (1977) statement that "it is obvious that where *Galaxias zebratus* is found, this minnow species forms a definite part of the trout diet." indicates that it has long been assumed that trout consume galaxias. McVeigh (1977) also makes the point that the spawning season of trout precedes that of galaxias, and therefore that young-of-the-year trout will have an abundant supply of galaxias fry on which to feed, as appears to be the case in New Zealand (Jellyman & Mcintosh 2010). Woodford & Impson (2004) found juvenile galaxiids in trout stomachs, which adds support to the hypothesis that trout are a contributing factor to the fragmented population structure of galaxias in CFR headwater streams. (McDowall 2006) was of the opinion that co-existence was unlikely between galaxias and introduced trout in South Africa based on the outcome of trout-galaxiid interactions documented in other parts of the world, and called for surveys of galaxias distributions in streams with and without trout to confirm this suspicion. A substantial body of evidence indicates that the introduction of salmonids, particularly brown and rainbow trout, has led to fragmentation, population declines and local extirpations of galaxiids in

other parts of the world including New Zealand (see review by McDowall 2006), Australia and Tasmania (see review by Cadwallader 1996), Chile (Young *et al.* 2010, Habit *et al.* 2010), Patagonia (Garcia de Leaniz *et al.* 2010), Argentina (Macchi *et al.* 1999) and the Falkland Islands (McDowall *et al.* 2001). Further work in this vein is required to clarify the extent of the impacts of trout on galaxias populations in the CFR.

My analysis of the factors most important in explaining variation in galaxias density among sites indicates that environmental factors were better predictors of variation in galaxias density than was trout density. Specifically, galaxias density tended to be highest at sites with the steepest gradient, where mean substrate size was large, and where riparian vegetation was abundant. However, the linear models constructed for galaxias should be treated with some caution since the species was absent from ~70% of the study sites. Despite the fact that galaxias density was log-transformed prior to analysis to improve normality, and that DISTLM is flexible with respect to the distribution of the response variable, the large number of zero data points meant that the effective sample size for galaxias was small. Interestingly, the only site where trout and galaxias co-occurred had the lowest trout density measured in this study (Kaaimansgat Stream, 0.72 trout/100 m^2) suggesting that galaxias populations are able to persist in streams where trout density is low. Clearly, further survey work is needed to increase the sample size of galaxias-containing sites in order to confirm the impact of trout on galaxias populations in the CFR.

2.4.2 Variation in trout density

The finding that trout impacts on native fish populations appear to be dependent on trout density could have important conservation implications. Specifically, sites supporting low-density trout populations may be of greater conservation value than sites supporting high-density trout populations. In streams, salmonid density can be affected by a range of biotic and abiotic features of the stream environment (Fausch *et al.* 2001). Trout populations can be influenced by physical disturbances such as bed-moving floods (Fausch *et al.* 2001), and McIntosh (2000) found that stable streams tend to support a higher density of brown trout (*S. trutta*) than streams that experience a high frequency of bed-moving floods. Salmonid abundance can also be influenced by various aspects of water chemistry. For example,

Olsson *et al.* (2006) found the density of brown trout (*S. trutta*) to be strongly influenced by natural variation in water acidity, and that trout density in acidic streams was lower than that in streams that had a higher pH. Water acidity may well influence trout abundance in the CFR where stream water is often naturally acidic because complex polyphenolic compounds such as tannins leach from decaying fynbos vegetation into streams (de Moor & Day 2013).

Salmonids are highly sensitive to variations in temperature, and in general they cannot tolerate high temperatures. The optimal temperature range for rainbow trout is 12-18°C (Raleigh *et al.* 1984), while the critical thermal maximum is ~25°C (Adams *et al.* 2008). At some of the sites where trout density was low, water temperatures over 23°C were recorded, which approaches the upper limit of their thermal tolerance. High summer temperatures may therefore constrain the density, and associated impact, of trout in some headwater streams in the upper Breede River catchment, and potentially in other similar systems in the CFR. Interestingly, early attempts to breed trout in the CFR were often unsuccessful, and high summer temperatures and associated low levels of dissolved oxygen in the water were assumed to be the main reasons for this (Scott 1902, 1905). Furthermore, reports following the introduction of trout into the Hex River (upper Breede River catchment) early in the 1900s document how trout "struggled to cope" with the high temperatures and low flows that prevail in summer: "During the hot weather in January and February part of the Hex River near the mountains dried up and a lot of fish (trout) were lost….only a few pools were left" (Scott 1904).

Developing an understanding of the factors influencing trout density was not an objective of my research, but would add valuably to our overall knowledge of the factors influencing trout impacts in the CFR, and is clearly an important avenue for future research.

2.4.3 Detection probability

Since snorkel-sampling tends to underestimate stream fish abundance, Thurow (1994) recommend calibrating snorkel estimates with a sampling method known to produce accurate estimates of stream fish populations, such as multi-pass electrofishing. This was

not possible in the present study for logistical reasons, and because of low water conductivity, but should be considered in future surveys. The shoaling behaviour of redfin, the small size of galaxias and the fact that kurper often conceal themselves in the substrate may have affected my density estimates of these species. However, Thurow (1994) found that snorkel estimates are usually within 70% of actual population sizes, and thus, even if our method underestimated abundance of one or more species, it was likely that this underestimate was consistent among sites, facilitating comparisons between sites with and without trout.

2.4.4 Evidence for size-selective predation by trout

The absence of small size classes (<40 mm) of native fish at sites with trout, but not at sites without trout, suggests that trout alter the size-structure, and thereby the overall density, of native fish in streams by selectively preying on small size classes of native fish. This is consistent with the finding of Cambray & Meyer (1988) that, in the Tsoelikane River in Lesotho, young-of the-year *P. quathlambae* were present at sites without trout, but not at sites with trout. My predation experiment revealed that both small (<150 mm), and large (>150 mm), trout were capable of consuming small (<30 mm) redfin, but that large trout had a significantly higher consumption rate than did small trout. These results are consistent with the fact the trout usually become piscivorous at approximately 150 mm (Mittelbach & Persson 1998). In general, information on the consumption of small cyprinids by trout is scarce, although Blinn *et al.* (1993), who conducted an experiment investigating predation by rainbow trout on little Colorado spinedace *Lepidomeda vittata* (a small, stream-dwelling cyprinid that is broadly ecologically similar to South African *Pseudobarbus* spp.), found that rainbow trout (190-270 mm in length) consumed approximately 30% of the spinedace (40-65 mm in length) available to them over a ten-day period.

Current fish distributions in the study area represent the outcome of historical interactions between trout and native fish, and the general lack of co-occurrence between trout and native fish makes it difficult to directly investigate predation in the natural stream environment. Although my data suggest that consumption of small native fish by trout has led to the fragmentation of native fish populations within the study area, conclusive

evidence would require the introduction of trout into a trout-free stream, and subsequent monitoring of native fish density. In the present study, this was not possible for ethical reasons, but reach-scale fish manipulations (of the type used by Fletcher 1979) may be an exciting prospect for further investigating the role of trout predation in declines of native fish populations in the future. Nevertheless, taken together, my results indicate that predation by trout is the best explanation for the differences in size distribution and density of redfin recorded during the field surveys. Further experimental work is needed to confirm size-selective predation on kurper and galaxias, although the presence of young galaxias in trout stomachs in the upper Berg River (Woodford & Impson 2004) shows that trout are certainly capable of feeding on galaxias. Additionally, experimental work conducted in New Zealand has demonstrated size-selective predation by brown trout on the native galaxiids (Fletcher 1979, McIntosh 2000, Woodford 2009).

The predation experiment suffered from several limitations. The tanks were small, closed systems, so both predator and prey were confined to a relatively small area. The density of redfin in the tanks was roughly an order of magnitude higher than the maximum density recorded at any of the study sites, potentially increasing their vulnerability to predation by trout. Redfin may also have been more vulnerable in the tanks than they would have been in the natural stream environment where they are able to avoid patches of stream occupied by trout. Although an attempt was made to mimic the natural structural complexity of the streambed, the 12 cobbles placed in each tank probably provided less shelter than would naturally be available, and this may also have increased the vulnerability of redfin to trout. Although tanks were seeded with invertebrates as a potential food source for fish, invertebrate prey available to trout may have been limited. Trout are known to be drift feeders (Nakano *et al.* 1999b, Baxter *et al.* 2004), and the quantity of aquatic and terrestrial invertebrates drifting through the tanks was likely lower than in the natural stream, since the windows in tanks were lined with a relatively fine mesh (2 mm). Finally, the experiment ran for a relatively short period of time (48 h), and it would have been interesting to see whether trout consumed larger redfin once the supply of smaller individuals available to them was exhausted.

Despite these shortcomings, the predation experiment does demonstrate that both small and large trout are capable of consuming redfin, and that predation is size-selective.

Predation experiments elsewhere have been conducted in similar small, closed systems and over time-scales comparable to that of my experiment. McIntosh (2000) examined predation by brown trout on galaxias over a period of 40 h in similar-sized plastic tanks (68 x 121 cm), and with similar sizes and densities of predator and prey. Garvey *et al.* (1994) used 200 L aquaria and 180 x 100 cm tanks to examine predation by largemouth bass (250-277 mm) on two species of freshwater crayfish, *Orconectes rusticus* and *O. propinquus*, and experiments ran for 4-6 days. Barr & Babbitt (2007) used 76 L tanks to examine predatory interactions between brook trout *Salvelinus fontinalis* and two-lined salamander larvae *Eurycea bislineata*, and in that case experimental trials were 20 h long.

The conclusions drawn from the predation experiment are lent support by historical records in South Africa documenting the presence of redfin minnows in the stomachs of trout caught by anglers. Harrison (1952) noted a "rainbow of about a pound chasing a shoal of minnows [Breede River redfin] at the tail of a pool in the Hex River. The trout took a fly and when landed disgorged a freshly-caught rooivlerk [Breede River redfin]". In an earlier article, (Harrison 1950b) claimed that "An essential element in the success of this big trout sanctuary [the Dwars River near the town of Ceres in the upper Breede River catchment] is the enormous number of rooivlerk minnows", and goes on to describe the experiences of Mr R Mayer who was a trout angler at the time: in a pool on the Dwars River containing "enormous shoals of rooivlerk minnows", Mr Mayer caught "three rainbows [trout]…all of which were crammed with minnows", and on another occasion in the same river, redfin "formed the main item in the stomachs of four trout". Interestingly, Harrison reports that several of the trout caught by Mr Mayer were over 50 cm long, and noted that "these trout had made exceptional growth since the winter of 1949 while living in an optimum environment and feeding on an unfailing supply of minnows and other food". There are additional reports of notably large trout being caught by anglers in the upper Breede River catchment in the early 1900s: trout weighing six pounds (2.7 kg) were caught in the Eerste River (Scott 1903) and trout weighing seven pounds (3.2 kg) in the Hex River (Scott 1900). Trout of this size are rarely caught in streams of the upper Breede River catchment any more (J.M. Shelton, pers. obs. 2010), and thus it may be that the abundance of large trout caught earlier in the 20th century was a result of once abundant populations of small-bodied native fish which provided an excellent food source for trout.

Although predation by trout appears to be the main process to have driven the fragmented population structure of native fish in headwater streams in the study area, competition for food and space may also have played a role. Competition between trout and native fish was not investigated in the present study, but may be an interesting prospect for future research in this field.

2.4.5 Conclusions and conservation implications

Taken together, these results indicate that (1) where trout have invaded, they have largely displaced native fish species' in headwater streams in the upper Breede River catchment; (2) native fish may be able to persist at a reduced density at invaded sites where trout density is low; and (3) predation on small size classes of native fish appears to be a key process driving observed patterns in native fish density and size distribution. These results are in line with predictions that naïve native species with an evolutionary history of isolation will be highly vulnerable to predation by a novel, introduced predator (Cox & Lima 2006, Sih *et al.* 2010). These findings have important implications for the conservation of native fish in the upper Breede catchment, and potentially also for native fish in other South African catchments where trout have been introduced. Although none of the native species included in this study are currently listed as threatened on the IUCN Red List, the fact that these species are highly fragmented and largely restricted to headwater tributaries suggests that predation by introduced trout may well be one of the greatest threats they will face in the future.

In the early twentieth century trout were initially stocked into larger tributaries, and subsequently spread into many of the smaller headwater streams, such as those surveyed in this study. The question of why trout have colonized some, but not all, of these streams is an interesting one and deserves some attention. As discussed above, trout density could be affected by a suite of environmental factors, yet the finding of no consistent difference in environmental conditions between sites with and without trout, suggests that some other factor must have prevented trout from invading.

At the majority of trout-free sites (Sandspruit, Stettyn, Tierhok, Tierkloof, Tierstel, Waaihoek, Wolwenberg and Titus tributary), it appears that trout invasion was prevented by

physical barriers such as waterfalls, weirs and dry/braided reaches of stream. However, at other sites (Amandel, Bobbejaans, Wolwekloof and Du Toits), it remains unclear why trout are not present. It may be that trout stocking in these areas was minimal, and as a result they were never able to establish. Physical barriers are important in limiting the spread of non-native trout in other parts of the world (Townsend & Crowl 1991, McDowall 2006), and may also play a key role in the persistence of native fish populations in CFR headwater streams.

Based on the present analysis, it seems likely that, if stocked into headwater streams that are presently trout-free, trout will establish self-sustaining populations with serious, negative consequences for the native fish that inhabit these streams. The effectiveness of headwater streams above trout barriers as native fish sanctuaries in partially-invaded riverscscapes may therefore depend on preventing the stocking of trout into these streams. Finally it is noted that the impact of introduced trout may extend beyond their impact on native fish populations, and the following chapter in this thesis focuses on community-level consequences of non-native trout in headwater streams in the upper Breede River catchment.

Chapter 3

Influence of non-native rainbow trout on benthic community structure in headwater streams of the upper Breede River catchment, South Africa

3.1 INTRODUCTION

Predators are functionally important components in biological systems. From their position at the top of the trophic web, they can regulate the structure and function of biological communities "below" them through a combination of direct and indirect effects (Terborgh & Estes 2010). They directly regulate prey populations by reducing their abundance, or by restricting their movements, and this can lead to indirect effects on other components of the food web to which the prey are linked (Townsend 2003, Simon & Townsend 2003). The modification of predator assemblages can therefore lead to the restructuring of communities through direct effects on prey populations as well as through indirect, knock-on effects that result from the disruption of predator-prey linkages (Allan & Castillo 2007). This phenomenon is known amongst ecologists as a "trophic cascade", and has been defined by Pace *et al.* (1999) as "reciprocal predator–prey effects that alter the abundance, biomass or productivity of a population, community or trophic level across more than one link in a food web".

The decimation of sea otter *Enhydra lutris* populations off the west coast of North America illustrates how changes in predator populations can produce cascading effects with community-level consequences (Estes *et al.* 1978). Sea otters prey on herbivorous sea urchins, which in turn are important grazers of kelp. The importance of the top-down effects of predation in this system became apparent when sea otter populations were depleted by hunters for the fur trade which began in the mid 1700s. The decline in sea otters released sea urchins from predation, which resulted in a proliferation of urchins on the sea bed. The abundant urchins rapidly mowed down kelp forests, transforming coastal areas without sea otters into barren, rocky reef ecosystems. This transformation in turn had further indirect

consequences for other species that depend on kelp forests for habitat. Interestingly, in areas where sea otter populations have recovered, kelp forests have since re-established because sea otters once again constrain urchin abundance (Estes & Duggins 1995).

In freshwater systems, fish are often important components of native predator assemblages (Allan & Castillo 2007), but unfortunately native fish populations have been negatively affected by human-related activities the world over (Moyle & Light 1996). Various forms of fishing have no doubt contributed to native fish declines in many lakes and streams (Allan *et al.* 2005), but perhaps the greatest threat facing native fish populations at present is posed by introduced predatory fish (Moyle & Light 1996, Eby *et al.* 2006). The direct effects of non-native predators on conspicuous native species like fish have been well studied, and in many cases they dramatically reduce native fish abundance, or even eliminate entire populations (Eby *et al.* 2006). On the other hand, comparatively little attention has been paid to the potential indirect effects of non-native predators (Townsend 2003) which may be, subtle, unintuitive, and difficult to measure (Flecker & Townsend 1994). As a result, impacts of non-native predators in freshwater systems may often be underestimated (Simon & Townsend 2003). Adopting a multi-trophic-level approach to assessing impacts of non-native predators, whereby both direct impacts on adjacent trophic levels, as well as indirect impacts on non-adjacent trophic levels, are measured, should enable a more comprehensive assessment of the consequences of predator introductions (Simon & Townsend 2003, Eby *et al.* 2006), and facilitate more effective management of invasive species (Townsend 2003).

In the Cape Floristic Region (CFR) of South Africa, non-native predatory fish have been widely introduced and appear to have largely replaced native fish as top predators in many streams (de Moor & Bruton 1988, Cambray 2003, Woodford *et al.* 2005, Weyl *et al.* 2010, RHP 2011). In particular, invasions by bass *Micropterus* spp. have led to dramatic declines in native fish populations in the foothill zones of many CFR rivers (de Moor & Bruton 1988, Woodford *et al.* 2005, Weyl *et al.* 2010), while rainbow trout *Oncorhynchus mykiss* (henceforth "trout") appear to have largely replaced native fish populations upstream in headwater habitats in many rivers (see Chapter 2). Our understanding of whether these perturbations at the level of the fish assemblage have cascaded down the food web to lower trophic levels is inadequate (de Moor & Bruton 1988, Cambray 2003), but this information is needed if we are to appreciate the full extent of the non-native fish impacts

in CFR streams, and manage non-native fish accordingly. Improving our knowledge of community-wide impacts of non-native predatory fish is especially important in biodiversity hotspots like the CFR where large numbers of unique taxa, confined to a relatively small geographical area, are under serious threat (Myers *et al.* 2000). A long history of geologic and climatic stability has no doubt contributed to the high levels of endemism found in the aquatic biota of the CFR (Wishart & Day 2002, Wishart *et al.* 2006, de Moor & Day 2013). The freshwater invertebrate fauna shows especially high levels of endemism in that roughly two thirds of all known species are endemic to the region (Wishart & Day 2002), with some species displaying extremely narrow distribution ranges (King & Schael 2001).

Streams in the CFR are nutrient-poor environments as a result of their underlying geology (Dallas & Day 2007, de Moor & Day 2013), and consequently support short and simple food chains. Two distinct trophic pathways operate in these systems: an autotrophic pathway in which algae growing in the stream is the primary energy source, and a heterotrophic pathway which is based on organic matter (or detritus) inputs from the adjacent riparian zone (Davies & Day 1998, Allan & Castillo 2007) (Figure 3.1). Both algae and detritus are fed upon by benthic invertebrates which are important primary consumers in these streams. The assignment of invertebrates to functional feeding groups (FFGs) is a useful way to conceptualize trophic relationships in streams (Cummins *et al.* 2008). The main FFGs found in CFR streams include grazer-scrapers, collector-gatherers, filter-feeders, shredders and predators (for information on the different groups, their feeding mechanisms and the food resources they consume, refer to Section 3.2.2). Algae are consumed by herbivorous invertebrates such as grazer-scrapers and collector-gatherers. Shredders feed on the coarse particulate organic matter (CPOM) that enters the stream from the riparian zone, and convert it to fine particulate organic matter (FPOM) which is then consumed by filter-feeders and collector-gatherers. Predatory invertebrates and insectivorous fish are secondary consumers that feed on invertebrate primary consumers, although fish may also feed on predatory invertebrates in which case they may be considered tertiary consumers. Additionally native fish (particularly *Pseudobarbus* spp.) may also feed directly on algae and particulate organic matter at the base of the food web. Since fish have trophic links to both alga-eating and detritus-eating invertebrates, they have the potential to influence both autotrophic and heterotrophic food chains in streams.

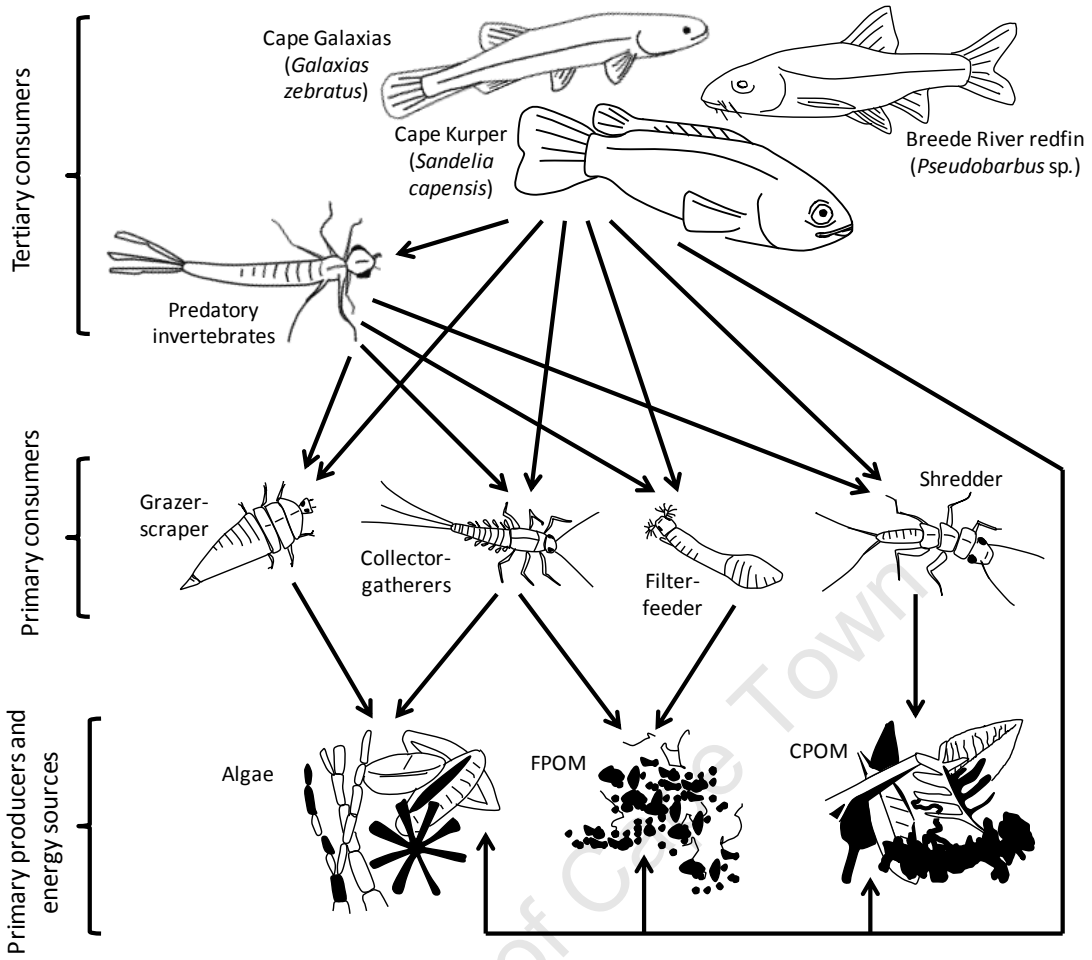

Figure 3.1 Hypothetical community structure and trophic relationships between dominant biota and resources in a CFR headwater stream. Arrows indicate major interactions between community components. Native fish feed on both predatory and non-predatory invertebrates, and may also feed directly on algae and particulate organic matter. Fish may therefore function as primary, secondary or tertiary consumers, but their main role in CFR stream communities appears to be as tertiary consumers. Predatory invertebrates are secondary consumers that feed on non-predatory invertebrates. Non-predatory invertebrates, including grazer-scrapers, collector-gatherers, filter-feeders and shredders, are primary consumers that feed on either algae, particulate organic matter, or a combination of these two food sources. At the base of the food web, algae are primary producers, while detritus, including coarse particulate organic matter (CPOM) and its breakdown product, fine particulate organic matter (FPOM), enters the stream as plant material from the riparian zone.

The results from two recent studies conducted in the CFR (Lowe *et al.* 2008, Weyl *et al.* 2010) suggest that the impact of bass invasions may extend beyond the replacement of native fish populations, in that the composition of aquatic invertebrate assemblages has been found to differ between sections of streams with and without bass. Moreover, notable

differences in algal biomass in relation to bass presence have also been observed (S.R. Lowe, pers. comm. 2010), suggesting that bass-related changes in invertebrate assemblages may have cascaded down to the base of the autotrophic pathway. On the other hand, the question of whether the replacement of native fish by trout (Chapter 2) has consequences for lower trophic levels in CFR streams has not yet been addressed, and forms the focus of this chapter.

On a global scale, the ecological effects of introduced trout (and other salmonids) have been relatively well studied (see reviews by Cambray 2003, Townsend 2003, Simon & Townsend 2003). In streams, trout introductions have been shown to initiate trophic cascades that increase the biomass of benthic algae as a result of reductions in the biomass of herbivorous invertebrates (e.g. Herbst *et al.* 2009, Buria *et al.* 2010), or by restricting invertebrate foraging behaviour (e.g. McIntosh & Townsend 1996). The nature and strength of trout-induced cascades appears to be dependent on how trout modify functional relationships between native predators and their prey. If trout simply replace ecologically similar native predators, then cascading effects on lower trophic levels may be weak or undetectable. However, in systems where trout present a functional novelty, strong cascading effects are likely to ensue, with measurable consequences at the community level (e.g. Dahl & Greenberg 1996, Schmitz 2008, Benjamin *et al.* 2011). The invasion of New Zealand streams by trout illustrates how non-native brown trout *Salmo trutta* can uncouple trophic links between native predatory fish *Galaxias* spp. and their prey, grazing benthic invertebrates, and initiate a trophic cascade that ultimately affects the biomass of benthic algae at the base of the food web (Townsend 2003, Simon & Townsend 2003, Eby *et al.* 2006).

In South Africa, evidence concerning the impact of trout on community components other than native fish is surprisingly scarce. De Moor & Bruton (1988) suggested that although trout may have little impact on the total biomass of stream invertebrates, they are likely to alter species composition by decreasing the abundance of active, visible species. On the other hand, observations made by (Crass 1960) suggested that trout had little effect on the abundance of aquatic invertebrates in streams in Kwa-Zulu Natal, South Africa. There has been a high level of uncertainty regarding the impact of trout on stream invertebrates in South Africa for some time, and de Moor & Bruton (1988) called for studies comparing invertebrate assemblages between streams where trout have replaced indigenous fish

species and those in which indigenous fish populations are still present. Despite intensive searching of the literature, I have not been able to find any reference to cascading effects of trout on basal trophic levels (i.e. standing stocks of algae or detritus) in South African streams.

The upper Breede River catchment in the CFR is partially invaded by non-native trout, with several tributaries that have been colonized by trout interspersed with similar tributaries that have not been invaded, and in general native fish are abundant in streams without trout, but rare, or absent, in streams with trout (Chapter 2). This situation presents a natural experiment that provides a valuable opportunity to study community-wide impacts of trout by comparing the structure of benthic communities in the presence and absence of trout (see Chapter 2 for details on the distribution of trout and native fish species in the upper Breede River catchment). In this chapter, I use a broad-scale comparative field study to ascertain whether impacts of non-native trout extent beyond the replacement of native fish populations, and alter the structure of CFR headwater stream communities at lower trophic levels. Specifically, the following questions were addressed:

1) Are their differences in the density, taxonomic composition and functional composition of invertebrate assemblages between sites with and without trout?
2) Does the biomass of benthic algae, fine particulate organic matter (FPOM) and coarse particulate organic matter (CPOM) differ between streams with and without trout?
3) Can differences, if any, in community structure between streams with and without trout be explained by environmental factors other than the presence of trout?

3.2 METHODS

Comparative studies conducted at broad spatial scales are useful for describing patterns that occur in nature, and can be used to formulate hypotheses and guide controlled experiments aimed at examining underlying mechanisms. Since variability in environmental conditions among streams is notoriously high (Power *et al.* 1988), and could potentially overshadow the influence of trout on benthic community structure, I made an effort to

select streams with similar environmental conditions, but differing in terms of trout presence. Furthermore, surveys of benthic community structure were accompanied by measurements of environmental characteristics of the study streams in order to assess whether any patterns observed were likely a result of trout presence, or differences in environmental conditions between streams. In this study, I used the same 24 sites (i.e. 12 sites with trout, 12 sites without trout) where fish and environmental conditions were sampled (Chapter 2), to make inferences about the influence of the replacement of native fish by trout on the structure of benthic communities. A detailed description of the study area, the procedure used to select study sites and the fish assemblage composition at each site are provided in Chapter 2.

3.2.1 Field sampling

Invertebrates, organic matter and periphyton were sampled at each 50 m site on the same day that fish abundance and other environmental factors were measured (16 February – 19 March 2010, see Chapter 2 for details of sampling methods for fish and physico-chemical variables). The samples for this study were collected under permit 0035-AAA 007-00057 issued from Cape Nature, and animal ethics clearance was obtained from the University of Cape Town. Sampling was conducted approximately 1 h after the fish surveys were completed to allow time for invertebrates disturbed by snorkelling to recover. Environmental conditions vary among different habitat types within streams, and this influences invertebrate assemblages as well as the biomass of benthic algae and the accumulation and breakdown of organic matter (Davies & Day 1998, Rosenfeld 2000, Allan & Castillo 2007). Headwater streams generally contain two main habitat types: erosional habitats (including runs and riffles) and depositional habitats (pools) (Rosenfeld 2000, Cummins *et al.* 2008). Erosional habitats are relatively shallow areas of stream, with high current velocities and coarse substrata, while depositional habitats tend to be deeper areas of stream with slower water flow and finer substrata. In the present study, benthic community components were sampled in both erosional and depositional habitats at each site so that overall estimates of community composition at the reach scale (i.e. over the 50 m site) incorporated the range of environmental conditions available. Sampling followed a

random, stratified approach (Biggs & Kilroy 2000), with samples being collected at five randomly-selected locations in each habitat type at each site. In this study, areas of stream where the water surface was broken or rippled (depth usually <50 cm) were considered erosional habitats, whilst areas with a smooth surface and minimal visibly-detectable flow (depth usually >50 cm) were considered depositional habitats (Cummins *et al.* 2008).

Invertebrates and particulate organic matter

Samples of benthic invertebrates and organic matter were collected with a box sampler (30 x 30 x 30 cm, 250 µm mesh). The box sampler had a steel frame lined with mesh on three sides and a 60 cm long net ending in a removable collecting bottle on the fourth side. At each sampling point, the box sampler was placed on the stream bed with the net extending downstream. The area of streambed falling within the frame was disturbed by hand for one minute, ensuring that all movable substrate particles were turned over and rubbed to dislodge invertebrates and other organic matter. In the erosional habitat, the stream current ensured that all dislodged benthic material was washed through the net into the collecting bottle, while in depositional habitats this process was aided by sweeping dislodged material into the net by hand. The contents of each box sample were preserved in 70% ethanol for later processing in the lab.

Algae

Five fist-sized stones were randomly collected from both erosional and depositional habitats at each site for assessment of algal biomass. Each stone was scrubbed in 500 ml stream water for two minutes with a toothbrush, after which the resulting slurry was homogenized and a 200 ml sub-sample collected, held on ice in the field, and frozen in the dark within three hours of collection (Biggs & Kilroy 2000). The x, y and z dimensions of each stone were measured using plastic callipers so that stone surface area could be estimated and linked to algal biomass (Biggs & Kilroy 2000).

Environmental conditions

Nineteen physico-chemical variables were measured in order to characterize the environmental conditions at each site and ascertain whether there were any consistent differences in environmental conditions between sites with and without trout. A detailed description of sampling methods and equipment can be found in Chapter 2, Section 2.2.5.

Preliminary survey

A preliminary survey was conducted at 16 of the 24 survey sites to assess patterns in invertebrate assemblage structure in relation to trout presence. The preliminary survey data were used to ascertain whether the general patterns detected in the main survey would also be detected in a different year. Invertebrate samples were collected between 16 March and 29 April 2009 from nine of the sites with trout and seven of the sites without trout. One of the sites containing trout (site 25) was sampled only in the preliminary survey, not in the main survey. The reason for this was that although rainbow trout was the only non-native fish species noted in 2009, subsequent visits revealed that smallmouth bass *M. dolomieu* had since become established, and therefore the site did not meet the site-selection criteria for the 2010 survey (Chapter 2, Section 2.2.2). A 20 m reach at each site was delineated for invertebrate sampling, and invertebrates were collected with a 30 x 30 cm net with 1 mm diameter mesh. Invertebrates were collected from both erosional and depositional habitats by disturbing substrate (by kicking with feet and brushing with hands) and holding the net just downstream to collect animals that became dislodged (active sweeping was used to collect animals where flow was too weak to carry them into the net). Erosional habitats were sampled for two minutes, whilst depositional habitats were sampled for one minute at each site.

3.2.2 Laboratory methods

Invertebrates

The contents of the box samples were sorted under a dissecting microscope. All invertebrates were removed from each sample, and remaining material set aside for further processing. Invertebrates were identified to lowest feasible taxonomic level and counted. When possible, invertebrates were identified to genus or species, although several taxa represented coarser levels of taxonomic resolution. The major references for keying out invertebrate taxa were the "Guides to the Freshwater Invertebrates of Southern Africa" (Day *et al.* 2001, 2003, Day & de Moor 2002a, b, de Moor *et al.* 2003a, b, Stals & de Moor 2007). Denise Schael (Nelson Mandela Metropolitan University, South Africa) assisted with identification of Ephemeroptera, Michael Samways (Stellenbosch University, South Africa) assisted with identification of Odonata, and Vere Ross-Gillespie (University of Cape Town, South Africa) assisted with identification of Plecoptera.

Table 3.1 Aquatic invertebrate functional feeding groups and associated foraging behaviours and food resources (adapted from Cummins *et al.* 2008). CPOM = coarse particulate organic matter, and FPOM = fine particulate organic matter.

Functional feeding group	Foraging behaviour	Dominant food resources	Particle size range of food (mm)
Collector-gatherers	Deposit feeders that ingest sediment or gather loose particles in depositional areas	FPOM - decomposing detrital particles, algae, bacteria and faeces	0.05 - 1.0
Filter-feeders	Suspension feeders that filter particles from the water column with nets or adapted body parts	FPOM - decomposing detrital particles, algae, bacteria and faeces	0.01 - 1.0
Grazer-scrapers	Graze mineral and organic surfaces	Periphyton - attached algae and associated detritus, microfauna and flora, and feces	0.01 - 1.0
Predators	Capture and engulf prey or ingest body fluids	Prey - living animal tissue	>0.5
Shredders	Chew conditioned or live vascular plant tissue, or gauge wood	CPOM – decomposing, or living, vascular plants	>1.0

Invertebrates were assigned to FFGs including collector-gatherers (CG), filter-feeders (FF), grazer-scrapers (GS), predators (P) and shredders (SH). The feeding behaviours and dominant food recourses of these five FFGs are detailed in Table 3.1. Major references used for designating invertebrate taxa to FFGs included the "Guides to the Freshwater Invertebrates of Southern Africa" listed above, and Cummins *et al.* (2008). The density (number/m^2) of each invertebrate taxon and each FFG was calculated based on the area of streambed incorporated in each box sample (0.09 m^2). Invertebrates collected during the preliminary survey were identified to family level using the texts listed above, and enumerated.

Particulate organic matter

The material remaining after invertebrates were removed from samples was used to estimate the biomass of FPOM and CPOM in each sample. Samples were elutriated to remove sand and gravel, and remaining organic matter was passed through a 1 mm sieve to separate organic matter into FPOM (250 – 1000 µm) and CPOM (>1000 µm). Ash-free dry mass (AFDM) of organic matter samples was obtained using the following procedure. Samples were dried at 60 °C for 24 h in a drying oven, weighed (to the nearest 0.01 mg), combusted at 500 °C for 1 h and weighed again. The AFDM of each sample was then calculated by subtracting the mass of the ashed sample from that of the oven-dried sample, and converted to AFDM/m^2 based on the area of streambed incorporated in each box sample (0.09 m^2).

Algae

The frozen algal samples were defrosted within 30 d of collection, and stored in the dark for a maximum of 12 h before being processed in the laboratory. Defrosted samples were homogenized and passed through Whatman GF/F 0.7 µm glass fibre filter papers, and the volume (usually approximately 150 ml) of filtered sample recorded. Chlorophyll *a* was extracted from filter papers using 90% ethanol and concentrations were measured using the spectrophotometric method of Sartory & Grobbelaar (1984), as summarized by Biggs &

Kilroy (2000). Absorbance (665 nm and 750 nm) was measured using a Merck Spectroquant Pharo 100 spectrophotometer. The x, y and z dimensions of each stone were used to estimate stone surface area as described by Biggs & Kilroy (2000), and the biomass (mg) of chlorophyll *a*/m^2 was calculated.

3.2.3 Data analyses

Environmental conditions

The analyses used to compare environmental conditions between sites with and without trout are explained in detail in Chapter 2, Section 2.2.8. Essentially, a combination of univariate and multivariate tests were used to ascertain whether any consistent differences in environmental conditions existed between the two groups of sites. Furthermore, a varimax-rotated principal components analysis (PCA) was used to visualize differences in environmental conditions among sites, and to reduce the 19 environmental variables to a limited number of independent, uncorrelated factors that summarize major axes of environmental variation among sites (Quinn & Keough 2002). These factors were then used, along with trout presence/absence, as predictors in non-parametric, multivariate regression models.

Benthic invertebrates

The density of each invertebrate taxon in each sample was calculated by dividing the number of individuals recorded by the area of stream bed covered by the box sampler (0.09 m^2). The mean density of each invertebrate taxon in erosional and depositional habitats at each site was then estimated from the five samples collected in each habitat at each site. The final density of each taxon for each study reach was obtained by weighting the mean density in each habitat by the proportional cover of each habitat at each site. The proportional cover of the two habitat types at each site was estimated by recording which habitat type occurred at each of the 30 points where environmental conditions were measured along transects (Chapter 2, Section 2.2.5), and scaling this up to obtain an estimate of proportional habitat coverage at the site level.

Multivariate analysis was used to assess differences in the taxonomic and functional composition of invertebrate assemblages between sites with and without trout. Invertebrate density data were ln(x+1) transformed prior to analysis to down-weight the influence of the most abundant taxa, and converted to a resemblance matrix using Bray-Curtis similarity (Anderson *et al.* 2008). Non-metric multidimensional scaling (nMDS) ordination was used to visualize differences in taxonomic and functional invertebrate assemblage composition between sites with and without trout. PERMANOVA, a semi-parametric, permutation-based analogue of traditional ANOVA/MANOVA, was used to test for significant differences in assemblage structure between sites with and without trout (Anderson *et al.* 2008). One-way PERMANOVA using Bray-Curtis similarity, 9999 permutations and unrestricted permutation of raw data were used to examine the effect of the fixed factor trout presence on both the taxonomic and functional composition of invertebrate assemblages. The main assumption of PERMANOVA is that there is no significant difference in dispersion between the groups being compared, and this was evaluated using permutational analysis of multivariate dispersion (PERMDISP, Anderson *et al.* 2008). Analysis of similarity percentages (SIMPER, Anderson *et al.* 2008) was then used to identify the taxa (or FFGs) contributing most to the overall dissimilarity in taxonomic and functional assemblage composition between the two groups of sites. An nMDS bubble plot (with bubbles scaled to taxon/FFG density) was generated for the taxon/FFG identified by SIMPER as contributing the most to the overall dissimilarity. Densities of the top ten taxa identified by SIMPER analysis were compared between sites with and without trout using independent sample *t* tests, since ln(x+1) transformed invertebrate density data met the assumptions of this test. Similarly, the densities of total invertebrates, collector-gatherers, filter-feeders, grazer-scrapers, predators and shredders were ln(x+1) transformed and compared between sites with and without trout using independent sample *t* tests.

Metrics of benthic invertebrate taxon abundance and diversity were computed for each sampling site, and each metric was compared between sites with and without trout conducted using Mann-Whitney *U* tests, since distributions of the data did not meet the assumptions of parametric *t* tests, even after transformation. The following five commonly-used diversity indices were computed:

1. Taxon richness (*S*), the total number of taxa;

2. Margalef's index (*d*), a richness index, given by the equation:

   $d = (S-1) / \log N$, where N is the total number of individuals;

3. Shannon diversity index (*H'*), given by the equation:

   $H' = - \Sigma_i p_i \log (p_i)$, where p_i is the proportion of the total count arising from the *i*th taxon;

4. Pielou's index of evenness (*J'*), given by the equation:

   $J' = H' / \log S$; and

5. Simpson diversity index (*1 - λ*), which once again expresses evenness and is given by the equation:

   $1-\lambda = 1-(\Sigma p_i^2)$.

Influence of trout presence and other environmental factors on invertebrate assemblage composition

Relationships between taxonomic and functional invertebrate assemblage composition and a set of predictor variables including environmental factors, as well as the presence of trout, were investigated using distance-based linear models (DISTLM, Anderson *et al.* 2008). DISTLM is a non-parametric multivariate multiple regression technique for analyzing and modeling the relationship between a multivariate response data cloud, as described by a resemblance matrix, and one of more predictor variables. Since DISTLM is a permutation-based technique performed on a resemblance matrix, it avoids the assumption of normality

associated with standard linear modeling approaches, and is thus an appropriate option for analyzing community datasets which often fail to meet this assumption.

Based on the recommendation of Quinn & Keough (2002), all factors with eigenvalues >1 were retained from the PCA and used as predictors that represent major axes of variation in environmental conditions among sites. Predictor variables were checked for multicollinearity, but the correlation coefficient *r* never exceeded 0.7 so no variables were dropped from the analysis (Anderson *et al.* 2008, Budaev 2010). Resemblance matrices were calculated using Bray-Curtis similarity, which is appropriate for models with multiple biotic response variables (Anderson *et al.* 2008). The density of each invertebrate taxon and, FFG, were ln(x+1) transformed prior to analysis to even out their skewed distributions.

The DISTLM routine was used achieve two primary objectives. Firstly, the proportion of the variation in assemblage composition explained by each predictor was assessed individually in marginal tests. Secondly, a step-wise procedure with the "adjusted R^2" selection criterion was used to identify the combination of predictors that produced the most parsimonious model explaining the variation in assemblage composition among sites. (In this context, parsimony refers to the trade-off between explaining the largest possible proportion of variation in assemblage composition, and minimizing the number of predictors included in the model.) The predictor explaining the greatest proportion of the variability is fitted first, and then predictors are sequentially added to, and subtracted from the model, in an attempt to improve the selection criterion (adjusted R^2). The procedure is complete when no further improvement to the selection criterion can be made by adding or deleting a term from the model. Distance-based redundancy analysis (dbRDA) ordination plots provided a visual representation the most parsimonious model (Anderson *et al.* 2008). Unlike nMDS, dbRDA is a constrained ordination technique, meaning that it produces axes that are directly linearly related to the predictor variables included in the model.

Lower trophic levels

The mean biomass (g/m^2) of chlorophyll *a*, FPOM and CPOM was estimated for both erosional and depositional habitats at each site by averaging the five samples collected from

each habitat. The final biomasses for each of these three community metrics for each study reach were obtained using the protocol described above for estimates of invertebrate density. The mean biomass of each metric in each habitat was weighted by the proportional cover of each habitat at each site. The proportional cover of the two habitat types at each site was estimated by recording which habitat type occurred at each of the 30 points where environmental conditions were measured along transects, and scaling this up to estimate proportional habitat coverage at the site level. Mean chlorophyll *a* concentration, and biomass of FPOM and CPOM, were ln(x+1) transformed to improve normality and homogeneity of variances, and compared between sites with and without trout using independent sample *t* tests.

Influence of trout presence and other environmental factors on the biomass of algae, FPOM and CPOM

Relationships between the biomass of algae, FPOM and CPOM, and a set of predictor variables including environmental factors, as well as the presence of trout, were investigated using DISTLMs. The procedure followed that described above for invertebrate assemblage composition, except that because response variables were univariate (as opposed to invertebrate assemblage composition which was a multivariate response variable), Euclidean distance was used instead of Bray-Curtis similarity, and therefore dbRDA plots were not produced (Anderson et al. 2008). Response variables were ln(x+1) transformed prior to analysis to even out their skewed distributions.

Preliminary survey

Although the area of streambed sampled was not quantified during the preliminary survey, sampling effort was standardized and therefore the relative abundance of invertebrates could be compared among sites. The family-level invertebrate data were ln(x+1) transformed and nMDS plots used to visually explore differences in assemblage composition between sites with and without trout. One-way PERMANOVA, with trout presence as a fixed factor, was used to ascertain whether overall differences in assemblage composition, in

relation to trout presence, were statistically significant. SIMPER analysis was used to establish which taxa contributed most to dissimilarity between the samples from sites with and without trout, and an nMDS bubble plot (with bubbles sizes scaled to taxon density) was generated for the taxon identified by SIMPER as contributing the most to the overall dissimilarity. Independent sample *t* tests were used to test for differences in the relative abundance of taxa identified by SIMPER analysis as important contributors to the overall dissimilarity.

Software used

All univariate analyses were carried out with SPSS 20.0 (SPSS 2011), and multivariate analyses were performed using PRIMER-E (Clarke & Gorley 2006) with the add-on package PERMANOVA+ (Anderson *et al.* 2008).

3.3 RESULTS

3.3.1 Environmental conditions

Detailed results for the comparisons of environmental conditions between sites with and without trout can be found in Chapter 2, Section 2.3.2. Essentially, both univariate and multivariate tests showed that there were no consistent differences in environmental conditions between sites with and without trout. The PCA produced seven principal components that had eigenvalues >1. Together, these components accounted for 74.10% of the variation in environmental conditions among sites (Chapter 2, Section 2.3.2), and were used, along with trout presence, as predictors in DISTLM models for invertebrate, algae and particulate organic matter response variables.

3.3.2 Invertebrates

Total density

The mean ± standard error (SE) total density of invertebrates at sites containing trout (3568 ± 315 individuals/m^2) was significantly higher (*t* test, t_{22} = -2.90, p = 0.005) than that at sites lacking trout (2238 ± 291 individuals/m^2) (Figure 3.2a).

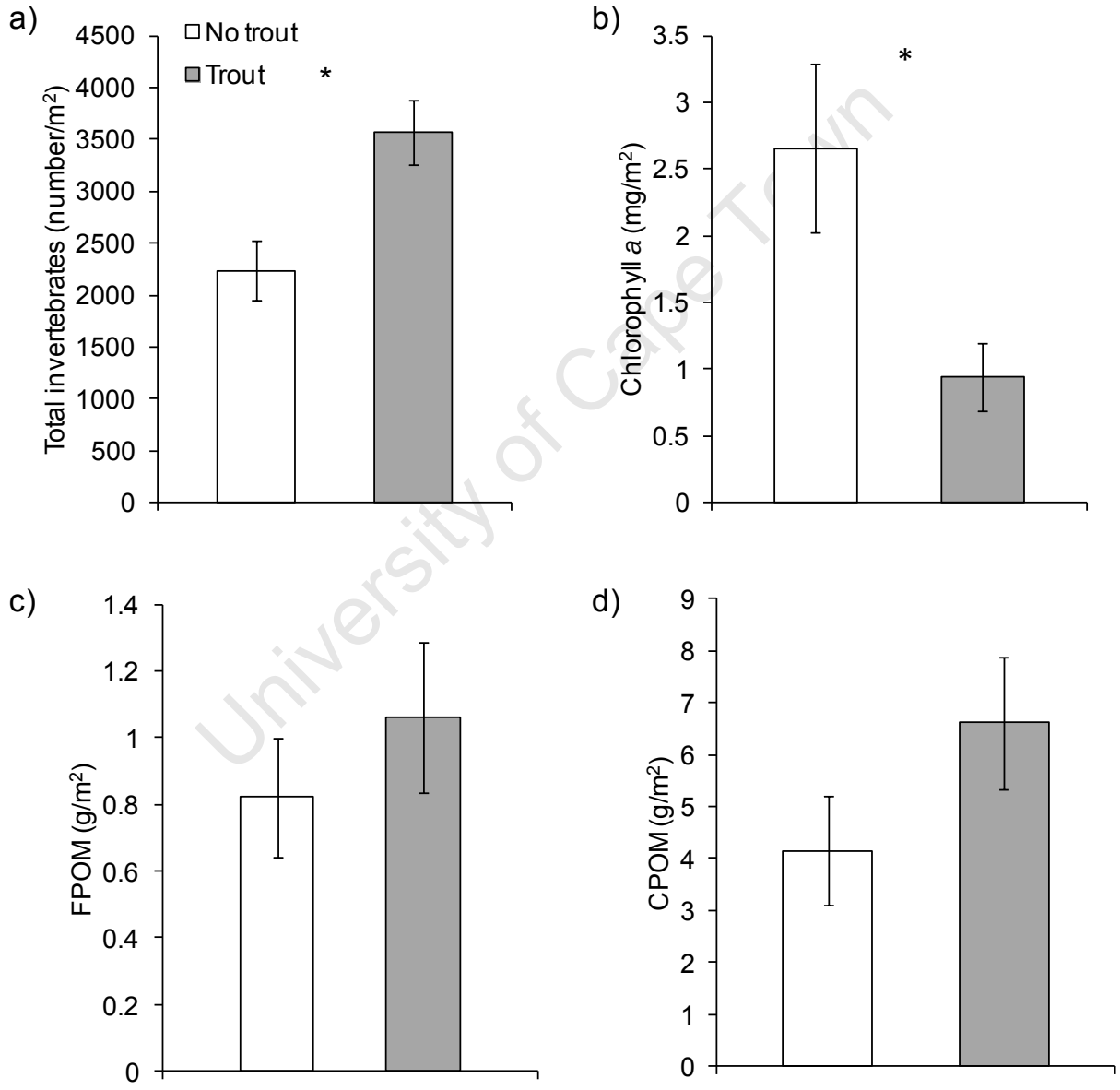

Figure 3.2 Mean ± SE of (a) total invertebrate density, (b) chlorophyll *a* biomass, (c) FPOM biomass and (d) CPOM biomass at sites without (white bar) and with (grey bar) trout. An asterisk indicates a significant difference resulting from an independent sample *t* test on ln(x+1) transformed data (α = 0.05).

Taxonomic composition

The nMDS ordination on the taxon-level invertebrate dataset revealed that most of the sites containing trout separated out from the sites lacking trout, indicating a consistent difference in assemblage composition between these two groups of sites (Figure 3.3a). One-way PERMANOVA showed that trout presence had a significant effect on the taxonomic composition of invertebrate assemblages ($F_{1, 22} = 3.01$, $P_{perm} = 0.002$), and the PERMDISP test showed that multivariate dispersion of the data clouds did not differ significantly between sites with and without trout ($F_{1, 22} = 0.04$, $P_{perm} = 0.831$).

SIMPER analysis revealed that the average dissimilarity in taxonomic composition between sites with and without trout was 59.06% and that the ten taxa most important in discriminating between these groups of sites accounted for 63.10% of that dissimilarity (Figure 3.4). *Baetis* was the taxon that contributed most to the dissimilarity in assemblage composition between sites with and without trout (18.47%), and Figure 3.3b shows differences in mean *Baetis* density among the 24 sampling sites using an nMDS bubble plot. *Baetis* was the most abundant taxon overall (mean proportional abundance across all sites = 14.01%, Appendix 3), and mean *Baetis* density at sites with trout (648 ± 143 individuals/m^2) was approximately four-fold higher than at sites without trout (165 ± 77 individuals/m^2). Other taxa making important contributions to the dissimilarity between the two groups of sites included the ephemeropterans *Lestagella penicillata* and *Demoreptus capensis*, the coleopterans Elmidae and Scirtidae, the dipterans *Simulium*, Orthocladiinae and Chironominae, the plecopteran *Aphanicercella* and the trichopteran *Athripsodes* which collectively contributed a further 44.64% of the overall dissimilarity. With the exception of *Athripsodes*, the densities of these taxa were higher at sites with trout than at sites without trout. *T* tests conducted on ln(x+1) transformed density data revealed that the density of *Baetis* ($t_{22} = -3.96$, $p = 0.001$), *L. penicillata* ($t_{22} = -3.03$, $p = 0.004$), *D. capensis* ($t_{22} = -3.03$, $p = 0.006$) and *Simulium* ($t_{22} = -2.270$, $p = 0.033$) was significantly higher at sites with trout than at sites without trout.

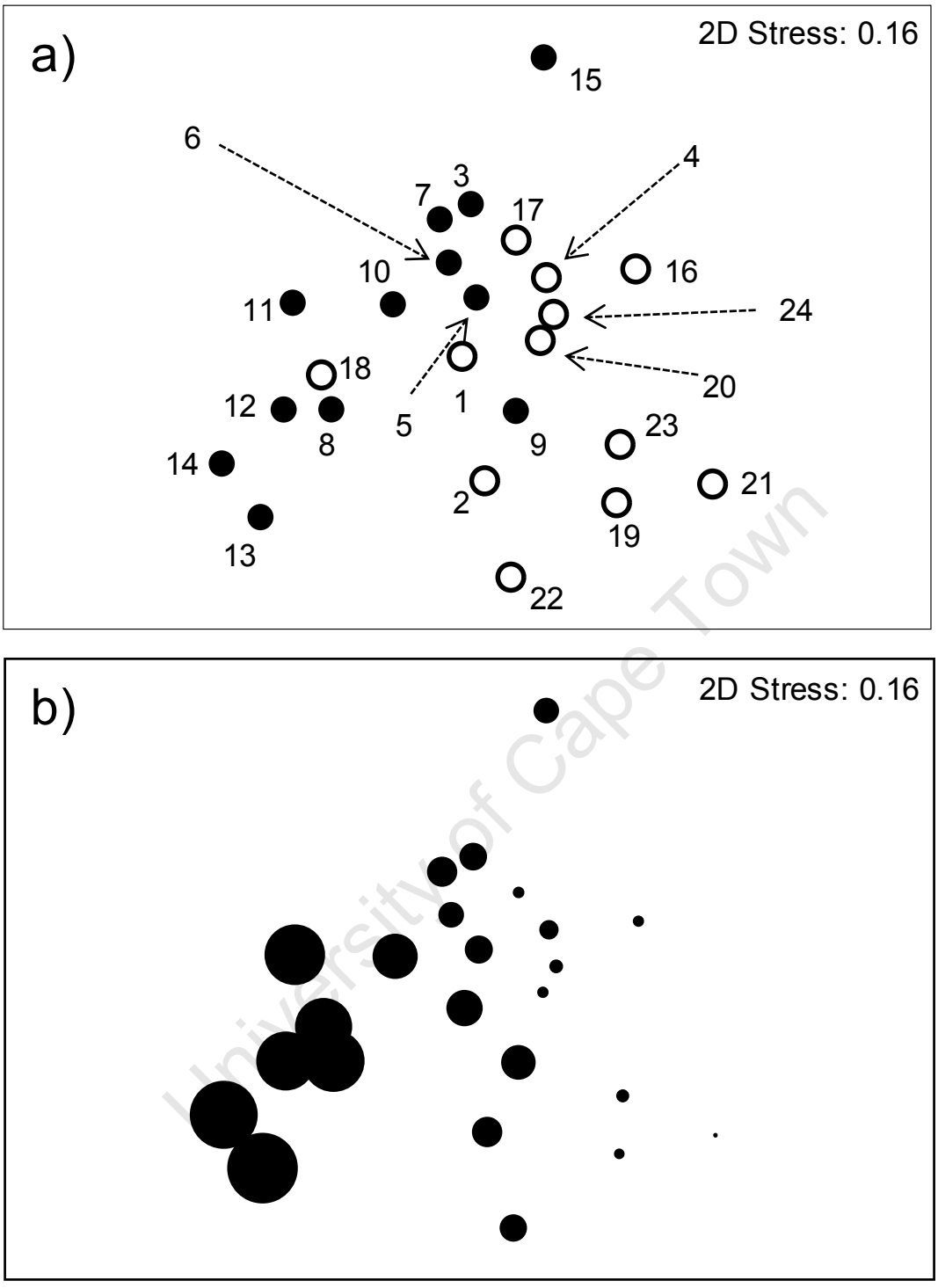

Figure 3.3 nMDS ordination plots of the taxonomic composition of invertebrate assemblages at the 24 study sites. Panel a) indicates sites without (white circles) and with (black circles) trout, and panel b) is a bubble plot on the same ordination indicating the density of *Baetis* mayflies at each study site (bubble size is scaled to *Baetis* density).

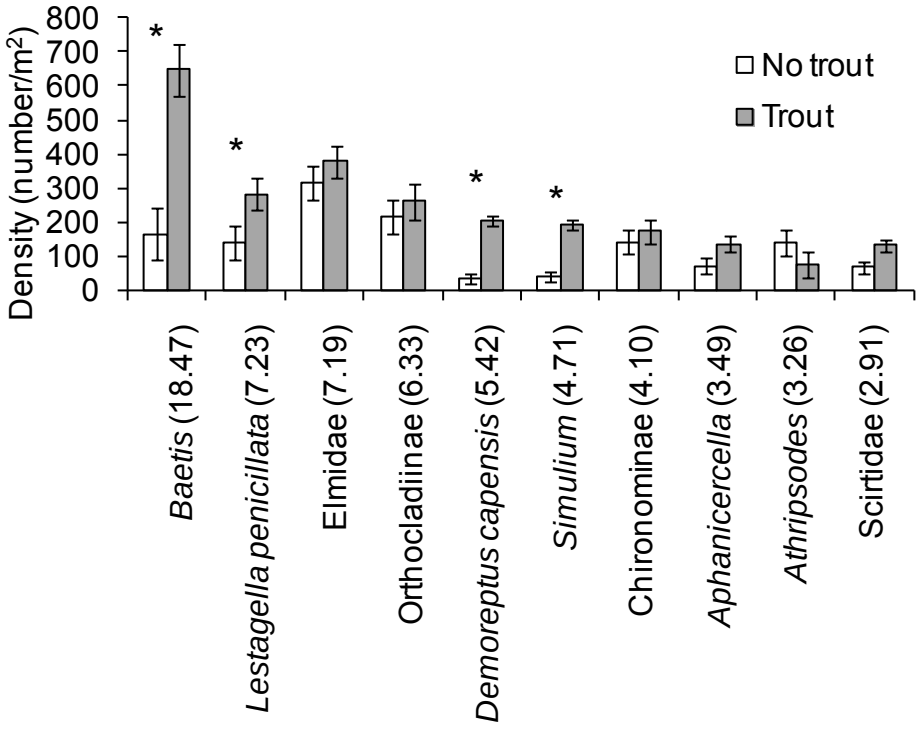

Figure 3.4 Mean ± SE of the density of the ten taxa identified by SIMPER analysis as contributing the most to the dissimilarity in taxonomic assemblage composition between sites with and without trout. The average dissimilarity between sites with and without trout was 59.06%, and values in parentheses indicate the percentage contribution of each taxon to this dissimilarity. An asterisk indicates a significant difference resulting from an independent sample *t* test on ln(x+1) transformed data (α = 0.05).

Functional composition

Collector-gatherers and grazer-scrapers were the dominant components of the invertebrate assemblages at the study sites, comprising 43.57% and 34.62% of the assemblage, when density-based compositional data were averaged across all 24 sites, respectively (Figure 3.5). Predators, shredders and filter-feeders featured less-prominently than collector-gatherers and grazer-scrapers, comprising 8.42%, 7.20% and 6.18% of the assemblage, when density-based compositional data were averaged across all 24 sites, respectively. The nMDS ordination on the functional composition of invertebrate assemblages revealed some level of separation between sites with and without trout, but that there was also some overlap between the two groups (Figure 3.6). One-way PERMANOVA revealed a significant effect of trout presence on the functional composition of invertebrate assemblages ($F_{1,\ 22}$ = 6.52,

p_{perm} = 0.002), and the PERMDISP test showed that multivariate dispersion of the data clouds did not differ significantly between sites with and without trout ($F_{1,22}$ = 1.44, p_{perm} = 0.272).

SIMPER analysis revealed that the average dissimilarity in functional composition between sites with and without trout was 36.26%. Collector-gatherers contributed by far the most to this dissimilarity (41.59%, Figure 3.7), and Figure 3.6b shows differences in mean *Baetis* density among the 24 sampling sites by means of an nMDS bubble plot. The mean density of collector-gatherers at sites containing trout (1553 ± 136 individuals/m^2) was nearly double that at sites lacking trout (808 ± 110 individuals/m^2) and this difference was statistically significant (t_{22} = -4.29, p < 0.001). Grazer-scrapers were also important in distinguishing between the two groups of sites, accounting for 32.22% of the dissimilarity, but mean grazer-scraper density did not differ significantly between the two groups of sites. Filter-feeders and shredders contributed 8.98% and 8.69% respectively to the dissimilarity between the two groups of sites, and were both more abundant at sites with trout than at sites without trout. The mean density of filter-feeders, but not shredders, differed significantly between sites with and without trout (*t* test, t_{22} = -1.98, p = 0.049). Predators contributed least to the overall dissimilarity (8.53%), and predator density at sites with trout was similar to that at sites without trout.

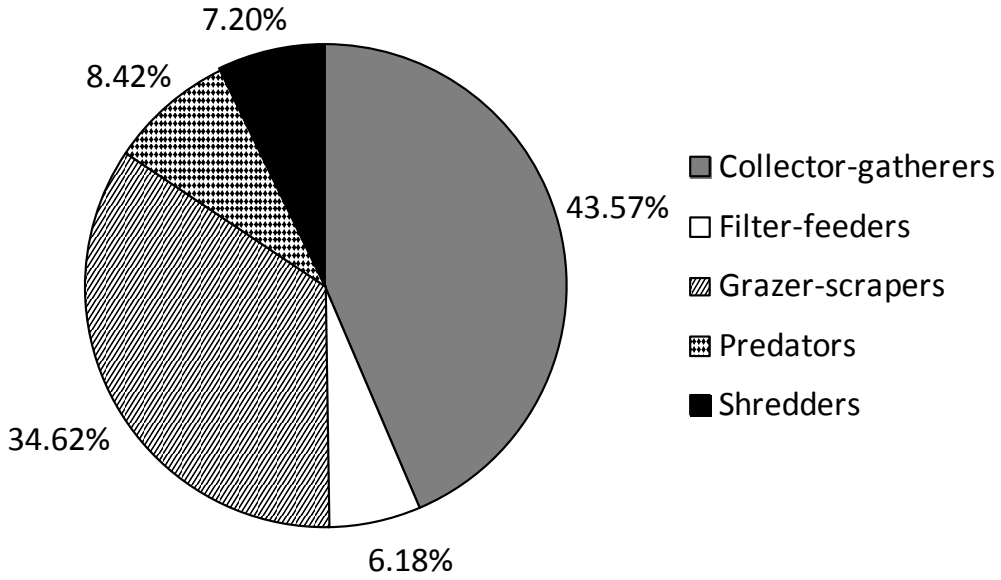

Figure 3.5 Functional composition of the benthic invertebrate assemblages based on mean proportional density of each FFG across all samples and sites.

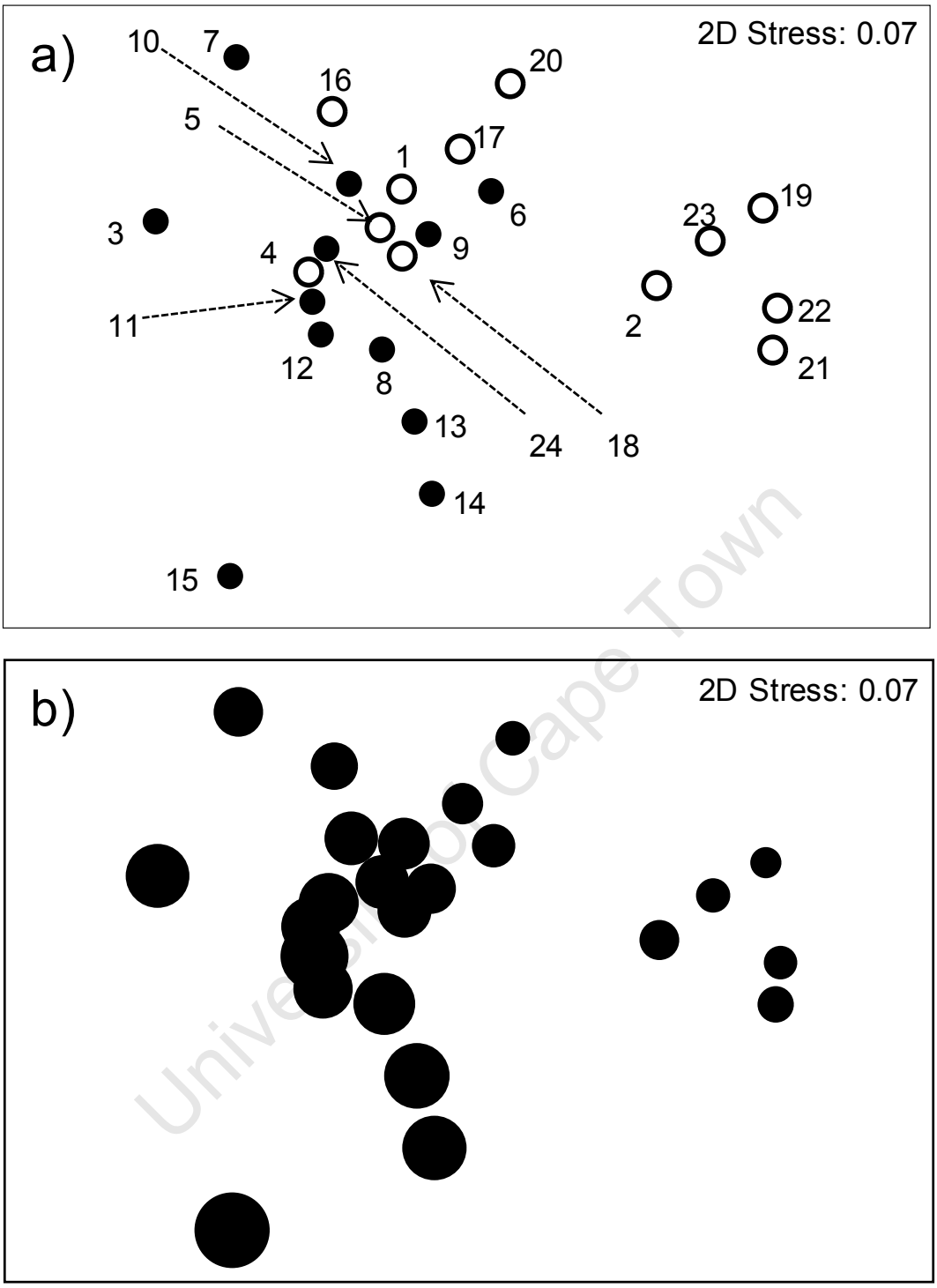

Figure 3.6 nMDS ordination plots of the functional composition of invertebrate assemblages at the 24 study sites. Panel a) indicates sites without (white circles) and with (black circles) trout, and panel b) is a bubble plot on the same ordination indicating the density of collector-gatherers at each study site (bubble size is scaled to collector-gatherer density).

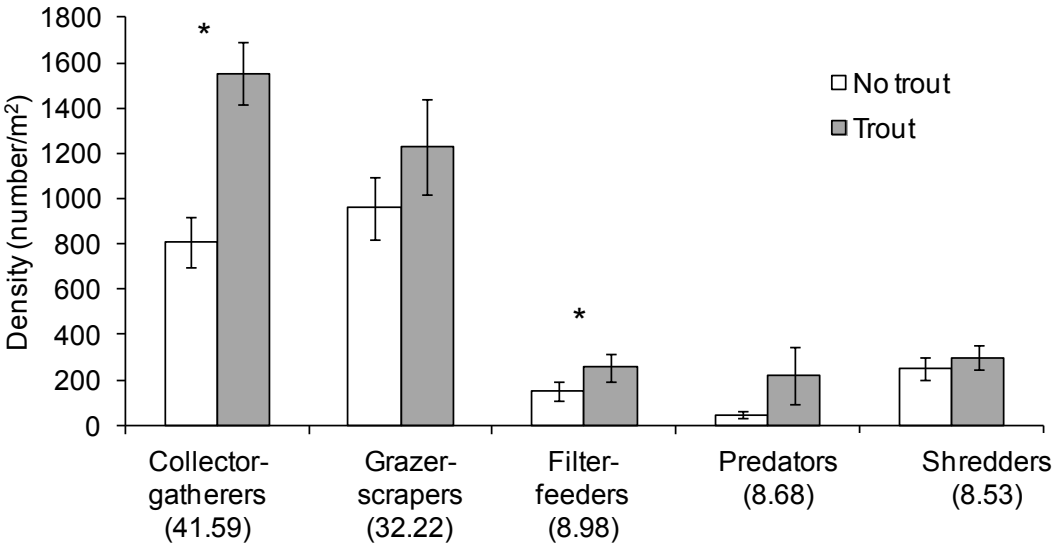

Figure 3.7 Mean ± SE density of functional feeding groups of aquatic invertebrates at sites with and without trout. Functional feeding groups are collector-gatherers (CG), filter-feeders (FF), grazer-scrapers (GS), predators (P) and shredders (SH). Average dissimilarity between sites with and without trout was 36.26%, and values in parentheses indicate the percentage contribution of each taxon to this dissimilarity. An asterisk indicates a significant difference resulting from an independent sample *t* test on ln(x+1) transformed data (α = 0.05).

Indices of diversity

The mean number of taxa (*S*) was higher at sites with trout, while Margalef's index (*d*), Shannon diversity (*H'*) and Simpson diversity (*1- λ*) were lower at sites with trout, than at sites without trout. None of these four measures differed significantly between sites with and without trout (Mann-Whitney *U* tests, Table 3.2). Pielou's evenness (*J'*), however, was significantly higher at sites without trout than at sites with trout. Five of the taxa present at sites lacking trout, were not recorded at sites where trout were present (Appendix 3). These included the dipteran *Forcipomyia*, the trichopterans *Hydrosalpinx*, *Leptecho* and Polycentropodidae and *Hydra*.

Table 3.2 Mean ± SE of diversity indicies computed for invertebrate assemblages at sites with and without trout. An asterisk indicates a significant difference at the $\alpha = 0.05$ level.

Index	No trout		Trout		Mann-Whitney *U* Test	
	Mean	SE	Mean	SE	*U*	*p*
Total species (*S*)	44.83	1.77	46.67	0.51	81.50	0.590
Species richness (*d*)	5.76	0.20	5.61	0.06	61.00	0.550
Pielou's evenness (*J'*)	0.74	0.02	0.67	0.01	31.00	0.017*
Shannon diversity (*H'*)	2.81	0.10	2.57	0.03	42.00	0.089
Simpson diversity (*1-λ*)	0.91	0.02	0.86	0.01	48.00	0.178

Influence of trout presence and other environmental factors on invertebrate assemblage composition

Table 3.3 summarizes the results of the step-wise DISTLM used to investigate the relative influence of trout presence and other environmental factors on variation in the taxonomic composition of invertebrate assemblages among the 24 sampling sites. The marginal tests show that the proportion of variation explained by each environmental predictor alone was low (<5 %), and that none of the environmental predictors explained a significant proportion of the variation in invertebrate assemblage among sites. Trout presence, on the other hand, explained 12.02% of the overall variation which was found, by permutation, to be statistically significant ($F_{1, 22} = 3.005$, $P_{perm} = 0.002$).

The sequential tests produced a final model that contained three predictors, namely trout presence, PC 3 and PC 1, but the overall proportion of variation explained by the model was relatively low (20.35%). Trout presence was fitted first, and was the only predictor that explained a significant proportion of the overall variation in the final model. PC 3, representing gradients in pH and elevation, and PC 1, representing gradients in phosphates and temperature, were also included but did not explain significant components of the overall variation, and contributed relatively little over and above the variation already accounted for by trout presence (<5% each). In the dbRDA plot (Figure 3.8), the first two axes accounted for ~90% of the variation among sites explained by the final model. Sites with and without trout separated out clearly along the axis 1. Multiple partial correlations indicated that axis 1 was highly correlated with trout presence ($r = 0.976$), while PC 1 ($r = -0.082$) and PC 3 ($r = -0.201$) were not well correlated with this axis. This implies that

differences in assemblage structure between the two groups of sites were driven largely by the presence of trout. Axis 2 accounted for variation among sites within the trout and no trout site groups, and was well-correlated with the environmental predictors PC 1 (r = 0.884) and PC 3 (r = -0.460), but not with trout presence (r = -0.082). This implies that variation in assemblage taxonomic structure among sites within the groups of sites containing and lacking trout was related to gradients in pH, elevation, phosphate concentration and temperature.

Table 3.3 Test statistics for distance-based linear model (DISTLM) analysis investigating relationships between the taxonomic composition of invertebrate assemblages and a set of predictor variables including seven principal components that represent major axes in variation in environmental conditions, as well as trout presence/absence. Marginal tests indicate the proportion of variation in taxonomic composition explained by each variable alone, while the sequential tests indicate the cumulative variation explained by each variable fitted to the final model in the order specified, and taking previously-fitted variables into account. Sequential tests were based on a step-wise selection procedure, and the selection criterion used was adjusted R^2. "Variation" = proportion of variation explained and "Cum. var." = the cumulative proportion of variation. An asterisk indicates a significant difference at the α = 0.05 level.

Variable	Adjusted R^2	SS	F	p_{perm}	Var. (%)	Cum. var. (%)	Residual *df*
Marginal tests							
PC 1	-	1621.60	0.96	0.467	4.17	-	-
PC 2	-	1237.40	0.72	0.732	3.18	-	-
PC 3	-	1066.70	0.62	0.810	2.74	-	-
PC 4	-	1353.60	0.79	0.669	3.48	-	-
PC 5	-	1379.00	0.81	0.625	3.55	-	-
PC 6	-	1205.00	0.70	0.733	3.10	-	-
PC 7	-	771.29	0.45	0.959	1.98	-	-
Trout presence	-	4673.50	3.01	0.002*	12.02	-	-
Sequential tests							
+Trout presence	0.08	4673.50	3.01	0.005*	12.02	12.02	22
+PC 3	0.08	1598.70	1.03	0.405	4.11	16.13	21
+PC 1	0.08	1641.50	1.06	0.361	4.22	20.35	20

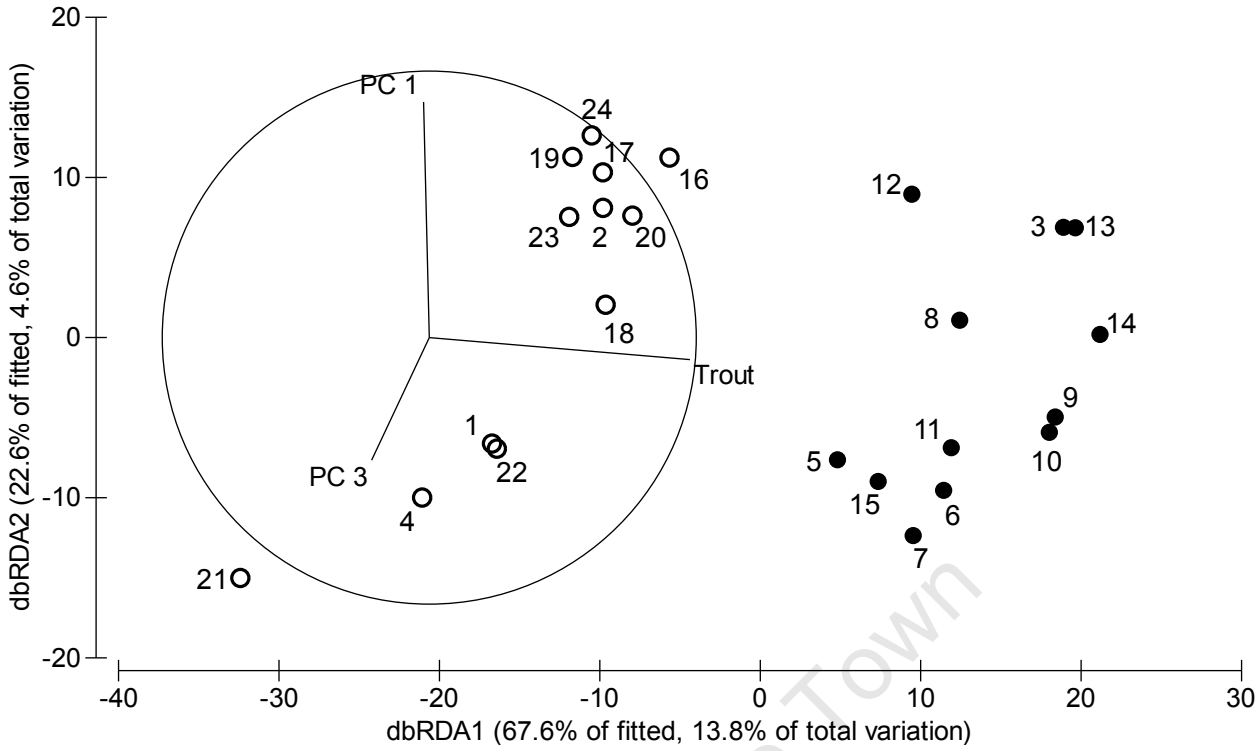

Figure 3.8 dbRDA ordination showing relationships between variation in taxonomic assemblage composition among the sampling sites and the predictors included in the top step-wise DISTLM model. The proportion of overall variation in assemblage composition explained by the model "% of total variation" and the proportion of that variation captured by each dbRDA axis "% of fitted" are indicated. The length and direction of each vector in the vector overlay represent the direction and strength of its influence on the variation in assemblage composition among sampling sites. Sites without trout are indicated by white circles, and sites with trout are indicated by black circles, and distances between sites represent dissimilarity in assemblage composition between sites.

Table 3.4 summarizes the results of the step-wise DISTLM used to investigate the relative influence of trout presence and other environmental factors on variation in the functional composition of invertebrate assemblages. The marginal tests indicate that the proportion of variation explained by each environmental predictor alone was low (<5%), and that none of the environmental predictors explained a significant proportion of the variation in the assemblage among sites. Trout presence, on the other hand, explained a significant amount of the variation (22.88%, $F_{1, 22} = 6.53$, $p_{perm} = 0.004$).

Table 3.4 Test statistics for distance-based linear model (DISTLM) analysis investigating relationships between the functional composition of invertebrate assemblages and a set of predictor variables including seven principal components that represent major axes in variation in environmental conditions, as well as trout presence/absence. Marginal tests indicate the proportion of variation in functional composition explained by each variable alone, while the sequential tests indicate the cumulative variation explained by each variable fitted to the final model in the order specified, and taking previously-fitted variables into account. Sequential tests were based on a step-wise selection procedure, and the selection criterion used was adjusted R^2. "Variation" = proportion of variation explained and "Cum. var." = the cumulative proportion of variation. An asterisk indicates a significant difference at the α = 0.05 level.

Variable	Adjusted R^2	SS	F	p_{perm}	Var. (%)	Cum. var. (%)	Residual *df*
Marginal tests							
PC 1	-	229.98	0.33	0.828	1.49	-	-
PC 2	-	672.25	1.00	0.357	4.37	-	-
PC 3	-	247.51	0.36	0.781	1.61	-	-
PC 4	-	370.68	0.54	0.632	2.41	-	-
PC 5	-	175.71	0.25	0.857	1.14	-	-
PC 6	-	696.92	1.04	0.357	4.53	-	-
PC 7	-	475.52	0.70	0.527	3.09	-	-
Trout presence	-	3522.30	6.53	0.004*	22.88	-	-
Sequential tests							
+Trout presence	0.19	3522.30	6.53	0.002*	22.88	22.88	22
+PC 2	0.21	702.50	1.32	0.247	4.56	27.44	21
+P 7	0.22	663.24	1.26	0.261	4.31	31.75	20

The sequential tests indicate that the most parsimonious model included trout presence, PC 2 and PC 7, and that this model explained 31.75% of the variation in functional composition among sites. PC 2 represents a gradient in ammonium, while PC 7 represents gradients in substrate length and site slope. In the dbRDA plot (Figure 3.9), the first two axes accounted ~100% of the variation among sites explained by the final model. Sites with and without trout separated out clearly along the axis 1. Multiple partial correlations indicated that axis 1 was highly correlated with trout presence (r = 0.992), while PC 2 (r = -0.092) and PC 7 (r = 0.092) were not well correlated with this axis. This implies that differences in assemblage structure between the two groups of sites were driven largely by the presence of trout. Axis 2 accounted for variation among sites within the trout and no trout site groups, and was

well-correlated with the environmental predictors PC 2 (r = 0.760) and PC 7 (r = -0.637), but not with trout presence (r = -0.082). This implies that variation in assemblage functional composition among sites within the groups of sites with and without trout was related to gradients in ammonium concentration, substrate length and site slope.

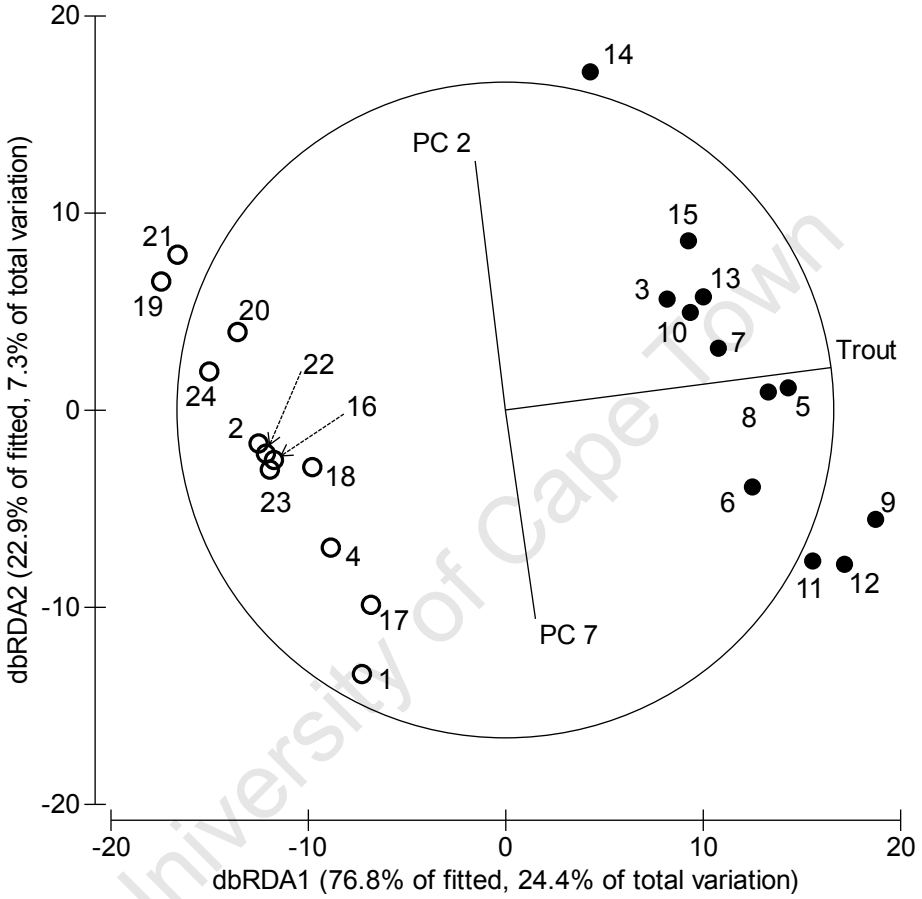

Figure 3.9 dbRDA ordination showing relationships between variation in functional assemblage composition among the sampling sites and the predictors included in the top step-wise DISTLM model. The proportion of overall variation in assemblage composition explained by the model "% of total variation" and the proportion of that variation captured by each dbRDA axis "% of fitted" are indicated. The length and direction of each vector in the vector overlay represent the direction and strength of its influence on the variation in assemblage composition among sampling sites. Sites without trout are indicated by white circles, and sites with trout are indicated by black circles, and distances between sites represent dissimilarity in assemblage composition between sites.

3.3.3 Lower trophic levels

The mean biomass of chlorophyll *a* at sites with trout (0.94 ± 0.24 mg/m^2) was less than half that at sites without trout (2.65 ± 0.63 mg/m^2), and this difference was statistically significant (*t* test, t_{22} = 2.77 p = 0.011, Figure 3.2b). The mean biomass of both FPOM (Figure 3.2c) and CPOM (Figure 3.2d) was somewhat higher at sites with trout relative to that at sites without trout, but these differences were not statistically significant.

Influence of trout presence and other environmental factors on the biomass of algae, FPOM and CPOM

Table 3.5 summarizes the results of the step-wise DISTLM used to investigate the relative influence of trout presence and other environmental factors on variation in the biomass of chlorophyll *a* among the 24 sampling sites. The marginal tests show that the proportion of variation explained by each environmental predictor alone was low (<10%), and that none of the environmental predictors explained a significant proportion of the variation in invertebrate assemblage among sites. Trout presence, on the other hand, explained 25.86% of the overall variation which was found, by permutation, to be statistically significant ($F_{1, 22}$ = 7.68, p_{perm} = 0.013).

The sequential tests produced a final model that contained six predictors, which together explained a cumulative total of 63.84% of the variation in chlorophyll *a* biomass among the study sites. Trout presence and PC 1 were the only predictors that explained a significant proportion of the variation in the final model. Trout presence, explaining 25.86% of the variation was fitted first, and PC 1, which represented gradients primarily in phosphates and temperature, was fitted next, and explained 15.55% of the overall variation in chlorophyll *a* biomass among the sites. Taken together, these results indicate that trout density was the single best predictor of chlorophyll *a* biomass, but that variation in phosphates and temperature among sites were also important. Figure 3.10 shows that chlorophyll *a* biomass tended to be greater at sites with relatively low phosphate concentrations, and low temperatures, but regression analysis revealed that the relationship between Log_e chlorophyll *a* and PC 1 was not statistically significant. Other environmental factors,

represented by PCs 3, 6, 5 and 4, improved the selection criterion R^2, but did not explain significant proportions of the variation in algal biomass among sites, and were therefore considered unimportant predictors relative to trout and PC 1.

Table 3.5 Test statistics for distance-based linear model (DISTLM) analysis investigating relationships between chlorophyll *a* biomass and a set of predictor variables including seven principal components that represent major axes in variation in environmental conditions, as well as trout presence/absence. Marginal tests indicate the proportion of variation in chlorophyll *a* explained by each variable alone, while the sequential tests indicate the cumulative variation explained by each variable fitted to the final model in the order specified, and taking previously-fitted variables into account. Sequential tests were based on a step-wise selection procedure, and the selection criterion used was adjusted R^2. "Variation" = proportion of variation explained and "Cum. Var." = the cumulative proportion of variation. An asterisk indicates a significant difference at the $\alpha = 0.05$ level.

Variable	Adjusted R^2	SS	*F*	*p*	Variation	Cum. var.	Residual *df*
Marginal tests							
PC 1	-	0.45	1.51	0.226	6.42	-	-
PC 2	-	0.01	0.03	0.872	0.14	-	-
PC 3	-	0.10	0.30	0.592	1.36	-	-
PC 4	-	0.48	1.62	0.217	6.87	-	-
PC 5	-	0.28	0.93	0.355	4.05	-	-
PC 6	-	0.21	0.68	0.410	3.01	-	-
PC 7	-	0.07	0.21	0.628	0.96	-	-
Trout presence	-	1.82	7.68	0.013*	25.86	-	-
Sequential tests							
+Trout presence	0.22	1.82	7.68	0.005*	25.86	25.86	22
+PC 1	0.36	1.09	5.58	0.020*	15.55	41.42	21
+PC 3	0.42	0.59	3.33	0.093	8.35	49.77	20
+PC 6	0.48	0.49	3.05	0.101	6.95	56.72	19
+PC 5	0.50	0.31	2.03	0.155	4.39	61.11	18
+PC 4	0.51	0.19	1.28	0.272	2.72	63.84	17

Table 3.6 and 3.7 summarize the results of the step-wise DISTLM used to investigate the relative influence of trout presence and other environmental factors on variation FPOM and CPOM respectively. The marginal tests indicated that, regardless of response variable, the proportion of variation explained by each predictor alone was relatively low (<15%), and not significant.

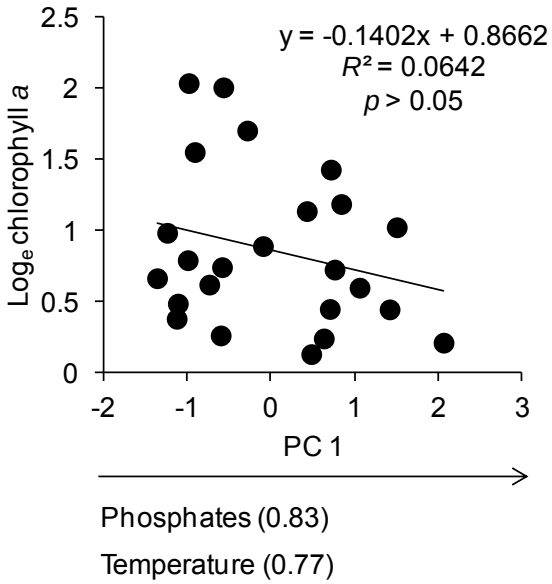

Figure 3.10 Relationship between log transformed chlorophyll *a* biomass and scores along principal component axis 1 (PC 1). The percentage of variation explained, and variables with loadings >0.7, is shown for PC 1 (non-significant regression line is shown).

Table 3.6 Test statistics for distance-based linear model (DISTLM) analysis investigating relationships between FPOM biomass and a set of predictor variables including seven principal components that represent major axes in variation in environmental conditions, as well as trout presence/absence. Marginal tests indicate the proportion of variation in FPOM biomass explained by each variable alone, while the sequential tests indicate the cumulative variation explained by each variable fitted to the final model in the order specified, and taking previously-fitted variables into account. Sequential tests were based on a step-wise selection procedure, and the selection criterion used was adjusted R^2. "Variation" = proportion of variation explained and "Cum. Var." = the cumulative proportion of variation. An asterisk indicates a significant difference at the α = 0.05 level

Variable	Adjusted R^2	SS	*F*	*p*	Variation	Cum. var.	Residual *df*
Marginal tests							
PC 1	-	0.21	2.14	0.157	8.87	-	-
PC 2	-	0.00	0.00	0.999	0.00	-	-
PC 3	-	0.26	2.69	0.114	10.90	-	-
PC 4	-	0.05	0.43	0.505	1.92	-	-
PC 5	-	0.02	0.15	0.716	0.69	-	-
PC 6	-	0.01	0.07	0.810	0.31	-	-
PC 7	-	0.04	0.33	0.566	1.46	-	-
Trout presence	-	0.08	0.73	0.388	3.20	-	-
Sequential tests							
+PC 3	0.07	0.26	2.69	0.121	10.90	10.90	22
+PC 1	0.12	0.21	2.32	0.146	8.87	19.77	21

Table 3.7 Test statistics for distance-based linear model (DISTLM) analysis investigating relationships between CPOM biomass and a set of predictor variables including seven principal components that represent major axes in variation in environmental conditions, as well as trout presence/absence. Marginal tests indicate the proportion of variation in CPOM biomass explained by each variable alone, while the sequential tests indicate the cumulative variation explained by each variable fitted to the final model in the order specified, and taking previously-fitted variables into account. Sequential tests were based on a step-wise selection procedure, and the selection criterion used was adjusted R^2. "Variation" = proportion of variation explained and "Cum. Var." = the cumulative proportion of variation. An asterisk indicates a significant difference at the $\alpha = 0.05$ level

Variable	Adjusted R^2	SS	*F*	*p*	Variation	Cum. var.	Residual *df*
Marginal tests							
PC 1	-	0.02	0.05	0.819	0.23	-	-
PC 2	-	0.23	0.67	0.414	2.96	-	-
PC 3	-	0.03	0.09	0.773	0.41	-	-
PC 4	-	0.02	0.04	0.816	0.20	-	-
PC 5	-	1.00	3.17	0.085	12.60	-	-
PC 6	-	0.02	0.05	0.811	0.23	-	-
PC 7	-	0.46	1.35	0.263	5.79	-	-
Trout presence	-	0.01	0.03	0.852	0.16	-	-
Sequential tests							
+PC 5	0.09	1.00	3.17	0.094	12.60	12.60	22
+PC 7	0.17	0.89	3.09	0.110	11.23	23.83	21
+PC 2	0.17	0.35	1.23	0.298	4.43	28.26	20

The sequential tests indicated that the most parsimonious model for FPOM included the environmental predictors PC 3 (representing gradients in pH and elevation) and PC 1 (representing gradients in phosphates and temperature), and that the most parsimonious model for CPOM included the environmental predictors PC 5 (representing gradients in riparian vegetation and canopy cover) and PC 7 (representing gradients in mean substrate length and site slope) and PC 2 (representing a gradient in ammonium). None of these predictors explained significant proportions of the variation in response variables, and the overall amount of variation captured by the final models was relatively low (19.77% for FPOM, and 28.26% for CPOM).

3.3.5 Preliminary survey

The nMDS ordination on the family-level invertebrate dataset from the preliminary survey revealed that the sites with trout separated out clearly from the sites without trout, indicating that there were consistent differences in the assemblage composition between these two groups of sites (Figure 3.11a). One-way PERMANOVA showed that trout presence had a significant influence on assemblage composition ($F_{1, 14}$ = 5.95, P_{perm} = 0.001), and the PERMDISP test showed that multivariate dispersion of the data clouds did not differ significantly between sites with and without trout ($F_{1, 14}$ = 2.52, P_{perm} = 0.217).

SIMPER analysis revealed that the average dissimilarity in assemblage composition between sites with and without trout was 71.43%, and that three ephemeropteran taxa, namely Teloganodidae, Leptophlebiidae and Baetidae, collectively accounted for 67.08% of the overall dissimilarity (Figure 3.12). Teloganodidae was the taxon that contributed most to the dissimilarity in assemblage composition between sites with and without trout (46.84%) and Figure 3.11b shows differences in mean teloganodid density among the 24 sampling sites. Teloganodidae was also the most abundant taxon overall, making up 45.84% of the assemblage when samples were averaged across all sites (Appendix 4), and was on average approximately fifteen times more abundant at sites with trout than at sites without trout (*t* test, t_{14} = -4.27, p = 0.001). The mean abundances of Leptophlebiidae and Baetidae across all sites were 14.96% and 7.59%, respectively (Appendix 4), and together these taxa contributed a further 20.24% to the overall dissimilarity between sites with and without trout. Both Leptophlebiidae (*t* test, t_{14} = -3.73, p = 0.002) and Baetidae (*t* test, t_{14} = -2.73, p = 0.016) were significantly more abundant at sites with trout that at sites without trout. Finally, the mean relative abundance of all invertebrates was significantly higher at sites with trout (321 ± 95 individuals/m^2) than at sites without trout (93 ± 43 individuals/m^2) (*t* test, t_{14} = -4.83, p < 0.001).

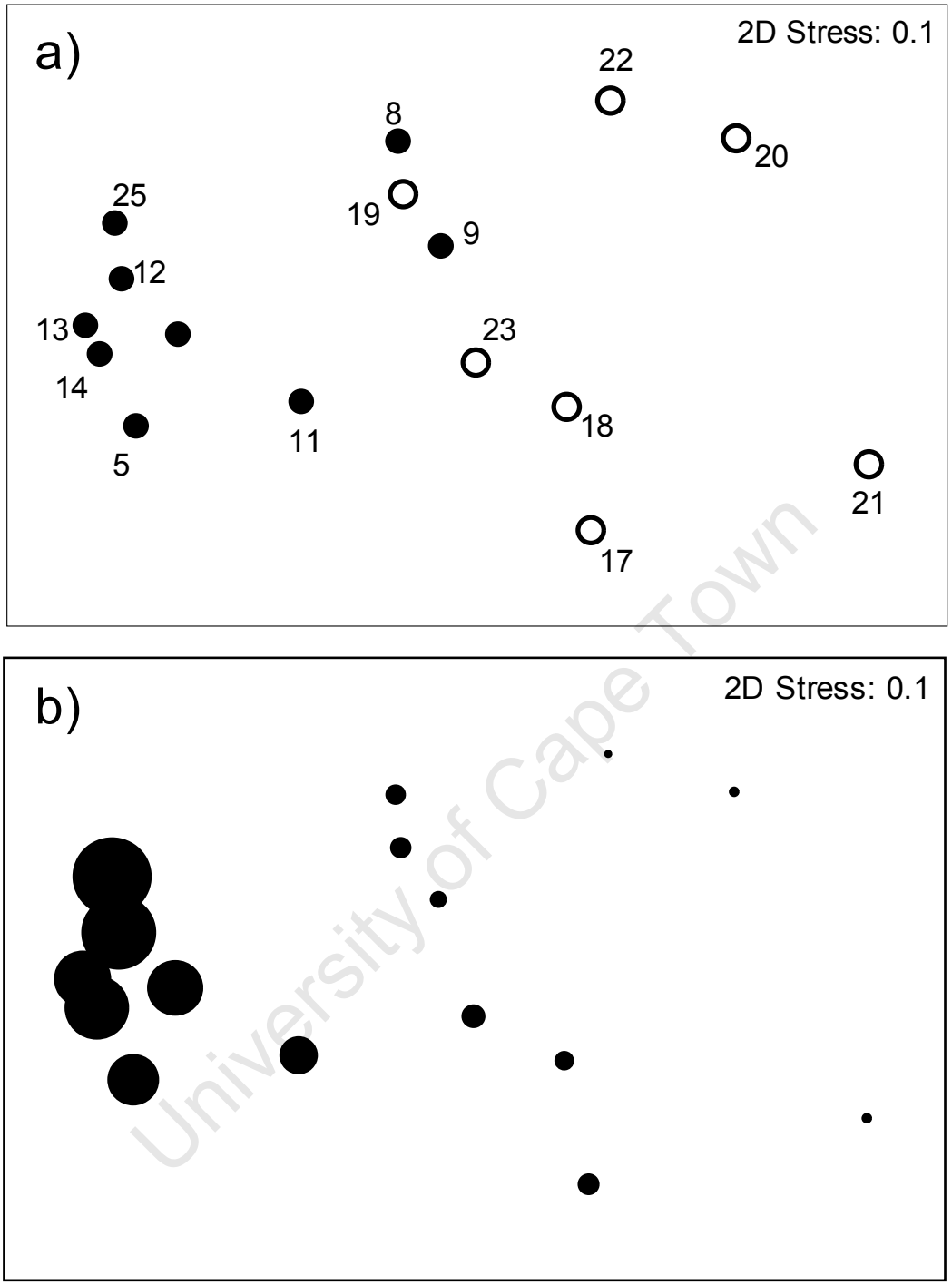

Figure 3.11 nMDS ordination plots of the family-level composition of invertebrate assemblages at the 16 study sites sampled during the preliminary study. Panel a) indicates sites without (white circles) and with (black circles) trout, and panel b) is a bubble plot on the same ordination indicating the density of teloganodid mayflies at each study site (bubble size is scaled to teloganodid density).

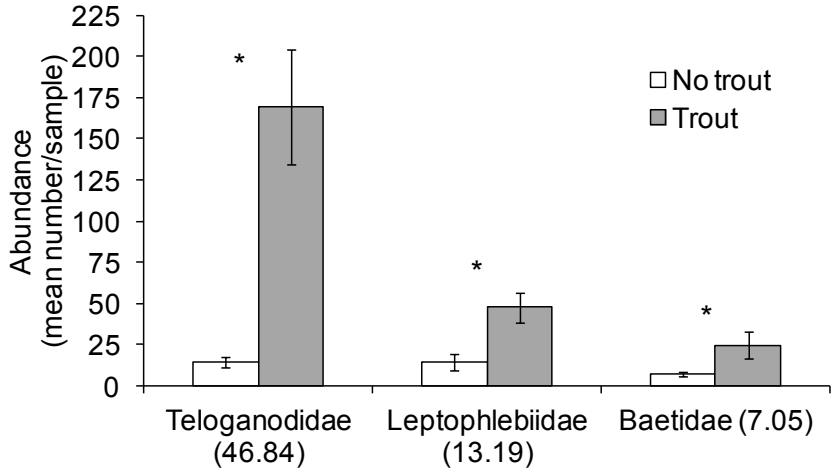

Figure 3.12 Mean ± SE number/sample of the three taxa identified by SIMPER analysis on the preliminary survey data as contributing the most to the dissimilarity in family-level assemblage composition between sites with and without trout. The average dissimilarity between sites with and without trout was 71.43%, and values in parentheses indicate the percentage contribution of each taxon to this dissimilarity. An asterisk indicates a significant difference resulting from an independent sample *t* test on ln(x+1) transformed data (α = 0.05).

3.4 DISCUSSION

3.4.1 Invertebrates

Total density

In headwater streams in the upper Breede River catchment, mean total invertebrate abundance at sites with trout was significantly higher than that at trout-free sites. One explanation for this pattern may be that at sites without trout, native fish are abundant and regulate the abundance of benthic invertebrates, while at sites where trout have become established, native fish have been eliminated which reduces the predation pressure on benthic invertebrates, allowing their abundance to increase. The patterns in fish and invertebrate abundance documented here are comparable to those recorded by Bowlby & Roff (1986), who examined invertebrate abundance in the presence and absence of large predatory fish (brown trout *S. trutta* and northern pike *Esox lucius*) in southern Ontario streams. In streams without large predators, smaller insectivorous fish (creek chub *Semotilus atromaculatus*, and brook trout *Salvelinus fontinalis*) were abundant, and reduced the abundance of benthic invertebrates. However, where large predators were present,

they reduced the abundance of smaller insectivorous fish, which released benthic invertebrates from predation, and allowed them to proliferate on the stream bed. On the other hand, the presence of large, introduced predatory fish (in particular *S. trutta* and *O. mykiss*) in New Zealand streams appears to have had the opposite effect. At sites where non-native trout have eliminated native fish (*Galaxias* spp.), benthic invertebrate density is generally lower than that in comparable streams that lack trout and consequently support healthy populations of native fish (Flecker & Townsend 1994, Simon & Townsend 2003). Whether the replacement of native predatory fish by non-native predators produces a net positive or a negative effect on invertebrate abundance appears to be linked to differences in the predation pressure exerted by native fish and the non-native fish species by which they are replaced (Benjamin *et al.* 2011). For example, in New Zealand, it has been demonstrated that non-native trout exert stronger top-down control on benthic invertebrates than do native galaxiids because trout attain a higher total biomass (Huryn 1998) and because of subtle differences in foraging mode between trout and galaxiids (McIntosh & Townsend 1995a). Extending this line of reasoning to the situation in CFR streams suggests that trout may have a less pronounced effect on benthic invertebrates than the native fish they replace. An investigation of differences in feeding biology between trout and native fish in CFR headwater streams would enable a better understanding of the mechanism by which the introduction of trout has resulted in a relatively high total invertebrate density, and such an investigation is undertaken in Chapter 4 of this thesis.

Assemblage composition

Consistent differences in the composition of invertebrate assemblages between streams with and without trout were found, and the components of the assemblage responsible for the differences identified. Compositional differences in relation to trout presence were driven largely by taxa falling within the collector-gatherer FFG. The *Baetis* group of mayflies, in addition to being the most abundant taxon when taxon density was averaged across all sites, contributed by far the most to the overall dissimilarity in assemblage structure between sites with and without trout. *Baetis* mayflies (Figure 3.13a) are integral components in many stream food webs, because they can numerically dominate primary

consumer assemblages, and thus form an important trophic link between predators and basal trophic levels (Barber-James & Lugo-Oritz 2003). The abundance of *Beatis* mayflies has been shown to be strongly influenced by top-down effects of predatory fish elsewhere (Bechara *et al.* 1992, Dahl & Greenberg 1998, Rosenfeld 2000, McIntosh *et al.* 2004, Ruetz *et al.* 2004), and it has been suggested that their behaviour as drift-prone, epibenthic foragers renders them especially vulnerable to visual predators such as insectivorous fish (Meissner & Muotka 2006). It is plausible that in streams without trout, predation by native fish keeps *Baetis* density in check, but that where trout have depleted native fish numbers, the predation pressure on *Baetis* is relaxed, which allows their density to increase.

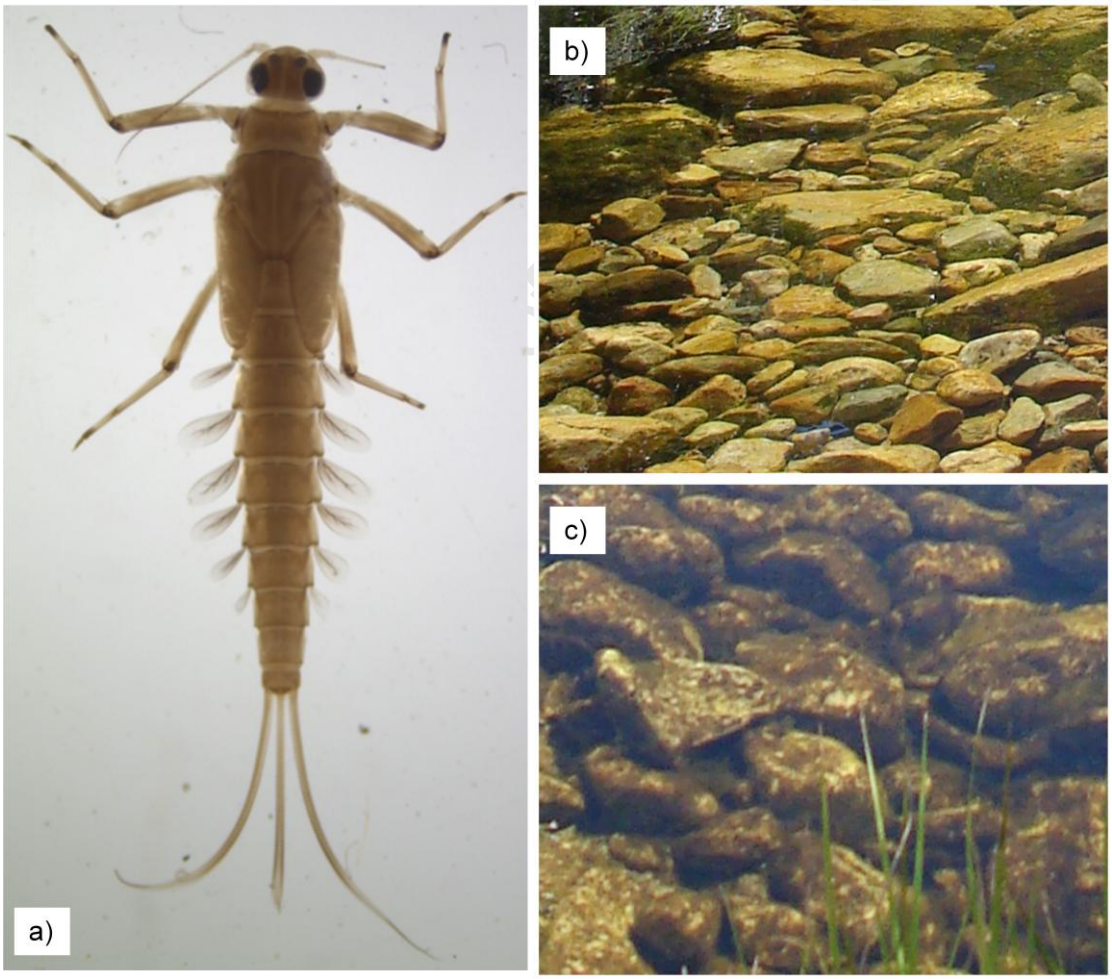

Figure 3.13 Ephemeropterans within the genus *Baetis* (panel a), were on average approximately four times more abundant in streams with trout than in streams without trout. Panel b shows a low biomass of benthic algae in a stream invaded by trout (Groothoek Stream) and panel c shows a relatively high biomass of benthic algae in a trout-free stream supporting healthy native fish populations (Wolwekloof Stream).

Another ephemeropteran collector-gatherer, *L. penicillata*, was also identified as important in differentiating between assemblages at sites with and without trout, and was the fourth most abundant taxon across all study streams. *L. penicillata* is a CFR endemic, and little is known about its trophic habits apart from the fact that it brushes diatoms and fine detritus from the surfaces and undersides of stones with its comb-like mouthparts in fast-flowing headwater streams (King & Schael 2001, Barber-James & Lugo-Oritz 2003). This feeding behaviour likely make it vulnerable to insectivorous fish foraging among the benthos, and its high abundance at sites where trout have depleted native fish abundance relative to sites with healthy native fish populations may be a result of relaxed top-down control. A third ephemeropteran collector-gatherer, *D. capensis*, was also significantly more abundant in streams with trout, and was identified as important in distinguishing between invertebrate assemblages in relation to trout presence. The ecology of this species is poorly known (Barber-James & Lugo-Oritz 2003), but it seems plausible that as a member of the family Baetidae, it may have a similar life history to that of *Baetis*, and its density may be similarly regulated by top-down control at sites where native fish populations remain intact. The chironomid sub-families Chironominae and Orthocladiinae were also among the collector-gatherer taxa identified as important contributors to the dissimilarity in assemblage composition between sites with and without trout. Although on average slightly more abundant at sites with trout, differences in the density of these chironomids were not statistically significant suggesting that trout presence had only a slight, if any, influence on the density of these taxa. Non-predatory chironomids feed mostly on algae and fine detritus, and are in turn consumed by most aquatic predators (Harrison 2003). As result of their high abundance in most streams, chironomids form important trophic links in stream food webs (Harrison 2003). The finding in the present study of no significant difference in chironomid density between streams with and without trout contrasts with the results of other studies (e.g. Meissner & Muotka 2006, Herbst *et al.* 2009) who found trout to have a measurable effect on chironomid density. Interestingly, while trout are generally found to have a negative effect on exposed taxa such as collector-gatherers and grazer-scrapers, their effect on chironomids has been shown to be positive (Meissner & Muotka 2006, Herbst *et al.* 2009). This appears to be an indirect response to trout-induced reductions in invertebrate predator density (Meissner & Muotka 2006), or to an indirect increase in algal biomass which translates into an increase in food and habitat availability for chironomids

(Power 1990b, Harrison 2003, Herbst *et al.* 2009). Of the taxa identified as important in distinguishing between invertebrate assemblages in the presence and absence of trout, *Athripsodes* (another collector-gatherer) was the only taxon which had a greater abundance at sites with trout than at sites without trout; a pattern which may be related to the fact that it builds hard cases of sand grains and/or detritus (de Moor & Scott 2003) which may render it less accessible to insectivorous fish than other taxa (Nyström *et al.* 2003).

Filter-feeders were also significantly more abundant in streams with trout, and the taxon *Simulium* was particularly important in distinguishing between assemblages in the presence and absence of trout in erosional habitats. Simuliids are important primary consumers in many South African streams and forage by filtering suspended particles from the water column (de Moor 2003). They often occur in high numbers on the exposed surfaces of rocks in fast-flowing water (de Moor 2003), and this foraging behaviour is likely to make them particularly vulnerable to predation by insectivorous fish, which may explain their high densities at sites where trout have depleted native fish relative to trout-free sites in the present study.

The grazer-scraper taxa Elmidae and Scirtidae were also among the taxa identified as important contributors to the differences in assemblage structure in relation to trout presence, and both tended to be more abundant in streams with trout. However, differences in the mean density between streams with and without trout were not statistically significant for these taxa, nor for grazer-scrapers as a group, indicating that they may be less strongly influenced by the presence of trout than are collector-gatherers and filter-feeders. This result is somewhat surprising considering that several other studies (Cheever & Simon 2009, Herbst *et al.* 2009, Buria *et al.* 2010) have documented strong, negative numerical responses of grazer-scrapers to trout invasions, presumably because they feed by scraping algae from the exposed surface of rocks making them vulnerable to visual predators like insectivorous fish. It is unclear why grazer-scraper density did not respond strongly to trout presence in the present study, but it may be that the predation pressure exerted by trout on this FFG is similar to that previously exerted by native fish.

No significant difference in mean shredder density was found between streams with and without trout indicating that this FFG was not strongly affected by the presence of trout,

and associated declines in native fish abundance. *Aphanicercella* was the only shredder taxon identified as an important contributor to differences in assemblage structure in relation to trout presence, and is largely responsible for the slightly higher shredder density in streams with trout relative to streams without trout. These results are consistent with those of Reice (1991), Rosenfeld (2000), Ruetz *et al.* (2002) all of whom found a lack of strong response of shredders to variations in fish predation pressure. Shredders feed on detritus accumulations in substrate interstices, and thus may be largely concealed from predators such as fish (Rosenfeld 2000). This may explain the lack of relationship between trout presence and shredder density, in comparison to the relationship between trout presence and the density of epibenthic taxa. On the other hand, studies conducted by Konishi *et al.* (2001), Greig & McIntosh (2006), Buria *et al.* (2010) recorded significant responses of shredders to variations in fish predation, suggesting that the vulnerability of shredders to insectivorous fish may be context-dependent.

The lack of difference in predator density between streams with and without trout, and the fact that no predatory taxa were identified as important contributors to the overall differences in invertebrate assemblage composition, suggests that predatory invertebrates were largely unaffected by the presence of trout in the study streams. This result is somewhat surprising given the wealth of studies that have reported a negative association between predatory invertebrate density and trout presence (see review in Meissner & Muotka 2006). The reason for this general pattern is that visual predatory fish like trout are known to favour exposed, large-bodied, conspicuous prey, and many invertebrates in the predator FFG fit these criteria. In the present study, however, several of the more abundant predatory taxa, such as the dipterans Tanypodinae, Athericidae, *Hemerodromia*, the tricherperans *Cheumatopsyche* and *Parecnomina resima*, and the taxa Acarina and Nematoda were not especially large or conspicuous, and therefore may not be particularly vulnerable to trout. Furthermore, trout undergo ontogenetic shifts in diet, consuming increasingly larger prey items as they grow (Mittelbach & Persson 1998, Macchi *et al.* 1999, Arismendi *et al.* 2012). The fact that the trout inhabiting the study streams were generally relatively small (<100 mm, Chapter 5, Figure 5.4) could thus potentially explain why large predatory invertebrates were not strongly influenced by trout presence.

Alternatively, if trout exert a similar predation pressure on predatory invertebrates to that exerted by native fish, then the replacement of native fish by trout may have little net effect on the net top-down control exerted by fish on predatory invertebrates. It could also be that the raised abundance of non-predatory invertebrates (such as collector-gatherers and filter-feeders) at sites containing trout increased prey availability for predatory invertebrates, allowing their numbers to increase and potentially offset the direct predatory effects by trout. Comparisons of the diet composition and relative predation pressure exerted by these native and non-native fish on benthic assemblages should enable a better understanding of the variation in trout effects upon different taxa and FFGs recorded in the study streams, and such investigations form the basis of Chapters 4 and 5 in this thesis.

Samways (1994) noted that in South Africa, the distribution of the synlestid dragonfly *Ecchlorolestes peringueyi* appeared to be negatively affected by trout presence, but the results from the present study were not consistent with this pattern in that the abundance of members of the synlestid *Chlorolestes* was not significantly influenced by trout presence (Appendix 3). However, a number of the larger, more conspicuous predatory taxa, including the coleopteran Gyrinidae and the Odonates *Aeshna*, *Ceratogomphus* and *Notogomphus*, were indeed less abundant at sites with trout, but their generally low abundance at the study sites likely affected their influence in my density-based estimates of community structure, and the likeliness of detecting statistically significant differences in their density. Although the abundance of these (and other predatory taxa such as Corixidae, Naucoridae, Notonectidae and Veliidae and Corydalidae) was generally low, the body sizes of these taxa are large in comparison to other invertebrates. Therefore, had assemblage composition analysis been based on estimates of biomass, rather than density, predators may have emerged a more prominent feature of the invertebrate assemblage, and differences in the predator component of the invertebrate assemblage between sites with and without trout may have been more pronounced. Analysis of invertebrate biomass was not included in the present study because length-weight relationships are not available for the majority of taxa occurring in CFR streams. Future studies of this type would therefore do well to base analyses of assemblage composition on biomass data as well as density data. The first step towards this would be to compile reliable length-weight relationships for the taxa unique to the CFR. Despite the fact that biomass was not assessed in the present study, the general

patterns detected in assemblage composition are likely to be robust, given that the taxa that differed in density between streams with and without trout would have had roughly the same body size in both groups of streams.

Indices of diversity

The lower evenness (*J'*) of invertebrate assemblages in streams with trout relative to streams without them was probably primarily attributable to large increases in the density of certain collector-gatherer taxa such as *Baetis*, *L. penicillata* and *D. capensis*, as well as the filter-feeder *Simulium*. It appears that these, and other functionally similar taxa, benefitted most from the disappearance of native fish at sites invaded by trout, but that other taxa, such as members of the predator and shredder FFGs were relatively unaffected, skewing the overall structure of the invertebrate assemblage.

Of the five taxa present at sites lacking trout, but not at sites containing trout, two of the trichopterans, *Leptecho* and *Hydrosalpinx sericea* are CFR endemics. The genus *Leptecho*, in the family Leptoceridae, is unique in that it constructs coiled, snail-shell-shaped cases of sand grains which enable it to adhere to stones from which it scrapes algae in swift-flowing headwater streams (de Moor & Scott 2003). The species *H. sericea* is the only known member of a family that is entirely endemic to the CFR, the Hydrosalpingidae, and this species builds cases of silk and feeds by scraping algae off the surface of stones in the upper reaches of headwater streams (de Moor & Scott 2003). The absence of these taxa from streams with trout suggests that in addition to their influence on invertebrate density and assemblage composition, trout may have eliminated certain endemic taxa from headwater streams in the upper Breede River catchment, and this should be an important consideration for those charged with the management of non-native trout in the CFR biodiversity hotspot.

Preliminary study

The pattern in invertebrate assemblages detected in the preliminary survey was broadly consistent with that found in the main survey, in that (1) the mean abundance of invertebrates at sites containing trout was significantly higher than at sites lacking trout, (2) there were consistent differences in the composition of invertebrate assemblages between the two groups of sites and (3) the taxa identified as important contributors to the overall assemblage dissimilarity between sites with and without trout were ephemeropteran taxa that had a high overall abundance at the study sites. The lower level of taxonomic resolution in the preliminary data set meant that many taxa could not be confidently assigned to FFGs. However, the four genera (*Ephemerallina*, *Lestagella*, *Lithogloea* and *Nadinetella*) falling within the family Teloganodidae (the taxon contribution most to the overall dissimilarity between sites with and without trout) are all known to be collector-gatherers (de Moor et al. April 2003, King and Schael 2001). Similarly, most of the genera within the families Baetidae and Leptophlebiidae (which also made important contributions to the overall dissimilarity) are also classified as collector-gatherers (King & Schael 2001, Barber-James & Lugo-Oritz 2003). This implies that a higher relative abundance of collector-gatherer taxa at sites where trout occurred, relative to sites where they were absent, was largely responsible for the high level of dissimilarity in assemblage composition between sites with and without trout. Taken together, these findings imply that the general patterns described for the main survey were also detectable in a different year, indicating that the influence of trout on headwater stream invertebrate assemblages in the study area is consistent across years.

3.4.2 Lower trophic levels

Algae

The lower chlorophyll *a* biomass in streams invaded by trout relative to trout-free streams suggests that the impacts of trout extend beyond native fish and invertebrate assemblages, and manifest at the base of the autotrophic food chain in CFR headwater streams (Figure 3.13b and c). Taken together, the results from my surveys of community structure in CFR

headwater streams suggest that by eliminating (or greatly reducing the abundance of) native fish, trout indirectly release certain abundant herbivorous invertebrate taxa (i.e. taxa that feed at least partly on algae) from predation, which increases the grazing pressure they exert on algae, and ultimately results in streams with trout having a low biomass of benthic algae relative to streams without trout (Figure 3.14).

Interestingly, this finding is the opposite to the general pattern recorded by studies examining multi-trophic level impacts of non-native trout elsewhere. The usual trend is that primary consumer abundance decreases, and algal biomass increases, when trout are added to stream communities (see Townsend 2003 and Simon & Townsend 2003 for reviews of community-wide effects of non-native trout). As alluded to above, the discrepancy between my results and those of other studies may be attributable to the fact that these studies were conducted either, in streams that lack prominent native predator assemblages (e.g. Herbst *et al.* 2009, Buria *et al.* 2010), or in streams where trout have replaced native predators that exert weak top-down control on primary consumers relative to that exerted by trout (e.g. Flecker & Townsend 1994, McIntosh & Townsend 1996, Biggs *et al.* 2000, Nyström *et al.* 2003). In both situations, the resulting predation pressure where trout occur is stronger than that where trout are absent, leading to a decrease in primary consumer abundance, and an increase algal biomass. My results, on the other hand, imply that native fish in CFR streams exert stronger control over invertebrate primary consumers than do trout, and therefore that trout do not functionally compensate for the loss of native fish populations. This could be a result of differences in total biomass attained, or feeding biology, between trout and native fish, and a detailed examination of these differences is the focus of Chapter 4 in this thesis.

The pattern found in the present study is comparable to that recorded in CFR streams where non-native bass have displaced native fish populations. Where bass have invaded, algal biomass is significantly lower than that at similar sites where healthy native fish populations persist in the absence of bass (S.R. Lowe, pers. comm. 2010). As in the present study, this appears to be a consequence of increases in the abundance of certain primary consumer taxa (e.g. Baetidae, Simuliidae, Leptophlebiidae) recorded at sites with non-native fish (Lowe *et al.* 2008, S.R. Lowe, pers. comm. 2010).

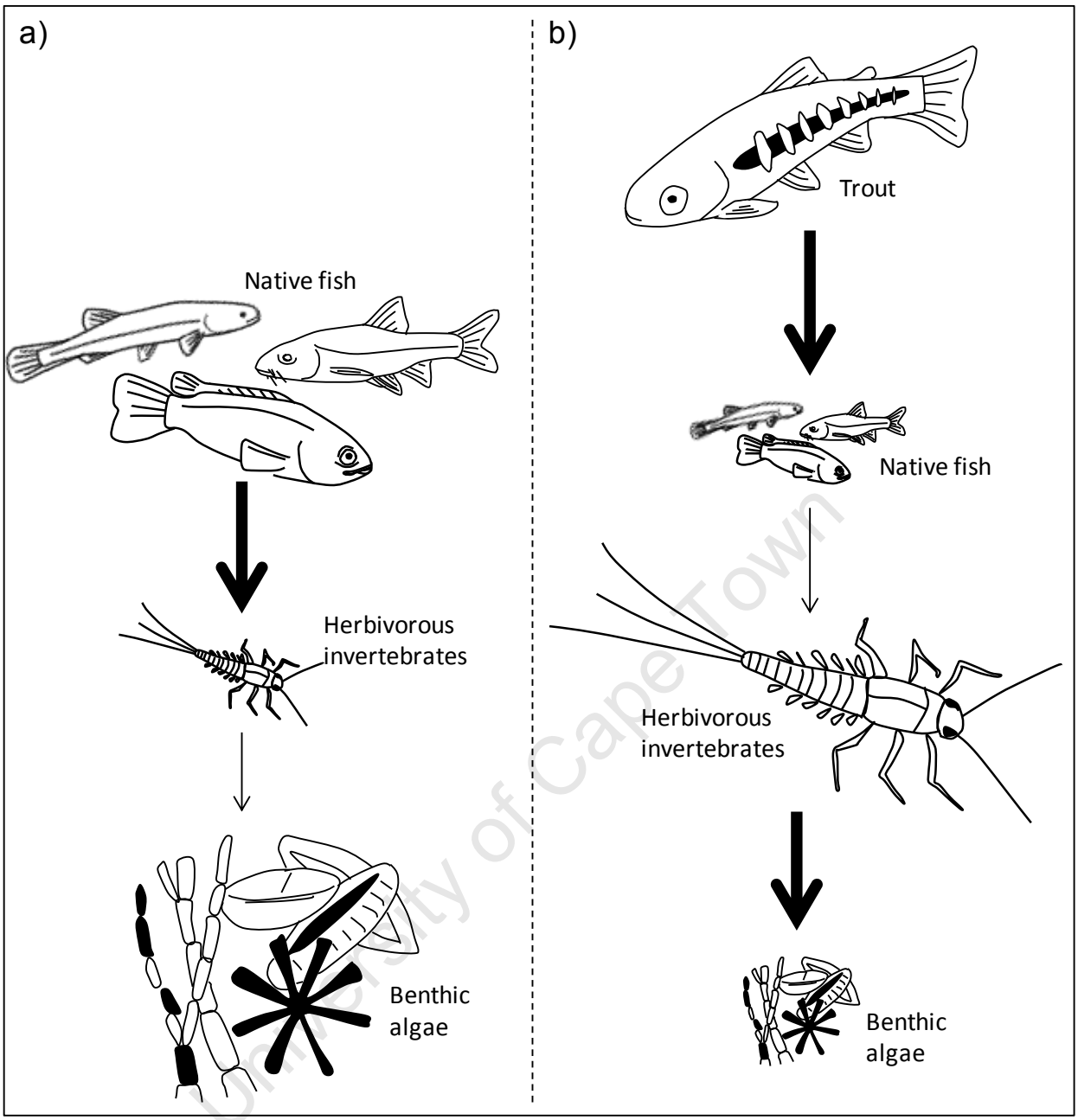

Figure 3.14 Conceptual diagram showing the strength of interactions between key community components, and patterns in community structure, at a) sites without trout, and b) sites invaded by trout. The size of each community component represents its relative density or biomass, and the thickness of the arrows represent the size of the effect exerted by each community component on the one below it (thick arrow = strong effect).

Particulate organic matter

Most studies investigating community-level effects of non-native predatory fish in streams have focused on effects on the autotrophic food chain, in which modification of the fish assemblage affects herbivorous invertebrates, and in turn alters the standing stock of algae

on which they graze (Buria *et al.* 2010). Trophic cascades can, however, also potentially affect the detritus-based trophic pathway (Ruetz *et al.* 2004, Greig & McIntosh 2006), but this has not been well-studied (Buria *et al.* 2010), and in general, cascading effects down detrital trophic pathways appear to be less common than cascades down algal trophic pathways (Rosenfeld 2000, Herbst *et al.* 2009, Buria *et al.* 2010). Rosenfeld (2000) attributed this phenomenon to the fact that herbivorous invertebrates feed on exposed rock surfaces where algae grows, rendering them more vulnerable to fish predation than detritus-feeders which inhabit, and feed on, leaf packs that accumulate in interstices in the stream bed, potentially concealing them from predators (Buria *et al.* 2010). As far as I know, the present study is the first assessment of the simultaneous effects of a top predator replacement (i.e. trout replacing native fish as the dominant predator by number) on both algal and detritus-based pathways in headwater streams.

The finding that levels of particulate matter in streams containing trout did not differ significantly from levels in trout-free streams adds further evidence hypothesis that algae-based food chains may be more susceptible to the cascading effects of predators than detritus-based food chains (Rosenfeld 2000). The lack of difference in CPOM biomass is consistent with the finding that shredders (which feed primarily on CPOM) had a similar density in streams lacking trout to that in streams where trout occurred. On the other hand, since collector-gatherer, and filter-feeder, density was higher at sites with trout, a decrease in FPOM (their main food source) biomass may have been expected at these sites, but this was not the case. This result implies that levels of FPOM are not strongly controlled by top-down effects (as appears to be the case for benthic algal biomass), and may instead be regulated from the bottom-up. Indeed, detrital food chains are donor-controlled systems that are based largely on leaf-litter inputs from the adjacent riparian ecosystem (Polis & Strong 1996). Although consumers in streams have the potential to reduce the biomass of detritus, they have no control over the amount of detritus entering the stream (Rosenfeld 2000), and thus variation in detrital inputs among streams may overwhelm the effects of collector-gatherers on standing stocks of FPOM. Furthermore, the accumulation of POM will be strongly influenced by hydrological conditions in the stream (Davies & Day 1998, Allan & Castillo 2007), and thus variations in flow and other hydrological variables may overshadow the influence of biotic top-down control.

Results from the multivariate regression models (DISTLMs) are in agreement with this, indicating that variations in both FPOM and CPOM biomass among the study sites were not related to the presence of trout, but rather were explained by a suite of environmental factors, including pH, elevation, phosphates and temperature in the case of FPOM, and riparian vegetation, canopy cover, substrate length, site slope and ammonium in the case of CPOM. The fact that the overall variation explained by the final models for both FPOM and CPOM was low (<30% of the total variation), indicates that there was a large proportion of unexplained variation in particulate organic matter biomass among the sites. Detritus supply from upstream reaches could have a strong influence on standing stocks of particulate organic matter at the study sites (Allan & Castillo 2007), but the factors governing detritus inputs upstream (such as distance to stream source and the nature of riparian vegetation) were not quantified in the present study.

3.4.3 Influence of trout and other environmental factors on the patterns detected in benthic community structure

Although clear differences in invertebrate assemblage composition, and algal biomass, were found between sites with and without trout, the study reported on here was a comparative survey, as opposed to a controlled experiment, and the possibility that variation in environmental conditions among sites may confound comparisons cannot be overlooked. Variability in environmental conditions, and the composition of biological communities, among streams is notoriously high, posing inherent difficulties in comparative studies of the type conducted here. In my study, measures were taken to account for the influence of high inter-stream variability in environmental conditions on comparisons of community structure between streams with and without trout. The stringent site selection criteria ensured that the final set of sites selected were as comparable as possible with regard to stream order, and proportional cover of canopy and bedrock, and that comparisons between streams with and without trout were not confounded by human-related environmental perturbations such as water abstraction, pollution, the presence of non-native plants and the presence of other non-native fish species.

Moreover, the surveys of biological community structure were accompanied by measurements of a set of environmental variables including most variables known to have a potentially important influence on the structure of stream assemblages. Regardless of whether univariate or multivariate techniques were used, analyses of environmental variables indicated that there was no consistent difference in environmental conditions between sites with and without trout. Furthermore, the multivariate regression models (DISTLMs), despite explaining a relatively small proportion of the overall variation (20.35-63.84%), indicated that trout presence was clearly a better predictor of the variation in the taxonomic and functional composition of invertebrate assemblages, and algal biomass, than were any of the other environmental factors. Although less important than trout presence, other environmental factors were included in the final models for both the taxonomic and functional composition of invertebrate assemblages, but they did not explain a significant proportion of the variation over and above that already accounted for by trout presence, and were therefore not examined further. Similarly, trout presence was the best predictor of variation in algal biomass, and the environmental predictors included in the final model for algal biomass did not explain a significant amount of variation, with the exception of PC 1. This result implies that although less important than trout presence, variations in temperature and phosphate levels were important in explaining the variation in algal biomass among sites. This finding is not surprising given the well-known link between algal biomass and temperature and nutrients in streams (Allan & Castillo 2007), and suggests that algal biomass is regulated both from the top-down by grazing, and from the bottom-up by resource availability.

The fact that the overall variation explained by the final models for taxonomic and functional composition was low (20.35-31.75%) indicates that there was a relatively large amount of unexplained variation in invertebrate assemblage structure among the sites. This is a common finding in correlative field studies conducted in highly variable environments such as aquatic systems (Anderson *et al.* 2008). Despite including measurements of many of the environmental variables known to have an important influence on stream biota, the possibility that some other unmeasured variable could be responsible for the patterns in invertebrate assemblages (and algal biomass) cannot be ignored, and it is recognized that cause-and-effect–type relationships can only be inferred through manipulative experiments.

Regardless, taken together, my findings here imply that trout presence, rather than variation in the environmental variables measured, is responsible for the differences detected in community structure between streams with and without trout. Finally, it is noted that other studies (e.g. McIntosh & Townsend 1995b, Nyström *et al.* 2003, Herbst *et al.* 2009) have used a similar comparative approach to that used here to infer impacts of non-native fish in streams.

3.4.4 Conclusions and conservation implications

In conclusion, the results from this survey indicate that by replacing native fish as the dominant top-predator in headwater streams in the upper Breede River catchment, trout initiate a trophic cascade down the algae-based, but not detritus-based trophic pathway. The total density, taxonomic composition and functional composition of invertebrate assemblages differed substantially between streams with and without trout. The density of herbiovorous primary consumers, including members of the collector-gatherer and filter-feeder, and to a lesser extent grazer-scraper, FFGs, was greater in the presence of trout, while shredders and predators appeared to be largely unaffected by trout presence. The elevated density of primary consumers at sites with trout apparently increased grazing pressure on benthic algae, resulting in reduced biomass of chlorophyll *a* at sites containing trout, relative to sites lacking trout. On the other hand, levels of particulate organic matter did not appear to be strongly influenced by trout presence: a pattern that may be attributable to the lack of response of shredder density to trout presence, as well as the fact that heterotrophic stream food-chains are highly variable, donor-controlled systems. Comparisons of environmental conditions between sites with and without trout, in combination with distance-based linear models used to evaluate the relative importance of trout and other environmental factors in explaining variation in invertebrate assemblage structure and algal biomass, indicated that variation in environmental conditions among sites was unlikely to be accountable for the patterns recorded, implicating trout as the main factor responsible for the differences in benthic community structure between sites with and without trout. It is, however, acknowledged that while correlative studies of the type conducted here are useful for formulating hypotheses based on data collected at broad,

realistic spatial scales, they cannot be used to identify mechanisms underlying the patterns observed. Collectively, the results from this study imply that trout do not functionally compensate for the loss of native fish from the study streams, and that they are weaker regulators of herbivorous invertebrate density than are native fish. This hypothesis is further explored in the remaining chapters of this thesis by characterizing and contrasting fish trophic niches (Chapter 4), and through a manipulative field experiment designed to compare the top-down effects of trout vs. native fish on benthic stream communities (Chapter 5).

From a conservation perspective it is important to recognize that the impact of non-native trout in the upper Breede River catchment (and potentially other similar catchments in the CFR) extends beyond its effect on native fish populations documented in Chapter 2. By strongly reducing native fish abundance, trout indirectly alter the structure of invertebrate assemblages which in turn alter the biomass (and potentially assemblage composition) of benthic algae. These perturbations could also have further consequences for other organisms that depend on algae for food and habitat (Power 1990b, Meissner & Muotka 2006, Herbst *et al.* 2009), or on consumers in adjacent riparian ecosystems that rely on emergent aquatic invertebrates for food (Nakano *et al.* 1999b, Fausch *et al.* 2010). In addition to their influence on the structure and function of headwater stream communities, trout may also have eliminated certain vulnerable invertebrate taxa from headwater streams in the CFR. Although difficult to prove using my data, the absence of the taxa *Leptecho* and *H. sericea* at sites containing trout, and the fact that they were recorded at sites where trout were absent, suggests that this may indeed be the case. This is of conservation concern, given the fact that these taxa are CFR mountain stream endemics.

Headwater streams are functionally important components of river networks. They are critical habitats for sediment export, organic matter processing, nutrient cycling, and the establishment of other chemical and biological characteristics that shape the ecology of downstream reaches into which they feed, and ultimately of entire river networks (Lowe & Likens 2005, Clarke *et al.* 2008). In the CFR, where downstream reaches are often highly degraded and/or heavily invaded by non-native plants and fish, headwater habitats perform particularly important roles as refugia for native biodiversity in river networks. At present, trout may be the greatest threats to the ecological integrity of headwater streams,

potentially compromising the value of these habitats as ecological sanctuaries in a highly degraded landscape. The distribution of trout in the upper Breede River catchment appears to be restricted by physical barriers to dispersal, such as impassable dry/braided reaches and waterfalls, as well as by variations in the intensity of stocking efforts across the landscape. Based on my analyses of environmental conditions in streams with and without trout, it seems likely that if introduced into headwater streams that are presently trout-free, trout will establish new self-sustaining populations with serious community-level consequences. I therefore re-emphasize that the value of headwater stream habitats as ecological refugia in river networks in this highly degraded Biodiversity Hotspot may rest largely on our ability to prevent further stocking of trout into headwater streams; particularly those that still remain trout-free.

Chapter 4

Characterising and contrasting the trophic niches of native Breede River redfin and non-native rainbow trout in headwater streams of the upper Breede River catchment, South Africa

4.1 INTRODUCTION

The introduction of species outside of their natural range is a principal driver of the human-induced biodiversity crisis (Mack *et al.* 2000, Dudgeon *et al.* 2006), causing species extinctions, habitat degradation and changes in the structure and functioning of ecosystems the world over (Leprieur *et al.* 2006). Introduced predators, in particular, have had devastating and far-reaching effects on recipient systems (Eby *et al.* 2006, Cox & Lima 2006, Salo *et al.* 2007). This is attributable to the fact that predators can regulate the structure of entire communities through a suite of direct and indirect top-down effects (Allan & Castillo 2007, Terborgh & Estes 2010). Predators not only keep prey populations in check, but can also influence non-adjacent trophic levels with which their prey interact (Pace *et al.* 1999).

The consequences of a predator introduction for the receiving community will depend upon how the introduced predator alters the native predator assemblage. While many introduced predators fail to establish in their new environments (Sih *et al.* 2010), those that do will either join the native predator assemblage, or replace native predators (Eby *et al.* 2006). In situations where native predators are replaced, the degree to which the rest of the community is affected may then depend on how closely the functional role performed by the introduced predator matches that previously performed by the native predator(s) (Chalcraft & Resetarits 2003). The functional role performed by a predator in a community is determined by the density/biomass of the predator population, as well as by the type and amount of food it consumes (Estes *et al.* 2001, Chalcraft & Resetarits 2003, Schmitz *et al.* 2004). If the introduced predator establishes at a biomass that is appreciably higher or lower than that of the native predator it replaces, then the magnitude of its effect on prey populations may differ from that of the native predator (Townsend 2003, Baxter *et al.* 2004,

Benjamin *et al.* 2011). Even if it establishes at a biomass comparable to that of the native predator, its influence in the community may differ from that of native predators as a result of differences in feeding behaviour between the two predators (Dahl & Greenberg 1996, Parker *et al.* 1999, Schmitz 2007, 2008). Understanding how the functional role of predators in a system changes following a predator invasion is thus critical for managing non-native predator invasions (Benjamin *et al.* 2011). Eradicating non-native species is a difficult and expensive process (Simberloff 2003), and may also be complicated by an array of socio-economic and biological factors (Dunham *et al.* 2002, McDowall 2006). So if a non-native predator replaces a functionally similar native predator at a similar biomass, so that only the identity of the predator changes but its function in the system retained, then perhaps management interventions may not be worthwhile (Townsend 2003, Benjamin *et al.* 2011). On the other hand, if the trophic niche occupied by the non-native predator is distinct from that occupied by the native predator(s), then the consequences of the invasion for the recipient ecosystem may be more serious, and management interventions may be required.

Although predator introductions have occurred in all major ecosystem types (Estes *et al.* 2011), they are especially common in freshwater ecosystems due to the popularity of predatory fish species among anglers and aquaculturalists (Eby *et al.* 2006). As a result, predator invasions have disproportionately transformed freshwater ecosystems relative to their marine and terrestrial counterparts (Cox & Lima 2006). Salmonids are among the most widely introduced predatory freshwater fish (Crawford & Muir 2008), and have modified native predator assemblages in many of the places where they have become invasive (Rahel 2000, Cambray 2003, Townsend 2003, Simon & Townsend 2003, McDowall 2006). In many of the systems where non-native salmonids perform a functional role that is different from that performed by native predators, community-wide effects have ensued. For example, in New Zealand brown trout *Salmo trutta* have replaced native galaxiids *Galaxias* spp. in many streams (Townsend & Crowl 1991), and because they have a foraging behaviour that differs from that of the galaxiids (McIntosh & Townsend 1995a), their impact there extended beyond the replacement of native fish, and ultimately changed the structure of the entire stream community (Flecker & Townsend 1994, McIntosh & Townsend 1996, Townsend 2003). Similarly, the replacement of native cutthroat trout *Oncorhynchus clarkii* by non-native brook trout *Salvelinus fontinalis* in streams in the Rocky Mountains, USA, has changed

the structure and function of stream food webs, due to differences in the biomass attained (Benjamin *et al.* 2011), and feeding biology (Lepori *et al.* 2012), between the two species.

Four species of salmonid have been introduced to South African waters over the last 120 years. They are brown trout *S. trutta*, Atlantic salmon *Salmo salar*, brook trout *S. fontinalis*, and rainbow trout *Oncorhynchus mykiss* (de Moor & Bruton 1988). Atlantic salmon and brook trout failed to establish self-sustaining populations, presumably because they could not tolerate environmental conditions in South African streams (and oceans) (de Moor & Bruton 1988). Self-sustaining populations of brown trout established in certain South African rivers, but the species also disappeared from several of the systems into which it was introduced (de Moor & Bruton 1988). The most successful of these salmonid introductions was that of the rainbow trout (de Moor & Bruton 1988, Cambray 2003). Rainbow trout (henceforth "trout") were brought to South Africa in 1897 for angling purposes and have since spread and established self-sustaining populations in river systems across the country (de Moor & Bruton 1988). The species survived well and has invaded many of the cold, clear-flowing headwater tributary streams within the Cape Floristic Region (CFR) of South Africa (de Moor & Bruton 1988) – a global hotspot of biological diversity (Myers *et al.* 2000). Recently, concern has been expressed that trout may have had a detrimental effect on native biota and on ecosystem functioning in the region (de Moor & Bruton 1988, Cambray 2003), but quantitative information on their impact in the CFR is scarce. Knowledge about how trout modify native predator assemblages and thereby alter predation dynamics in streams is essential if we are to effectively manage this non-native species in the CFR, and indeed in South Africa.

Results from field surveys conducted in 24 headwater tributaries of the upper Breede River in the CFR revealed that trout have largely replaced once-abundant native fish species as the dominant predators in these streams (Chapter 2). The surveys indicated that these changes at the level of the fish assemblage appear to have released aquatic invertebrates (particularly herbivorous taxa) from predation, and altered the composition of benthic invertebrate assemblages (Chapter 3). Moreover, the raised abundance of herbivorous invertebrates at sites invaded by trout appears to have led to an increase in the grazing pressure exerted, since the biomass of benthic algae was significantly greater than at sites without trout (Chapter 3). These results imply that trout do not functionally compensate for

the native fish species which they have replaced. Rather, they suggest that native fish and trout do not have equivalent top-down effects on benthic invertebrate assemblage composition, and that trout do not regulate aquatic invertebrate density as strongly as native fish do.

Changes in predation pressure following trout invasions might be due to the fact that trout have altered the overall predator density or biomass, or because the trophic niche that they occupy is different to that occupied by the native predator(s). (The use of the term "trophic niche" here follows Silvertown's (2004) definition: "the place of an organism in the environment in relation to its food") Estimates of fish biomass in the 24 streams where fish populations were surveyed, indicated that there was no significant difference in mean fish biomass between invaded and uninvaded streams, suggesting that trout invasions have not altered overall predator biomass. Therefore, differences in the trophic niche occupied by native fish and trout, rather than differences in total predator biomass, may be responsible for the differences in invertebrate density and assemblage composition detected between streams with and without trout. On the other hand, fish density at sites invaded by trout was significantly lower than that at sites with no trout, implying that trout invasions decrease overall fish density. A lower overall density of fish at sites invaded by trout is therefore another possible explanation for the high density of benthic invertebrates relative to that at sites without trout. The focus of the present chapter was on ascertaining whether trout and native fish occupied different trophic niches, and whether any differences were likely to be responsible for the differences in benthic community composition detected between streams with and without trout.

Studies on the dietary habits of the native fish species in the CFR are relatively scarce, but available information suggests that their feeding behaviour and diet may differ substantially from that of the non-native trout that have replaced them. Literature on the trophic ecology of *Pseudobarbus* sp. "Burchelli Breede" (henceforth "redfin"), one of the three indigenous species present in the study streams, suggests that it is primarily an active benthic forager (Cambray & Stewart 1985, de Wet 1990, Skelton 2001). Redfin are known to consume benthic invertebrates, as well as plant material such as algae and detritus, but the relative importance of these different food sources in redfin diet varies among studies (Esterhuizen 1978, Cambray & Stewart 1985, de Wet 1990). Trout, on the other hand, are known to be

passive drift feeders that, in addition to feeding on aquatic invertebrates, can acquire a substantial proportion of their diet from terrestrial invertebrates that fall into the stream from the riparian zone (Kido *et al.* 1999, Nakano *et al.* 1999b, Nakano & Murakami 2001, Skelton 2001, Baxter *et al.* 2004, 2007, White & Harvey 2007). If terrestrial invertebrates constitute a substantial proportion of trout diet in the CFR, their reliance on benthic invertebrates as a food source may be reduced, and the predation pressure that they exert on benthic invertebrates may therefore be weaker than that exerted by benthic-foraging redfin.

The fish surveys (Chapter 2) revealed that, although Cape kurper *Sandelia capensis* (henceforth "kurper") and Cape galaxias *Galaxias zebratus* (henceforth "galaxias") were frequently present, redfin was the dominant component of the native fish assemblage at uninvaded sites, comprising >75% of the assemblage on average by both number and weight. On the other hand in streams where trout occurred, it was the dominant fish species, making up >85% of the fish assemblage by both number and weight at these sites. Thus, predator assemblages in these streams were either "redfin-dominated", or "trout-dominated", and the broad aim of the present chapter was therefore to characterize and contrast the trophic niches of redfin and trout in these headwater streams. It was hypothesized that the foraging mode and diet of trout would differ from that of redfin, and specifically, that redfin would rely more strongly on benthic invertebrate prey than would trout. These hypotheses were addressed through a combination of complementary approaches including focal animal watching (FAW), gut contents analysis (GCA) and stable isotope analysis (SIA). FAW was used to compare the foraging behaviours of trout and redfin, while GCA and SIA were used to characterize and compare the diets of these two species.

4.2 METHODS

4.2.1 Study area

This study was conducted at a subset of the sites sampled during the surveys of fish populations and community structure in headwater streams of the upper Breede River

catchment reported in Chapters 2 and 3. Three streams with trout and three streams without trout, but containing native fish, were selected for surveys of foraging behaviour and fish diet (Figure 4.1). In this study I did not investigate the diets of trout and redfin in sympatry because the broad-scale surveys of fish populations indicated that fish assemblages were either trout-dominated, or native fish-dominated, and that co-occurrence of native and non-native fish was relatively uncommon and, that where it did occur, fish densities were low. Interest lay therefore in comparing the trophic niches occupied by the two fish species in allopatry. The feeding habits of trout and redfin were assessed in three separate streams each in order to incorporate some level of among-stream variation in diet composition. The sampling sites used in this study were chosen based on accessibility and on the abundance of the fish species being sampled. I chose streams that were easily accessible by roads so that sampling equipment could be easily and rapidly transported to and from the site, and streams that supported abundant populations of either trout or redfin so that adequate sample sizes could be obtained, and power of statistical analyses maximized.

Samples for this study were collected on the same day that fish populations, invertebrate assemblages, algae and particulate organic matter were surveyed (Chapters 2 and 3), while behavioural observations of fish foraging mode were conducted the following day to avoid recording the behaviours of fish that may had been recently disturbed. Behavioural observations and samples for examining fish diets were collected from a 200 m reach directly upstream of, the 50 m reach where fish abundance and community structure were surveyed (Chapters 2 and 3). Samples for the study of fish diets were collected after all other sampling at a site had been completed, so as to avoid disturbing the 50 m site downstream where fish, invertebrates and other community components were surveyed (Chapters 2 and 3). The samples for this study were collected under permit 0035-AAA 007-00057 issued from Cape Nature, and animal ethics clearance was obtained from the University of Cape Town.

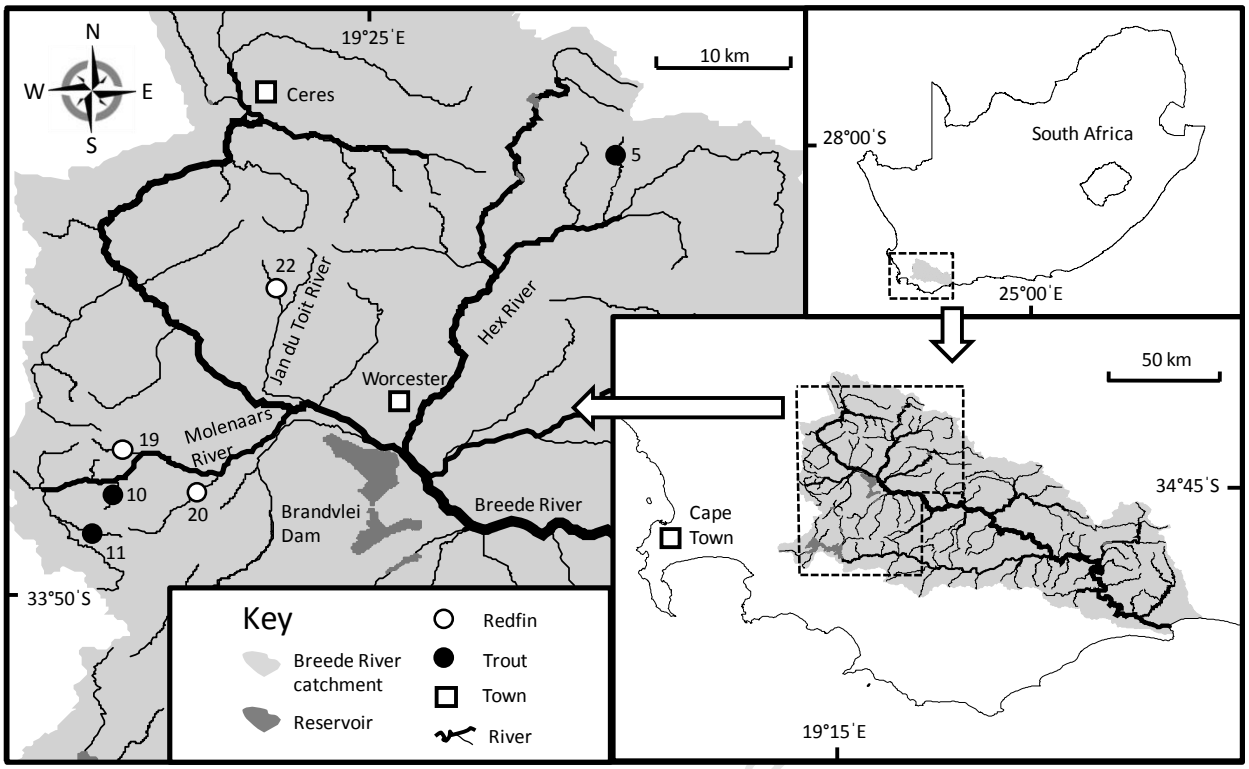

Figure 4.1 Location of sampling sites in the upper Breede River catchment in the CFR of South Africa. White circles represent sites where redfin were sampled, and black circles represent sites where trout were sampled. Sampling site numbers correspond to numbers in Table 2.1, Chapter 2.

4.2.2 Data collection

Focal animal watching

The foraging behaviour of trout and redfin was characterized using focal animal watching, a technique that involves selecting a single animal within a group of animals and observing it for a set length of time in order to make deductions about its behaviour (Dawkins 2007). The foraging behaviours of both fish species were examined in three separate streams by snorkelling (Macneale *et al.* 2010). Beginning at the downstream boundary of the 200 m reach, the snorkeler proceeded slowly upstream by swimming/crawling until a fish was located. Once located, the snorkeler observed the fish (i.e. focal animal) until it made a definitive feeding attempt. A feeding attempt, regardless of whether or not it was successful, was defined as an abrupt opening and closing of the mouth and operculum (Bechara *et al.* 1993). The snorkeler recorded whether the feeding attempt was made in one of three foraging habitats: on the stream bottom, in the water column or at the water

surface. The length (total length, TL) of each focal animal was estimated (to the nearest 5 mm) with a ruler, and foraging behaviour and fish length were recorded underwater with a pencil on a perspex slate. Once a foraging attempt was recorded, the snorkeler proceeded slowly upstream in search of the next focal fish. A minimum distance of 2 m was left between two focal fish to minimize the possibility of observing the same focal fish twice (Macneale *et al.* 2010). All observations at all sites were made between 14h00 and 17h00, so that diel variations in foraging behaviour would not confound inter-species comparisons.

Gut contents Analysis

GCA was used to obtain a high resolution, summer snapshot (Gelwick & Matthews 2007) of trout and redfin diets at the study sites. Fish for GCA were collected from each stream by actively seining through the 200 m study site in an upstream direction with a 3 m seine net. The net was operated by two fieldworkers, and sampling continued until 20-30 individuals had been collected, or until the upstream boundary of the site was reached. Upon capture, fish were euthanased, measured (TL) to the nearest 0.1 mm using plastic callipers, and weighed to the nearest 0.01 g using an Ohaus Scout Pro 400 g scale. Fish were held on ice in the field for a maximum of two hours and then frozen and stored until gut contents were examined later in the laboratory.

In order to relate the gut contents of fish to the prey availability in the stream, both benthic and drifting invertebrates were sampled at each study site on the same day that fish were collected. Benthic invertebrates were sampled prior to fish collection in the 50 m survey reach directly below the 200 m reach where fish foraging behaviour and diet were sampled. The protocol for sampling benthic invertebrates is described in detail in Chapter 3. In summary, 10 benthic invertebrate samples were collected at each site using a 30 x 30 cm box sampler (250 µm mesh): five samples came from randomly-selected locations in erosional habitats, and five from randomly-selected locations in depositional habitats. Samples were preserved in 70% ethanol for processing in the laboratory. Drifting invertebrates were sampled using three drift nets that had square 30 x 30 cm openings, a net depth of 60 cm and 250 µm mesh diameter. Nets were anchored to the streambed in erosional habitats along-side each other (at right angles to the direction of flow) at the

upstream end of each 200 m study site, using metal stakes. Nets were positioned such that they extended 5 cm above the water surface to ensure that invertebrates drifting on the water surface, as well as those drifting in the water column, were captured. Invertebrate drift was sampled for one hour, beginning at 16h00, after which nets were removed from the stream and their contents collected using a 250 µm sieve and squeegee. Drift samples were preserved separately in 70% ethanol for processing back at the laboratory.

Stable isotope analysis

In addition to GCA, which provides detailed information about foods recently ingested, SIA was used to obtain a coarser-resolution, time-integrated measure of fish dietary habits (Gelwick & Matthews 2007). These two approaches are complementary in that each provides a level of resolution not offered by the other (Clarke *et al.* 2005). When animals feed, the carbon and nitrogen present in their food is assimilated into their body tissues (Vander Zanden & Rasmussen 1999, Post 2002). The ratio of the heavy stable nitrogen isotope ^{15}N to that of the lighter isotope ^{14}N in an animals' tissue can be used to estimate the trophic level at which that animal feeds because ^{15}N typically increases predictably from one trophic level to the next - a phenomenon called "trophic fractionation". In contrast, the ratio of the heavy stable isotope of carbon ^{13}C to that of the lighter isotope ^{12}C can be used to examine the food sources used by a consumer, because ^{13}C changes little as it moves through food webs (Post 2002, Clarke *et al.* 2005). Together, the stable isotopes of carbon and nitrogen can be used to evaluate the trophic niche occupied by different species in a biological community (Cucherousset *et al.* 2007, Olsson *et al.* 2008), and to resolve trophic relationships among major community components (Phillips & Gregg 2003, Clarke *et al.* 2005, Schmidt *et al.* 2007).

In this study I wished to ascertain whether information on trout and redfin diets gleaned from SIA was broadly consistent with patterns in fish diets detected using CGA. Specifically, I collected stable isotope samples from trout and redfin tissue, as well as from other major food web components (including benthic invertebrates, detritus and algae), in order to examine trophic relationships in the study streams and to ascertain whether the trophic niche exploited by non-native trout was distinct from that exploited by native redfin. A

subset of the fish collected for GCA were subjected to stable isotope analysis: 16 fish from each species from each site were randomly selected (i.e. n = 48 for trout, and n = 48 for redfin), and a 5 mm^2 plug of dorsal muscle tissue was removed from behind the dorsal fin (Clarke *et al.* 2005) and frozen in a plastic vial.

Aquatic invertebrates and detritus were collected by kick-sampling using a 30 x 30 cm square frame net with 250 µm mesh diameter. Samples were collected from five random locations at each site by disturbing the substrate (by kicking with feet and brushing with hands) and holding the net just downstream to collect animals that became dislodged (active sweeping was used to collect animals where flow was too weak to carry them into the net). Each sample was transferred into a 1 L plastic bottle containing stream water and transported back to a field laboratory and processed within 2 h of collection.

Algal samples were collected from five randomly-selected stones at each study site. Each stone was removed from the stream, and associated organic material (i.e. invertebrates and detritus) was removed by rinsing the stone gently with a squeegee containing stream water, or by picking with fine forceps. Algae were then removed from a 50 x 50 mm square on each stone using a razor blade, stored in a plastic vile, frozen within 2 h of collection and stored in the dark.

Terrestrial invertebrates were collected by sweeping vegetation in the riparian zone with a hand-held sweep net (30 x 30 cm square frame, 1 mm mesh diameter). Ten sweep samples were randomly collected from random locations within the riparian zone, and I treated all vegetation within 5 m of either stream bank as part of the riparian zone. For each sample, riparian vegetation was swept for 60 s, and the contents of the net were transferred into a plastic vial and frozen within two hours of collection.

4.2.3 Laboratory procedures

Gut contents analysis

The fish samples were defrosted and their stomachs were removed and dissected so that recently-consumed food items could be examined. The procedure for fish dissection and gut

content examination was based on that outlined by Gelwick & Matthews (2007). First, a small incision was made on the ventral side of the fish behind the isthmus of the gills, posterior to the anal fin. Next, two transverse cuts were made at each end of the first cut in order to open the coelom and expose the visceral mass. Then, using sharp scissors, the oesophagus, posterior end of the intestine, and dorsal mesentery at its point of attachment were severed, allowing the visceral mass to be removed from the coelom. The digestive tract was separated from other digestive organs, and the foregut section removed for further examination since this section contained the most recently ingested prey items. In trout, the foregut comprises a true stomach containing gastric caeca (fingerlike foldings of stomach walls), and is easily distinguishable from other sections of the digestive tract. Redfin, on the other hand, have a less distinct stomach, and in this study I focused on the section from the oesophagus to the end of the first U-shaped bend in the foregut, since this is where the most recently-eaten, and most easily-identifiable, prey items occur (Cambray 1983, Whitehead *et al.* 2007). The foregut was then opened by making a longitudinal slit using fine scissors and all food items removed using fine forceps and a squeegee bottle, and placed in a petri dish containing a small amount of water. Food items were then sorted and identified under a dissecting microscope.

Initially, gut contents were sorted into the following broad categories: aquatic invertebrates, terrestrial invertebrates, unidentifiable invertebrate remains, algae, detritus, sand and other (which included invertebrate pupae and eggs). Adult stages of aquatic invertebrates that no longer feed within the stream were considered as "terrestrial" in this study because interest lay in distinguishing between invertebrates that fed within the stream, and those that did not. Aquatic invertebrates were then identified to the lowest feasible taxonomic level using the identification guides listed in Chapter 3, as well as the reference collection compiled from the broad-scale invertebrate surveys (Chapter 3). Taxa were then assigned to one of five functional feeding groups (FFGs) including collector-gatherers (CG), grazer-scrapers (GS), filter-feeders (FF), shredders (SH) and predators (P) were identified, using "Guides to the Freshwater Invertebrates of Southern Africa" (Day *et al.* 2001, 2003, Day & de Moor 2002a, b, de Moor *et al.* 2003a, b, Stals & de Moor 2007), and Cummins *et al.* (2008). Terrestrial invertebrates were identified to order, or class when order could not be distinguished (Picker *et al.* 2004).

For both aquatic and terrestrial invertebrates, the number of individuals in each taxon in each stomach was recorded. Next, all individuals within a taxon in each stomach were then combined, blot-dried for 30 s on filter paper and weighed to the nearest 0.0001 g as an estimate of the wet weight of that taxon in the fish's stomach. Blotted wet weight was also estimated for the non-animal prey categories using the procedure outlined for the invertebrate prey taxa. Although a less accurate measure than dry weight, wet weight is generally highly correlated with dry weight, and is recommended by Hyslop (1980) for situations where large amounts of material need to be processed. Blotting removes the majority of excess water from samples, and blotted wet weight is therefore a less erroneous measure of gut content weight than is wet weight (Hyslop 1980).

Stable isotope analysis

In the field laboratory, live invertebrate samples were examined under a dissecting microscope and all invertebrates were removed from the sample and placed on a sorting tray. The numerically dominant taxa within each of the five FFGs (listed above) were identified, and between five and 30 individuals of each dominant taxon were collected and placed in separate 1 L plastic bottles (i.e. one bottle per FFG per site) containing aerated stream water. I decided to use representative taxa for each FFG because it was expected that the different FFGs would have distinct isotopic signatures, since they utilize distinct food resources within the stream (Zah *et al.* 2001). The isotope signatures of the different FFGs, if distinct from one another, could then be used to resolve stream food web structure, and to partition fish diets. Invertebrates were kept alive for 24 h to ensure clearance of their guts (Cucherousset *et al.* 2007), and then euthanased and preserved by freezing. The material remaining after invertebrates had been removed from each sample was elutriated to remove sand and gravel, and a 10 ml subsample of the remaining organic matter was collected as a sample of detritus and frozen.

All isotope samples were freeze-dried for 24 h and then ground to a fine powder using mortar and pestle. Approximately 1 mg of dried sample of fish and invertebrates, or approximately 2 mg dried sample for algae and detritus, was packaged in a tin cup and analysed for carbon and nitrogen isotopic ratios at the University of Cape Town Archaeology

Department Stable Isotope Laboratory. Samples were combusted, and isotope signatures measured in a Thermo 1112 Elemental Analyser (Germany, Italy) interfaced via a Thermo Conflo II to a Thermo Delta XP Plus stable light isotope mass spectrometer, and reported relative to international standards. Results are reported as δ (which refers to deviation) in parts per thousand ($^{o}/_{oo}$, also termed "per mil") difference between sample ratio (R_{sample}) and standard ratio ($R_{standard}$), using the following equation:

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

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Stable isotope ratios for each community component at each site were averaged, and these averages used to further examine the structure of the stream community at each site.

4.2.4 Data analysis

Foraging behaviour

For each fish species, observational data collected from the three sampling sites were aggregated so that feeding behaviour could be examined at the species level. Fisher's exact test was used to test for differences between trout and redfin in the frequency of feeding attempts among the three habitat types, since the number of observations in certain habitat types was low (Zar 1999).

Gut contents

Aquatic invertebrates were aggregated into FFGs since my main interest lay in whether trout and redfin differ in their effect on the functional role they perform in stream communities. The non-aquatic, adult stages of aquatic invertebrates, and invertebrates of terrestrial origin, were aggregated into the single category "terrestrial invertebrates"

because these taxa no longer fed within the stream food web. This resulted in a total of eleven different food types, including the five FFGs of aquatic invertebrate, terrestrial invertebrates, invertebrate remains, algae, detritus, sand and other. The "other" category consisted of invertebrate pupae and invertebrate eggs. The proportion of these eleven food types in the guts of redfin and trout were then compared at both the whole sample (i.e. the frequency of occurrence across all guts for each species) and the individual fish level (i.e. proportional contribution of each food category to the gut contents of each individual fish) in order to investigate major differences in diet composition between species.

Feeding selectivity was investigated by relating the proportional abundance of each invertebrate group to the proportional availability of the same invertebrate group in the stream environment. The categories invertebrate remains, algae, detritus, sand and other were measured exclusively by weight, since abundance estimates at the individual fish level were not relevant, and were therefore only included in analyses that were exclusively weight-based.

Finally, multivariate analysis of gut contents was conducted on the full weight-based data set (i.e. at the level of individual invertebrate taxa, as opposed to taxa being aggregated into functional groups), to investigate differences in fish diet in greater detail.

Diet composition at the whole sample level

Descriptions of gut contents were based on the procedure outlined by (Cortés 1997). Fish diet was summarized at the whole-sample level by calculating the percentage frequency occurrence (*% O*) of each food type across all stomachs of each species. A Chi-square test was then used to test for differences in the frequency of occurrence of the major prey categories between trout and redfin.

Diet composition at the individual fish level

The gut content composition of each individual fish was summarized by calculating percentage by number (*% N*) and percentage by weight (*% W*) of each food type.

PERMANOVA, a semi-parametric, permutation-based analogue of traditional ANOVA/MANOVA was then used to test for significant differences in the number- and weight-based percentage composition of gut contents between species. Multivariate PERMANOVA, using Bray-Curtis similarity and permutation of residuals under a reduced model, was used to test for differences in the overall composition of gut contents between species. Univariate PERMANOVAs, using Euclidian distance and unrestricted permutation of the raw data, were then used to assess differences between species in the percentage composition of each food source separately. A two-way nested design was adopted for both multivariate and univariate models, in order to examine the effect of the fixed factor fish species and the random, nested factor site on fish diet composition. I considered site to be a nested factor because the three streams where redfin were sampled were not the same three streams where trout were sampled, and thus inter-stream variation in fish diet needed to be accounted for (Quinn & Keough 2002).

Index of relative importance for invertebrate prey

The index of relative importance (*IRI*), a composite measure of prey importance incorporating *% O*, *% N* and *% W*, was calculated for each invertebrate group, *i*, using the formula of Pinkas *et al.* (1971):

$$IRI = \%\, O_i\, (\%\, N_i\, +\, \%\, W_i\,)$$

In order to standardize this index and facilitate comparisons with other studies, the *% IRI* value for each prey category was also calculated following the equation of Cortés (1997):

$$\%\, IRI = \frac{\%\, O_i\, (\%\, N_i\, +\, \%\, W_i\,)}{(\Sigma \%\, IRI_i)}$$

Multivariate analysis of unaggregated gut content data

Multivariate analysis was used to examine differences in the composition of trout and redfin diets in greater detail. A similarity matrix was calculated from ln(x+1) transformed, unaggregated *% W* dietary data set (Anderson *et al.* 2008) and non-metric multidimensional scaling (nMDS) ordination was used to visualize differences in trout and redfin diet, as well as dietary differences within a species among study sites. Nested, two-way PERMANOVA (a semi-parametric, permutation-based analogue of traditional ANOVA/MANOVA, (Anderson *et al.* 2008)) using Bray-Curtis similarity, 9999 permutations and permutation of residuals under a reduced model, was used to examine the effects of species and site on diet composition as described above. Permutational multivariate analysis of dispersion (PERMDISP, Anderson *et al.* 2008), which compared multivariate dispersion between the trout and redfin data clouds, was used to ascertain whether there was a significant difference in diet breadth between redfin and trout. Finally, analysis of similarity percentages (SIMPER, Anderson *et al.* 2008) was used to identify the food items contributing most to the dissimilarity in diet composition between trout and redfin.

Feeding selectivity

Prey abundance in fish guts was related to prey availability in the benthos and in the drift using Strauss' (1979) linear electivity index, *L*:

$$L_i = r_i - p_i,$$

where r_i and p_i are the proportional abundances of the different prey types in fish diet and environment respectively. *L* ranges from -1 to +1, with negative values indicating avoidance of a prey item, and positive numbers indicating selection for a prey item. Index values -0.25 < *L* < +0.25 were considered to indicate weak selection/avoidance, values between 0.25 and 0.50, or between -0.25 and -0.50, to indicate moderate selection/avoidance and values

between 0.50 and 0.75, or between -0.50 and -0.75, to indicate strong selection/avoidance (Schleuter & Eckmann 2008).

The procedure for estimating the proportional abundance of each FFG in the benthic invertebrate samples followed that outlined in the Section 3.2.3 in Chapter 2. In summary, the samples collected from erosional and depositional habitats were weighted by the proportional cover of these two habitat types at each site and then combined to produce a final estimate of the functional composition of the benthic invertebrate assemblage at each site. For the drift samples, I used the mean relative abundance of terrestrial and aquatic invertebrates in the three drift samples taken at each site to relate to the proportional abundance of these food types in fish guts at the sites. Selectivity values based on box sample availability estimates were used to ascertain whether trout and redfin differed in their selection of each of the five FFGs of aquatic invertebrates. On the other hand, selectivity values based on prey availability in the drift were used to ascertain whether selection of aquatic invertebrates (i.e. all FFGs combined) vs. terrestrial invertebrates differed between trout and redfin. Univariate nested PERMANOVA tests (as described above) were used to test for the effects of species and site on prey selectivity.

Stable isotopes

Visualizing the structure of CFR headwater stream food webs

Carbon-nitrogen bi-plots are a convenient and informative means of visualizing the trophic structure of biological communities (Hershey *et al.* 2007). Separate bi-plots were constructed for each of the six sampling sites to explore trophic relationships between different community components at the within-site scale. Additionally, a bi-plot, based on means of samples from all six sites, was constructed in order to summarize trophic relationships among community components across all sites.

Stable isotope-based estimates of fish trophic niches

The locations of samples in a carbon-nitrogen bi-plot provide a representation of the trophic niche occupied by a species (Layman *et al.* 2007a, b). I therefore plotted all fish samples on axes of $\delta^{15}N$ and $\delta^{13}C$ in order to examine differences in the trophic niche occupied by trout and redfin (Vander Zanden & Rasmussen 1999, Olsson *et al.* 2009, Taylor & Soucek 2010). Separate univariate two-way nested PERMANOVAs were performed on the $\delta^{15}N$ and $\delta^{13}C$ data sets to ascertain whether isotope signatures differed significantly between species, and among sampling sites for each species. Fish species was treated as a fixed factor and site was treated as a random, nested factor. Models were run using 9999 permutations and unrestricted permutation of the raw data was used.

The resources at the base of food webs (i.e. algae and detritus in the case for stream food webs) are naturally variable in $\delta^{15}N$ signature, and some studies have thus recommended that this natural variation should be taken into account when conducting among-site comparisons of the trophic level occupied by consumers (Cabana & Rasmussen 1996, Vander Zanden & Rasmussen 1999, Anderson & Cabana 2007). I therefore conducted an additional assessment of differences in fish trophic niches after inter-site differences in $\delta^{15}N$ of basal resources among sampling sites had been accounted for. To achieve this, I calculated the trophic position (*TP*) of each individual fish using the equation of Anderson & Cabana (2007):

$$TP_{\text{fish}} = \frac{(\delta^{15}\text{N}_{\text{fish}} - \delta^{15}\text{N}_{\text{baseline}})}{4.1} + 2,$$

where TP_{fish} is the trophic position of a fish, $\delta^{15}N_{\text{fish}}$ is the nitrogen isotope signature of the fish, $\delta^{15}N_{\text{baseline}}$ is the nitrogen isotope signature of primary consumers (assumed to be trophic level 2), and 4.1 is an estimate of nitrogen trophic fractionation for the study streams (see below).

Because there was variation in fractionation of $\delta^{15}N$ among the study sites, the correction value for this isotope was estimated by calculating the median of all site-specific differences

in $\delta^{15}N$ between secondary consumers and primary consumers, and between primary consumers and primary producers (Taylor & Soucek 2010). I used the mean value of fish at each site as a measure of secondary consumer $\delta^{15}N$, the mean value of collector-gatherer invertebrates as a measure of primary consumer $\delta^{15}N$, and the mean value of all algae and detritus values as a measure of primary producer $\delta^{15}N$. This resulted in a median value of 4.1 $^{o}/_{oo}$, which is relatively similar to commonly-reported estimates of trophic fractionation in freshwater systems (Cabana & Rasmussen 1996). Collector-gatherer invertebrates were used as a baseline (Post 2002, Olsson *et al.* 2008), since longer-lived primary consumers, such as grazing snails and filter-feeding mussels, recommended by other studies (Cabana & Rasmussen 1996, Post 2002) were not present in the study streams.

The *TP* and $\delta^{13}C$ of each fish were then plotted in a stable isotope bi-plot so that differences in the $\delta^{15}N$-corrected trophic niche of redfin and trout could be examined visually. A univariate two-way nested PERMANOVA was conducted on fish *TP* to examine the effects of species and sampling site. Fish species was treated as a fixed factor, site was treated as a random, nested factor, and the model was run using 9999 permutations and unrestricted permutation of the raw data. PERMDISP analysis, which compared multivariate dispersion between the trout and redfin data clouds, was used to ascertain whether or not there was a significant difference in trophic niche breadth between species based on both the corrected and uncorrected $\delta^{15}N$ data sets.

Partitioning redfin and trout diets

The mixing model IsoSource (Phillips & Gregg 2003) was used to estimate contributions of different food sources to the diets of redfin and trout. Since the carbon and nitrogen isotope signatures of basal resources may have differed among sites, separate mixing models were constructed for each site. Furthermore, because I was interested in estimating variation in food source contributions among individual consumers, a separate model was run for each individual fish at each study site. Mixing models were constructed using the carbon and nitrogen signatures of individual fish, as well as the mean isotope signatures of the different potential food items at each site. The food sources included in the mixing models were based on the gut content composition of redfin and trout (Clarke *et al.* 2005). In order to

reduce the number of potential food sources included in the model, individual taxa within FFGs were aggregated (by averaging) *a priori* since they have similar trophic habits and were found to have similar carbon and nitrogen isotope signatures (Phillips *et al.* 2005). This resulted in eight potential food sources, including algae, detritus, the five FFGs of aquatic invertebrates (collector-gatherers, filter-feeders, grazer-scrapers, predators and shredders), and terrestrial invertebrates.

The $\delta^{15}N$ signature of each fish was corrected for trophic fractionation prior to IsoSource analysis (Post 2002, Phillips & Gregg 2003) by subtracting 4.1 $^{o}/_{oo}$ from the actual $\delta^{15}N$ (see above for details regarding the estimation of trophic fractionation in the study streams). Source increment was set at 2% and tolerance was set at 0.1%. If no solution was found, the tolerance was increased in 0.1% increments up to a maximum of 1% (Bellchambers 2010). If no feasible solution was found following this approach, then the mixture (isotope signature of a fish) was considered to be too far outside of the mixing polygon (i.e. smallest polygon encapsulating mean data points of each food source in the 2-dimensional isotope niche space) for the model to yield reliable information on food source contribution to that fish, and it was excluded from the analysis (Phillips & Gregg 2003).

Percentage source contributions are presented as medians of all iterations for each individual fish, and medians, 25th and 75th percentiles, data ranges and outliers were then calculated and presented at the species level. Since IsoSource outputs are modeled medians, it is not appropriate to conduct statistical comparisons of source contributions between the two fish species (Phillips and Gregg 2003), and therefore no further statistical analyses were conducted on the model outputs.

Software used

All univariate analyses were carried out with SPSS 20.0 (SPSS 2011), and multivariate analyses were performed using PRIMER-E (Clarke & Gorley 2006) with the add-on package PERMANOVA+ (Anderson *et al.* 2008). Stable isotope mixing models were computed using IsoSource 1.3 (Phillips & Gregg 2003).

4.3 RESULTS

4.3.1 Focal animal watching

Foraging observations were conducted on a total of *n* = 129 trout and *n* = 144 redfin, and observations were considered to be independent since measures were taken to prevent observing the same fish more than once. Trout and redfin differed significantly in terms of frequency of foraging attempts among the three habitat types (Fishers exact test, $p < 0.001$, Figure 4.2).

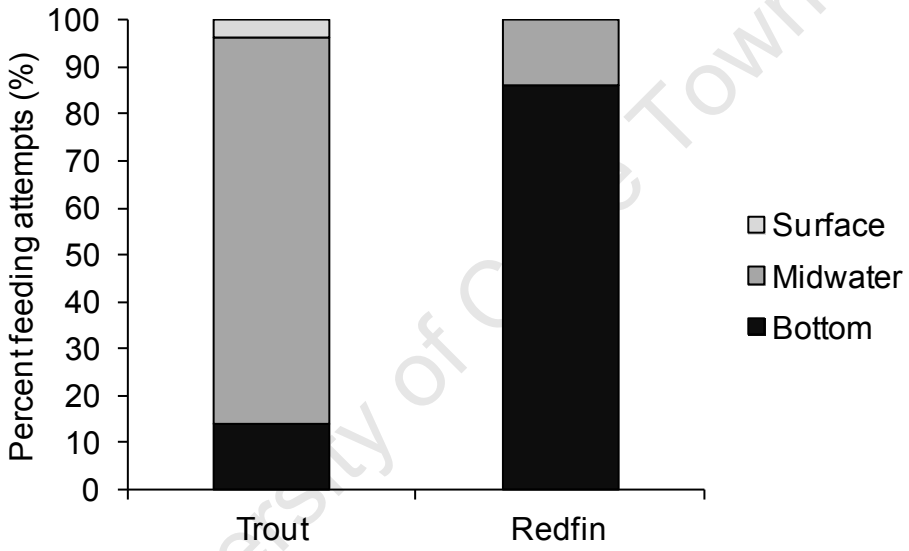

Figure 4.2 Percentage of feeding attempts by trout (*n* = 129) and redfin (*n* = 144) in "surface", "midwater" and "bottom" foraging habitats.

While 86.11% of redfin feeding attempts occurred on the stream bottom, only 13.95% of trout feeding attempts occurred in this habitat type. On the other hand, 82.17% of trout feeding attempts occurred in the midwater habitat whilst the percentage frequency of redfin feeding attempts in this habitat type was only 13.89%. For trout, the frequency of feeding attempts from the surface was low (3.87%), while no redfin were observed feeding at the stream surface. While recording fish foraging attempts, it was noted that in general, redfin were observed actively searching the stream bed for prey, while trout fed passively by holding their position in the water column and only consumed prey items that drifted

into their general vicinity. Figure 4.3 is an underwater photograph showing differences in the foraging mode of these two species.

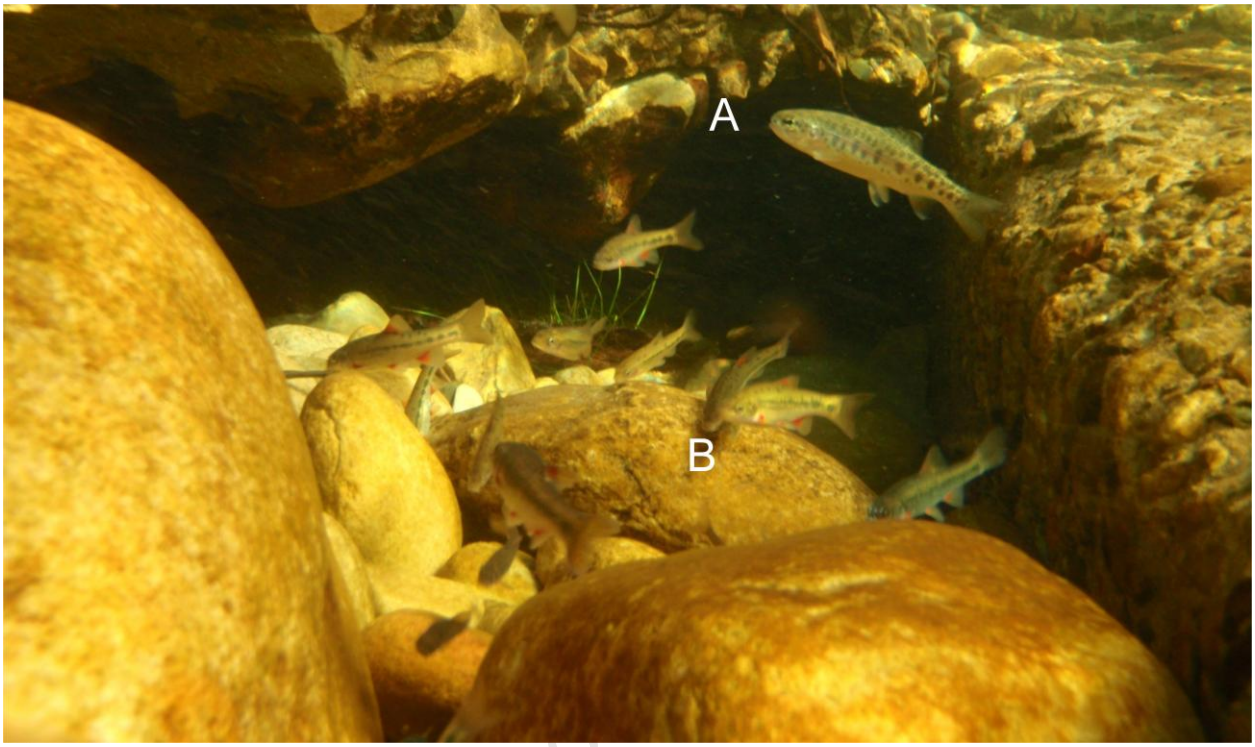

Figure 4.3 Photograph of a rainbow trout feeding on drifting prey in the "midwater" habitat (A), and redfin feeding on the stream bottom (B) in Jan du Toit Stream in the upper Breede River catchment, CFR.

4.3.2 Gut contents analysis

A total of *n* = 89 trout and *n* = 102 redfin were collected and subjected to GCA. Of these, nine trout and six redfin had empty stomachs, and were therefore excluded from further analyses, leaving sample sizes of *n* = 80 for trout and *n* = 96 for redfin. The number of fish collected at each study site, as well as the total length (TL, mm) and weight (g) of each individual fish sampled can be found in Appendix 5. Appendix 6 provides a full list of food items found in the guts of redfin and trout at the finest level of taxonomic resolution, and includes estimates of the frequency of occurrence (*% O*), mean proportional weight (*% W*), mean proportional abundance (*% N*) and the index of relative importance (*% IRI*) of each food item for both species. The diets of redfin and trout were broadly similar both in the

frequency of occurrence, and in the proportional composition, of the various food sources, but closer examination of the data set revealed some important dietary differences between the two species. Figure 4.4 and Table 4.1 summarize the frequency of occurrence (Figure 4a), proportional composition by weight (Figure 4.4b), and the proportional composition by number (Figure 4.4c), of the different food sources in fish diets.

Diet composition at the whole sample level

Overall, the frequency of occurrence of the eleven different food categories in the guts of redfin (Figure 4,4a, Table 4.1) differed significantly from that in the guts of trout (Chi-square test, $\chi^2_{10} = 183.16$, $p < 0.001$). However, >20% of cells in the contingency table had expected frequencies <5, and therefore one of the main assumptions of the χ^2 test was violated (Zar 1999, Quinn & Keough 2002). I therefore aggregated food sources *post-hoc* into meaningful categories so that the assumption of the test was met. The new categories were aquatic invertebrates (all aquatic invertebrate FFGs combined), terrestrial invertebrates, plant material (algae and detritus combined) and all remaining material (invertebrate remains, sand and other). The frequency of occurrence of these "aggregated" categories in the guts of redfin was significantly different to that in the guts of trout (Chi-square test, $\chi^2_3 = 95.02$, $p < 0.001$). Aquatic invertebrates were found in the vast majority of fish guts examined, occurring in 88.54% of redfin guts and 95.00% of trout guts. Collector-gatherers were the most frequently-recorded FFG of aquatic invertebrate in the guts of both fish species, occurring in 83.33% of redfin guts and 88.75% of trout guts. The remaining four aquatic invertebrate FFGs were generally found in <50% of trout and redfin guts, and were more commonly recorded in the guts of trout than in the guts of redfin. Filter-feeders, which were frequently found in trout guts (*% O* = 41.25%) were only found in 17.71% of redfin guts, while grazer-scrapers, which were present in 43.75% of trout guts were found in only 33.33% of redfin guts. Shredders were not commonly found in the guts of either fish species (*% O* trout = 5.00%, *% O* redfin = 1.04%), while predators were more frequently recorded in the guts of trout (*% O* = 51.25%) than in the guts of redfin (*% O* = 30.21%). Terrestrial invertebrates were found in 78.75% of trout guts, but only 26.04% of redfin guts, while algae and detritus, were present in ~60% of redfin guts, but <20% of trout guts. Finally, sand

was relatively common in the guts of redfin (*% O* = 34.38%), but was only found in 3.75% of trout guts.

Diet composition at the individual fish level

Although there were some broad similarities in the gut content composition of redfin and trout (Table 4.1, Figure 4.4b and c), the overall proportional composition of redfin and trout diet was significantly different both by weight (Table 4.2), and by number (Table 4.3). The proportion of aquatic invertebrates in the diet of redfin (*% W* = 54.63%, *% N* = 87.37%, Figure 4.4, Table 4.1) was significantly greater than that in the diet of trout (*% W* = 43.78%, *N* = 69.46%) both by weight (Table 4.2) and by number (Table 4.3). Of the different FFGs of aquatic invertebrates, collector-gatherers constituted by far the greatest proportion of redfin and trout gut contents, both by number, and by weight, and the proportion of collector-gatherers in the diet of redfin (*% W* = 32.05%, *% N* = 63.06%, Figure 4.4, Table 4.1) was significantly greater than that in the diet of trout (*% W* = 16.13%, *% N* = 42.33%) both by weight (Table 4.2) and by number (Table 4.3). Filter-feeders, grazer-scrapers, shredders and predators were less-important components in the diets of both species than were collector-gatherers. Each of these groups constituted <10% of the gut contents of redfin and trout (Figure 4.4, Table 4.1), and no significant inter-species differences in the proportional contribution of these groups were detected, regardless of whether analyses were based on estimates by weight (Table 4.2) or by number (Table 4.3). The proportion of terrestrial invertebrates in the diet of trout (*% W* = 38.57%, *% N* = 30.54%, Figure 4.4, Table 4.1) was significantly greater than that in the diet of redfin (*% W* = 11.80%, *% N* = 12.63%), regardless of whether weight- or number-based estimates of dietary composition were used (Tables 4.2 and 4.3). Non-invertebrate foods made up ~20% of redfin diet, but only ~5% of trout diet by weight (Figure 4.4b, Table 4.1). The mean proportional weights of algae and detritus in the guts of redfin (7.42% and 9.72% respectively,) were significantly greater than the mean proportional weights of these food items in the guts of trout (<2% for both algae and detritus) (Table 4.2). The mean proportional weight of sand in redfin guts (4.76%, Figure 4.4b, Table 4.1) was significantly greater than the mean proportional weight of sand in the guts of trout (<1%, Table 4.2). Finally, it is noted that there was a significant site effect on

the percentage by weight of grazer-scrapers, predators and on the "other" category, as well as on the percentage by number of filter-feeders, grazer-scrapers and predators, indicating that the contributions of these food items to fish diets varied significantly among sites (Tables 4.2 and 4.3).

Estimates of the index of relative importance revealed some clear differences in the importance of the different invertebrate prey groups in the guts of redfin and trout (Figure 4.5, Table 4.1). For redfin, aquatic invertebrates (*% IRI* = 93.48%) were a far more important prey type than were terrestrial invertebrates (*% IRI* = 6.52%). Collector-gatherers were clearly the most important FFG of aquatic invertebrates in the diet of redfin (*% IRI* = 81.29%), while the importance of each of the other four FFGs was in the diet of redfin was far lower (*% IRI* <6%). Terrestrial invertebrates were far more important in the diet of trout than in redfin. With a *% IRI* of 44.17%, terrestrial invertebrates were nearly as important in the diet of trout as aquatic invertebrates were (*% IRI* = 55.83%). As for redfin, collector-gatherers were the most important FFG of aquatic invertebrate (*% IRI* = 42.10%) in the diet of trout. Next most important in trout diet were predators (*% IRI* = 8.34%), while the remaining FFGs had *% IRI* values <5.

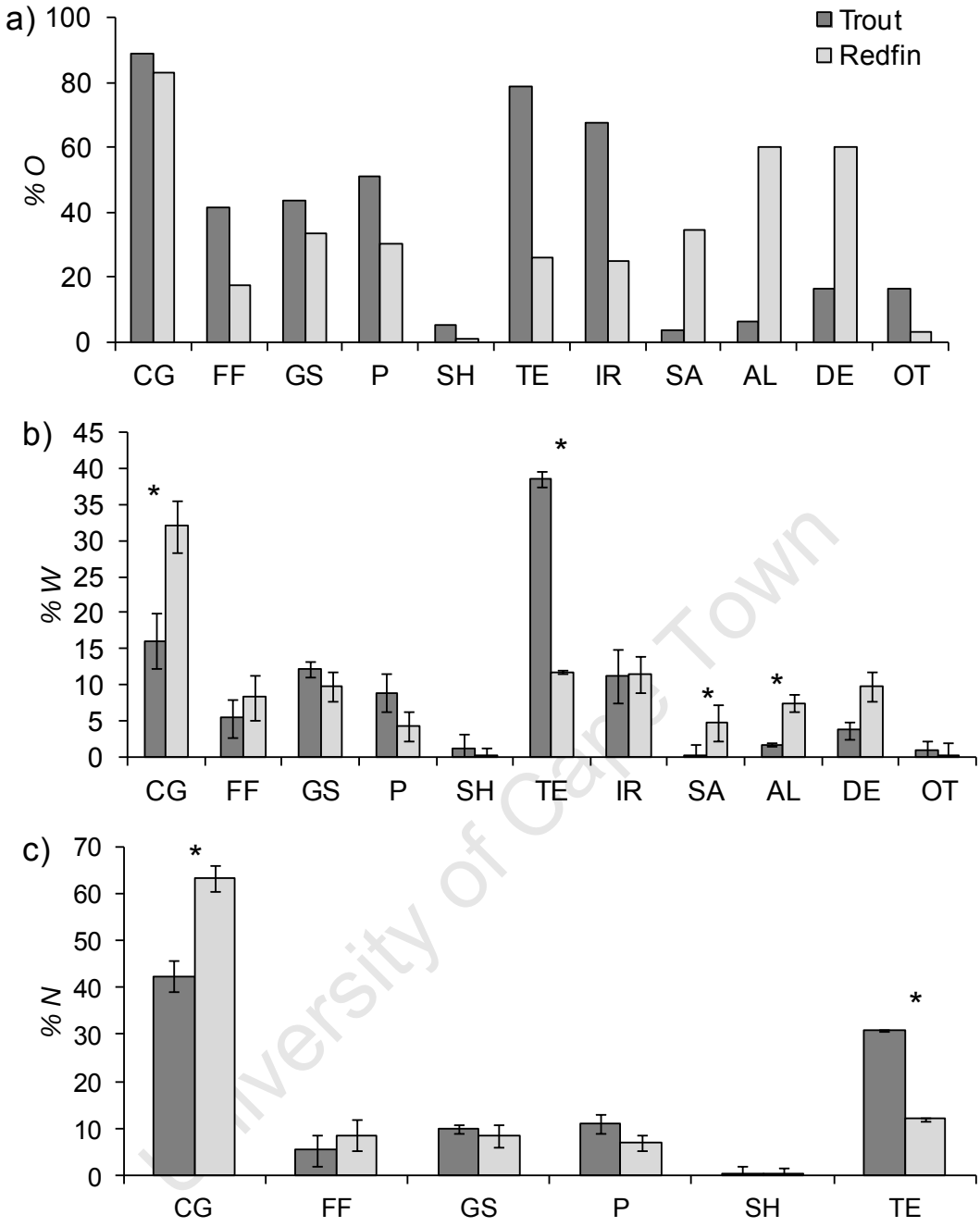

Figure 4.4 (a) Percentage frequency of occurrence (*% O*), (b) mean ± standard error (SE) proportional weight (*% W*), and (c) mean ± SE proportional abundance (*% N*) of food items in the guts of trout (dark grey bars, *n* = 80) and redfin (light grey bars, *n* = 96). Codes indicate "CG" = collector-gatherers, "FF" = filter-feeders, "GS" = grazer-scrapers, "P" = predators, "SH" = shredders, "TE" = terrestrial invertebrates, "IR" = invertebrate remains, "SA" = sand, "AL" = algae, "DE" = detritus, "OT" = other. An asterisk indicates a significant difference at the α = 0.05 level.

Table 4.1 Composition of trout and redfin diets by frequency of occurrence (*% O*), mean ± SE proportional weight (*% W*) and mean ± SE proportional abundance (*% N*). Estimates of these parameters are based on samples from all three sites where each species was sampled. The index of relative importance (*% IRI*) is a composite measure of the importance of invertebrate food sources in the diets of trout and redfin.

Food source	Trout						Redfin					
	% O	*% W*		*% N*		*% IRI*	*% O*	*% W*		*% N*		*%I RI*
		Mean	SE	Mean	SE			Mean	SE	Mean	SE	
Total aquatic invertebrates	95.00	43.78	19.32	69.46	19.73	55.83	88.54	54.63	18.94	87.37	23.49	93.48
Collector-gatherers	88.75	16.13	5.79	42.33	9.90	42.10	83.33	32.05	8.69	63.06	14.11	81.29
Filter-feeders	41.25	5.40	1.49	5.56	1.51	3.67	17.71	8.25	3.21	8.56	3.47	3.05
Grazer-scrapers	43.75	2.97	1.79	1.73	0.80	1.67	33.33	8.59	3.14	7.90	2.40	5.64
Shredders	5.00	1.13	1.08	0.23	0.19	0.06	1.04	0.18	0.18	0.37	0.36	0.01
Predators	51.25	8.90	5.78	11.16	4.46	8.34	30.21	4.33	2.68	6.93	2.69	3.49
Terrestrial invertebrates	78.75	38.57	11.46	30.54	8.07	44.17	26.04	11.80	5.31	12.63	5.74	6.52
Invertebrate remains	68.75	11.16	1.51	-	-	-	25.00	11.50	2.42	-	-	-
Algae	6.25	1.71	1.25	-	-	-	60.42	7.42	2.06	-	-	-
Detritus	16.25	3.74	1.52	-	-	-	60.42	9.72	1.92	-	-	-
Sand	3.75	0.24	0.17	-	-	-	34.38	4.76	1.23	-	-	-
Other	16.25	0.79	0.37	-	-	-	3.13	0.18	0.11	-	-	-

Table 4.2 Multivariate and univariate nested PERMANOVA models examining effects of fish species and sampling site on proportional weight (*% W*) of food sources. The multivariate model tested for overall differences in diet composition, while the univariate models tested for differences in each food source separately. Asterisks indicate significant effect at α = 0.05.

Response variable	Source	df	SS	MS	Pseudo-F	P(perm)
Multivariate PERMANOVA						
	Species	1	326.96	326.96	5.68	0.044*
	Site(species)	4	232.52	58.13	2.81	0.001*
	Residual	170	3519.00	20.70		
	Total	175	4082.80			
Univariate PERMANOVA						
Total aqauatic invertebrates	Species	1	26.57	26.57	8.09	0.042*
	Site(species)	4	13.16	3.29	1.45	0.234
	Residual	170	372.57	2.27		
	Total	175	414.68			
Collector-gatherers	Species	1	25.30	25.30	31.05	0.040*
	Site(Species)	4	3.18	0.79	0.36	0.817
	Residual	170	376.05	2.21		
	Total	175	404.56			
Filter-feeders	Species	1	3.12	3.12	0.44	0.513
	Site(Species)	4	28.98	7.25	4.18	0.005*
	Residual	170	295.00	1.74		
	Total	175	327.45			
Grazer-scrapers	Species	1	0.24	0.24	0.01	0.951
	Site(Species)	4	82.36	20.59	10.20	0.001*
	Residual	170	343.16	2.02		
	Total	175	426.76			
Predators	Species	1	5.73	5.73	1.10	0.363
	Site(Species)	4	20.99	5.25	3.23	0.017*
	Residual	170	275.77	1.62		
	Total	175	303.64			
Shredders	Species	1	0.30	0.30	1.13	0.371
	Site(Species)	4	1.06	0.27	1.44	0.205
	Residual	170	31.37	0.18		
	Total	175	32.64			
Terrestrial invertebrates	Species	1	245.88	245.88	71.62	0.038*
	Site(Species)	4	13.84	3.46	2.06	0.111
	Residual	170	285.97	1.68		
	Total	175	542.40			
Algae	Species	1	147.09	147.09	40.56	0.036*
	Site(Species)	4	14.60	3.65	1.85	0.137
	Residual	170	336.09	1.98		
	Total	175	506.98			
Sand	Species	1	36.18	36.18	16.16	0.016*
	Site(Species)	4	9.02	2.26	1.97	0.085
	Residual	170	194.64	1.15		
	Total	175	237.49			
Invertebrate remains	Species	1	30.26	30.26	15.10	0.088
	Site(Species)	4	8.00	2.00	0.86	0.473
	Residual	170	393.07	2.31		
	Total	175	431.28			
Detritus	Species	1	34.82	34.82	11.48	0.048
	Site(Species)	4	12.22	3.05	1.95	0.094
	Residual	170	265.64	1.56		
	Total	175	312.51			
Other	Species	1	1.86	1.86	2.24	0.203
	Site(Species)	4	3.37	0.84	3.85	0.005*
	Residual	170	37.16	0.22		
	Total	175	42.03			

Table 4.3 Multivariate and univariate nested PERMANOVA models examining effects of fish species and sampling site on proportional abundance (*% N*) of food sources. The multivariate model tested for overall differences in diet composition, while univariate models tested for differences in each food source separately. Asterisks indicate significant effect at $\alpha = 0.05$.

Response variable	Source	*df*	SS	MS	*F*	p_{perm}
Multivariate PERMANOVA						
	Species	1	157.61	157.61	4.05	0.084
	Site(species)	4	156.56	39.14	3.44	0.001*
	Residual	170	1863.40	11.36		
	Total	175	2179.00			
Univariate PERMANOVA						
Total aquatic invertebrates	Species	1	1.52	1.52	0.51	0.049*
	Site(species)	4	12.03	3.01	1.93	0.186
	Residual	170	264.85	1.56		
	Total	175	277.82			
Collector-gatherers	Species	1	26.57	26.57	8.09	0.042*
	Site(Species)	4	13.16	3.29	1.45	0.234
	Residual	170	372.57	2.27		
	Total	175	414.68			
Filter-feeders	Species	1	5.29	5.29	0.71	0.322
	Site(Species)	4	30.16	7.54	4.31	0.004*
	Residual	170	286.78	1.75		
	Total	175	322.16			
Grazer-scrapers	Species	1	0.36	0.36	0.02	0.680
	Site(Species)	4	86.41	21.60	12.88	0.001*
	Residual	170	275.10	1.68		
	Total	175	362.76			
Predators	Species	1	10.37	10.37	1.53	0.252
	Site(Species)	4	27.29	6.82	3.35	0.009*
	Residual	170	333.66	2.03		
	Total	175	372.64			
Shredders	Species	1	0.08	0.08	0.59	0.618
	Site(Species)	4	0.52	0.13	0.96	0.450
	Residual	170	22.03	0.13		
	Total	175	22.61			
Terrestrial invertebrates	Species	1	245.14	245.14	86.37	0.035*
	Site(Species)	4	11.38	2.84	1.39	0.206
	Residual	170	335.08	2.04		
	Total	175	587.36			

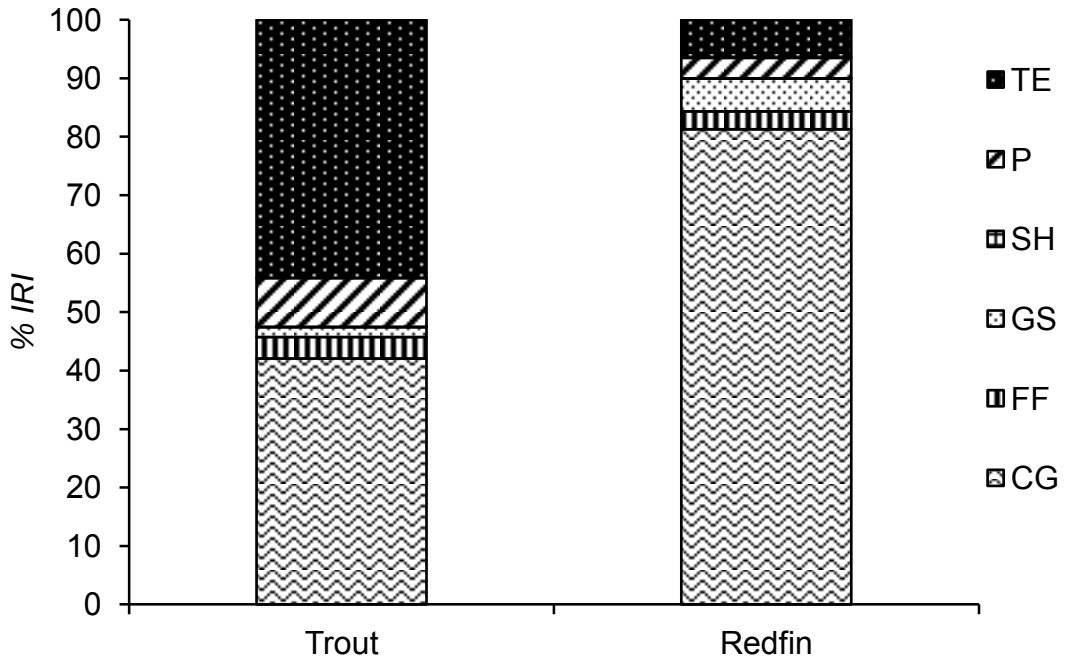

Figure 4.5 Index of relative importance (*% IRI*) of invertebrate taxa in the guts of trout and redfin. Codes are "CG" = collector-gatherers, "FF" = filter-feeders, "GS" = grazer-scrapers, "P" = predators, "SH" = shredders, "TE" = terrestrial invertebrates.

Multivariate analysis of unaggregated gut content data

The nMDS ordination of unaggregated (i.e. high taxonomic resolution), weight-based gut content data revealed that in general trout samples separated out clearly from redfin samples, but that there was also some overlap between the redfin and trout data clouds (Figure 4.6). This pattern indicates that, despite some dietary overlap, there were consistent differences in gut content composition between the two species, and the nested two-way, PERMANOVA indicated that both the fixed factor species and the random, nested factor site had a significant effect on gut content composition (Table 4.4). The interpretation here is that there was significant variation in the gut content composition within each species among sampling sites, but that there was a significant species effect over and above the effect of site.

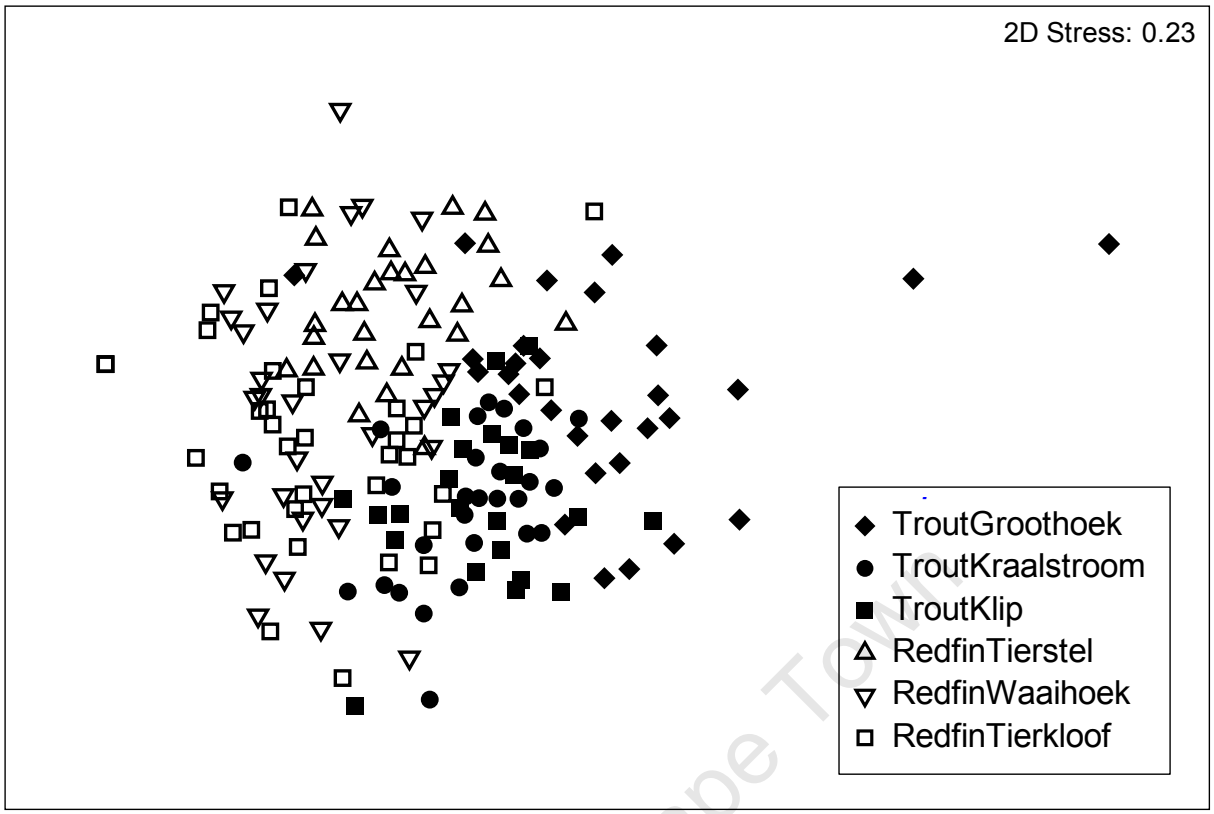

Figure 4.6 nMDS ordination of unaggregated (i.e. finest level of taxonomic resolution), weight-based gut content composition for trout (*n* = 80) and redfin (*n* = 96) at the six sampling sites.

Table 4.4 Multivariate nested PERMANOVA model examining the effect of the fish species and sampling site on fish diet composition based on the percentage weight of un-aggregated food sources. Asterisks indicate a significant difference at $\alpha = 0.05$.

Source	*df*	SS	MS	*F*	p_{perm}
Species	1	60708.00	60708.00	3.78	0.044*
Site(Species)	4	65075.00	16269.00	5.39	0.001*
Residual	170	513270.00	3019.30		
Total	175	639270.00			

SIMPER analysis revealed that the average dissimilarity in gut content composition between trout and redfin was high (88.11%), and Figure 4.7 shows the mean proportional weights of the food items identified as the most important contributors to the overall dissimilarity between trout and redfin diets. Invertebrate remains was identified as the single most

important food item in discrimination between the diet of trout and redfin, contributing 8.48% to the overall dissimilarity, yet there was little difference in the mean percentage weight of this food type between species. Terrestrial invertebrates, in particular those within the families Diptera, Hemiptera and Coleoptera, were among the set of food items that contributed strongly to the overall dissimilarity between species, and the mean percentage weight of each of these food items in the guts of trout was notably greater than in the guts of redfin. Collector-gatherer invertebrates, including Baetidae, Orthocladiinae, other Chironomidae (non-Tanypodinae) and *Aprionyx peterseni* were also identified as important, and the mean percentage weight of these food items in the guts of redfin tended to be greater than in the guts of trout. The grazer-scrapers Elmidae and *Hydroptila*, and the filter-feeder *Simulium*, also featured in the set of important contributors, and while Elmidae and *Simulium* had a greater mean percentage weight in the guts of redfin, *Hydroptila* had a greater mean percentage weight in the guts of trout. Finally, the non-invertebrate food items sand, algae and detritus were also identified as important in discriminating between the diets of redfin and trout, and all had a notably greater mean percentage weight in the guts of redfin than in the guts of trout.

The PERMDISP test revealed no significant difference in multivariate dispersion between the trout and redfin data clouds ($F_{1, 174} = 2.97$, $p = 0.144$), implying that diet breadth did not differ significantly between species when dietary composition was analysed by weight. Furthermore, this result indicates that the significant *p*-value detected in the PERMANOVA test should be attributed to interspecies differences in dietary composition rather than differences in the variability of gut content composition among individuals within a species.

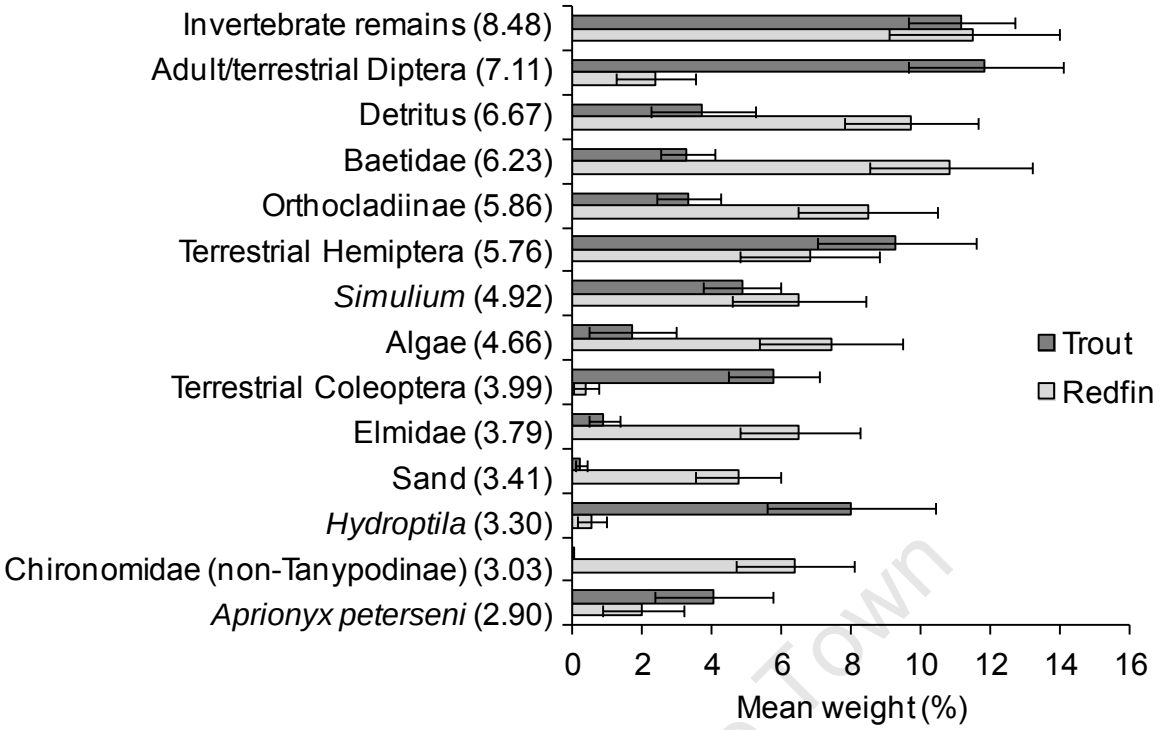

Figure 4.7 Mean ± SE percentage weight of the taxa contributing the most to the overall dissimilarity in gut content composition between trout and redfin. The average dissimilarity between trout and redfin gut contents was 88.11%, and values in parentheses indicate the percentage contribution of each taxon to this dissimilarity.

Feeding selectivity

The patterns of feeding selectivity displayed by trout and redfin were broadly similar, although there were also some clear differences in their preferences for certain prey types (Figure 4.8). Collector-gatherers and grazer-scrapers dominated the benthic invertebrate samples (Appendix 7), and trout and redfin differed in their selectivity for these FFGs (Figure 4.8a). Redfin displayed a moderate ($0.25 < L < 0.5$) preference for collector-gatherers, while this FFG was only weakly selected by trout ($0 < L < 0.25$), and this difference was statistically significant (Table 4.5). Both species displayed a moderate avoidance for grazer-scrapers ($-0.5 < L < -0.25$). However, this FFG was avoided more strongly by redfin than it was by trout. Differences in selectivity for grazer-scrapers by redfin and trout were, however, not statistically significant (Table 4.5). The abundance of filter-feeders, predators and shredders in the benthic box samples was relatively low (Appendix 7), and these groups were

consumed roughly in proportion to their availability (i.e. -0.25 < *L* < +0.25) (Figure 4.8a). The significant site effect detected for collector-gatherers, grazer-scrapers, shredders and predators implies that selectivity for these FFGs was highly variable among sites (Table 4.5).

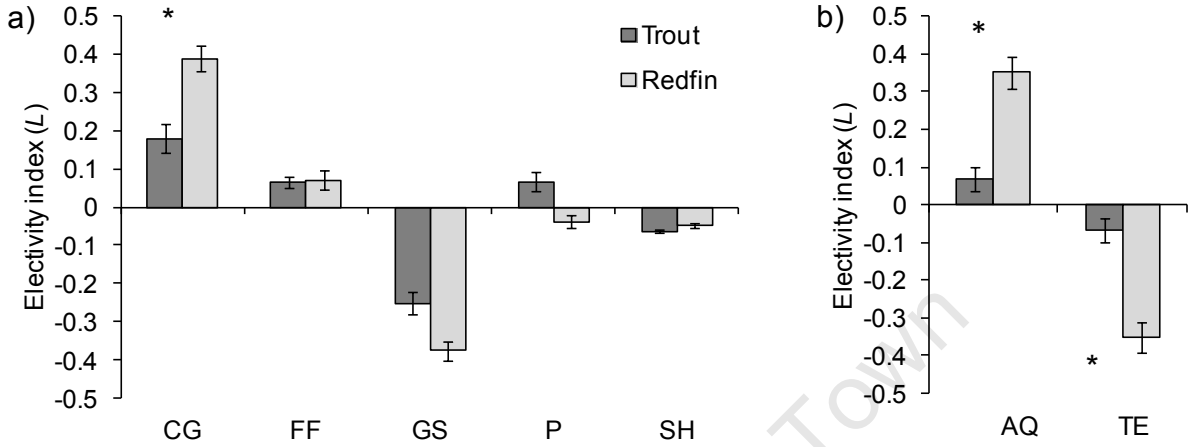

Figure 4.8 Mean electivity values (Strauss' *L*, ± SE) for trout (dark grey bars, *n* = 80) and redfin (light grey bars, *n* = 96) for (a) the aquatic invertebrate functional feeding groups relative to their abundance in the benthos, and (b) for terrestrial and aquatic invertebrates relative to their abundance in the drift. Codes indicate "CG" = collector-gatherers, "FF" = filter-feeders, "GS" = grazer-scrapers, "P" = predators, "SH" = shredders, "TE" = terrestrial invertebrates and "AQ" = aquatic invertebrates. An asterisk indicates a significant difference at the α = 0.05 level.

The drift samples were dominated by aquatic invertebrates at most sites, although terrestrial invertebrates were also relatively common (Appendix 7). Aquatic invertebrates were moderately selected by redfin, but only weakly selected by trout (Figure 4.8b), and the difference in selectivity between fish species for total aquatic invertebrates was statistically significant (Table 4.6). Terrestrial invertebrates, on the other hand, were strongly avoided by redfin, but only weakly avoided by trout (Figure 4.8b), and the difference in selectivity between fish species for terrestrial invertebrates was also statistically significant (Table 4.6).

Table 4.5 Univariate nested PERMANOVA models examining the effect of the fish species and sampling site on selection (Strauss' *L*) for aquatic invertebrate FFGs. An asterisk indicates a significant difference at $\alpha = 0.05$.

Response variable	Source	*df*	SS	MS	*F*	p_{perm}
Collector-gatherers	Species	1	1.56	1.56	6.44	0.017*
	Site(species)	4	0.97	0.24	2.65	0.041*
	Residual	170	14.11	0.09		
	Total	175	16.85			
Filter-feeders	Species	1	0.00	0.00	0.02	0.939
	Site(species)	4	0.25	0.06	1.89	0.113
	Residual	170	5.19	0.03		
	Total	175	5.45			
Grazer-scrapers	Species	1	0.57	0.57	0.65	0.546
	Site(species)	4	3.51	0.88	23.27	0.001*
	Residual	170	5.80	0.04		
	Total	175	9.93			
Predators	Species	1	0.36	0.36	3.59	0.104
	Site(species)	4	0.41	0.10	3.04	0.020*
	Residual	170	5.14	0.03		
	Total	175	5.99			
Shredders	Species	1	0.01	0.01	0.07	0.697
	Site(species)	4	0.28	0.07	77.57	0.001*
	Residual	170	0.14	0.00		
	Total	175	0.42			

Table 4.6 Univariate nested PERMANOVA models examining the effect of the fish species and sampling site on selection (Strauss' *L*) for aquatic and terrestrial invertebrates. Asterisks indicate a significant difference at $\alpha = 0.05$.

Response variable	Source	*df*	SS	MS	*F*	p_{perm}
Aquatic invertebrates	Species	1	8.25	2.06	27.62	0.001*
	Site(species)	4	3.05	3.05	1.49	0.273
	Residual	170	12.02	0.07		
	Total	175	23.63			
Terrestrial invertebrates	Species	1	8.25	2.06	27.62	0.001*
	Site(species)	4	3.05	3.05	1.49	0.277
	Residual	170	12.02	0.07		
	Total	175	23.63			

4.3.3 Stable isotope analysis

A total of *n* = 48 redfin samples, *n* = 48 trout samples, *n* = 180 aquatic invertebrate samples (*n* = 30 for each FFG), *n* = 30 terrestrial invertebrate samples, and *n* = 30 samples of each of algae and detritus were subjected to SIA, giving a total of *n* = 366 samples.

Visualizing the structure of CFR headwater stream food webs

The stable isotope bi-plot of mean ± SE values of all measured community components from all sampling sites combined (Figure 4.9) provides a visual representation of the trophic structure of headwater stream communities in the study area. Appendix 8 lists the representative taxa for the different aquatic invertebrate FFGs at each of the sampling sites. Algae and detritus were most depleted in both $\delta^{15}N$ and their positions in the plot indicate that they are situated at the base of the stream food web. Algae were, however, more enriched in $\delta^{13}C$ than was detritus, indicating that autotrophic and heterotrophic resources in the study streams differed in their $\delta^{13}C$ signatures.

The non-predatory aquatic invertebrate FFGs collector-gatherers, grazer-scrapers, filter-feeders and shredders, were situated approximately one trophic level above algae and detritus. The $\delta^{13}C$ signatures of collector-gatherers and shredders were associated with that of detritus, suggesting that these FFGs derive the majority of their carbon from heterotrophic resources. On the other hand, the $\delta^{13}C$ signatures of grazer-scrapers and filter-feeders were more closely associated with that of algae than that of detritus, indicating that these FFGs rely more strongly on autotrophic resources for their carbon. The $\delta^{15}N$ signature of predatory aquatic invertebrates indicated that they were roughly one trophic level above the non-predatory invertebrates, and the fact that their $\delta^{13}C$ signature was intermediate relative to that of the non-predatory invertebrate groups, suggests that they could potentially derive their carbon from any of these groups. Terrestrial invertebrates were slightly enriched in both $\delta^{15}N$ and $\delta^{13}C$ relative to predatory aquatic invertebrates, suggesting that this group feeds at a similar, but slightly higher, trophic position than do predatory invertebrates.

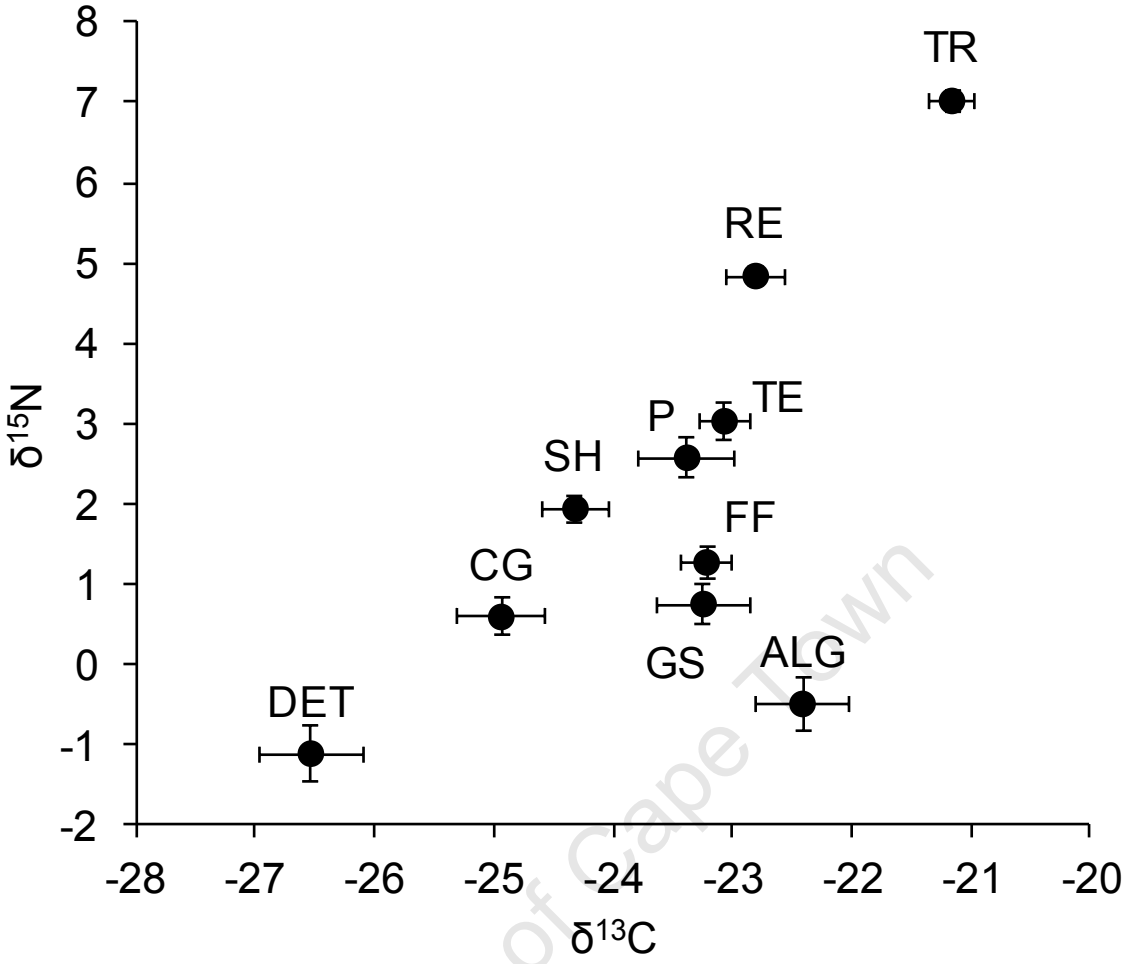

Figure 4.9 Stable isotope bi-plot of mean ± SE $\delta^{15}N$ and $\delta^{13}C$ signatures of stream community components based on samples from all six sites combined. Codes and sample sizes of community components are as follows: "TR" = trout (*n* = 48), "RE" = redfin (*n* = 48), "CG" = collector-gatherers (*n* = 30), "FF" = filter-feeders (*n* = 30), "GS" = grazer-scrapers (*n* = 30), "P" = predators (*n* = 30), "SH" = shredders (*n* = 30), "TE" = terrestrial invertebrates (*n* = 30), "ALG" = algae (*n* = 30) and "DET" = detritus (*n* = 30).

The $\delta^{15}N$ signature of redfin was approximately one trophic level above most of the non-predatory aquatic invertebrate groups, and their $\delta^{13}C$ signature suggests that redfin potentially derive the majority of their carbon from aquatic invertebrates and algae. The mean $\delta^{15}N$ of trout was enriched by approximately 2 $^{o}/_{oo}$ relative to that of redfin, indicating that trout feed at a higher trophic position than do redfin. The $\delta^{13}C$ of trout was slightly enriched relative to redfin, and their isotopic signature indicates that terrestrial invertebrates and predatory invertebrates are likely important sources of carbon in their diet.

Stable isotope-based estimates of fish trophic niches

Figure 4.10a shows the $\delta^{15}N$ and $\delta^{13}C$ signature of each fish sampled and provides a representation of the trophic niche occupied by redfin and trout in the stream food web (see Appendix 9 for $\delta^{15}N$ and $\delta^{13}C$ values of individual fish). The clear separation of redfin and trout samples in the dual-isotope space suggests that the trophic niche occupied by redfin was largely distinct from that occupied by trout, and that there was very little trophic overlap between species. On average, trout were more enriched in both $\delta^{15}N$ and $\delta^{13}C$ than were redfin (Figure 4.11), and differences between fish species in $\delta^{15}N$ and $\delta^{13}C$ signatures were statistically significant (Table 4.7).

These results suggest that trout foraged at a higher trophic level than did redfin, and that $\delta^{13}C$-enriched food sources, such as terrestrial invertebrates and predatory aquatic invertebrates, contributed more to the diet of trout than to the diet of redfin. The significant site effect for $\delta^{15}N$ (Table 4.7) indicates that the variation in nitrogen signature of fish among sites was significant, but that a significant species effect was, however, detected over and above this variation. Figure 4.10b shows that after differences in $\delta^{15}N$ of basal resources among streams had been corrected for, the differences in trophic niche occupied by redfin and trout were less pronounced. Specifically, the two species did not separate out clearly along the axis of trophic position, and although the mean trophic position of trout was higher than that of redfin (Figure 4.11), this difference was not significant (Table 4.7). Finally, there was no significant difference in the dispersion between the data clouds of redfin and trout for both the corrected (PERMDISP test, $F_{2,\ 94} = 1.58$, $p = 0.221$), and uncorrected (PERMDISP test, $F_{2,\ 94} = 0.42$, $p = 0.567$) data sets indicating that the trophic niche breadth of redfin was not significantly different from that of trout.

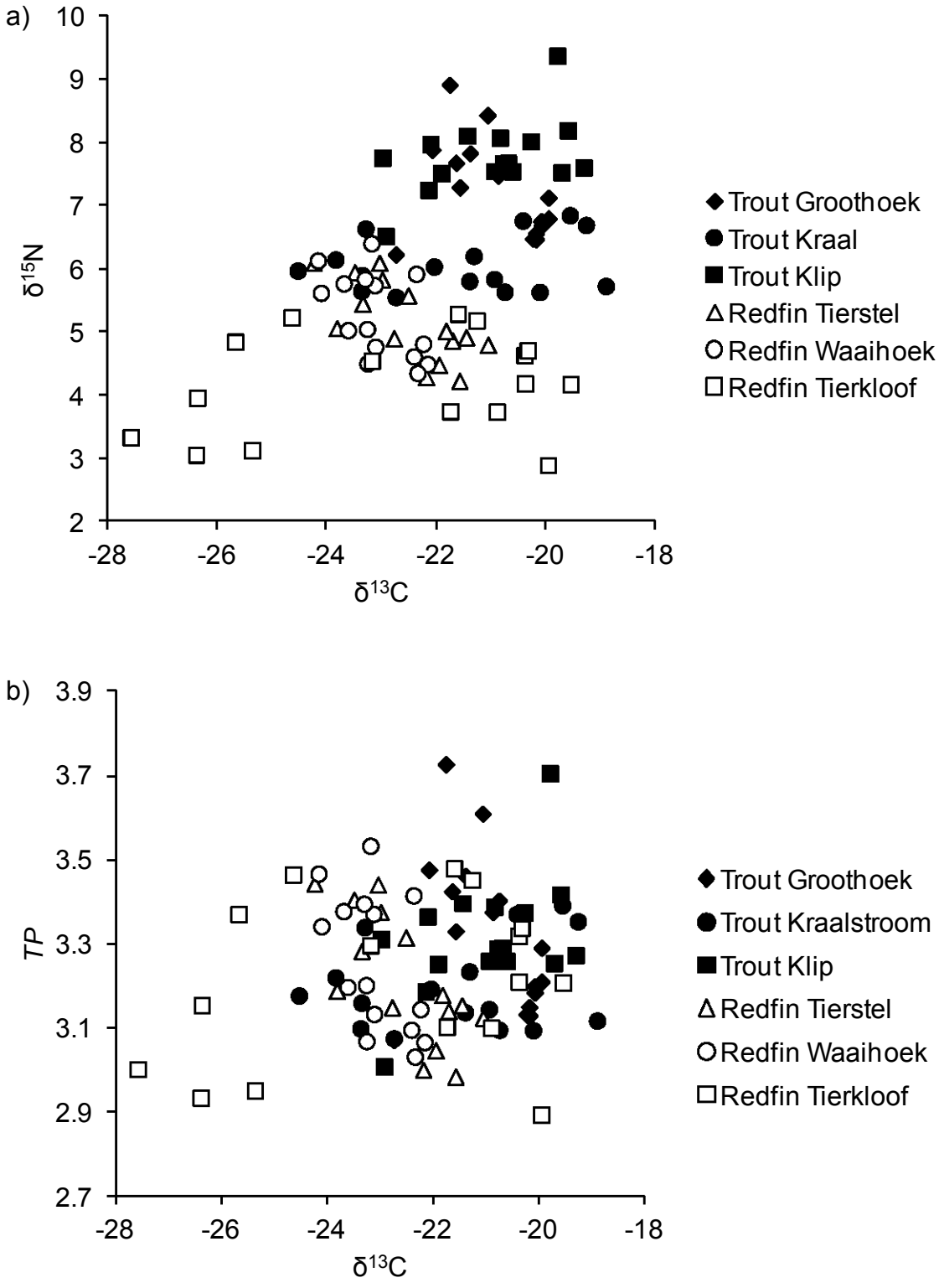

Figure 4.10 Stable isotope bi-plots of (a) the $\delta^{15}N$ and $\delta^{13}C$ signature, and (b) trophic position (*TP*) and $\delta^{13}C$ signature, of each individual fish sampled from each study site. *N* = 16 for each species at each of the three sites where it was sampled.

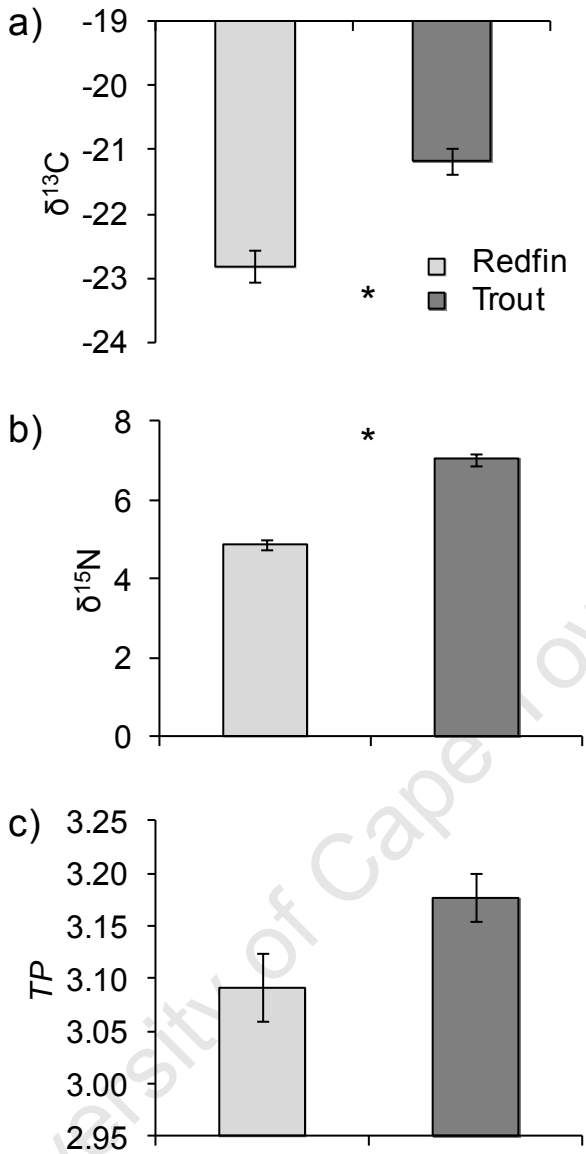

Figure 4.11 Mean ± SE $\delta^{13}C$ (a), $\delta^{15}N$ (b) and trophic position (*TP*) (c) for redfin (*n* = 48) and trout (*n* = 48) as estimated from all samples combined. Asterisks indicate a significant difference at the $\alpha = 0.05$ level.

Table 4.7 Univariate nested PERMANOVA models examining the effect of the fish species and sampling site on $\delta^{13}C$, $\delta^{15}N$ and trophic position (*TP*). Asterisks indicate a significant difference at $\alpha = 0.05$.

Response variable	Source	*df*	SS	MS	*F*	p_{perm}
$\delta^{13}C$	Species	1	63.48	63.48	35.72	0.039*
	Site(species)	4	7.11	1.78	0.75	0.557
	Residual	91	208.25	2.37		
	Total	96	278.89			
$\delta^{15}N$	Species	1	110.09	110.09	12.17	0.017*
	Site(species)	4	36.22	9.05	20.76	0.001*
	Residual	91	38.39	0.44		
	Total	96	186.30			
TP	Species	1	0.16	0.16	0.60	0.667
	Site(species)	4	1.03	0.26	9.94	0.001*
	Residual	91	2.28	0.03		
	Total	96	3.49			

Partitioning redfin and trout diets

Figure 4.12 shows separate stable isotope bi-plots for each of the six sampling sites. The isotopic signature of each individual fish is shown, while potential food sources are represented by the mean ± SE of all samples collected at the site (see Appendices 10a and b for mean ± SE $\delta^{15}N$ and $\delta^{13}C$ values for each community component at sites containing trout and redfin respectively). Once corrected for trophic fractionation, the positions of individual fish in these bi-plots were related to the positions of the potential food sources using the mixing model IsoSource, and source contributions estimated for each individual fish. Of the 48 trout and 48 redfin subjected to IsoSource analysis, no feasible solutions could be generated for five of the trout samples, and seven of the redfin samples, and thus the results presented here are based on a sample size of $n = 43$ for trout and $n = 41$ for redfin.

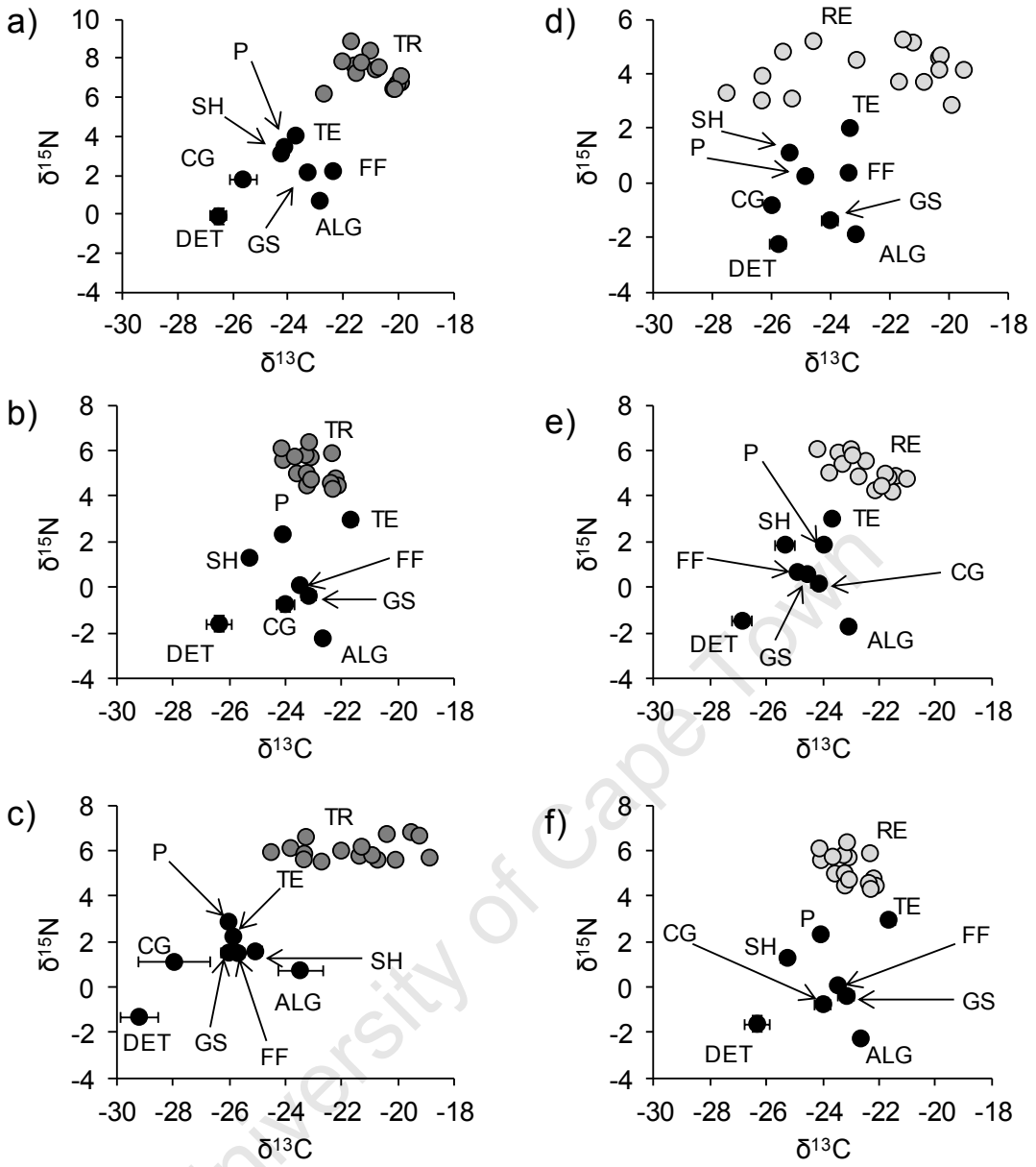

Figure 4.12 Stable isotope bi-plots depicting the trophic structure of stream communities at sites containing trout (a = Groothoek, b = Kraalstroom, c = Klip) and sites containing redfin (d = Tierkloof, e = Tierstel, f = Waaihoek). The $\delta^{15}N$ and $\delta^{13}C$ signatures of individual fish at each site are shown (dark grey circles = trout, light grey circles = redfin), while other community components (black circles) are presented as the mean ± SE of all samples collected at the site. Codes and sample sizes of fish and other community components sampled at each site are as follows: "TR" = trout (*n* = 16), "RE" = redfin (*n* = 16), "CG" = collector-gatherers (*n* = 5), "FF" = filter-feeders (*n* = 5), "GS" = grazer-scrapers (*n* = 5), "P" = predators (*n* = 5), "SH" = shredders (*n* = 5), "TE" = terrestrial invertebrates (*n* = 5), "ALG" = algae (*n* = 5) and "DET" = detritus (*n* = 5).

Figure 4.13a summarizes the median percentage contribution of each food source to each species, based on the median of all iterations for each individual fish. The contributions of the different food sources to the diets of redfin and trout were broadly similar, and generally low (median contributions <15%). With the exception of terrestrial invertebrates, which clearly contributed more to the diet of trout (median = 23.77%) than to the diet of redfin (median = 8.57%), none of the food sources stood out as particularly important contributors to the diet of either fish species.

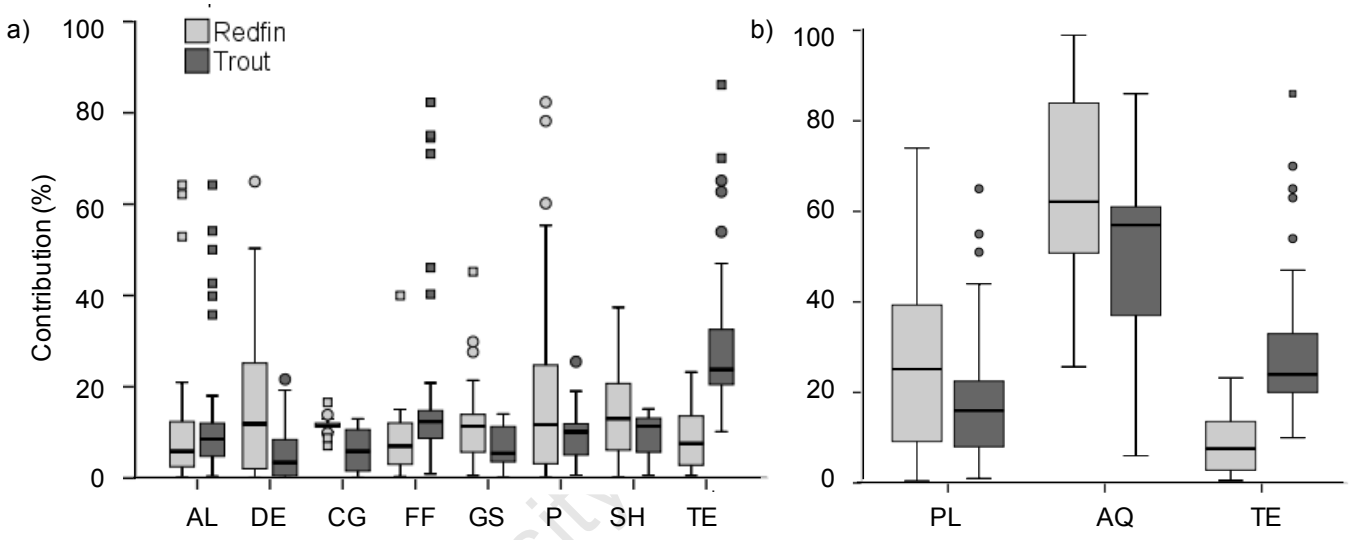

Figure 4.13 Estimates derived from the mixing model IsoSource of the percentage contribution of (a) each food source separately, including algae (AL), detritus (DE), collector-gatherers (CG), filter-feeders (FF), grazer-scrapers (GS), predators (P) and shredders (SH), and (b) sources aggregated into plant material (PL), aquatic invertebrates (AQ) and terrestrial invertebrates (TE) to the diet of redfin (n = 48) and trout (n = 48). Horizontal lines inside boxes indicate medians, and the upper and lower edges of boxes indicate 25th and 75th percentiles. Error bars indicate minimum and maximum values for source contributions (excluding outliers), and data points outside of error bars are outliers. Circles indicate outliers that fall between 1.5 and 3 times the interquartile range (i.e. data points between the 25th and the 75th percentiles), and squares indicate outliers that fall beyond three times the interquartile range.

4.4 DISCUSSION

Differences in food web structure between streams supporting healthy native fish populations and streams where trout have replaced native fish as the major top predators may be attributable to differences in the trophic niches exploited by non-native trout and the native fish that they have replaced. Specifically, the high abundance of benthic invertebrates in streams invaded by trout, relative to streams lacking trout, suggests that trout may be weaker regulators of benthic invertebrates than are native fish. Previous studies suggest that redfin, the dominant member of the native fish assemblage, is a benthic forager and may rely strongly on aquatic invertebrates for food, while trout is known to be a drift feeder that, in addition to feeding on aquatic invertebrates, can acquire a substantial proportion of its diet from terrestrial invertebrates that fall into the stream from the riparian zone. In the present study I used a blend of complementary approaches to characterize and compare the foraging behaviour, and diet, of trout and redfin in headwater streams in the upper Breede River catchment, and thereby address two predictions regarding the dietary habits of these two fish species. First, I used focal animal watching to address the prediction that redfin would feed more frequently from the stream bed than would trout, while trout would feed more frequently on drifting invertebrates than would redfin. Second, I used gut contents and stable isotope analysis to address the prediction that these contrasting foraging behaviours would translate into differences in the diet consumed by these two fish species. Specifically, it was predicted that redfin would be more strongly reliant on aquatic invertebrate prey than would trout, because terrestrial invertebrate subsidies from the riparian zone would offset their demand on aquatic invertebrates for food.

4.4.1 Foraging behaviour

Results from the focal animal watching study revealed clear differences in foraging mode between trout and redfin in the study streams, and supported the hypothesis that redfin is primarily an active benthic feeder, while trout is predominantly a passive drift feeder. Two features of redfin mouth morphology are consistent with the view that the species is a benthic feeder. Firstly, the orientation of the mouth is subterminal (downward-facing),

which enables effective consumption of food items beneath the fish on the stream bed, and secondly, the mouth is flanked by two pairs of sensory barbels, an adaptation for locating food items upon substrate (Skelton 2001). Additional support for the benthic feeding habits of redfin is provided by underwater observations made by Avenant (1989), who noted that all individuals observed were located close to the stream substrate.

In contrast with redfin, the mouth position of trout is terminal (frontward-facing), a mouth orientation generally associated with fish that feed on prey drifting in the water column in front of them (Skelton 2001). Indeed, trout are widely known to be visual predators that feed primarily by selectively consuming prey items drifting in the water column (Rader 1997, Allan & Castillo 2007, Albariño & Buria 2011), although they will also feed from the stream bed (Nakano *et al.* 1999b), or water surface (Baxter *et al.* 2004), depending on prey availability. The results from the present study indicate that the foraging behaviour of the trout in my study streams is consistent with the general view that trout is primarily a drift feeder. The discussion that follows focuses on whether these differences in foraging mode translate into differences in the diet consumed by native redfin and non-native trout, and if so, how the diets of these two species differ.

4.4.2 Trophic niche

Gut content composition and $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures were used to examine differences in the trophic niches occupied by redfin and trout in the study streams. Multivariate analysis of gut content data revealed consistent differences in dietary composition between trout and redfin, suggesting that the trophic niches occupied by these two species in the stream food web are not equivalent. This finding is consistent with the contrasting foraging strategies observed for these two fish species. The fact that there was not complete separation of trout and redfin data points in the nMDS plot (Figure 4.6) indicates that there was also some level of dietary overlap. However the high overall dissimilarity in gut content composition between trout and redfin (average dissimilarity between trout and redfin data points = 88.11%) suggests that dietary overlap was not substantial.

Results from the stable isotope analysis partially corroborated the findings of the gut contents analysis. The $\delta^{13}C$ and $\delta^{15}N$ bi-plot of redfin and trout samples (Figure 10a) suggested that despite some overlap, the trophic niche of trout was largely distinct from that of redfin, and the nested PERMANOVA models confirmed that inter-species differences along both the $\delta^{13}C$ and $\delta^{15}N$ axes of the bi-plot were statistically significant. These results corroborated the gut contents analysis finding that the trophic niches occupied these two species in the stream food web are not equivalent. However, when differences in $\delta^{15}N$ of basal resources among sites were corrected for, the bi-plot of $\delta^{13}C$ and trophic position indicated that the distributions of trout and redfin data points in the stable isotope bi-plot largely overlapped (Figure 10b). This result suggests that the clear inter-species differences in $\delta^{15}N$ may, to some extent, have been an artifact of differences in $\delta^{15}N$ of basal resources among streams (Cabana & Rasmussen 1996, Vander Zanden & Rasmussen 1999, Anderson & Cabana 2007). However, even after correction, subtle differences in the trophic niches of redfin and trout were evident, particularly with regard to their $\delta^{13}C$ signatures. Taken together, the results produced by these contrasting, yet complementary, approaches indicated that although there appeared to be some level of trophic similarity between these two species, they could not be considered as functionally equivalent predators in the study streams, and important differences in fish diet composition are discussed in the following section.

4.4.3 Diet composition

The dietary habits of trout and redfin in the study streams were broadly similar in that both species appeared to feed predominantly on animal prey, while non-animal food types such as algae, detritus and sand constituted a relatively small proportion of their overall diets. Detailed comparisons of dietary composition between species revealed that there were subtle differences in the importance of aquatic invertebrates (particularly collector-gatherers), terrestrial invertebrates and certain non-animal food types in the diet of trout and redfin.

Aquatic invertebrates

Gut contents analysis revealed that aquatic invertebrates, which formed an important component of the diet of both species (occurring in >85% of the stomachs of both species, Figure 4.4, Table 4.1), contributed more strongly to the diet of redfin than to the diet of trout. Differences between species in the proportional contribution of aquatic invertebrates to fish diet were significant for both number- and weight-based estimates of dietary composition, and the *% IRI* of aquatic invertebrates for redfin was roughly double that of trout (Figure 4.5, Table 4.1). Furthermore, analysis of fish feeding selectivity revealed that selection of aquatic invertebrates by redfin was significantly stronger than that by trout (Figure 4.8b, Table 4.5). These inter-species differences appeared to be driven largely by differences in the proportional contribution of collector-gatherer invertebrates to overall gut contents. Collector-gatherers were by far the most abundant FFG of aquatic invertebrates in the guts of both species, yet they were significantly more abundant in the guts of redfin than in the guts of trout (Tables 4.2 and 4.3). In particular, the collector-gatherer taxa Baetidae, *A. peterseni*, Orthocladiinae and other chironomids (non-Tanypodinae) featured prominently in the set of food items identified as important contributors to the overall dissimilarity in diet between fish species (Figure 4.7). Furthermore, analysis of fish feeding selectivity revealed that selection for collector-gatherer invertebrates by redfin was significantly stronger than that by trout (Figure 4.8a).

Results for the stable isotope mixing models corroborated the finding that aquatic invertebrates were an important food source for both fish species, and that they generally contributed more strongly to the diet of redfin to that of trout (Figure 4.13). Although the IsoSource models indicated that the contribution of collector-gatherers to redfin diet was generally greater than the contribution of this FFG to trout diet, they did not show that collector-gatherers were a more important food source than were the other aquatic invertebrate FFGs, as indicated by gut contents analysis. The congruence between the results from the gut contents and stable isotope analyses constitutes strong support for the hypothesis that benthic-feeding redfin were more strongly reliant on aquatic invertebrate prey than were trout. Furthermore, these results imply that the greater quantity of collector-gatherer invertebrates in the diet of redfin compared to trout was at least partially responsible for this trend.

The finding that redfin was strongly reliant on aquatic invertebrate prey is supported by the study of de Wet (1990), who examined the foregut contents 142 specimens of Breede River redfin collected from the Hex and Steenboks Rivers (upper Breede River catchment, CFR). In that study it was found that although redfin utilized a wide variety of food sources including algae, detritus and sand, it was primarily an opportunistic carnivore that fed mostly on benthic invertebrates. She found that aquatic invertebrates comprised roughly 50% of gut contents by volume, which is relatively similar to the mean proportional weight of aquatic invertebrates recorded in the present study (*% W* = 54.63%, Table 4.1). Furthermore, de Wet (1990) found Ephemeroptera and Diptera to be the most important aquatic invertebrate prey occurring in redfin guts. The majority of taxa within these orders are collector-gatherers (Cummins *et al.* 2008), and therefore it is likely that the aquatic invertebrate component of redfin diet in that study was dominated by collector-gatherers, as was the case in the present study. Interestingly, the two most important taxa in redfin guts in my study were Baetidae, a family of Ephemeroptera, and non-predatory chironomids within the order Diptera. This congruency between these two studies suggests that redfin may have a widespread preference for these taxa. Indeed, ephemeropterans such as the Baetidae, and dipterans such as the Chironomidae, are generally epibenthic foragers (Barber-James & Lugo-Oritz 2003, Harrison 2003), and would therefore be susceptible to predation by a benthic-feeding fish such as redfin. Additional support for the view that redfin are primarily carnivorous comes from studies on the diet of the closely related fiery redfin, *P. phlegethon*. Whitehead *et al.* (2007) found that aquatic invertebrate material was the dominant food item in the foreguts of 158 individuals collected from the Noordhoeks River (Olifants River catchment, CFR). Similarly, Lowe *et al.* (2008) found that aquatic invertebrates formed the dominant prey items in the guts of 30 individuals of *P. phlegethon* collected from the Rondegat River (Olifants River Catchment, CFR).

The view that redfin are primarily benthic invertivores is, however, not in agreement with results from earlier studies investigating their diet. In an examination of the digestive tracts of 30 individuals collected from the Keisers River near McGreggor (middle Breede River catchment, CFR), Esterhuizen (1978) found mostly detritus and very little animal material. Cambray & Stewart (1985), who examined the guts of 20 individuals captured in the Kogmanskloof River near Montague (middle Breede River catchment), found that they

contained mostly detritus, but that ostracods, copepods and chironomids were also present. Similarly, Shelton (2003) found algae and detritus to be the dominant food items in the guts of nine redfin from the Witte River in the upper Breede River catchment. All three studies concluded that Breede River redfin were primarily detritivorous.

The discrepancy between these earlier studies and the results of de Wet (1990) and the present study is potentially attributable to several factors. The composition of redfin diet varies on both a diel and a seasonal scale (de Wet 1990), and differences in timing of sample collection could therefore be at least partially responsible for the variation in diet composition among studies. The incorporation of stable isotope data in the present study, adds confidence to the conclusions drawn, however, since stable isotopes provide a more time-integrated picture of diet than that provided by snapshot surveys of fish gut contents (e.g. Esterhuizen 1978, Cambray & Stewart 1985).

Alternatively, variations in environmental conditions among sampling sites could influence the availability of different food sources, and thereby also influence the composition of redfin diet. Interestingly, while the present study and that of de Wet (1990) were conducted in swift-flowing upper reaches of tributaries, the studies of Esterhuizen (1978) and Cambray & Stewart (1985) were conducted in lower-lying river reaches where current velocity would likely have been slower. Well documented relationships between current velocity and the biomass of plant material that accumulates on the stream bed (Davies & Day 1998, Allan & Castillo 2007) suggest that the availability of detritus and algae may have been greater at the study sites sampled by Esterhuizen (1978) and Cambray & Stewart (1985). The slower flows, and relatively stable physical environments, at lower-lying sites could potentially explain the dominance of detritus in the guts of redfin collected at these sites. Despite the variation in redfin diet among studies, the balance of evidence indicates that in headwater stream environments, redfin feed primarily on benthic invertebrates.

Although trout are capable of consuming considerable quantities of aquatic invertebrates (Allen 1951, Huryn 1998), as drift feeders, their diet is influenced by the availability of different food sources in the drift (Nakano *et al.* 1999b, Laudon *et al.* 2005). For example, the contribution of aquatic invertebrates to trout diet is expected to be large where the drift is dominated by aquatic invertebrates, but may be lower in situations where non-aquatic

prey constitute a prominent proportion of the drift (Nakano *et al.* 1999b, Kawaguchi & Nakano 2001, Laudon *et al.* 2005). In the present study, the finding that aquatic invertebrates were less important in the diet of trout than they were in the diet of redfin could be a result of an abundant alternative food source that was available in the drift. In the streams in which the present study was conducted, terrestrial invertebrates were common in the drift, and this appears to have influenced the contribution of aquatic invertebrates to trout diet.

Terrestrial invertebrates

Terrestrial invertebrates, although generally less abundant in fish guts than were aquatic invertebrates, formed an important component of the diet of trout, but constituted only a relatively small component of the diet of redfin. Although present in the vast majority of trout guts (78.75%), terrestrial invertebrates were found only in 26.04% of redfin guts (Figure 4.4a, Table 4.1), and differences between species in the contribution of this food type to fish diet were significant for both number- and weight-based estimates of dietary composition (Tables 4.2 and 4.3). Furthermore, the *% IRI* of this food type for trout (44.17%) was more than six times greater than the *% IRI* of this food type for redfin (6.52%, Figure 4.5), and analysis of fish feeding selectivity revealed that, while redfin displayed moderate avoidance of terrestrial invertebrates, trout consumed them roughly in proportion to their availability in the drift (Figure 4.8b).

The results of the stable isotope mixing models were consistent with this pattern in that terrestrial invertebrates were found to contribute more strongly to the diet of trout than they did to the diet of redfin (Figure 4.13). Moreover, the relatively high $\delta^{15}N$ and trophic position of trout (Figure 4.11), in comparison to that of redfin, appeared to be driven largely by the consumption of terrestrial invertebrates (and potentially also aquatic predatory invertebrates), which tended to be notably enriched in $\delta^{15}N$ relative to other food sources. Taken together, my gut contents and stable isotope analyses indicate that the contribution of terrestrial invertebrates to the diet of trout was substantially greater than their contribution to the diet of redfin.

As benthic feeders, redfin would not be expected to consume substantial quantities of terrestrial invertebrates, and the finding that terrestrial invertebrates were generally not an prominent feature in their diet is consistent with the results of earlier studies investigating their diet (Esterhuizen 1978, Cambray & Stewart 1985, de Wet 1990). Interestingly, the present study found that terrestrial invertebrates were present in roughly a quarter of the redfin guts analysed, and contributed ~10% to redfin diet (7.58% by isotope mixing models, *% W* = 11.80%, *% N* = 12.63% by gut content analysis). Thus, although terrestrial invertebrates were not a major component of their diet, redfin were apparently capable of accessing and utilizing them as a food source.

Drift-feeding trout, on the other hand, have access to terrestrial invertebrates, as well as non-aquatic adult stages of aquatic invertebrates, that have fallen into the stream from the riparian zone and it is therefore not surprising that terrestrial invertebrates were an important food source for trout. Elsewhere, it has been found that terrestrial invertebrates can constitute a large proportion of the diet of trout (Kido *et al.* 1999, Nakano *et al.* 1999a, Kawaguchi & Nakano 2001, Nakano & Murakami 2001, Baxter *et al.* 2004, 2007, White & Harvey 2007), but this is not necessarily the case, and in some instances their occurrence in trout diet is rare (Huryn 1998, Buria *et al.* 2009). The degree to which trout rely on terrestrial prey appears to be linked to the relative availability of aquatic vs. terrestrial prey in the drift (Nakano *et al.* 1999a, Buria *et al.* 2009), which in turn is influenced by a suite of environmental factors such as the productivity of the aquatic system (Huryn 1998) and the productivity of adjacent riparian habitats (Edwards & Huryn 1996). The productivity in headwater streams in the CFR is generally relatively low (de Moor & Day 2013), and many of the aquatic invertebrate taxa inhabiting these streams are univoltine (one generation per year) (King & Day 1988, Davies & Day 1998, Dallas 2004). Furthermore, benthic invertebrate abundance in these streams is relatively low, and the body size of most invertebrate species at emergence relatively small, in comparison to other systems (King 1983). Thus, in these systems where the availability of aquatic invertebrate prey is relatively low, terrestrial invertebrates may be expected to constitute a relatively large proportion of the diet of drift-feeding fish. Indeed, the relatively high abundance of terrestrial invertebrates recorded in drift samples collected during the present study (Appendix 7) indicates that terrestrial invertebrates may well represent a potentially important food source for drift-feeding trout,

and the results from the gut contents and stable isotope analyses indicate that the consumption of terrestrial invertebrates by trout appears to have offset their consumption of aquatic invertebrates. This finding could potentially explain why benthic invertebrate abundance on the stream bed was greater in streams where trout have replaced native fish than uninvaded streams where native benthic-feeding fish are still plentiful (see Figure 4.14).

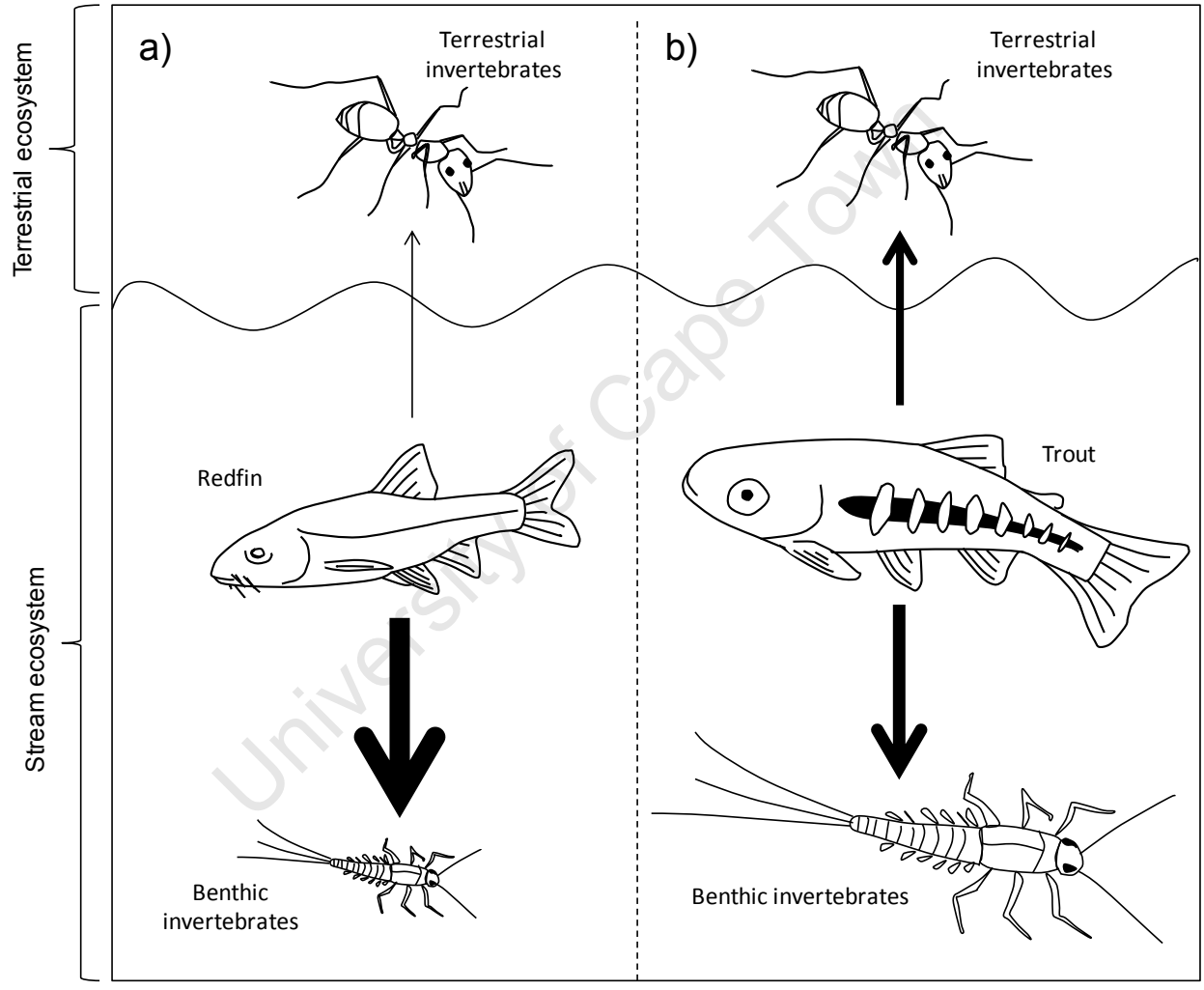

Figure 4.14 Relative predation pressure exerted by a) trout and b) redfin on the benthic and terrestrial invertebrates. Arrow thickness represents relative consumption of aquatic and terrestrial invertebrates by redfin and trout. Size of aquatic invertebrate image corresponds to the relative density of aquatic invertebrates on the stream bed.

Reliable data on the diet of trout in South African streams is surprisingly scarce, and to my knowledge, just two quantitative studies exist. Woodford (2002), who analysed the gut contents of 33 rainbow trout captured in autumn and 12 rainbow trout captured in spring 2002 in the upper Berg River (CFR) found that 96% of their guts contained terrestrial invertebrates, and that terrestrial prey accounted for 54% and 14% of their gut contents by number in spring and autumn, respectively. The remaining gut contents consisted of aquatic invertebrates, with Baetidae and Chironomidae featuring prominently, as was found in the present study. Lamberth (2001) who examined the gut contents of 11 trout collected from the Molenaars River (Breede River catchment, CFR), during summer 2000, found that gut contents were dominated by the ephemeropteran family Baetidae, and noted that all individuals were final instars that were preyed upon as they were emerging from the water surface. Unfortunately the data of Lamberth (2001) were presented only as the frequency of occurrence of individual prey items in all trout guts, and consequently assessment of the contribution of terrestrial invertebrates to total gut contents is not possible. It was, however, recorded that terrestrial Hymenoptera and Araneae both occurred in ~50% of the guts examined, which is consistent with the view that terrestrial invertebrates constitute a considerable proportion of the diet of trout in CFR headwater streams. Finally, additional support for this view comes in the form of numerous anecdotal reports from anglers that terrestrial invertebrates are frequently consumed by trout in CFR headwater streams (for examples, see Rolston 2004, Flemming 2009, Krige 2010).

Taken together, these limited data and observations are broadly consistent with the results of the present study and suggest that terrestrial invertebrates frequently form an important part of the diet of trout in headwater streams in the CFR, as is the case in other parts of the world (Kido *et al.* 1999, Nakano *et al.* 1999a, Kawaguchi & Nakano 2001, Nakano & Murakami 2001, Baxter *et al.* 2004, 2007, White & Harvey 2007).

Non-animal food sources

Algae and detritus, which were not often present in the guts of trout (*% O* <20%), were recorded in ~60% of redfin guts (Figure 4.4, Table 4.1), and although the mean proportional contribution of these dietary components was relatively small (<10%) for both species, redfin guts had a significantly greater mean proportional weight of both algae and detritus

than had trout guts (Table 4.2). These results suggest that, while trout consumed animal prey almost exclusively, redfin utilized a wider range of food types, and consumed a more omnivorous diet. The comparisons of niche breadth based on gut contents and stable isotopes, however, were not in support of the view that redfin occupied a broader trophic nice than trout did.

Plant material is probably a relatively non-nutritious food source relative to benthic invertebrates, but it may be that detritus becomes a "fall-back" food source at times when benthic invertebrates are scarce. Alternatively, other studies, including de Wet (1990) and Whitehead *et al.* (2007) have argued that plant material present in the guts of closely-related *P. phlegethon*. is ingested accidentally while foraging on benthic invertebrates, rather than being targeted directly as a food source, and therefore may not be digested and assimilated. If one considers the large number of benthic invertebrates consumed by redfin, and the behaviour and habitat of these invertebrates, this seems a plausible explanation for the presence of plant material, as well as sand, in the guts of redfin found during the present study. For example, several of the benthic invertebrate taxa recorded in redfin guts inhabit algal turfs that grow upon rocky stream substrates, and benthic invertebrates such as the dipterans Orthocladiinae and *Simulium*, both of which were frequently consumed by redfin, live in silken cases that have a tendency to trap detritus (de Moor 2003, Harrison 2003).

The hypothesis of accidental ingestion is lent further support by studies investigating relationships between the length of redfin bodies and the length of their guts. In general, the ratio of gut length to fork length (GL:FL) of fish increases with an increasingly herbivorous diet. Carnivorous fish have a GL:FL of approximately 1, herbivorous species generally have GL:FL ratios >10, and the GL:FL ratio of omnivorous species lies somewhere between these two values (Kruger & Mulder 1973). De Wet (1990) estimated the mean ± SE GL:FL of redfin to be 0.89 ± 0.33 for specimens shorter than 40 mm, and 1.24 ± 0.19 for specimens longer than 40 mm, indicating a predominantly carnivorous diet. On the other hand, an earlier study conducted by Skelton (1980) estimated the gut length to body length ratio (in this instance standard length was used instead of fork length) of redfin to be approximately 2-2.5, suggesting a somewhat more omnivorous diet, although the author noted that the variation among individuals was high.

The inclusion of stable isotope analysis in the present study allowed some insight into whether the plant material ingested was assimilated into the fish's body, or whether it was simply passed through the gut as undigested material. Results from the stable isotope mixing models were broadly consistent with the gut content results in that plant material was found to contribute more to the diet of redfin than to the diet of trout, and that the contributions of both algae and detritus to the diet of both fish species were relatively small (<10%, Figure 4.13a). The finding that the median contribution of plant material (i.e. algae and detritus combined) to redfin diet was ~25% (Figure 4.13b), suggests that redfin do in fact digest and assimilate at least some of the plant material that they ingest. This result should, however, be treated with some caution since the mixing models also estimated that plant material comprised nearly 20% of the diet of trout, a species that is widely known to be an obligate carnivore (de Moor & Bruton 1988, Skelton 2001). Perhaps the similarity of the isotopic signatures of algae and certain aquatic invertebrate FFGs at some of the sampling sites (Figure 4.12) made it difficult for the mixing models to distinguish between the contributions of these sources fish diets (Phillips & Gregg 2003), and the contribution of algae to fish diets may therefore have been overestimated. Regardless of whether or not plant material is utilized by redfin as a food source, the fact remains that it made up a relatively minor proportion of the gut contents in comparison to invertebrate prey, and the balance of evidence supports the view that redfin is primarily a benthic invertivore.

4.4.4 Strengths, weaknesses and future directions

This study suffered from several weaknesses that need to be recognized and taken into consideration when interpreting the results, but it also had some important strengths that deserve to be highlighted. As already touched on above, there were some notable temporal and spatial limitations in the sampling design employed in my study. Furthermore, the fact that energetic requirements of redfin and trout were not quantified, the fact that ontogenetic shifts in diet were not considered, the fact that the trophic niches of the other indigenous species kurper and galaxias were not investigated, and the difficulties inherent in decisions regarding the choice of food sources to include in stable isotope mixing models need to be acknowledged.

Temporal scale

The diet of stream fish is temporally variable, both on a diel and on a seasonal scale (Angradi & Griffith 1990, Gelwick & Matthews 2007, Buria *et al.* 2009). The present study only captured a mid-afternoon, summer snapshot of redfin and trout gut contents in the study streams, and thus did not to take into account temporal variations in the availability and consumption of different food sources. The incorporation of stable isotope data, however, added a time-integrated perspective to the high resolution snapshot obtained from the gut content data. The fact that the results of the gut contents analysis were, for the most part, corroborated by the results of the stable isotope analysis constitutes good evidence that the differences in diet detected between trout and redfin were authentic and not just some artifact of an imperfect sampling design. Regardless, future studies of this type would do well to incorporate diel and seasonal components into the sampling design, because this would enable a more comprehensive understanding of temporal fluctuations in fish diets, and in the relative functional roles that they perform in stream food webs.

Spatial scale

Although the sample size for each species at each site was respectable (23-36 individuals per species per stream), the fact that each species was only sampled in three streams raises the question of how well the results from the present study would "scale up" to the landscape level. This issue could be addressed by collecting samples of each species from a greater number of streams, although the threatened status of redfin may pose an ethical obstacle to acquiring such samples. Despite this limitation, the present study nonetheless represents an expansion in spatial scale relative to previous studies of redfin (Esterhuizen 1978, Cambray & Stewart 1985, de Wet 1990) and trout (Lamberth 2001, Woodford & Impson 2004) diets in CFR streams which only collected samples from a maximum of two streams.

Variation in fish diet among streams

Because the focus of the present study was on characterizing and comparing the feeding habits of redfin and trout in allopatry, rather than in sympatry, differences in the availability of different food types among sites could potentially confound inter-specific differences in gut content composition. Indeed, the nested PERMANOVA on fish gut content composition by weight detected a significant site effect (Table 4.2) indicating that variation in fish diet among streams was significant. In the case of invertebrate prey, inter-site differences in availability were accounted for by relating the abundance of prey items in fish guts to their availability in the stream. Similarly, natural inter-site differences in isotope signatures of fish, invertebrates and lower trophic levels could potentially confound comparisons of trophic niche and food source contribution between species. I was, however, able to control for this by correcting for inter-site differences in the $\delta^{15}N$ of basal resources prior to analysis.

Differences in energy requirements

In addition to variations in predator population biomass and predator diets, the top-down effects of predators in a community can also be influenced by species-specific energy requirements (Williams *et al.* 2004). The energy requirements (i.e. consumption rate) of redfin and trout were not measured in the present study, but differences in the energy requirements among fish species have been shown to influence their net top-down effects in stream ecosystems (Huryn 1996, 1998). The fact that redfin are active foragers, while trout largely feed passively from the drift, suggests that the *per capita* energy requirements of redfin may exceed that of trout. This could potentially be an additional factor contributing to stronger top-down control by redfin than by trout. Furthermore, although the mean fish biomass in invaded and uninvaded streams was relatively similar, the mean fish density at sites without trout (i.e. redfin-dominated sites) was significantly greater than that at sites where trout had invaded (i.e. trout-dominated sites). Differences in the mean density of redfin and trout populations could also potentially influence the predation pressure experienced by aquatic invertebrates in these streams. Future studies should therefore look to quantify the *per capita* energetic requirements of native vs. non-native

fish predatory fish in CFR streams. Despite these alternative explanations, the differences found in the trophic niches occupied by redfin and trout are likely an important driver of the differences in the trophic structure of communities in streams with and without trout.

Ontogenetic shifts

Although most fish species undergo dietary changes as they grow, ontogenetic shifts in feeding behaviour were not taken into account in the present study. Studies investigating ontogenetic changes in the diet of trout are common (Mittelbach & Persson 1998, Macchi *et al.* 1999, Arismendi *et al.* 2012), and in general show that while juvenile trout feed mostly on invertebrates, larger individuals are increasingly likely to consume vertebrate prey such as fish and amphibians. Redfin also appear to display an ontogenetic shift in diet, in that larger individuals appear to consume a more omnivorous diet than do smaller individuals (de Wet 1990). Incorporating body size as a factor in future analyses of feeding habits could provide additional insight into the functional roles performed by redfin and trout in the study streams. The fact that weight and length measurements were taken for all fish sampled in the present study means that such an analysis could potentially be conducted on the present data set in the future. The focus of the present study was, however, on inter-specific differences in diet, and the fact that samples were randomly collected and encompassed the full size range of individuals observed in the wild suggests that the sample of fish analysed is probably a good representative of the populations occurring in the study streams.

Kurper and Galaxias

It is important to keep in mind that only one of the three indigenous fish species commonly found in headwater streams of the upper Breede River Catchment was included in the present study. Thus, to fully appreciate the functional role of the native fish assemblage as whole, future studies should look to incorporate the dietary habits of kurper and galaxias as well. Quantitative data on the feeding habits of these two species is scarce. Kurper are ambush hunters that favour slow-glowing habitats where aquatic vegetation is abundant

(Harrison 1952b, Cambray 1990, Skelton 2001). They feed mostly on aquatic invertebrates from the benthos, but larger individuals are capable of consuming larger prey items such as fish and amphibians (Cambray 1990, Lamberth 2001, Skelton 2001, Shelton 2003). Consumption of aquatic invertebrates by kurper probably contributes to the relatively low density of benthic invertebrates at sites without trout. Galaxias, on the other hand, prefer faster-flowing habitats (Gore *et al.* 1991, Shelton *et al.* 2008) and feed primarily on small aquatic invertebrates drifting in the water column (Harrison 1952c, Lamberth 2001, Skelton 2001). As drift feeders, terrestrial invertebrates that fall into the stream may constitute an important component of the diet of galaxias as has been found for other galaxias species elsewhere (McIntosh & Townsend 1995a, McDowall 2006), and consequently the predation pressure that they exert on benthic invertebrate prey may be weaker than that exerted by benthic-feeding redfin and kurper, but their diet remains to be thoroughly studied. Despite the fact that kurper and galaxias were not included in my study, the fact that redfin was by far the numerically and gravimetrically dominant member of the native fish assemblage suggests that it was likely responsible for a large proportion of the predation pressure exerted by the native fish assemblage in these streams.

Food sources in stable isotope mixing models

One of the greatest challenges in using stable isotope data to partition the diet of consumers is the decision of which food sources to include in mixing models (Post 2002, Phillips & Gregg 2003, Moore & Semmens 2008). In the present study, source inclusion was based on the major food items present in the guts of redfin and trout, as recommended by (Clarke *et al.* 2005). However, the fact that no solution could be found for five trout and seven redfin samples suggests that food sources other than those included may also be utilized by redfin and trout. For example, in addition to the food sources included, trout are known to consume fish, amphibians and crabs (Skelton 2001, Woodford & Impson 2004), and failure to include such prey types may well have been a source of error in the mixing model estimates of food source contributions. A potential additional source of error is the fact that the aerial stages of aquatic invertebrates were not included in the mixing models, and this may have led to an underestimation of the contribution of non-aquatic invertebrate

prey to fish diets. Future studies using mixing models to partition fish diets could include additional food sources such as those suggested here, although model computation becomes increasingly complex as the ratio of sources relative to the number of isotope elements used increases (Phillips & Gregg 2003).

4.4.5 Significance of the findings

The results from the present study show that trout and redfin (the dominant member of the native fish assemblage) differ in foraging mode, and in the diet that they consume, suggesting that the replacement of native fish by non-native trout has altered the functional role performed by top predators in these streams. This finding has important implications for managers charged with conserving aquatic biodiversity, and the integrity of aquatic ecosystems, in the CFR, because changes in the functioning of predator assemblages can potentially alter the structure and function of entire aquatic communities. Indeed, differences in community structure between streams with and without trout in the upper Breede River catchment (see Chapter 3) appear to be linked to differences in the trophic niches occupied by trout and redfin (Figure 4.14). The relatively low density of benthic invertebrates in streams supporting healthy native fish populations (i.e. lacking trout) could be attributed to the fact that redfin are strongly reliant on aquatic invertebrate prey. Specifically, the relatively strong predation pressure exerted by redfin on herbivorous invertebrates, such as collector-gatherers, could account for their relatively low abundance at sites lacking trout, and also for the relatively high biomass of benthic algae at these sites. On the other hand, the high density of benthic invertebrates (and correspondingly low algal biomass) at sites where trout have established and replaced native fish could be a consequence of the fact that the importance of terrestrial invertebrates in trout diet offsets the predation pressure they exert on aquatic invertebrates. In the following chapter, a manipulative field experiment is used to test the hypothesis that these functionally-distinct predators (i.e. an active benthic feeder vs. a passive drift feeder) have different top-down effects on the trophic organization of stream food webs as alluded to by the results of comparative field surveys.

Chapter 5

Relative top-down effect of native benthic-feeding redfin vs. non-native drift-feeding trout on benthic community structure in a Cape Floristic Region headwater stream

5.1 INTRODUCTION

Insectivorous fish are commonly the apex predators in streams, but their role as organizers of stream community structure remains unresolved (Williams *et al.* 2003, Meissner & Muotka 2006, Winckler-Sosinski *et al.* 2008, Cheever & Simon 2009, Winkelmann *et al.* 2011). Through selective predation, fish can suppress the abundance of certain invertebrate taxa, and thereby regulate the density (Flecker & Townsend 1994, Olsson *et al.* 2006, Herbst *et al.* 2009), and composition (Harvey 1993, Rosenfeld 2000, Herbst *et al.* 2009, Winkelmann *et al.* 2011), of invertebrate assemblages on the stream bed. In some cases, fish effects on invertebrate assemblages can translate into cascading effects on resources, such as algae (Flecker & Townsend 1994, Biggs *et al.* 2000, Nyström *et al.* 2003, Herbst *et al.* 2009, Buria *et al.* 2010), and detritus (Ruetz *et al.* 2002, Greig & McIntosh 2006, Buria *et al.* 2010), at the base of the food web. On the other hand, predatory fish sometimes have little influence over the trophic organization of stream communities at all (Allan 1982, Ruetz *et al.* 2004, Meissner & Muotka 2006, Zimmerman & Vondracek 2007, Winckler-Sosinski *et al.* 2008). These varying results raise the question: why are fish sometimes important regulators of stream community structure, but sometimes not?

Variation in fish effects among studies has been attributed to a range of environmental and biological factors, and also to methodological differences. Temporal and spatial variability in environmental factors such as habitat structure (Power 1992a, Rosenfeld 2000), temperature (Cheever & Simon 2009), pH (Olsson *et al.* 2006), current velocity (Rosenfeld 2000, Cheever & Simon 2009, Ludlam & Magoulick 2009) and ecosystem productivity (Power 1992b) have all been linked to variations in the strength of fish impacts on stream communities. For example, the structural complexity of stream habitats has been found to

strongly influence the strength of top-down effects of predatory fish. Predator impacts on prey in simple habitat types, such as smooth boulders or bedrock, tend to be stronger than in complex habitat types such as cobble or gravel beds (Power 1992a, Diehl 1993, Dahl & Greenberg 1998, Rosenfeld 2000). The reason for this is that the interstitial spaces that occur in complex habitats increase the availability of refugia for prey, rendering prey less vulnerable to predation by fish than in simpler habitats offering no such refugia.

The biological characteristics of both predator and prey assemblages can also influence the outcome of fish predation in streams. Although studies examining top-down fish effects generally focus on the role of an individual fish species, streams often support more than one type of predator. In situations where different species of predator co-occur, interactions between predators can influence the foraging behaviour, and ultimately the top-down effect, exerted by an individual predatory species (Sih & Wooster 1994, Nyström *et al.* 2001, Nilsson *et al.* 2008). Interspecific interactions may benefit the predatory species involved (facilitation), or affect them negatively (competition), depending on species-specific behavioural traits (Sih *et al.* 1998). Although such interactions are most commonly reported to occur between different species of predatory fish, interactions between fish and predatory invertebrates can also influence fish effects in stream food webs (Harvey 1993, Dahl 1998a, Nilsson *et al.* 2008). Benthic invertebrates are commonly important prey for stream fish (Allan & Castillo 2007), and variation in the functional composition of benthic invertebrate assemblages among systems can influence the vulnerability of invertebrates to predation by fish (Rosenfeld 2000). For example, invertebrates that feed on algae and associated material on the surfaces of stones are more vulnerable to fish predation than invertebrates that feed on accumulations of detritus within substrate interstices (Rosenfeld 2000). Thus, invertebrate assemblages dominated by herbivorous (i.e. algae-consuming) species may be more susceptible to the effects of predatory fish than are assemblages dominated by detritivorous species (Bechara *et al.* 1992, Molineri 2008).

Differences in methodologies among studies examining top-down effects of predatory fish are no doubt an important source of variation in reported impacts (Cooper *et al.* 1990, 1998, Dahl 1998b, Meissner & Muotka 2006, Winkelmann *et al.* 2011). In cases where small-scale experiments are used to evaluate fish effects, differences in prey exchange rates between experimental enclosures that hold fish and the natural stream may be an

important source of variation in how prey populations respond to fish predation (Cooper *et al.* 1990). Cages with large mesh sizes allow high rates of prey migration into, and out of, enclosures, and this can overshadow the top-down influence of predatory fish. On the other hand, the movement of prey into, and out of, enclosures with small mesh sizes will be low, and fish effects consequently likely to be exacerbated. Furthermore, it may not be possible to establish whether observed effects on prey assemblages are a result of direct predation, prey emigration or a combination of these two processes (Meissner & Muotka 2006). Experimental results can also be influenced by the spatial and temporal scale of the experiment (Englund & Cooper 2003). For example, experiments conducted in small enclosures, and for short time periods, are less likely to capture natural spatial and temporal heterogeneity in abiotic and biotic processes than larger-scale, longer-term, investigations are, enhancing the probability of detecting significant predation effects (Peckarsky *et al.* 1997).

An alternative explanation for the inconsistent effects of fish among studies is that the foraging mode of a fish dictates its top-down influence in the stream community. Streams generally contain two types of fish predators, benthic feeders and drift feeders (Dahl 1998b). While benthic-feeding fish mostly consume benthic prey on the stream bed, drift feeders consume the majority of their prey in the water column and consequently may consume large numbers of terrestrial invertebrates that fall into the stream from the riparian zone (Nakano *et al.* 1999a). Based on these observations, Dahl & Greenberg (1996) proposed the hypothesis that benthic-feeding fish should have a stronger impact on benthic prey than do drift-feeding fish, because terrestrial invertebrates often constitute an important component of the drift. To evaluate this hypothesis, they conducted a meta-analysis incorporating the results from ten studies that manipulated these two types of predators in streams, and found that, indeed, benthic-feeding fish had stronger impacts on benthic prey assemblages than did drift-feeding fish (Dahl & Greenberg 1996). However, the results of subsequent studies evaluating Dahl & Greenberg's (1996) “foraging mode” hypothesis have been equivocal. For example, while the results of some studies are consistent with the hypothesis (e.g. Dahl 1998a, Cheever & Simon 2009), other studies (Ruetz *et al.* 2004, Inoue & Miyayoshi 2006, Zimmerman & Vondracek 2007) have found

that benthic feeders did not suppress the density of benthic prey more strongly than did drift feeders.

Improving our understanding about the role of fish in stream trophic dynamics is becoming more and more important as native fishes worldwide become increasingly threatened by factors such as habitat loss and fragmentation, hydrologic alteration, climate change, overexploitation, pollution and invasions by non-native species (Dudgeon *et al.* 2006, Vitule *et al.* 2009). Indeed, a capacity to predict the consequences of changes in the structure and function of fish assemblages, and manage associated aquatic systems accordingly, will rely heavily on a thorough understanding of the factors dictating the strength of top-down effects of fish on stream community dynamics (Cheever & Simon 2009).

In South Africa, non-native rainbow trout have partially invaded the upper Breede River Catchment in the Cape Floristic Region (CFR), in that some streams have been invaded by trout, but others have not. In streams where trout have established, they have largely replaced once-abundant native fish species and are now the dominant component of the stream fish assemblage (Chapter 2). The fact that the dominant native fish species Breede River redfin *Pseudobarbus* sp. "Burchelli Breede" (henceforth "redfin") is primarily a benthic feeder, while non-native rainbow trout *Oncorhynchus mykiss* (henceforth "trout") that have replaced them is a drift feeder (Chapter 4), presents a valuable opportunity to compare top-down effects of fish with different foraging modes within the same catchment, and thereby evaluate the "foraging mode" hypothesis proposed by Dahl & Greenberg (1996).

Field surveys conducted in 24 headwater streams in the upper Breede River catchment have revealed that the structure of benthic communities in trout-free streams supporting healthy native fish populations is different to that in streams where non-native trout had established and replaced the native species as the dominant top predator (Chapter 3). Differences in total density, and in the taxonomic and functional composition, of benthic invertebrate assemblages, as well as differences in the biomass of benthic algae, were detected between streams with and without trout. Importantly, the density of certain herbivorous invertebrates (particularly collector-gatherer taxa) at sites dominated by trout was significantly higher than that at sites with native fish only. Furthermore, this pattern appeared to be driven by the fact that trout are weaker regulators of these invertebrates

than are the native fish (Chapter 4). Additionally, the surveys showed that the biomass of benthic algae in streams where trout are absent is significantly greater than that in streams where trout are present, presumably a consequence of increased grazing pressure in the streams invaded by trout. These findings are consistent with Dahl & Greenberg's (1996) "foraging mode" hypothesis in that the suppression of benthic invertebrates by drift-feeding trout appears to be weaker than that by the native fish assemblage which is dominated by benthic-feeding redfin. Furthermore, the findings imply that these differential top-down effects on invertebrate assemblages cascade down to the base of the food web, resulting in a reduction in the biomass of benthic algae in streams containing trout.

While studies of this type are useful for describing ecological patterns at broad, realistic spatial scales, they cannot be used to resolve cause-and-effect type relationships between predators and their prey, because of the possibility that an unmeasured variable correlated with prey abundance may be responsible for the observed patterns (Park 2004, Greenlees *et al.* 2006). Resolving the mechanisms underlying such patterns requires the use of experiments that allow the manipulation of predatory fish while controlling for all other potential sources of variation (Kats & Ferrer 2003, Park 2004). In the present chapter I therefore used a small-scale, manipulative field experiment to ascertain whether native redfin (the dominant member of the native fish assemblage) and non-native trout differ in their top-down effect on the stream community structure, as hypothesized from results of the broad-scale surveys of community composition. Specifically, the "foraging mode" hypothesis proposed by Dahl & Greenberg (1996) was evaluated by testing the prediction that benthic-feeding redfin should have a stronger predatory impact on benthic invertebrate assemblages than should drift-feeding trout. An additional prediction was that the differential effects of redfin and trout on invertebrate assemblages would cascade down to the base of the food web and result in a lower biomass of benthic algae in the presence of trout than in the presence of redfin, as implied by the results of the broad-scale field survey.

5.2 METHODS

5.2.1 Study site

The experiment was conducted in Morainekloof Stream, a short, steep, clear-flowing tributary in the north-east corner of the upper Breede River catchment. It sources in the Hex River Mountains at an elevation of ~1700 m, and flows in a southerly direction for ~5 km before it joins Buffelshoek Stream (Figure 5.1). Buffelshoek Stream then joins Sandriftkloof Stream which then flows into the Hex River, one of the largest tributaries of the upper Breede River.

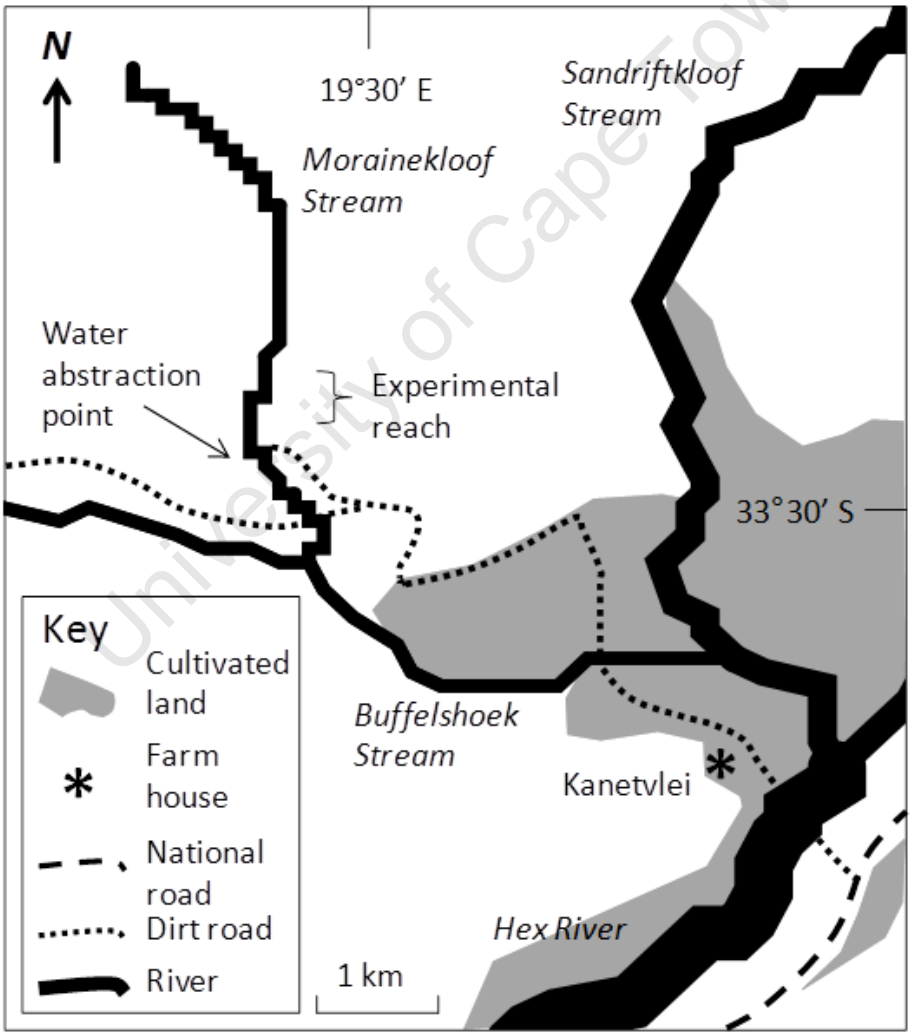

Figure 5.1 Location of the experimental reach on Morainekloof Stream, a headwater tributary in the upper Breede River catchment. Other streams in the area, as well as key features of the landscape, such as cultivated land, roads and farm houses, are also indicated.

The location of the experimental site within the context of the broader study area is indicated in Figure 2.1, Chapter 2, and the GPS coordinates of the site can be found in Appendix 1b. Morainekloof Stream was chosen for the experiment because it has road access, and because it was one of the few sites where trout and native fish species co-occur, enabling both species to be manipulated within the same stream. Redfin and trout were the most abundant species at the site, with an average (mean ± standard error (SE)) density of 2.25 ± 0.16 fish/100 m^2 and 1.93 ± 0.50 fish/100 m^2 respectively (see Chapter 2, Table 2.1). Cape kurper *Sandelia capensis* (henceforth "kurper") was also present but its density (0.80 ± 0.16 fish/100 m^2) was lower than that of trout or redfin. Cape galaxias *Galaxias zebratus* (henceforth "galaxias"), although not detected during the field surveys, was recorded during the experiment, but the density of this species appeared to be very low. In general, environmental conditions in Morainekloof Stream are typical of headwater streams in the upper Breede River catchment. The experimental reach consisted of alternating sections of erosional (including runs and riffles) and depositional habitats (pools).

The following information is based on measurements taken at the site during March 2010 (see Chapter 2, Section 2.2.5 for methodological details), roughly one year before the experiment was conducted, but during the same season (summer). The average stream width at the site was 4.14 ± 1.23 m, and the average depth was 0.28 ± 0.02 m. Flows ranged from no detectable flow in depositional habitats to a maximum of 0.70 m/s in erosional habitats, and the average flow at the study site was 0.54 ± 0.13 m/s. Substrate at the site consisted predominantly of cobbles and boulders, and the mean substrate particle length was 385.67 ± 70.09 mm. The water was acidic, with a mean pH of 4.83 ± 0.09, and conductivity (mean conductivity: 10.35 ± 0.04 µS/cm) and turbidity (mean turbidity: 0.35 ± 0.11 NTU) were low, which is typical of headwater streams in the area (Dallas and Day 2007). Water at the study site was well oxygenated, with the mean % oxygen saturation estimated at 83.17 ± 0.78%. Mean water temperature was 21.07 ± 0.02 °C, and canopy cover at the site was approximately 33%. The elevation at the study site was 458 m asl, and stream gradient at the experimental reach was 0.044. Morainekloof is an oligotrophic stream, with levels of phosphates (PO_4^{3+}), nitrates + nitrites (NO_3^- and NO_2^-) and ammonium (NH_4^+) recorded as 0.026, 0.0006 and 0.053 mg/L, respectively. For most of its length, the stream is unaffected by human activities. However, water is abstracted from a weir directly

downstream of the experimental reach, ~1 km upstream from its confluence with Buffelshoek stream (Figure 5.1). Vegetation in the Morainekloof catchment is predominantly indigenous Sandstone Fynbos (Rebelo *et al.* 2006), but non-native plant species (*Acacia* and *Pinus* spp.) were also present, but at a very low density. Riparian vegetation at the study site was entirely indigenous, and consisted mostly of broad-leaved woody species of scrub, perennial shrubs and small trees, with *Salix mucronata* (the Cape silver willow) and *Metrosideros* spp. featuring prominently in the riparian zone.

5.2.2 Experimental design

This experiment was designed with the objective of examining the relative influence of native redfin and non-native trout on benthic community structure in the study stream. Therefore, three treatments were established, including a treatment containing trout, a treatment containing redfin and a treatment without any fish which acted as a control against which fish effects could be assessed. The experiment was set up according to a randomized complete block design (Quinn & Keough 2002), with a total of four experimental blocks, each containing all three treatments (thus the total number of experimental units was n = 12, Figure 5.2a). Because of the narrow width of the stream over the experimental reach, the treatments within each block were arranged longitudinally (such that each treatment was either upstream or downstream of the other treatments within the same block), and the order of treatments within blocks was assigned randomly. All four blocks were situated in erosional habitat, and blocks were separated by a minimum of 20 m of stream.

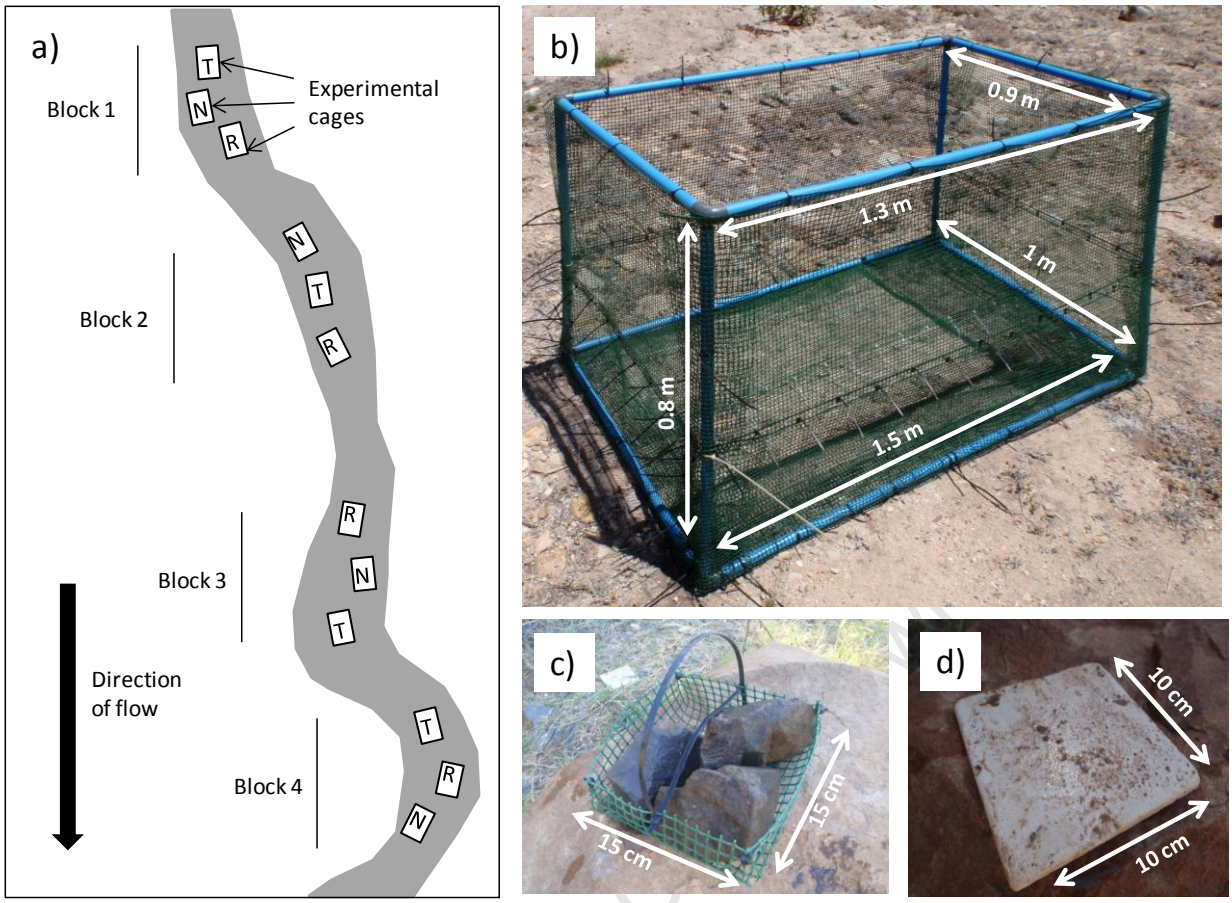

Figure 5.2 a) Aerial view of the experimental reach, showing locations of blocks and treatments within each block (N = no fish, T = trout, R = Redfin), b) experimental cage, c) mesh basket for invertebrate and organic matter sampling, and d) stone tile used for sampling algae.

Fish were manipulated using plastic cages that were 1.5 m long and 1 m wide at the base, 1.3 m long and 0.9 m wide on top and 0.8 m high (Figure 5.2b). This design allowed the cages to be stacked vertically upon one other, which was advantageous for logistical reasons. Cage frames were constructed from 40 mm diameter Polyvinyl chloride (PVC) pipe, and lined on the bottom and sides with 10 mm diameter plastic mesh using plastic cable ties. A 10 mm mesh size was chosen because it was sufficiently small to contain fish within the cages, yet large enough to allow invertebrates occurring in the stream to move freely in and out of the cages. Each cage was installed by clearing a layer of substrate from the streambed using a 1 x 1.5 m template, and then inserting the cage into the cleared rectangle of streambed (Zimmerman & Vondracek 2007). The substrate particles removed during clearing were then replaced on top of the cage base so that cages were lined with a layer of

natural substrate particles flush with the surrounding streambed. The stones inside the cages anchored them to the streambed, and provided natural substrate and cover for fish and invertebrates (Meissner & Muotka 2006). The cages were installed in such a way that water depth within each cage was 0.25 – 0.30 m, roughly standardizing the volume of water contained by each cage. Cages were fitted with removable 50 mm diameter plastic mesh covers on top using cable ties. The covers were used to prevent other animals (such as birds, otters and baboons) from accessing and disturbing the experimental cages. Fish were placed into the cages on 28 January 2011, marking the beginning of the experimental period. The experiment was left to run for 30 d, and was terminated on 26 February 2011. Samples of invertebrates and algae were collected at the end of the experiment, while environmental parameters were measured midway through the experimental period. Cages were checked twice a week to make sure that they were still intact, and any debris that had accumulated on the cage walls was removed to promote natural current flow through the cages. Figure 5.3 shows one of the four experimental blocks after cage installation.

Figure 5.3 Experimental block 2 showing three cages within one experimental block arranged parallel to the direction of flow in erosional habitat.

Fish

Fish for the experiment were captured using fyke nets set in the study reach overnight on the two nights before the experiment begun (26 and 27 January 2011). Two fyke nets, with a basal diameter of 600 mm and a mesh size of 2 mm, were set each night and checked the following morning. All redfin and trout captured were removed from the fyke nets using a small hand net, and held in aerated plastic buckets containing stream water, cobbles and invertebrates (for food) for a maximum of 48 h before being processed. Any kurper and galaxias captured were returned to the stream. On the day that the experiment was initiated (28 January 2011), fish were removed from the plastic buckets using a hand net and anesthetized with 2-Phenoxy-ethanol (MS222) so that minimal stress was incurred by fish during processing. Once under anesthesia, fish were weighed to the nearest 0.01 g and measured (TL) to the nearest 1 mm. After processing, fish were revived, and those that had been selected for use in the experiment were transported in buckets of fresh stream water, and released into the cages to which they had been assigned. All other fish were released back into Morainekloof Stream.

The fish treatments consisted of either two redfin, or two trout, and I attempted to match the total combined weight of the two redfin to that of the two trout placed in cages within a block as closely as possible, so that any differences in benthic community structure between treatments at the end of the experiment could be attributed to species, rather than biomass, or density, effects. The density of redfin in the cages (133.33 fish/100 m^2) fell within, but towards the upper and of, natural redfin densities in headwater streams in the broader study area (see Chapter 2, Table 2.1). The density of trout in the cages (133.33 fish/100 m^2) was, however, somewhat higher than the maximum trout density recorded in headwater streams in the study area (102.32 fish/100 m^2). The biomass of both redfin (10.94 – 14.13 g/m^2) and trout (10.03 – 14.12 g/m^2) in the cages was somewhat higher than the maximum biomass estimates of natural populations for these species in the broader study area (redfin: 4.45 g/m^2, trout: 4.90 g/m^2).

The length of the fish stocked into the cages was based on the size distributions of naturally occurring populations of redfin and trout in the broader study area. The size distribution of redfin, as based on snorkel-based length estimates of all individuals encountered at the 12

sites lacking trout, was bimodal, with distribution peaks occurring at roughly 30 – 50 mm and 60 – 80 mm TL (Figure 5.4a). On the other hand, the size distribution of trout, based on measurements of all individuals encountered at the 12 sites where they occurred, was unimodal, with a broad distribution peak occurring roughly between 50 – 90 mm TL (Figure 5.4b). I therefore attempted to select redfin and trout that were roughly 80 mm TL, because this length fell within size distribution peaks of both species. Actual lengths of fish used were influenced by the size ranges of fish captured for the experiment, and ranged from 77 – 96 mm for redfin, and 83 – 103 mm for trout.

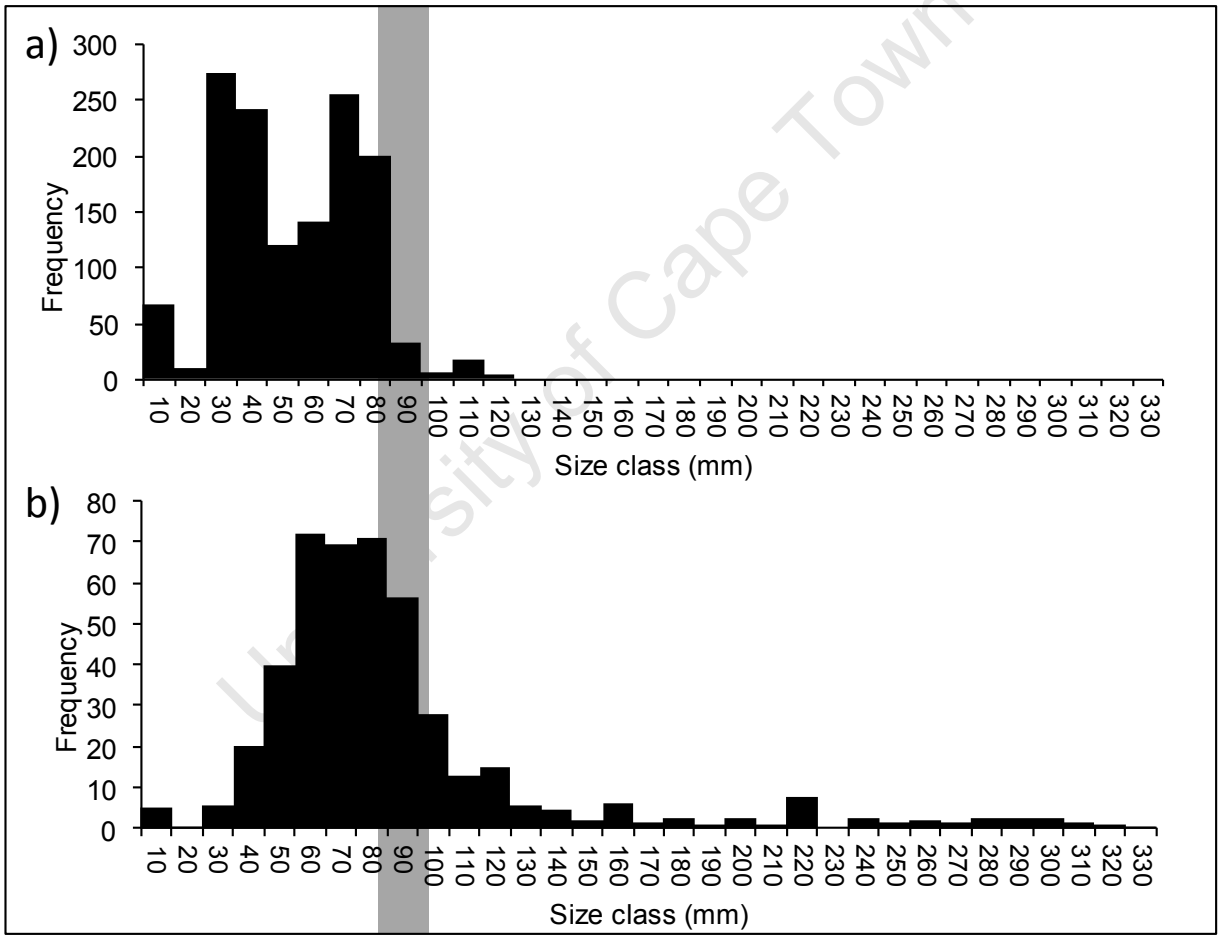

Figure 5.4 Size frequency distribution for (a) redfin (*n* = 1404) and (b) trout (*n* = 447) based on snorkel-survey estimates of fish populations in 24 streams in the upper Breede River catchment. The grey bar indicates the size ranges of fish used in the experimental cages.

Invertebrates

Plastic mesh baskets containing substrate particles from the stream were used as a standardized sampling unit for benthic invertebrates and organic matter within the cages (Peckarsky 1986). Baskets had square 150 x 150 mm bases, were 50 mm tall, were constructed from 10 mm plastic mesh, and were fitted with a plastic handles so that at the end of the experiment they could be easily removed from cages with minimal disturbance to surrounding substrate (Figure 5.2c). Each basket received three fist-sized stones (maximum diameter: 80 – 120 mm) which had been randomly collected from erosional habitats within the study reach. The stones were cleaned of invertebrates using a squeegee and fine forceps, but the periphyton layer was left intact (Zimmerman & Vondracek 2007). Four stone-containing baskets were randomly assigned to each cage on 12 January 2011, 16 days prior to the beginning of the experiment so that they could be colonized by invertebrates. This was deemed a sufficient colonization period since other studies (Rosenfeld 2000, Cheever & Simon 2009) have shown that invertebrate assemblages in cobble baskets represent the assemblage on the natural stream bed after seven days.

Algae

Stone tiles were used to sample the biomass of benthic algae in the cages (Figure 5.2d). Tiles were used instead of stones from the stream because they offer a standardized size, shape and surface texture, and therefore reduce natural variation in algal biomass (Rosenfeld 2000). The biomass of algae on tiles placed in streams is usually representative of that on natural stones after a one month period of incubation in the stream (Lamberti & Resh 1983, Dahl 1998b). I placed all tiles in an erosional reach within the study area 44 days before the experiment begun so that they could be colonized by algae. At the onset of the experiment, tiles were carefully removed from the erosional reach, transported while submerged in water in plastic trays, and four tiles were randomly assigned to each of the 12 experimental cages.

5.2.3 Sample collection

The samples for this study were collected under permit 0035-AAA 007-00057 issued from Cape Nature, and animal ethics clearance was obtained from the University of Cape Town.

Environmental parameters

A set of physico-chemical parameters were measured inside each cage between 11h00 and 14h00 on 14 February 2011, roughly halfway through the experiment. All parameters were measured at three random locations within each cage, with the exception of canopy cover which was estimated as total percentage cover. Depth was recorded using a calibrated depth rod placed vertically on the streambed at each point. The stone on which the depth rod was placed was then measured by recording maximum particle diameter using plastic callipers or a tape measure. The average flow of the water column at each point was measured with a Global FP101 Digital Water Velocity Meter. Temperature and dissolved oxygen were measured with a Crison OXI45 oxygen meter, pH with a Crison pH25 meter, conductivity with a Crison CM35 conductivity meter, and turbidity with a Hach 2100P turbidimeter. Percentage canopy cover was estimated from photographs taken directly upwards from the centre of each cage.

Invertebrates and organic matter

Beginning at block 4 (the downstream-most block) and working upstream, the cage covers were removed and samples of benthic community components were collected on 26 February 2011. The mesh baskets were individually removed from each cage by lifting them off the cage bottom into a square-framed hand net (300 x 300 mm frame, 250 µm mesh size). The contents of the net were then transferred into a plastic sorting tray containing 5 L of stream water, and all invertebrates clinging to the stones and baskets were removed using a squeegee and fine forceps, and retained in the tray. The contents of the tray were then passed through a 250 µm sieve and preserved in 70% ethanol for later processing of invertebrates and organic matter in the lab.

Algae

The stone tiles were carefully removed from each cage so that samples for algae analysis could be collected. Each tile was placed into a plastic tray containing 300 ml stream water and scrubbed for two minutes with a toothbrush. The resulting slurry was then poured into a plastic sample jar, held on ice in the field, and frozen in the dark within three hours of collection (Biggs & Kilroy 2000).

Fish

Once all tiles and mesh baskets had been removed from a cage, the remaining substrate particles were removed and returned to the stream. Each cage was then lifted from the stream bed, and the fish were removed using a hand net, euthanased with a lethal dose of MS222, measured and weighed to the nearest 1 mm and 0.01 g, respectively, and preserved in 70% ethanol for processing back at the laboratory. No fish disappeared from any of the experimental cages and all fish were alive at the end of the experiment.

5.2.4 Laboratory methods

Environmental parameters

The photographs of canopy cover were superimposed over a grid consisting of 200 grid squares and the number of grid squares in which canopy was present was divided by 200 in order to estimate percentage canopy cover over each cage.

Fish

Once back in the laboratory, fish were defrosted and their stomachs removed and dissected so that recently-consumed food items could be examined. Data on the gut contents of the fish from the cages were not examined further in this thesis.

Invertebrates

The contents of the samples collected from the mesh baskets were sorted under a dissecting microscope. All invertebrates were removed from each sample, and remaining material set aside for further processing. Invertebrates were identified to lowest feasible taxonomic level and counted. When possible, invertebrates were identified to genus or species, although several taxa represented coarser levels of taxonomic resolution. The major references for keying out invertebrate taxa were the "Guides to the Freshwater Invertebrates of Southern Africa" (Day *et al.* 2001, 2003, Day & de Moor 2002a, b, de Moor *et al.* 2003a, b, Stals & de Moor 2007). Denise Schael (Nelson Mandela Metropolitan University, South Africa) assisted with identification of Ephemeroptera, Michael Samways (Stellenbosch University, South Africa) assisted with identification of Odonata, and Vere Ross-Gillespie (University of Cape Town, South Africa) assisted with identification of Plecoptera. Invertebrates were assigned to functional feeding groups (FFGs) including collector-gatherers (CG), filter-feeders (FF), grazer-scrapers (GS), predators (P) and shredders (SH). Major references used for designating invertebrate taxa to FFGs included the "Guides to the Freshwater Invertebrates of Southern Africa" listed above, and Cummins *et al.* (2008). The density (number/m^2) of each invertebrate taxon and each FFG was calculated based on the area of streambed incorporated in each mesh basket (0.0225 m^2). Lastly, I calculated an estimate of the mean biomass of each invertebrate taxon and FFG. Between five and 253 individuals of each taxon were randomly collected from the samples, dried in an oven at 40°C for 48 h on pre-weighed foil dishes and then weighed to the nearest 0.001 mg so that estimates of mean dry mass could be computed. Per taxon dry mass estimates (mg/m^2) were then calculated based on the on estimates of taxon density.

Particulate organic matter

The material remaining after invertebrates had been removed from the mesh baskets was used to calculate estimates of fine (FPOM) and coarse (CPOM) particulate organic matter. Samples were elutriated to remove sand and gravel, and remaining organic matter was passed through a 1 mm sieve to separate organic matter into FPOM (250 – 1000 µm) and

CPOM (>1000 µm). Ash-free dry mass (AFDM) of organic matter samples was then obtained using the following procedure: samples were dried at 60 °C for 24 h in a drying oven, weighed (to the nearest 0.001 mg), combusted at 500 °C for 1 h, and then weighed again. The AFDM of each sample was then calculated by subtracting the mass of the ashed sample from that of the oven-dried sample, and converted to AFDM/m^2 based on the area of streambed incorporated in each mesh basket (0.0225 m^2).

Algae

Frozen periphyton samples were defrosted in the dark within 30 d of collection. Once defrosted, samples were homogenized and split into two 150 ml portions, one for measurement of AFDM, and one for measurement of Chlorophyll *a* (Biggs & Kilroy 2000). Total dry weight was measured by filtering the first sample portion through a Whatmann GFF 4 glass fibre filter paper, which was then dried at 60 °C for 24 h. Samples were then ashed in an oven at 400 °C for 4 h. The difference between the dry weight and the weight of the ash is the organic component (i.e. AFDM) of the periphyton. The second portion of the sample was then passed through a Whatman GF/F 0.7 µm glass fibre filter paper, and Chlorophyll *a* (a measure of live algal biomass in each sample) was extracted from filter papers using 90% ethanol. Pigment concentrations were then measured using the spectro-photometric method of (Sartory & Grobbelaar 1984), as summarized by Biggs & Kilroy (2000). Absorbance (665 nm and 750 nm) was measured using a Merck Spectroquant Pharo 100 spectrophotometer. The dimensions of the stone tiles were then used to calculate AFDM/m^2, and the biomass (mg) of chlorophyll *a*/m^2.

5.2.5 Statistical analysis

Fish

Matched pairs *t* tests were used to ascertain whether or not there was a significant difference in total fish biomass between the redfin and trout treatments within each block, at both the onset and the conclusion of the experiment. Matched pairs *t* tests were also

used to compare total fish biomass at the onset of the experiment to that at the conclusion of the experiment within each block for each species separately. Fish biomasses were ln(x+1) transformed so that they met the assumptions of the analysis.

Invertebrates and basal resources

Univariate statistics were used to test for treatment effects on a set of biotic response variables, as well as on measured physico-chemical parameters. Multivariate analysis was then used to further explore treatment effects on the taxonomic composition of invertebrate assemblages in the cages in greater detail.

Univariate analysis

Biotic response variables for the univariate tests included a set of density- and biomass-based invertebrate metrics, as well as a set of metrics representing basal resources (i.e. algae and detritus). The invertebrate metrics included total invertebrate density/biomass, the density/biomass of each benthic invertebrate FFG, as well as taxon richness (*S*), Margalef's richness index (*d*), Pielou's index of evenness (*J'*), Shannon diversity index (*H'*) and Simpson diversity index (*1-λ*). The metrics representing lower trophic levels included chlorophyll *a* biomass and periphyton AFDM, as well as the AFDM of FPOM and CPOM.

Univariate mixed model ANOVAs, with treatment as a fixed factor and block as a random factor, were used to ascertain whether any of the biotic response variables, and physico-chemical parameters, differed significantly among treatments, and whether effects were consistent among experimental blocks (Quinn & Keough 2002). Type 3 main effects model ANOVAs were used, and tests were run without the intercept term included because the block x treatment interaction was not relevant in this model (Quinn & Keough 2002). The four mesh baskets, and four stone tiles, collected from each cage were treated as sub-samples, and thus mean value derived from the four samples for each invertebrate and lower trophic level metric from each cage was used as a single, independent data point in ANOVA tests (Flecker 1996). Similarly, the mean of the three measurements of each

environmental parameter within each cage was used as single, independent data point in ANOVA tests (with the exception of percentage canopy cover which consisted of only a single measurement for each cage). Response variables were ln(x+1) transformed as needed to meet the assumptions of the analysis. *Post-hoc* pair-wise comparisons between treatments were performed using Tukey's honestly significant difference (HSD) tests.

Predator impact ratio and cascade strength

While absolute differences in the density or biomass of the various biotic response variables between the fish and no-fish treatments provide a measure of changes in those metrics in response to a fish, the log ratio of the density (or biomass) of these community components in fish treatments versus treatments without fish (i.e. ln[(x+1) fish ⁄ (x+1) no fish]) provides a standardized index of proportionate fish effects. This ratio, referred to as the predator impact index (*PI*) for invertebrate metrics (Cooper *et al.* 1990), and cascade strength index (*CS*) for basal resources (Herbst *et al.* 2009), is useful in that it standardizes the magnitude of predator impacts, allowing the direct effects of predators on prey populations, as well as indirect effects on basal resources, to be compared among studies (Hedges *et al.* 1999, Herbst *et al.* 2009). *PI* and *CS* ratios were therefore calculated for each community metric for each fish species, and matched pairs *t* tests used to test for significant differences between species. All response variables were ln(x+1) transformed so that they met the assumptions of the analysis.

Multivariate analysis

Multivariate analysis was used to test for differences in the taxonomic composition of invertebrate assemblages, based on both density and biomass data, among treatments. The untransformed invertebrate density data set was converted to a resemblance matrix using Bray-Curtis similarity (Anderson *et al.* 2008), and non-metric multidimensional scaling (nMDS) ordination was used to visualize differences in invertebrate assemblage composition among treatments. PERMANOVA, a semi-parametric, permutation-based analogue of traditional ANOVA/MANOVA (Anderson *et al.* 2008) was then used to test for significant

differences in assemblage structure between treatments. Two-way mixed model PERMANOVA (model type 3), using 9999 permutations and permutation of residuals under a reduced model, was used to examine the effect of the fixed factor treatment, and the random factor block, on variation in assemblage composition among cages. The treatment x block interaction term was not computed since it was not relevant in this model (Anderson et al. 2008). PERMANOVAs were followed by permutational *post-hoc* tests to examine pair-wise differences among treatments (Anderson et al. 2008). Analysis of similarity percentages (SIMPER, Anderson *et al.* 2008) was used to identify the taxa contributing most to the overall dissimilarity in taxonomic assemblage composition among treatments.

Software used

All univariate analyses were carried out with SPSS 20.0 (SPSS 2011), and multivariate analyses were performed using PRIMER-E (Clarke & Gorley 2006) with the add-on package PERMANOVA+ (Anderson *et al.* 2008).

5.3 RESULTS

5.3.1 Fish

Total fish biomass in fish-containing cages ranged between 15.05 and 21.20 g (Table 5.1), and no significant difference in fish biomass was detected between trout and redfin treatments within blocks at both the onset (matched pairs *t* test, t_3 = -1.77, p = 0.184), and conclusion (matched pairs *t* test, t_3 = -0.88, p = 0.444), of the experiment. The biomass of both species decreased slightly over the duration of the experiment (Table 5.1), but this decrease was not statistically significant for trout (matched pairs *t* test, t_3 = 0.79, p = 0.486), or for redfin (matched pairs *t* test, t_3 = 1.52, p = 0.226).

Table 5.1 Length and weight of individual trout and redfin at the start and at the end of the experiment.

Block	Trout			Redfin		
		Weight (g)			Weight (g)	
	Length (mm)	Start	End	Length (mm)	Start	End
1	83	7.10	7.30	77	5.59	5.81
	89	7.95	8.24	95	10.82	10.48
2	103	12.58	11.64	94	11.40	11.96
	89	8.61	7.79	90	9.80	8.08
3	89	8.24	8.24	89	10.80	9.86
	93	8.78	9.33	81	6.60	6.68
4	90	8.00	8.07	96	12.58	12.35
	87	12.40	11.21	83	8.10	8.54

5.3.2 Invertebrates

Assemblage description

Density

Density-based estimates of invertebrate assemblage composition averaged across all experimental cages indicated that collector-gatherers were the numerically dominant aquatic invertebrate FFG in the cages (Figure 5.5a), comprising 82.24% of the assemblage by number. This FFG was dominated by the dipterans Chironominae and Orthocladiinae, the ephemeropterans *Aprionyx peterseni*, *Labiobaetis/Pseudocloeon*, *Lestagella penicillata*, *Afroptilum* and *Baetis*, and the trichopteran *Athripsodes* (Appendix 11). Predators were the second most abundant functional component, but only comprised 9.08% of the assemblage by number. The dipteran Tanypodinae, the odonate *Pseudagrion* and the trichopterans *Oecetis* and *Cheumatopsyche* were the numerically dominant predatory taxa in the samples. Grazer-scrapers were the next most abundant FFG, and on average comprised 6.64% of the invertebrate assemblage. The coleopteran family Elmidae, the ephemeropteran *Afronurus* and the trichopterans *Hyrdoptila* and *Orthotrichia* were the numerically dominant taxa within this FFG. Shredders and filter-feeders were not abundant in the samples, both comprising <2% of the assemblage by number.

Biomass

Biomass-based estimates of the functional composition of invertebrates also indicated that the assemblage was dominated by collector-gatherers (57.85%), but to a lesser extent than found for density-based estimates (Figure 5.5b). The ephemeropterans *A. peterseni* and *Labiobaetis/Pseudocloeon*, and the dipteran Chironominae were the gravimetrically dominant components of the collector-gatherer FFG (Appendix 12). The grazer-scraper and predator FFGs were more strongly represented in biomass-based estimates than in density-based estimates, comprising 21.55% and 20.21% of the assemblage by weight respectively. The grazer-scraper FFG was dominated by the ephemeropteran *Afronurus* and the coleopteran Elmidae, while the predator FFG was dominated by the trichopteran *Cheumatopsyche* and the odonate *Pseudagrion*. As found with density-based estimates, the biomass of shredders and filter-feeders in the samples was low (<1% each).

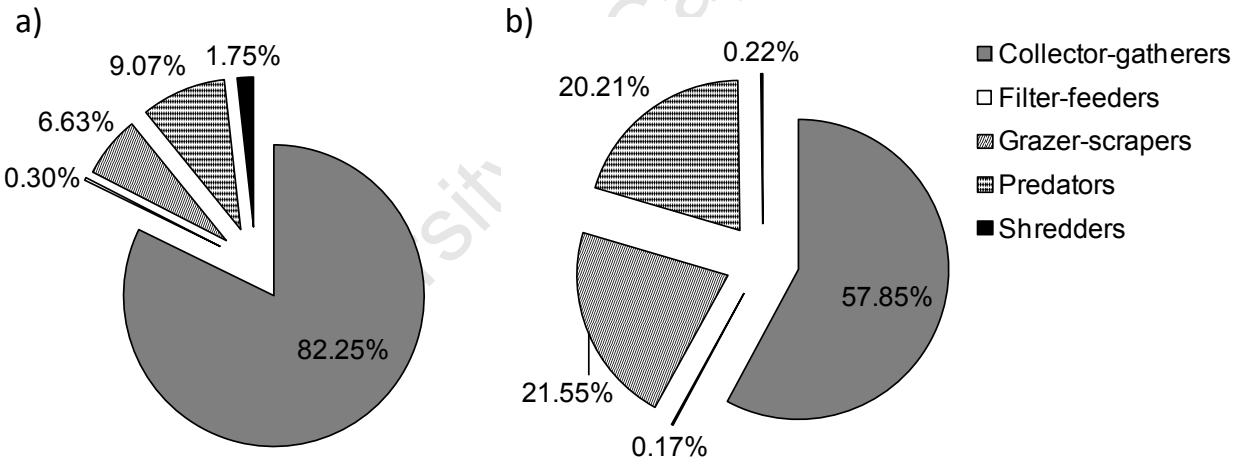

Figure 5.5 Functional composition of the benthic invertebrate assemblage by (a) density and (b) biomass based on the mean proportional abundance of each FFG across all cages.

Functional composition

Density

The distribution of filter-feeder and predatory invertebrate densities did not meet the assumptions of ANOVA and were therefore ln(x+1) transformed prior to statistical analysis. Mixed model ANOVA tests revealed significant treatment effects on total invertebrate density, and on the density of some, but not all, FFGs (Figure 5.6, Table 5.2). Total invertebrate density in the redfin treatment was approximately half that in the other two treatments (Figure 5.6a), and pair-wise tests confirmed that while no difference existed between the trout and no-fish treatments, total invertebrate density was significantly lower in the redfin treatment than in both the trout and no-fish treatments (Table 5.2).

Treatment effects were significant for collector-gatherers, grazer-scrapers and predators, indicating that these FFGs contributed to the difference in overall invertebrate density among treatments (Table 5.2). Collector-gatherer density in the redfin treatment was on average roughly half that of treatments containing either trout or no fish (Figure 5.6b), and pair-wise tests confirmed that while no difference existed between the trout and no-fish treatments, collector-gatherers were significantly less abundant in the redfin treatment than in both the trout and no-fish treatments (Table 5.2). Similarly, grazer-scraper density in the redfin treatment was significantly lower than that in treatments containing either trout or no fish (Table 5.2, Figure 5.6d). Predatory invertebrate density in the redfin treatment was significantly lower than that in the no-fish treatment, but predator density in the trout treatment did not differ significantly from that in either no fish or redfin treatments (Table 5.2, Figure 5.6e). A significant block effect was also detected for predatory invertebrates (Table 5.2), indicating that the treatment effect on this FFG was not consistent among blocks. Although the density of both filter-feeders and shredders in the redfin treatment was somewhat lower than that in the trout and no-fish treatments (Figure 5.6c and f), treatment effects for these FFGs were not significant (Table 5.2).

Table 5.2 Results of mixed model ANOVAs, and Tukey's HSD pair-wise tests, conducted on total invertebrate density and on the density of each invertebrate functional feeding group. For Tukey tests, "N" = no fish, "T" = Trout and "R" = Redfin. Variables marked with the symbol † were ln(x+1) transformed prior to analysis. Asterisks indicate significant differences at $\alpha = 0.05$.

Response variable	Mixed model ANOVA tests						Tukey *post-hoc* tests		
	Source	*df*	SS	MS	*F*	*p*	N vs. T	N vs. R	T vs. R
Total invertebrates	Treatment	2	33997800.08	16998900.04	11.91	0.008*	0.962	0.011*	0.015*
	Block	3	6356244.52	2118748.17	1.48	0.311			
	Error	6	8566565.19	1427760.86					
Collector-gatherers	Treatment	2	24542364.49	12271182.25	10.56	0.011*	0.987	0.016*	0.019*
	Block	3	2979043.76	993014.59	0.85	0.513			
	Error	6	6974752.49	1162458.75					
Grazer-scrapers	Treatment	2	85987.65	42993.83	7.44	0.024*	0.964	0.031*	0.042*
	Block	3	35915.64	11971.88	2.07	0.205			
	Error	6	34670.78	5778.46					
Filter-feeders†	Treatment	2	0.73	0.36	0.71	0.530	0.972	0.662	0.535
	Block	3	1.44	0.48	0.94	0.480			
	Error	6	3.09	0.52					
Predators†	Treatment	2	0.26	0.13	5.30	0.047*	0.214	0.041*	0.436
	Block	3	0.42	0.14	5.76	0.034*			
	Error	6	0.15	0.02					
Shredders	Treatment	2	45617.29	22808.64	2.93	0.129	0.298	0.765	0.122
	Block	3	25298.35	8432.79	1.09	0.424			
	Error	6	46646.10	7774.35					

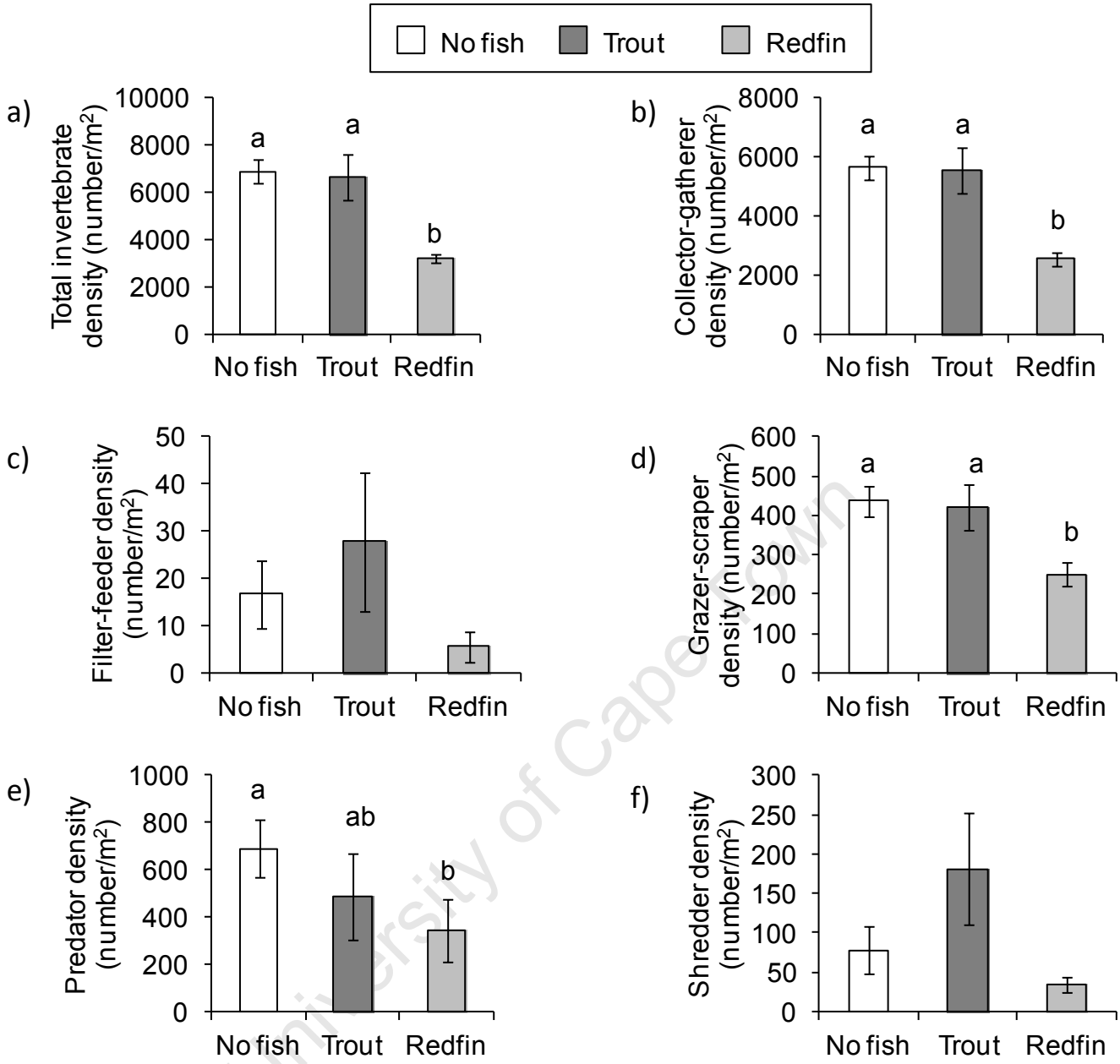

Figure 5.6 Mean ± SE density (number/m^2) of (a) total invertebrates, (b) collector-gatherers, (c) filter-feeders, (d) grazer-scrapers, (e) predators and (f) shredders recorded in cages at the end of the experiment. Different letters indicate significant differences based on mixed model ANOVA and Tukey's HSD pair-wise tests.

Biomass

The distributions of filter-feeder and shredder biomass data did not meet the assumptions of the mixed model ANOVA tests and these variables were therefore ln(x+1) transformed prior to statistical analysis. As was the case for density-based estimates, fish treatment had a significant effect on total invertebrate biomass (Table 5.3), and the biomass of invertebrates in the redfin treatment was on average roughly half that in the treatments containing either trout or no fish (Figure 5.7a). Pair-wise tests confirmed that the redfin treatment had a significantly lower biomass of invertebrates than had the trout and no-fish treatments (Table 5.3).

Similarly, there was a significant treatment effect on collector-gatherer biomass (Table 5.3), and the redfin treatment had a significantly lower biomass of collector-gatherers than did the other two treatments (Figure 5.7b). The biomass of filter-feeders, grazer-scrapers, predators and shredders in the redfin treatment was, on average, somewhat lower than in the trout and no-fish treatments (Figure 5.7c-f), but the treatment effect was not significant for any of these four invertebrate FFGs (Table 5.3). These results suggest that the differences in total invertebrate biomass among treatments were driven largely by differences in collector-gatherer biomass among treatments.

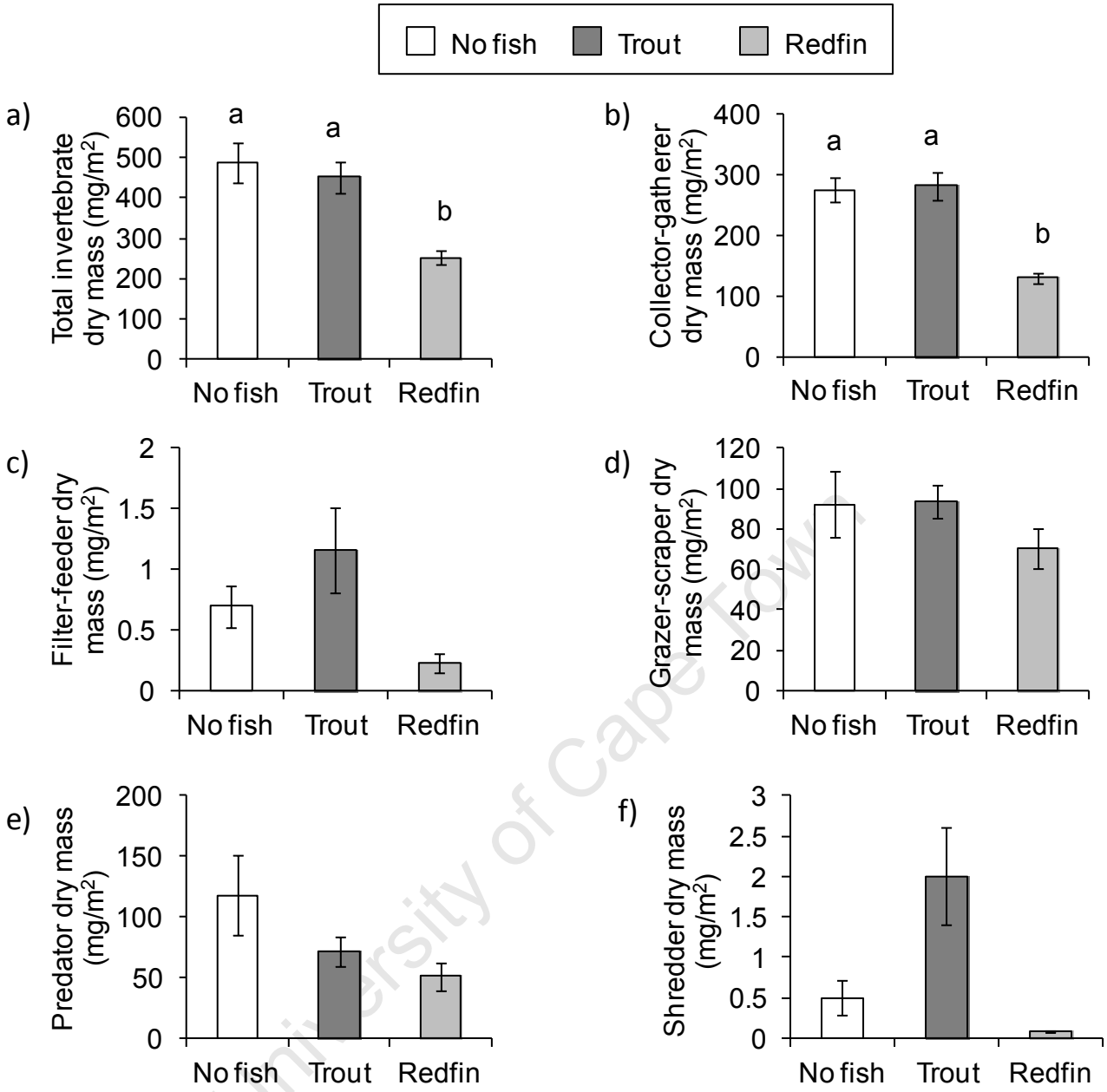

Figure 5.7 Mean ± SE biomass (mg/m^2) of (a) total invertebrates, (b) collector-gatherers, (c) filter-feeders, (d) grazer-scrapers, (e) predators and (f) shredders recorded in cages at the end of the experiment. Different letters indicate significant differences based on mixed model ANOVA and Tukey's HSD pair-wise tests.

Table 5.3 Results of mixed model ANOVAs, and Tukey's HSD pair-wise tests, conducted on total invertebrate biomass and on the biomass of each invertebrate functional feeding group. For Tukey tests, "N" = no fish, "T" = Trout and "R" = Redfin. Variables marked with the symbol † were ln(x+1) transformed prior to analysis. Asterisks indicates significant differences at $\alpha = 0.05$.

Response variable	Mixed model ANOVA tests						Tukey *post-hoc* tests		
	Source	*df*	SS	MS	*F*	*p*	N vs. T	N vs. R	T vs. R
Total invertebrates	Treatment	2	128114.00	64057.00	5.70	0.044*	0.932	0.047*	0.048*
	Block	3	28891.88	9630.63	0.49	0.571			
	Error	6	119115.33	19852.55					
Collector-gatherers	Treatment	2	58853.98	29426.99	6.08	0.036*	0.990	0.048*	0.049*
	Block	3	6550.82	2183.61	0.45	0.726			
	Error	6	29051.65	4841.94					
Grazer-scrapers	Treatment	2	1351.66	675.83	0.37	0.707	0.998	0.762	0.737
	Block	3	4102.91	1367.64	0.75	0.564			
	Error	6	11020.30	1836.72					
Filter-feeders†	Treatment	2	1.72	0.86	1.29	0.343	0.716	0.716	0.315
	Block	3	1.79	0.60	0.28	0.497			
	Error	6	4.00	0.67					
Predators	Treatment	2	9445.75	4722.87	0.78	0.499	0.695	0.484	0.923
	Block	3	13951.60	4650.53	0.77	0.551			
	Error	6	36230.41	6038.40					
Shredders†	Treatment	2	8.23	4.12	3.25	0.111	0.221	0.858	0.112
	Block	3	7.11	2.37	1.87	0.236			
	Error	6	7.61	1.27					

Predator impact ratios

Density

The *PI* values for redfin were consistently more negative than those for trout for all density-based invertebrate response variables (Table 5.4). These results indicate that, relative to the no-fish treatment, invertebrate density was more strongly reduced by redfin than by trout. Matched pairs *t* tests conducted on ln(x+1) transformed data indicated that redfin had significantly lower *PI* values than had trout for total invertebrates, collector-gatherers, grazer-scrapers and filter-feeders, but not for shredders or predators. In general, *PI* values were negative for both fish species, indicating that both redfin and trout reduced the density of most benthic invertebrates relative to the control cages. However, *PI* values in the trout treatment were positive for filter-feeders and shredders, indicating that the density of these FFGs increased in the presence of trout relative to the treatment with no fish.

Biomass

All biomass-based invertebrate response variables were ln(x+1) transformed prior to analysis of *PI* values. *PI* values for redfin and trout based on invertebrate biomass data were broadly similar to those calculated from invertebrate density data, in that the *PI* values for redfin were consistently more negative than those for trout for all invertebrate response variables (Table 5.4). As was the case with density-based estimates, the biomass-based *PI* values for total invertebrates and collector-gatherers for redfin were significantly lower than those for trout. On the other hand, no significant differences were detected for any of the other biomass-based invertebrate response variables. The positive *PI* values for collector-gatherers, filter-feeders, grazer-scrapers and shredders in the trout treatment indicated that the biomass of these FFGs increased relative to their biomass in the control cages.

Table 5.4 Mean ± SE predator impact ratios (*PI*) for trout and redfin on density- and biomass-based benthic invertebrate response variables. Results of matched-pairs *t* tests comparing predator impact ratios between trout and redfin for each response variable are shown. Asterisks indicate significant differences at α = 0.05. All response variables were ln(x+1) transformed to meet the assumptions of the analysis.

Response variable	Trout		Redfin		Matched pairs *t* test		
	Mean	SE	Mean	SE	*df*	*t*	*p*
Density							
Total invertebrates	-0.06	0.13	-0.76	0.07	3	4.07	0.026*
Collector-gatherers	-0.04	0.14	-0.80	0.11	3	3.39	0.042*
Filter-feeders	0.27	1.34	-1.05	0.74	3	3.69	0.034*
Grazer-scraper	-0.05	0.15	-0.57	0.08	3	3.19	0.048*
Predators	-0.49	0.32	-0.82	0.22	3	1.70	0.187
Shredders	0.98	0.59	-0.66	0.43	3	0.99	0.394
Biomass							
Total invertebrates	-0.06	0.31	-0.63	0.18	3	4.43	0.021*
Collector-gatherers	0.02	0.24	-0.74	0.07	3	3.65	0.035*
Filter-feeders	0.17	0.38	-0.29	0.17	3	1.25	0.299
Grazer-scraper	0.14	0.45	-0.25	0.54	3	1.23	0.306
Predators	-0.32	0.59	-0.75	0.52	3	1.55	0.218
Shredders	0.55	0.30	-0.26	0.22	3	1.98	0.141

Diversity indices

Table 5.5 shows the average (mean ± SE) values of five measures of taxon abundance and diversity computed for each of the three treatments. Average taxon richness (*S*) for the redfin treatment 20.50 ± 1.32 was lower than that in both the trout (26.25 ± 2.29) and no fish (25.25 ± 1.80) treatments. However, differences in *S* among treatments were not statistically significant. The mean values of Margalef's index (*d*), Pielou's evenness (*J'*), Shannon-Wiener Diversity (*H'*) and Simpson diversity (*1-λ*) were similar among treatments, and no significant treatment or block effects were detected for any of these metrics.

Table 5.5 Mean ± SE of diversity indices computed for invertebrate assemblages in treatments containing no fish, trout and redfin. Results of mixed model ANOVA tests examining the effect of treatment (fixed factor) and block (random factor) on each metric are shown.

Response variable	No fish		Trout		Redfin		Mixed model ANOVA tests					
	Mean	SE	Mean	SE	Mean	SE	Effect	*df*	SS	MS	*F*	*p*
Taxon richness (*S*)	25.25	1.80	26.25	2.29	20.50	1.32	Treatment	2	75.500	37.750	2.60	0.154
							Block	3	35.333	11.778	0.81	0.533
							Error	6	87.167	14.528		
Margalef's index (*d*)	2.74	0.18	2.87	0.22	2.42	0.15	Treatment	2	0.003	0.001	0.23	0.803
							Block	3	0.038	0.013	0.14	0.932
							Error	6	0.136	0.023		
Pielou's evenness (*J'*)	0.63	0.02	0.62	0.02	0.68	0.04	Treatment	2	0.008	0.004	0.91	0.452
							Block	3	0.002	0.001	0.13	0.940
							Error	6	0.028	0.005		
Shannon diversity (*H'*)	2.03	0.07	2.00	0.04	2.03	0.09	Treatment	2	0.003	0.001	0.06	0.945
							Block	3	0.038	0.013	0.57	0.658
							Error	6	0.136	0.023		
Simpson diversity (*1-λ*)	0.76	0.02	0.75	0.02	0.78	0.04	Treatment	2	0.002	0.001	0.32	0.741
							Block	3	0.005	0.002	0.49	0.701
							Error	6	0.021	0.004		

Taxonomic composition

Density

nMDS ordination on the taxon-level invertebrate density dataset revealed that the invertebrate samples from cages containing redfin separated out clearly from the samples from cages containing trout and those containing no fish (Figure 5.8a). This result indicates that there were consistent differences in taxonomic assemblage composition between the redfin treatment and the other two treatments. Mixed model PERMANOVA confirmed that treatment had a significant effect on assemblage composition in the cages (Table 5.6), and permutational pair wise tests confirmed that this difference was driven by compositional differences between the redfin treatment and the other two treatments. The random factor block had no significant effect on assemblage composition, indicating that the significant differences between treatments were consistent among experimental blocks. A PERMDISP test revealed that there was no difference in dispersion among treatments ($F_{2, 9} = 0.16$, $p_{perm} = 0.917$), indicating that the significant PERMANOVA result was attributable to differences in taxonomic composition, rather that multivariate dispersion, among treatments.

SIMPER analysis revealed that the average dissimilarity in taxonomic composition between the no fish and redfin treatments was 43.19% and that the ten taxa most important in discriminating between these treatments collectively accounted for 84.06% of the overall dissimilarity (Table 5.7). Chironominae was the taxon that contributed most to the dissimilarity in assemblage composition between these treatments, single-handedly accounting for 38.75% of the overall dissimilarity. Chironominae was the most abundant taxon in the invertebrate samples overall (mean proportional abundance across all cages = 43.36%) (Appendix 11), and the mean density of this taxon in the redfin treatment was approximately half that in the no-fish treatment. The taxa *Labiobaetis/Pseudocloeon* and Orthocladiinae were also important contributors to the overall dissimilarity between these two treatments. *Labiobaetis/Pseudocloeon*, which had a mean proportional abundance across all treatments of 13.27%, contributed 14.42% to the overall dissimilarity, and the mean density of this taxon in redfin treatment was less than half that in the no-fish treatment. Orthocladiinae had a mean proportional abundance across all treatments of 10.83%, and contributed 8.77% to the overall dissimilarity, and the mean density of this

taxon in the redfin treatment was approximately half that in the treatment with no fish. Other taxa making important contributions to the dissimilarity between these two treatments included the ephemeropterans *Afroptilum*, *L. penicillata* and *Baetis*, the dipteran Tanypodinae, the odonate *Pseudagrion*, the trichopteran *Oecetis* and the coleopteran Elmidae. Collectively, these taxa contributed a further 22.13% to the overall dissimilarity. With the exception of *Pseudagrion*, the mean densities of these taxa were lower in redfin treatment than in the no-fish treatment.

The average dissimilarity between redfin and trout treatments was 43.67%, and the ten taxa most important in discriminating between these treatments collectively accounted for 85.56% of that dissimilarity (Table 5.7). Chironominae was the taxon that contributed the most (40.82%) to the dissimilarity in assemblage composition between these treatments, and the mean density of this taxon in the redfin treatment was less than half that in the treatment with trout. *Labiobaetis/Pseudocloeon* was the next most important contributor to the overall dissimilarity between these two treatments, accounting for 13.04% of the overall dissimilarity, and the mean density of this taxon in the redfin treatment was also less than half that in the trout treatment. Other taxa making important contributions to the dissimilarity between the two groups of sites included the ephemeropterans *Afroptilum*, *L. penicillata* and *Baetis*, the dipterans Tanypodinae and Orthocladiinae, the odonate *Pseudagrion*, the plecopteran *Aphanicercella* and the coleopteran Elmidae. These taxa collectively contributed a further 31.71% to the overall dissimilarity. With the exception of *Pseudagrion* and *L. penicillata*, the mean densities of these taxa were lower in the redfin treatment than in the treatment without fish.

Biomass

The nMDS ordination of the taxon-level invertebrate matrix based on biomass data was similar to that produced when density data were used. Invertebrate samples from cages containing redfin separated out from the samples from cages containing trout and those lacking fish (Figure 5.8b), indicating that there were consistent differences in taxonomic assemblage composition between the redfin treatment and the other two treatments. A mixed model PERMANOVA test confirmed that treatment had a significant effect on

assemblage composition in the cages (Table 5.6), and permutational pair-wise tests revealed that this difference was driven predominantly by compositional differences between the redfin treatment and the other two treatments. The random factor block had no significant effect on assemblage composition, indicating that the significant differences between treatments were consistent among experimental blocks. A PERMDISP test revealed that there was no difference in dispersion among treatments ($F_{2, 9}$ = 0.41, p_{perm} = 0.765), indicating that the significant PERMANOVA result was attributable to differences in taxon composition, rather than multivariate dispersion among treatments.

SIMPER analysis revealed that the average dissimilarity in taxonomic composition between the no fish and redfin treatments was 49.96% and that the ten taxa most important in discriminating between these treatments collectively accounted for 82.55% of that dissimilarity (Table 5.8). The ephemeropterans *Labiobaetis/Pseudocloeon* and *A. peterseni* were the taxa that contributed the most to the overall dissimilarity, accounting for 14.66% and 13.83% of the overall dissimilarity respectively. The mean biomass of both taxa in the redfin treatment was roughly double that in the treatment with no fish. The trichopteran *Cheumatopsyche*, the ephemeropteran *Afronurus* and the dipteran Chironominae had greater mean biomasses in the treatment with no fish than in the redfin treatment, and were also identified as important contributors to the overall dissimilarity, contributing >10% each. The odonate *Pseudagrion*, the ephemeropteran *Lithogloea harrisoni*, the coleopteran Elmidae and the dipterans Tanypodinae and Orthocladiinae collectively contributed a further 18.86% to the overall dissimilarity. Of the top ten taxa identified by SIMPER analysis, only *Pseudagrion* and *L. harrisoni* had higher mean biomasses in the redfin treatment than in the other two treatments.

Finally, the average dissimilarity between redfin and trout treatments was 48.64%, and the ten taxa most important in discriminating between these treatments collectively accounted for 81.44% of that dissimilarity (Table 5.8). The ephemeropterans *A. peterseni* and *Labiobaetis/Pseudocloeon* contributed the most to the overall dissimilarity, accounting for 14.43% and 13.89% of the overall dissimilarity respectively, and the mean biomass of both taxa in the redfin treatment was roughly half that in the trout treatment. The dipteran Chironominae and the ephemeropteran *Afronurus* were also identified as important contributors to the overall dissimilarity, contributing >9% each. The odonate *Pseudagrion*,

the trichopteran *Cheumatopsyche*, the lepidopteran Crambidae, the ephemeropterans *Baetis* and *L. harrisoni* and the dipteran Tanypodinae collectively contributed a further 31.86% to the overall dissimilarity. Of the top ten taxa identified by SIMPER analysis, only *Afronurus*, *Pseudagrion* and *L. harrisoni* had a higher mean biomass in the redfin treatment than in the no-fish treatment.

Table 5.6 Results of mixed model multivariate PERMANOVA tests examining the effect of treatment (fixed factor) and block (random factor) on density- and biomass-based taxonomic composition of invertebrate assemblages at the end of the experiment. Results of permutational tests examining pair-wise differences among treatments ("N" = no fish, "T" = Trout and "R" = Redfin) are also shown. Asterisks indicate significant differences at α = 0.05.

Response	Mixed model PERMANOVA tests						Pairwise tests		
variable	Source	*df*	SS	MS	*F*	*p*	N vs. T	N vs. R	T vs. R
Density	Treatment	2	3569.30	1784.60	4.47	0.018*	0.718	0.045*	0.046*
	Block	3	1568.40	522.80	1.31	0.184			
	Residual	6	2393.30	398.88					
	Total	11							
Biomass	Treatment	2	3707.60	1853.80	2.40	0.035*	0.680	0.046*	0.039*
	Block	3	2897.20	965.73	1.25	0.250			
	Residual	6	4631.70	771.95					
	Total	11	11237.00						

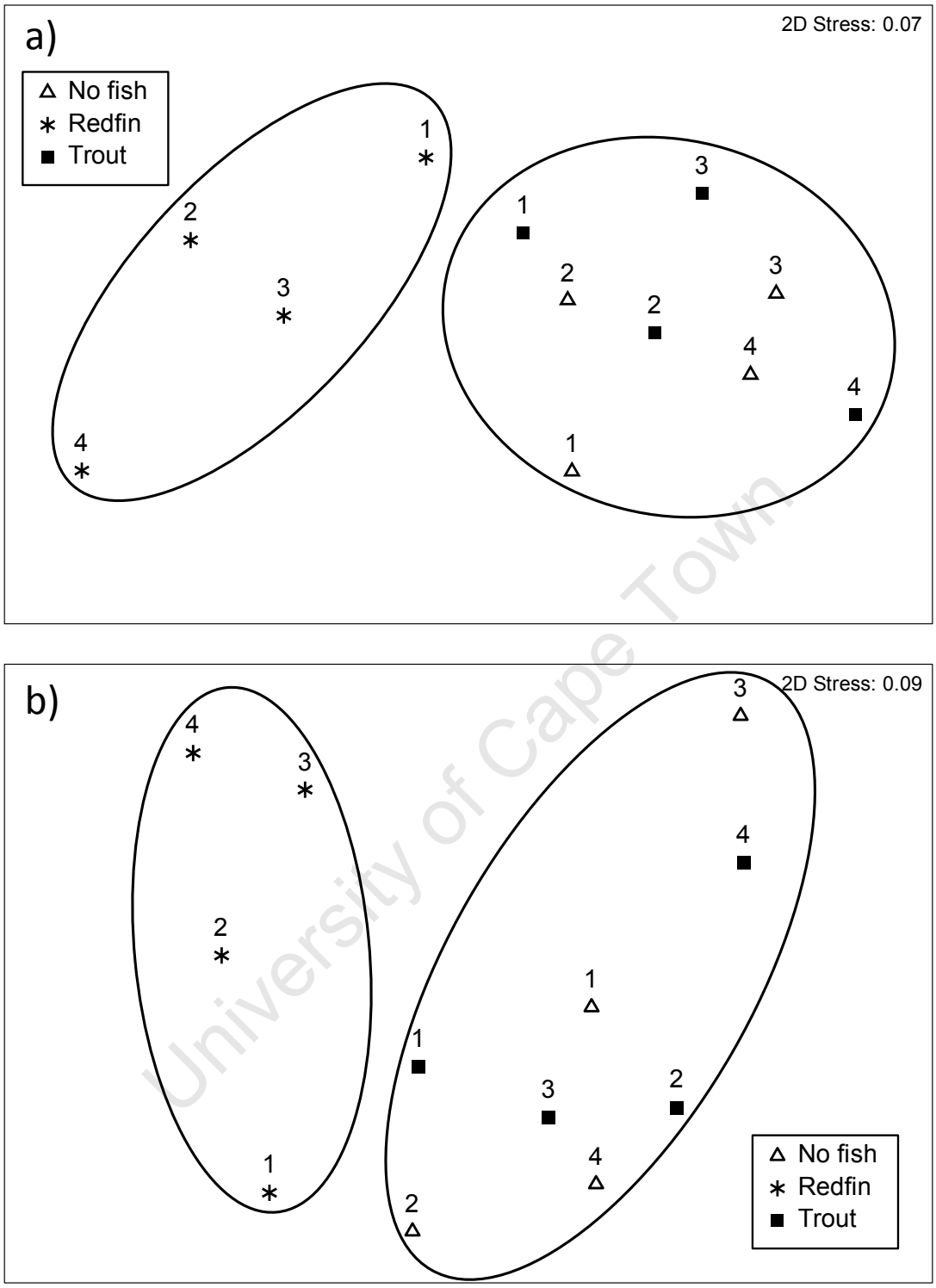

Figure 5.8 Non-metric multidimensional scaling (nMDS) ordination plots of (a) density- and (b) biomass-based benthic invertebrate assemblage composition at the end of the experiment in cages containing no fish, trout and redfin. Encircled sets of data points indicate the separation of samples in redfin cages from samples in cages with no fish and trout.

Table 5.7 Mean ± SE density (number/m^2) of taxa contributing most to the dissimilarity in invertebrate assemblage composition between the no-fish and redfin treatments, and between the redfin and trout treatments. "Contrib.%" is the percentage contribution of each taxon to the overall dissimilarity, and "Cum.%" indicates the cumulative percentage contribution.

No fish vs. redfin	No fish		Redfin			
(43.19% dissimilarity)	Mean	SE	Mean	SE	Contrib.%	Cum.%
Chironominae	2952.78	242.27	1241.67	147.79	38.75	38.75
Labiobaetis/Pseudocloeon	980.56	127.94	341.67	34.88	14.42	53.17
Orthocladiinae	769.44	86.24	438.89	51.95	8.77	61.94
Tanypodinae	241.67	52.07	77.78	14.10	3.80	65.74
Afroptilum	169.44	47.53	5.56	3.21	3.73	69.47
Lestagella penicillata	377.78	58.15	302.78	28.37	3.50	72.96
Pseudagrion	122.22	11.42	208.33	69.11	3.29	76.26
Oecetis	147.22	9.21	27.78	6.14	2.81	79.07
Elmidae	200.00	34.25	136.11	14.90	2.51	81.58
Baetis	130.56	36.42	75.00	12.39	2.49	84.06
Redfin vs. trout	Redfin		Trout			
(43.67% dissimilarity)	Mean	SE	Mean	SE	Contrib.%	Cum.%
Chironominae	1241.67	147.79	3047.22	237.53	40.82	40.82
Labiobaetis/Pseudocloeon	341.67	34.88	894.44	32.55	13.04	53.86
Orthocladiinae	438.89	51.95	600.00	116.03	6.44	60.30
Baetis	75.00	12.39	333.33	42.63	5.71	66.01
Tanypodinae	77.78	14.10	275.00	69.60	4.15	70.15
Pseudagrion	208.33	69.11	75.00	18.95	3.98	74.14
Afroptilum	5.56	3.21	166.67	47.21	3.38	77.52
Lestagella penicillata	302.78	28.37	241.67	40.69	3.31	80.82
Aphanicercella	30.56	15.80	163.89	39.23	2.82	83.64
Elmidae	136.11	14.90	177.78	25.26	1.92	85.56

Table 5.8 Mean ± SE dry mass (g/m^2) of taxa contributing most to the dissimilarity in invertebrate assemblage composition between the no-fish and redfin treatments, and between the redfin and trout treatments. "Contrib.%" is the percentage contribution of each taxon to the overall dissimilarity, and "Cum.%" indicates the cumulative percentage contribution.

No fish vs. redfin	No fish		Redfin			
(49.96% dissimilarity)	Mean	SE	Mean	SE	Contrib.%	Cum.%
Labiobaetis/Pseudocloeon	81.92	10.69	28.54	2.91	14.66	14.66
Aprionyx peterseni	70.79	13.99	33.18	5.27	13.83	28.49
Cheumatopsyche	54.49	31.46	8.72	3.56	13.11	41.60
Afronurus	53.70	13.56	43.37	9.21	11.39	53.00
Chironominae	68.86	5.65	28.96	3.45	10.70	63.70
Pseudagrion	16.44	1.54	28.02	9.29	4.93	68.63
Lithogloea harrisoni	2.36	1.36	16.53	2.61	4.04	72.67
Elmidae	25.29	4.33	17.21	1.88	3.61	76.27
Tanypodinae	16.58	3.57	5.34	0.97	3.24	79.51
Orthocladiinae	21.25	2.38	12.12	1.43	3.04	82.55
Redfin vs. trout	Redfin		Trout			
(48.64% dissimilarity)	Mean	SE	Mean	SE	Contrib.%	Cum.%
Aprionyx peterseni	33.18	5.27	73.01	21.00	14.43	14.43
Labiobaetis/Pseudocloeon	28.54	2.91	74.73	2.72	13.89	28.32
Chironominae	28.96	3.45	71.06	5.54	11.82	40.15
Afronurus	43.37	9.21	37.17	7.91	9.43	49.57
Pseudagrion	28.02	9.29	10.09	2.55	6.19	55.77
Cheumatopsyche	8.72	3.56	23.97	6.29	6.06	61.83
Crambidae	7.64	1.80	28.65	3.31	5.83	67.66
Baetis	5.83	0.96	25.93	3.32	5.66	73.32
Lithogloea harrisoni	16.53	2.61	2.36	1.36	4.25	77.58
Tanypodinae	5.34	0.97	18.87	4.78	3.87	81.44

5.3.3 Lower trophic levels

Algae

On average, chlorophyll *a* and periphyton AFDM on the tiles in the no-fish treatment was somewhat higher than that in the trout treatment, which, in turn, was somewhat higher than that in the redfin treatment (Figure 5.9a and b). Mixed model ANOVA tests revealed that treatment did not have a statistically significant effect on either chlorophyll *a* or on periphyton AFDM (Table 5.9). There was a significant block effect for AFDM, indicating that there was significant variation in AFDM among blocks, but not treatments. Tukey's post-hoc tests found no significant pair-wise differences between any of the treatments.

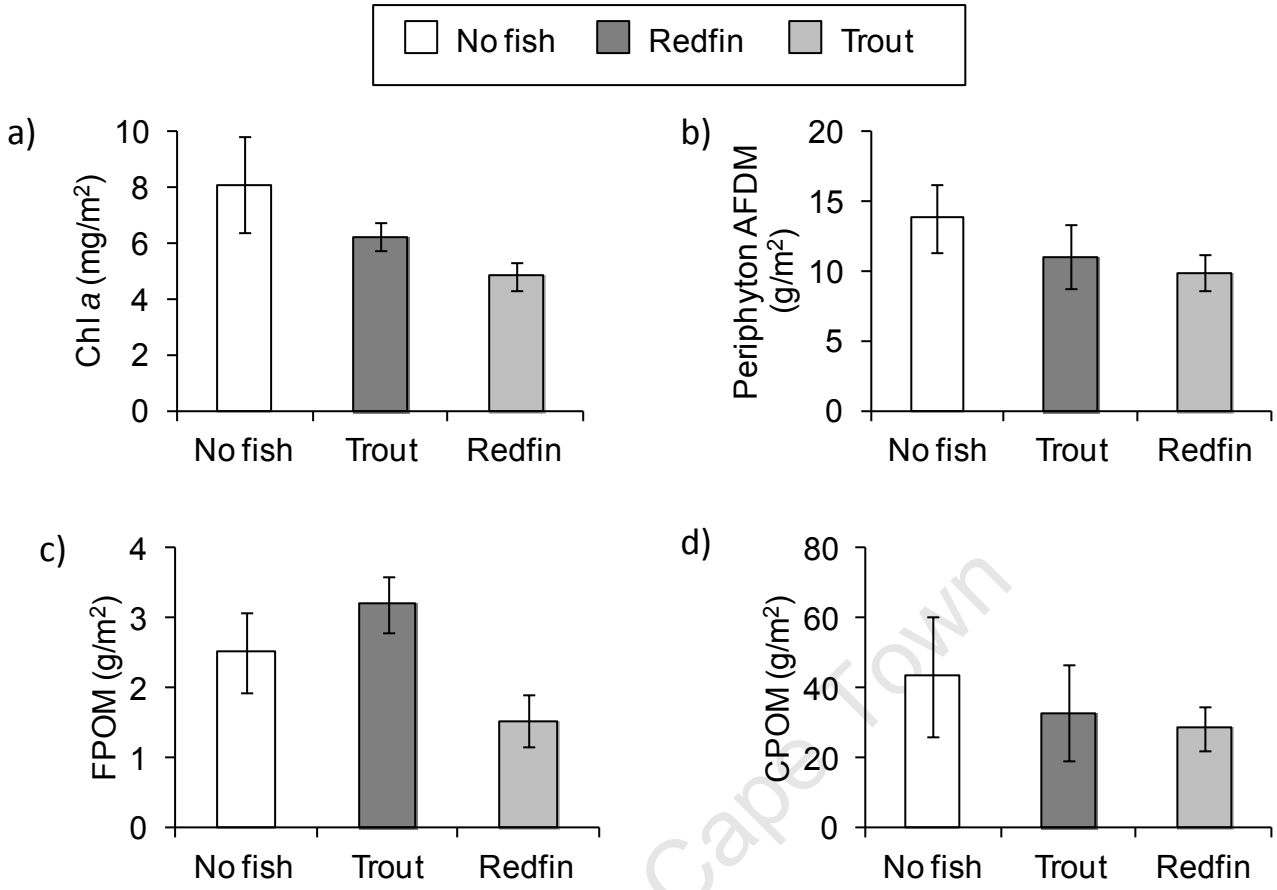

Figure 5.9 Mean ± SE biomass (mg/m^2) of (a) chlorophyll *a*, (b) periphyton AFDM, (c) fine particulate organic matter (FPOM) and (d) coarse particulate organic matter (CPOM) recorded in cages at the end of the experiment. Mixed model ANOVA detected no significant treatment effect for any of these four response variables.

Particulate organic matter

The distribution of CPOM biomass data did not meet the assumptions of mixed model ANOVA and was therefore ln(x+1) transformed prior to statistical analysis. Mean FPOM biomass was highest in the trout treatment, intermediate in the no-fish treatment, and lowest in the redfin treatment (Figure 5.9c). CPOM biomass, on the other hand was highest in the no-fish treatment, but somewhat lower in the other two treatments (Figure 5.9d). Mixed model ANOVA tests revealed that neither treatment nor block had a statistically significant effect on either FPOM or CPOM, and Tukey's post-hoc tests showed no significant pair-wise differences between any of the treatments for both resources (Table 5.9).

Table 5.9 Results of mixed model ANOVA, and Tukey's HSD pair-wise tests, conducted on stream resource metrics. For Tukey tests, "N" = no fish, "T" = Trout and "R" = Redfin. Variables marked with the symbol † were ln(x+1) transformed prior to analysis. Asterisks indicate significant differences at $\alpha = 0.05$.

Response variable	Mixed model ANOVA tests						Tukey *post-hoc* tests		
	Source	*df*	SS	MS	*F*	*p*	N vs. T	N vs. R	T vs. R
Chlorophyll *a*	Treatment	2	21.83	10.91	3.37	0.105	0.367	0.091	0.540
	Block	3	20.72	6.91	2.13	0.198			
	Error	6	19.45	3.24					
Periphyton AFDM	Treatment	2	32.26	16.13	2.46	0.166	0.350	0.157	0.799
	Block	3	116.10	38.70	5.91	0.032*			
	Error	6	39.31	6.55					
FPOM	Treatment	2	5.67	2.83	2.92	0.130	0.609	0.392	0.115
	Block	3	1.90	0.63	0.65	0.610			
	Error	6	5.82	0.97					
CPOM†	Treatment	2	0.05	0.03	0.23	0.803	0.847	0.821	0.998
	Block	3	0.05	0.02	0.14	0.932			
	Error	6	0.70	0.12					

Cascade strength ratios

On average, *CS* values for both chlorophyll *a* and periphyton AFDM were somewhat lower for the redfin treatment than for the trout treatment, but matched pairs *t* tests on ln(x+1) transformed data, revealed that these differences were not statistically significant (Table 5.10). For FPOM, the mean *CS* value for the redfin treatment was negative, while that for the trout treatment was positive, and this difference was statistically significant by matched pairs *t* test (Table 5.10). This result indicates that, relative to the no-fish treatments, FPOM biomass was more strongly reduced by redfin than by trout. Finally, for CPOM, the mean *CS* value for redfin was similar to that for trout, and no significant difference was detected with a matched pairs *t* test (Table 5.10).

Table 5.10 Mean ± SE cascade strength ratios (*CS*) for trout and redfin on stream resource metrics. Results of matched-pairs *t* tests comparing *PI* values between trout and redfin for each response variable are shown. Asterisks indicate a significant difference at $\alpha = 0.05$. The distributions of all four response variables were ln(x+1) transformed to meet the assumptions of the analysis.

Response variable	Trout		Redfin		Matched pairs *t* tests		
	Mean	SE	Mean	SE	*df*	*t*	*p*
Algae							
Chlorophyll *a*	-0.128	0.127	-0.263	0.131	3	1.98	0.141
AFDM	-0.225	0.098	-0.300	0.239	3	0.53	0.631
Particulate organic matter							
FPOM	0.003	0.004	-0.004	0.003	3	5.97	0.009*
CPOM	-0.038	0.087	-0.053	0.085	3	0.24	0.828

5.3.4 Environmental parameters

The distributions of all environmental parameters were ln(x+1) transformed prior to statistical analysis to improve normality and homogeneity of variances. The mean values of all measured environmental variables were similar among treatments, and no significant treatment effects were detected for any of the variables (Table 5.11). Significant block effects were found for the variables pH, conductivity and water temperature, however, indicating that there was significant variation in these variables among experimental blocks, but not among treatments.

Table 5.11 Mean ± SE of environmental parameters measured in cages containing no fish, trout and redfin. Results of mixed model ANOVA tests examining the effect of treatment (fixed factor) and block (random factor) on each parameter are shown. Asterisks indicate significant differences at $\alpha = 0.05$. All environmental response variables were ln(x+1) transformed to meet the assumptions of the analysis.

Dependent variable	No fish		Trout		Redfin		Mixed model ANOVA tests					
	Mean	SE	Mean	SE	Mean	SE	Effect	*df*	SS	MS	*F*	*p*
Depth (m)	23.00	0.72	24.92	1.66	22.83	0.78	Treatment	2	10.72	5.36	0.88	0.463
							Block	3	9.94	3.31	0.54	0.671
							Error	6	36.65	6.11		
Flow (m/s)	0.09	0.02	0.08	0.01	0.12	0.02	Treatment	2	0.01	0.01	0.92	0.447
							Block	3	0.01	0.01	0.30	0.826
							Error	6	0.010	0.01		
Canopy (%)	8.75	5.54	2.00	1.22	13.25	7.94	Treatment	2	256.50	128.25	0.83	0.482
							Block	3	211.33	70.44	0.45	0.724
							Error	6	932.17	155.36		
pH	4.61	0.14	4.48	0.15	4.57	0.17	Treatment	2	0.03	0.02	3.60	0.094
							Block	3	0.83	0.27	56.99	<0.001*
							Error	6	0.03	0.01		
Oxygen saturation (%)	86.51	3.95	83.03	2.60	84.35	2.07	Treatment	2	0.20	0.10	0.83	0.481
							Block	3	1.42	0.48	3.95	0.072
							Error	6	0.72	0.12		
Conductivity (µS/cm)	11.55	0.17	11.69	0.10	11.60	0.13	Treatment	2	0.04	0.02	2.27	0.184
							Block	3	0.62	0.21	22.11	0.001
							Error	6	0.06	0.01		
Turbidity (NTU)	0.53	0.06	0.51	0.09	0.64	0.08	Treatment	2	0.04	0.02	1.24	0.354
							Block	3	0.14	0.05	2.97	0.119
							Error	6	0.09	0.02		
Temperature (°C)	22.69	0.42	22.58	0.39	22.56	0.47	Treatment	2	0.040	0.02	0.80	0.491
							Block	3	6.44	2.15	85.75	<0.001*
							Error	6	0.15	0.03		

5.4 DISCUSSION

In this study, I used a manipulative field experiment to test the hypothesis generated by surveys of stream community structure and fish diets that benthic-feeding redfin are stronger regulators of benthic community structure than are drift-feeding trout. At the end of the experiment, total invertebrate density/biomass, as well as the functional and taxonomic structure of invertebrate assemblages, in cages containing redfin differed consistently from that in cages containing trout, indicating that these two fish species perform different predatory roles in the stream community. On the other hand, no significant differences in standing stocks of algae or organic matter were detected between treatments, suggesting that the differential effects of redfin and trout on invertebrate assemblages did not cascade down to the base of the stream food web. To my knowledge, the results of the present study constitute the first experimental evidence that the introduction of a non-native fish is responsible for changes in stream invertebrate assemblage structure in South Africa.

5.4.1 Benthic invertebrate assemblage

Total density and biomass

Total invertebrate density and biomass in the redfin treatment was on average approximately half that in the treatments with trout and no fish, indicating that redfin had a stronger top-down effect on benthic invertebrates than did trout. The fact that trout caused only a slight (and statistically non-significant) reduction in the total density, and biomass, of benthic invertebrates relative to the treatment with no fish implies that their effect on benthic invertebrates was barely detectable, and weak relative to that of redfin. Additionally, analysis of *PI* values confirmed that redfin had a stronger impact on both density and biomass of benthic invertebrates than did trout. These findings are consistent with the results from the broad-scale field survey which showed that total invertebrate density in redfin-dominated streams was on average roughly half that in trout-dominated streams (Chapter 3). Agreement between the experimental and survey results constitutes good evidence that benthic-feeding redfin are stronger regulators of benthic invertebrate

abundance than are trout. This conclusion is in agreement with other studies that also found strong effects of benthic-feeding fish relative to drift-feeding fish (e.g. Dahl 1998a, Cheever & Simon 2009), but contrasts with studies that found benthic- and drift-feeding fish to have equivalent predatory impacts on benthic stream invertebrates (e.g. Ruetz *et al.* 2004, Zimmerman & Vondracek 2007). Importantly, the results of my experiment support Dahl & Greenberg's (1996) "foraging mode" hypothesis, and lend support to the notion that differences in fish feeding behaviour can explain at least some of the variation in fish effects among studies.

The extent to which the predatory impact of drift feeders diverges from that of benthic feeders may be linked to the availability of alternative food sources, such as terrestrial invertebrates, in the drift. In situations where drifting terrestrial invertebrates are abundant, they are likely to contribute strongly to the diet of drift-feeding fish such as salmonids (Nakano *et al.* 1999a, Kawaguchi & Nakano 2001, Laudon *et al.* 2005), which could reduce the strength of the predatory impact exerted on benthic invertebrate prey (Dahl 1998b, Dahl & Greenberg 1998). On the other hand, in situations where terrestrial invertebrates are scarce, drift-feeding and benthic-feeding fish are likely to consume similar quantities of aquatic invertebrates (Zimmerman & Vondracek 2007, Buria *et al.* 2009), and consequently the strength of their predatory impacts on benthic invertebrates will be similar.

Surveys of invertebrate drift conducted in six CFR headwater streams (Chapter 4) revealed that terrestrial invertebrates were relatively abundant in the drift during summer (Chapter 4), comprising ~40% on average of the drifting invertebrate prey assemblage by number (although among-stream variation was high, Appendix 7). Analysis of fish diets based on data collected from the same six streams where drift was surveyed indicated that drift-feeding trout did indeed utilize terrestrial invertebrates as a food resource, and that this apparently offset their consumption of benthic invertebrates. On the other hand, benthic-feeding redfin were found to rely more strongly on benthic invertebrates as prey, and terrestrial invertebrates were only occasionally recorded in their guts (Chapter 4, Table 4.1 and Figure 4.4). Thus, differences in the utilization of terrestrial invertebrates as a food source by drift-feeding trout vs. benthic-feeding redfin could potentially explain the differences in predation strength on benthic invertebrates exerted by these two fish species observed in the present study.

Assemblage composition

In addition to their distinct effects on total invertebrate density and biomass, trout and redfin were also found to have non-equivalent top-down effects on invertebrate assemblage composition. The assignment of invertebrates to FFGs provided a convenient means by which to summarize important differences in the effects of trout and redfin on assemblage composition, and revealed that redfin had a stronger predatory impact than trout on some, but not all, functional components of the assemblage. The density of collector-gatherers (which comprised ~80% of the assemblage by number) and grazer-scrapers, but not filter-feeders and shredders, was significantly lower in the redfin treatment than in the other two treatments. This indicated that redfin had a stronger effect on collector-gatherers and grazer-scrapers than did trout, and this result was corroborated by analysis of *PI* values. On the other hand, both redfin and trout failed to significantly reduce the density of filter-feeders and shredders relative to the no-fish treatment, indicating that the predatory impact of both fish species on these FFGs was relatively weak. However, analysis of *PI* values indicated that redfin had a significantly stronger impact on filter-feeders than did trout. The reason for the discrepancy between the ANOVA and the *PI* analysis results for the filter-feeder FFG is unclear but could be linked to the fact that filter-feeder abundance was low and highly variable among treatments. Predatory invertebrate density was reduced by redfin, but not by trout, relative to the treatment with no fish, suggesting that redfin had a stronger impact on this FFG than did trout. However, no significant difference in predatory impact on this FFG between fish species was detected, and analysis of *PI* values corroborated this result.

Analysis of functional assemblage composition based on biomass data indicated that although collector-gatherers were the dominant functional component of the assemblage (comprising ~60% by weight), they constituted a smaller proportion of the assemblage than that estimated from invertebrate density data (~80%). On the other hand, grazer-scrapers and predators made up a greater proportion of the assemblage when composition estimates were based on biomass (comprising ~20% each) than when estimates were based on density (comprising <10% each). Differences between density- and biomass-based estimates of assemblage composition were due to the fact that several of the most abundant collector-gatherers (such as Chironominae and Orthocladiinae) were small-bodied

taxa that had a relatively low mean dry mass, while certain dominant grazer-scrapers (such as *Afronurus*) and predators (such as *Pseudagrion*) had larger body sizes and consequently larger mean dry masses (Appendix 13). Comparisons of redfin and trout impacts on biomass-based estimates of functional composition generally mirrored the density-based results, except that treatment effects on grazer-scrapers and predators were slightly weaker, and found not to be statistically significant. Furthermore, biomass-based analysis of *PI* values indicated that redfin and trout differed in the strength of the predatory impact that they exerted on collector-gatherers, but not on any of the other FFGs.

The finding here that benthic-and drift-feeding fish influenced the functional composition of benthic invertebrate assemblages differently, is consistent with the study of Cheever & Simon (2009). In mesocosms placed in a small stream in Virginia, USA, Cheever & Simon (2009) found that mottled sculpin *Cottus bairdi* reduced grazer-scrapers, but not any other benthic invertebrate FFGs, more strongly than did drift-feeding brook trout *Salvelinus fontinalis*. Interestingly, grazer-scraper abundance was also reduced by benthic, but not drift, feeders in the present study, suggesting that the behavioural attributes of this FFG may influence its vulnerability to benthic feeding fish (see discussion below).

Differences in assemblage composition were also evident at the taxonomic level. Taxonomic composition of invertebrate assemblages in cages with redfin was consistently different from that in cages containing trout and no fish, while no clear compositional differences were detected between the trout and no-fish treatments. These results indicate that while redfin had a strong influence on taxonomic composition, trout did not cause significant compositional shifts in the assemblage relative to the treatment with no fish. The clear compositional differences between the trout and redfin treatments were attributable to the fact that redfin reduced the density and biomass of some, but not all, invertebrate taxa, thereby skewing the overall structure of the invertebrate assemblage. In particular, the dipterans Chironominae and Orthocladiinae, and the ephemeropterans *Labiobaetis/Pseudocloeon*, *Afroptilum*, *L. penicillata* and *Baetis*, were strongly reduced by redfin, and were largely responsible for overall compositional differences between the redfin and trout treatments. Interestingly, these taxa are all collector-gatherers, which is consistent with the survey finding (Chapter 3) that differential fish effects on the collector-

gatherer FFG were largely responsible for the overall differences in assemblage composition between redfin and trout treatments.

My results here corroborate the results of Dahl (1998) who found, in flow-through enclosures placed in a small stream southern Sweden, that benthic-feeding bullhead *C. gobio* altered the taxonomic composition of benthic invertebrate assemblages more strongly that did drift-feeding brown trout *Salmo trutta*. Specifically, while bullhead significantly reduced the abundance of seven taxa of benthic invertebrate, trout only significantly reduced the abundance of one taxon. On the other hand, my findings here contrast with the results of Zimmerman & Vondracek (2007), who failed to detect differential effects of benthic-feeding slimy sculpin *C. cognatus* and drift-feeding brown trout *S. trutta* on the taxonomic composition of invertebrate assemblages in flow-through enclosures in Valley Creek, a small stream in Minnesota. In that study, it was suspected that a high production rate of aquatic invertebrates masked the top-down influence of fish in Valley Creek. The low production rates of aquatic invertebrates in CFR streams (de Moor & Day 2013), on the other hand, could potentially explain why strong top-down fish effects were recorded in the present study. Thus, the importance of fish in structuring assemblages of benthic invertebrates is probably strongly influenced by system productivity (Huryn 1998).

My experimental findings are broadly consistent with patterns in invertebrate assemblage structure detected during the broad-scale surveys of streams with and without trout (Chapter 3). Assemblage composition in trout-dominated streams was consistently different from that in redfin-dominated streams, and differences were driven largely by a lower abundance of herbivorous (i.e. taxa that feed at least partly on algae) invertebrates at redfin-dominated sites. In particular, collector-gatherers, including *Baetis*, *L. penicillata*, *Demoreptus capensis*, Chironominae and Orthocladiinae, and the filter-feeder *Simulium*, were consistently less abundant in redfin-dominated than in trout-dominated streams, indicating that redfin had a stronger predatory impact on these taxa than did trout. Taken together, the experimental and survey findings imply that redfin influence invertebrate assemblage structure differently from the ways that trout do, and that selective predation by redfin on certain herbivorous invertebrate taxa (especially collector-gatherers) was largely responsible for their divergent top-down effects.

My findings here, and those of other studies documenting selective predation by benthic-feeding fish on herbivorous invertebrates (e.g. Cheever & Simon 2009), suggest that the vulnerability of benthic invertebrates to fish predation may be strongly influenced by their feeding habits. The taxa reduced most strongly by redfin in the present study tended to be taxa that feed (at least in part) on algae and associated organic material (i.e. collector-gatherers and grazer-scrapers) in exposed habitats such as the surfaces of stones, and this epibenthic foraging behaviour likely renders such taxa especially vulnerable to predation by benthic feeding fish (Rosenfeld 2000). On the other hand, taxa that feed in more complex habitats such as the interstices between stones and leaf packs (i.e. shredders) are less accessible to benthic feeding fish (Rosenfeld 2000), which could explain why such taxa were not strongly influenced by treatment in my experiment. Results from studies investigating impacts of benthic-feeding stream fish on benthic invertebrate assemblages elsewhere are consistent with the view that herbivorous taxa with epibenthic foraging habits tend to be the most strongly reduced components of the benthic invertebrate assemblage (Dahl 1998b, Miyasaka & Nakano 1999, Ruetz *et al.* 2004, Cheever & Simon 2009), indicating that the findings of the present study may be more broadly applicable.

Finally, it is of interest that, of the taxa identified as important in discriminating between invertebrate assemblages in cages with redfin and trout, the predator *Pseudagrion* was the only taxon that had a greater mean density and biomass in the redfin cages than in the trout cages. This result indicates that trout consistently preyed more strongly on *Pseudagrion* than did redfin. This finding is consistent with the survey results in that large conspicuous predatory invertebrates (especially those within the order Odonata) tended to be less abundant in trout-dominated streams than in redfin-dominated streams. Furthermore, it is consistent with the observation of Samways (1994) that the distribution of other large predatory odonates appeared to be negatively related to the presence of non-native trout. Indeed, there is general consensus that trout selectively feed upon large, conspicuous aquatic invertebrate taxa (see review by Meissner & Muotka 2006), and *Pseudagrion* was one of the largest, most abundant taxa recorded in the experimental cages. Furthermore, large *Pseudagrion* larvae were frequently observed clinging to the exposed surfaces of stones on the stream bed, a behaviour which likely enhanced their vulnerability to predation by trout.

Diversity indices

Despite these clear compositional differences in invertebrate assemblages between the redfin and trout treatments, the experimental results provided little evidence for differences in taxon evenness or diversity among treatments. Taxon richness, on the other hand, tended to be somewhat lower in the redfin treatment that in the other two treatments, suggesting that redfin depleted not only the abundances of individual taxa, but also the total number of taxa, within the cages. The treatment effect on taxon richness was, however, found not to be statistically significant. The broad-scale surveys also found no significant differences in taxon richness or diversity between redfin-dominated and trout-dominated streams, but did record a significantly higher mean assemblage evenness in the presence of trout than in the presence of redfin. The reason for the discrepancy between experimental and survey results is not entirely clear, but it could be that invertebrate assemblages in the mesh baskets were somehow differently affected by fish predation than were assemblages occurring on the substrates of natural streams.

5.4.2 Basal resources

Algae

The reduction of herbivorous invertebrates in the redfin treatment relative to the other two treatments may have been expected to release benthic algae from grazing pressure, but this was not the case and cascading effects on chlorophyll *a* and periphyton AFDM were not detected in my experiment. These results, which were corroborated by analysis of *CS* values, contrast with the survey results (Chapter 3) which revealed that chlorophyll *a* biomass in redfin-dominated streams (where herbivorous invertebrate density was relatively low) was significantly greater than that at trout-dominated sites (where herbivorous invertebrate density was relatively high). Furthermore, these findings contrast with other experimental studies that detected significant cascading effects on benthic algae in cases where benthic-feeding fish reduced the abundance of herbivorous invertebrates more strongly than did drift-feeding fish. For example, in an enclosure experiment conducted in a small stream in southern Sweden, (Dahl 1998b) found that strong suppression of herbivorous invertebrates

by benthic-feeding bullhead *C. gobio,* relative to drift-feeding brown trout *S. trutta*, resulted in a significant increase in chlorophyll *a* biomass in cages containing bullheads relative to cages containing trout. Similarly, in a channel experiment conducted in a small stream in South Island New Zealand, Flecker & Townsend (1994) found that algal biomass in channels with native *G. vulgaris* was greater than that in channels containing non-native trout *S. trutta*, presumably as a result of the fact that trout suppressed grazing invertebrates more strongly than native did the native galaxiid.

On the other hand, differential fish effects on stream invertebrate assemblages do not always translate into changes at lower trophic levels. For example, in a mesocosm experiment conducted in a headwater stream in Virginia (USA), Cheever & Simon (2009) found that despite the fact that benthic-feeding mottled sculpin *C. bairdi* suppressed herbivorous invertebrate density more strongly than did drift-feeding brook trout *S. fontinalis*, no knock-on effects on benthic algae were detected. Similarly, in a cage experiment conducted in a headwater stream in north-eastern Finland, Meissner & Muotka (2006) found that strong suppression of herbivorous invertebrates by *S. trutta* did not lead to changes in the biomass of benthic algae.

There are several factors that might explain why the differential fish effects on invertebrate assemblages in the cages did not cascade down to benthic algae in my experiment. Firstly, the experimental period of one month may not have been sufficient for invertebrate suppression by redfin to manifest as differences in algal biomass among treatments, and it is possible that, had the experiment run for longer, such cascading effects may indeed have developed. However, other experiments of similar duration (e.g. Bechara *et al.* 1993, Flecker & Townsend 1994, Dahl 1998a) have detected significant invertebrate-mediated fish effects on algal biomass, suggesting that trophic cascades in streams can develop relatively rapidly. Secondly, the expression of differential fish effects on algal biomass may have been offset by the effects of intermediate predators such as predatory invertebrates (Cooper *et al.* 1990, Nyström *et al.* 2001, Cheever & Simon 2009). For example, redfin, but not trout, caused a decrease in the overall density of predatory invertebrates relative to the no-fish treatment, which could have caused a relaxation in predation pressure on non-predatory invertebrates, and a corresponding increase in grazing pressure. However, the fact that herbivorous invertebrate abundance was lower in the redfin treatment than in the other

two treatments, despite a reduction in predatory invertebrates, indicates that this explanation is unlikely, and suggests that the effects of fish predation overwhelmed the impact of predatory invertebrates in my experiment. Thirdly, despite the fact that redfin feed primarily on aquatic invertebrates, there is still some debate as to whether or not they also utilize plant material as food (see Cambray & Stewart 1985, de Wet 1990, Chapter 4). At present, the balance of evidence suggests that in situations where invertebrate prey is scarce, redfin diet may be supplemented by non-animal material such as algae and detritus. Thus, once redfin had reduced invertebrate abundance in the cages to the extent where invertebrate prey became difficult to locate, they may have switched to feeding directly on algae which could potentially have masked indirect invertebrate-mediated cascading effects. Fourthly, in some aquatic systems, algal biomass is regulated more strongly by bottom-up factors such as light, nutrients and temperature, than by top-down factors like grazing and predation (Hunter & Price 1992, Power 1992b, Biggs *et al.* 2000). It is therefore plausible that algal biomass in Morainekloof Stream was regulated primarily by abiotic factors from the bottom-up, rather than by biotic interactions from the top-down, which could potentially explain the lack of algal response to the fish treatment. Indeed, measurements of eight biologically important physico-chemical variables indicated that there was little difference in measured environmental conditions among treatments, which would be expected if algal biomass was regulated from the bottom-up. Fifthly, the tiles used for sampling algal biomass were simple, exposed habitats that offered invertebrates minimal refuges from predation by fish (Rosenfeld 2000). Thus, herbivorous invertebrates may have avoided feeding on the tiles because of an elevated risk of predation, which could potentially explain the absence of cascading effects on algal biomass on the tiles. However, other studies investigating cascading effects of stream fish have recorded significant effects on both invertebrate abundance, and algal biomass, on tile substrates (Bechara *et al.* 1992, McIntosh & Townsend 1996, Rosenfeld 2000, Nyström *et al.* 2001, Kurle & Cardinale 2011), and biotic interactions on tiles have been shown to accurately represent those occurring on natural stream substrata (Lamberti & Resh 1983). Lastly, cascading fish effects on algae may not necessarily manifest as changes in biomass, but rather as compositional shifts in the algal assemblage. Not all forms of benthic algae are equally palatable to invertebrate grazers (Holomuzki *et al.* 2010), and the possibility exists that top-down effects on edible taxa were masked by increases in the biomass of unpalatable taxa. Since algal assemblage structure

was not analysed in the present study, the potential for compositional shifts remains uncertain, and should be addressed in future studies of this type. The data collected during this study do not allow for discrimination among these hypotheses, but it is clear that fish had little influence over algal biomass in the cages, despite their strong suppression of herbivorous invertebrates.

Organic matter

As was the case for benthic algae, treatment effects on FPOM and CPOM were not significant, indicating that differential fish effects on benthic invertebrate assemblages did not translate into changes in standing stocks of organic matter in the experimental cages. These findings are consistent with the survey results which showed that FPOM and CPOM biomass at redfin-dominated sites was similar to that at sites dominated by trout. Agreement between the experimental and survey results constitutes good evidence that the replacement of redfin by trout does not induce cascading effects down the detrital trophic pathway in the study area.

These results are consistent with other studies reporting that fish had no detectable effect on standing stocks of organic matter in headwater streams (Reice 1991, Rosenfeld 2000, Rosemond *et al.* 2001, Herbst *et al.* 2009, Buria *et al.* 2010). Rosenfeld (2000) suggested that trophic cascades in detritus-based food chains are uncommon because detritivorous invertebrates feed on detrital accumulations in substrate interstices and are therefore not vulnerable to predation by fish. Indeed, shredder abundance did not differ among treatments in my experiment, implying that fish were unable to exploit shredders as a food source, which is consistent with the lack of a significant treatment effect on CPOM. Interestingly, studies that have reported significant fish effects on CPOM dynamics (Ruetz *et al.* 2002, Nyström *et al.* 2003, Greig & McIntosh 2006) also reported changes in the abundance of detritivorous invertebrates, suggesting that detritus-based cascades in streams may be dependent on the ability of fish to suppress key detritivores in the system.

On the other hand, the fact that collector-gatherers were significantly reduced by redfin relative to the other two treatments, may have been expected to result in a buildup of

FPOM in cages containing redfin, but this was not the case. Thus FPOM biomass was apparently regulated by factors other than top-down biotic interactions. Indeed, detrital food chains are donor-controlled systems driven by leaf-litter inputs from the adjacent riparian ecosystem (Polis & Strong 1996), and although detritivorous invertebrates have the potential to reduce the biomass of detritus (Ruetz *et al.* 2002, Nyström *et al.* 2003, Greig & McIntosh 2006), they have no control over the amount of detritus entering the stream (Rosenfeld 2000). Detrital inputs from the riparian zone may therefore have overwhelmed the effects of collector-gatherers on standing stocks of FPOM in this experiment. Furthermore, the rate at which particulate organic matter is transported downstream, away from headwater habitats, is mostly determined by physical conditions in the stream, rather than biotic interactions (Davies & Day 1998, Allan & Castillo 2007).

Although the effect of treatment on FPOM was not statistically significant, FPOM biomass in the redfin treatment was on average roughly half that in the other two treatments, and analysis of *CS* values revealed that redfin had a significantly stronger cascading effect on FPOM than did trout. This result is somewhat surprising given that redfin reduced collector-gatherer abundance which would be expected to cause an increase, rather than a decrease, in FPOM biomass. It may be, however, that while foraging for invertebrates among the benthos redfin disturbed FPOM in the experimental baskets resulting in the transport of FPOM from the cages downstream. Finally, it is noted that while a number of studies have used field experiments to investigate cascading effects of fish replacements on either algae-based or detritus-based trophic pathways, the present study is, to my knowledge, the first such experiment to measure simultaneous effects on both trophic pathways.

5.4.3 Strengths and shortcomings

In addition to the caveats already discussed, my experiment had some additional shortcomings, but also some important strengths, that need to be highlighted. The fact that biomass of both fish species in the experimental cages fell above the range of mean biomass values estimated for naturally-occurring populations of these species in the upper Breede River catchment may have led to overestimation of fish effects on aquatic invertebrate assemblages. However, extensive underwater observations in CFR headwater streams have

revealed that fish tend to aggregate in certain habitat patches (J.M. Shelton, pers. obs. 2010), and thus patch-specific fish biomass could potentially be far greater than estimates of mean biomass over the 50 m reaches sampled in my surveys. Furthermore, the fact that the density and biomass of trout and redfin within each block was closely matched meant that even if fish impacts were overestimated, they would have been overestimated to the same degree for both species, and thus comparisons of top-down species effects should remain informative.

While naturally occurring stream fish are free to move among habitat patches and forage over large areas of stream, the fish used in my experiment were confined to a relatively small area of erosional habitat which may have had some effect on their foraging behaviour. However, observations conducted during the experiment suggested that fish were behaving normally (J.M. Shelton, pers. obs. 2011), and the fact that decreases in fish weight were small and non-significant, and that no fish died, indicate that fish had access to a reasonable quantity of food. The use of larger cages (e.g. Flecker 1996), or fenced off sections of stream (e.g. Baxter *et al.* 2004, Winkelmann *et al.* 2011, Lepori *et al.* 2012), would mitigate the issue of artificially confining fish to small areas of stream, but the logistical challenges associated with such large-scale experiments may outweigh their potential benefits, especially in mountainous areas where access to study sites is difficult.

The low number of replicates used (i.e. $n = 4$ for each of the three treatments) limited the statistical power of the analyses, so my estimates of fish effects were likely to be conservative. Regardless, significant treatment effects on invertebrate density/biomass and assemblage composition were detected by the mixed model ANOVA/PERMANOVA tests, indicating that the differential top-down effects of redfin and trout on invertebrate assemblages were pronounced. The low level of replication used in my experiment was a consequence of logistical constraints, and future experiments of this type should look to increase replication. However, it should be noted that other field experiments evaluating similar hypotheses to those addressed here based conclusions on similar, low numbers of replicates (e.g. Flecker & Townsend 1994, Dahl 1998a, Cheever & Simon 2009).

Benthic community structure in the cages was broadly similar to that in natural streams in the study area, but there were also some important differences. Total invertebrate density

and functional composition in the mesh baskets were comparable to that in benthic samples collected from natural headwater streams during the broad-scale field survey (Chapter 3, Figure 3.5). On the other hand, mean taxon richness in natural streams was approximately double that in the cages. Thus the variety of aquatic invertebrate prey available to fish in the cages was lower than that in natural streams, and this may have influenced estimates of fish predatory impacts on invertebrate assemblages. The differences in taxon richness between the experimental cages and natural streams may be attributable to the fact that while the box sampler used in the surveys collected invertebrates from multiple layers of stream substrate, the mesh baskets used in the cages had only a single layer of stones. Chlorophyll *a* and FPOM biomass in the cages was slightly, but not substantially, higher than in natural streams in the study area, however CPOM biomass in the cages was approximately an order of magnitude higher than that in natural streams (Chapter 3, Figure 3.2). This discrepancy indicates that the cages accumulated unnaturally large quantities of leaves and other coarse plant material, despite the fact that the cage walls were cleaned twice a week, and this may have had some effect on benthic trophic dynamics. Indeed, experimental cages do tend to reduce stream flow (Zimmerman & Vondracek 2007), and this was likely to be the case in my experiment, since mean current velocity within the cages (0.09 - 0.12 m\s) was roughly five times slower than the mean flow velocity estimated in the natural stream during the 2010 survey (Chapter 2).

Finally, because fluxes of prey moving into, and out of, the cages were not measured in my experiment, I was unable to ascertain whether the changes in invertebrate abundance were a consumptive effect, a behavioural effect, or a combination of both (Cooper *et al.* 1990, McIntosh & Townsend 1996). Fish effects on prey abundance in experiments conducted at small spatial scales tend to reflect behavioural responses to predator presence (i.e. increased emigration), while prey responses at larger spatial scales are generally indicative of consumption (Sih & Wooster 1994, Englund 1997, Meissner & Muotka 2006). Although I cannot rule out the possibility that emigration may be partly responsible for the observed effects on invertebrate abundance in my experiment, the fact that the experimental results matched patterns in invertebrate assemblages measured at the landscape scale (Chapter 3) implies that consumption was probably also important. Furthermore, analysis of fish gut contents revealed that redfin selected collector-gatherers (the functional group most

strongly depleted in the redfin treatment) more strongly than did trout (Chapter 4), adding further support to the view that consumptive effects were important in the cages.

5.4.4 Conclusions and conservation recommendations

In conclusion, the differences in invertebrate density and assemblage structure, but not algal biomass, between redfin-dominated streams and trout-dominated streams could be explained by differences in the top-down effects exerted by redfin and trout. Redfin were stronger regulators of benthic invertebrate abundance and assemblage composition than were trout, and this appears to be responsible for the relatively low density of invertebrates in redfin-dominated streams relative to trout-dominated streams in the upper Breede River catchment. Specifically, redfin suppressed the abundance of certain herbivorous invertebrates (particularly collector-gatherer and grazer-scraper taxa) more strongly than did trout, which seems to be the primary reason for the differences in invertebrate assemblage composition between these two types of streams. These findings support Dahl & Greenberg's (1996) "foraging mode" hypothesis, and are consistent with the view that differences in fish foraging mode can drive variation in top-down fish effects among systems.

Whether or not the differences in algal biomass detected between redfin- and trout-dominated streams were a result of differences in herbivore-mediated top-down fish effects, or some other unmeasured factor, is not entirely clear, and will require additional studies to resolve. Despite the fact that the reduction of herbivorous invertebrates by redfin did not cause an increase in either chlorophyll *a* or periphyton AFDM in my experiment, there is general consensus that grazing by aquatic invertebrates can strongly affect algal biomass in streams (Rosemond 1993, Feminella & Hawkins 1995, Holomuzki *et al.* 2010). Furthermore, a recent small-scale tank experiment in the upper Berg River, a headwater stream in the CFR, has revealed that herbivorous invertebrates (in particular mayflies in the family Baetidae) exert strong top-down control over periphyton biomass (Ewart-Smith 2012). So if trout invasions are responsible for dramatic increases in the abundance herbivorous invertebrates, and shifts in assemblage structure, it follows that they also have

the potential to modify herbivore-algae interactions and ultimately influence the standing biomass of algae on the stream bed.

Not only do aquatic invertebrates regulate levels of basal resources in streams, but they can also constitute an important food source for other consumers in adjacent terrestrial ecosystems. Therefore, because trout alter the abundance of aquatic invertebrates in streams, the consequences of trout invasions potentially extend beyond intuitive effects on organisms with which trout directly interact. For example, studies elsewhere have shown that changes in aquatic invertebrate abundance, and the corresponding flux of aquatic invertebrates from aquatic to terrestrial systems, caused by non-native trout, can affect the abundance of riparian consumers such as spiders (Baxter *et al.* 2004, 2007, Benjamin *et al.* 2011) birds (Nakano & Murakami 2001) and bats (Baxter *et al.* 2005). Whether such cross-ecosystem effects occur as a result of trout invasions in CFR streams is not yet known, but is an interesting avenue for future research in this field.

Finally, the fact that differences in foraging behaviour can drive variations in top-down effects of predatory fish has important implications for how we manage fish invasions in streams, and highlights the need for a thorough understanding of the feeding biology of both native, and non-native species if accurate predictions about the community-level consequences of species invasions are to be made. In situations where an invasion by a non-native predatory fish results in the replacement of a native fish species, the degree to which impacts extend to other trophic levels will be influenced by how well the invader compensates for the trophic role previously performed by the native. If the invader and native species have the same foraging mode, then additional impacts on other food web components may be minimal. On the other hand, if the non-native has a foraging mode that is clearly different from that of the native fish being replaced, impacts are likely to extend to other trophic levels in the community and management interventions should be prioritized.

Chapter 6

General discussion

6.1 INTRODUCTION

Predators can have strong effects on the organization of biological communities through a combination of direct and indirect top-down interactions (Terborgh & Estes 2010). When introduced outside of their native range, predators can alter the functional composition of native predator assemblages (McIntosh & Townsend 1995b, Eby *et al.* 2006, Ricciardi & MacIsaac 2011, Benjamin *et al.* 2011), which can have consequences for prey populations and other organisms and resources at lower trophic levels (Chalcraft & Resetarits 2003, Schmitz 2007, 2008). Ultimately, predator introductions can lead to the re-organization of entire biological communities (Townsend 2003, Simon & Townsend 2003). On the other hand, introduced predators sometimes do not have strong effects in recipient systems (Mack *et al.* 2000, Ricciardi & Atkinson 2004).

It has been hypothesized that the extent to which native species (including native predators) are affected by an introduced predator will be influenced by whether or not they have prior experience with a predator that is functionally similar to that which is introduced (Cox & Lima 2006, Sih *et al.* 2010). Furthermore, it has been hypothesized that the prevalence of community-wide impacts may be linked to the degree to which an introduced predator changes the predation pressure exerted by the predator assemblage (Chalcraft & Resetarits 2003, Schmitz 2008, Benjamin *et al.* 2011). The factors driving variation in impacts of non-native predator are not sufficiently understood (Moyle & Light 1996, Parker *et al.* 1999, Salo *et al.* 2007), limiting our ability to forecast dangerous invasions and respond accordingly.

The broad aim of my thesis was to improve knowledge and understanding about population- and community-level impacts of novel predators in insular systems where biological communities evolved in the absence of functionally similar native predators. River catchments in the Cape Floristic Region (CFR) of South Africa are considered to be insular systems because they have a long history of geographic and biological isolation (Wishart &

Day 2002, Wishart *et al.* 2006, de Moor & Day 2013). Non-native predatory rainbow trout *Oncorhynchus mykiss* (henceforth "trout") were introduced into all of the major river systems in the region (de Moor & Bruton 1988, Scott *et al.* 2006), and have subsequently spread and established in countless small headwater streams where they represent a functional novelty. I studied the impacts of non-native trout in these headwater streams with a focus on how trout have modified the structure and function of a naïve native predator assemblage, and what the consequences of this are for community organization at lower trophic levels.

6.2 SYNTHESIS

The fact that trout have invaded some, but not all, headwater tributaries of the upper Breede River (in the CFR) presented a natural experiment that facilitated comparisons of stream food webs in the presence and absence of a functionally novel predator. Hypotheses about impacts generated by broad-scale comparative surveys were then evaluated using small-scale manipulative field experiments. Additionally, fish diets and feeding behaviours were studied to ascertain whether or not native and non-native fish perform similar predatory roles, and whether variation in community structure among streams could be linked to differences in the trophic niches occupied by native and non native fish. In the present chapter I summarize and synthesize the key findings emerging from my research, discuss conservation implications based on these findings, and highlight fruitful avenues for future research.

6.2.1 Surveys and experiments reveal strong predatory impact of trout on a naïve native predator assemblage

In Chapter 2 it was hypothesized that small-bodied native fish species which are common top predators in CFR headwater streams would be vulnerable to predation by trout because trout represent a novel threat in that environment. Broad-scale surveys comparing fish populations in streams with (n = 12) and without (n = 12) trout showed that, while generally

abundant in streams lacking trout, native fish were absent, or present at a very low density, in streams containing trout.

The mean density and biomass of the native fish Breede River redfin *Pseudobarbus* sp. "Burchelli Breede" (henceforth "redfin"), Cape kurper *Sandelia capensis* (henceforth "kurper") and Cape galaxias *Galaxias zebratus* (henceforth "galaxias"), was substantially higher in streams without trout than in streams with them, and although present at all 12 sites without trout, native fish were only recorded at five of the 12 sites with trout present. Multivariate analysis of variance revealed no consistent difference in environmental conditions between sites with and without trout, and distance-based linear models identified trout density as the best predictor of variation in redfin and kurper densities among sites. Galaxias density, on the other hand, was best predicted by other environmental variables including mean substrate size, site slope and riparian vegetation cover, but analyses for this species may have been compromised by the low frequency of galaxias occurrence.

The fish surveys also showed that the size structure of native fish populations in the presence of trout was different from that in trout-free streams. Importantly, small-sized individuals of all three species (<40 mm) tended to be abundant at sites without trout, but were largely absent at sites with trout. This pattern generated the hypothesis that size-specific predation by trout was responsible for the absence, or relatively low abundance, of native fish at sites containing trout. A small-scale predation experiment was used to evaluate this hypothesis, and revealed that, as predicted, large trout preyed selectively upon small-sized individuals of native redfin (Chapter 2).

Taken together, these results indicate that trout have replaced native fish as dominant top predators in streams in the study area, and that predation by trout on small sizes-classes of native fish appears to be an important mechanism driving this pattern. My findings are in agreement with other studies documenting strong negative impacts of novel predators on naïve native fish populations both in South Africa (e.g. Skelton 1993, Shelton 2003, Woodford & Impson 2004, Woodford *et al.* 2005, Weyl *et al.* 2010, Ellender *et al.* 2011), and in other parts of the world (e.g. Townsend & Crowl 1991, White & Harvey 2001, Sih *et al.* 2010, Habit *et al.* 2010). Furthermore, my findings here support the "naïve prey" hypothesis

(Cox & Lima 2006) that predicts strong impacts of introduced predators in insular systems supporting native species that are naïve to the hunting tactics of the introduced predator.

6.2.2 The threat posed by predator invasions to freshwater endemism on a global scale

Because it is an unrealistic aim to protect all species and habitats on Earth from human-related impacts, conservation biologists are faced with the challenge of deciding which areas should be prioritized for conservation (Margules & Pressey 2000, Brooks *et al.* 2006, Vörösmarty *et al.* 2010). Areas supporting high concentrations of endemic species have been identified as important targets for global conservation efforts (Lamoreux *et al.* 2006), because it is in such areas where the greatest number of species can be protected with the least effort (Myers *et al.* 2000). Endemism can result from a variety of physical, climatic and biological processes (Begon *et al.* 1996), and endemic species are especially likely to evolve in insular systems such as islands or water bodies isolated within terrestrial landscapes (Courchamp *et al.* 2003, Lamoreux *et al.* 2006, Tedesco *et al.* 2012). Their evolutionary isolation may render endemic species particularly naïve and vulnerable to the threat posed by introduced predators (Cox & Lima 2006), especially if such predators represent a functional novelty in the system into which they are introduced (Salo *et al.* 2007, Ricciardi & MacIsaac 2011). Thus, the impacts of introduced predators may be especially severe in areas supporting high concentrations of endemic species – areas of unusually high conservation value. The situation for freshwater endemics is of particular concern because, relative to their terrestrial and marine counterparts, freshwater systems have received a disproportionately large number of predator introductions through angling and aquaculture activities (Eby *et al.* 2006, Cox & Lima 2006). Therefore, quantifying and mitigating predator impacts in hotspots of freshwater endemism like the CFR is of critical importance from the perspective of conserving global biodiversity, because it is in such areas that the consequences of predator introductions are likely to be most severe.

6.2.3 Evidence for multi-trophic level impacts of trout from a broad-scale field survey

Studies investigating impacts of introduced predatory fish often focus on direct effects on populations of large, conspicuous native taxa (such as fish and amphibians), but introduced predators can also have subtle effects that propagate down through multiple trophic levels, which can result in the restructuring of entire communities (Flecker & Townsend 1994, Simon & Townsend 2003, Eby *et al.* 2006). In Chapter 3, I studied benthic community structure in the same streams where fish populations and environmental variables were surveyed to ascertain whether trout impacts have extended beyond the replacement of native fish and cascaded down through lower trophic levels of the stream community. It was found that the structure of benthic communities in trout-free streams supporting healthy native fish populations was different from that in streams where non-native trout have established and replaced native fish species as the dominant top predator, implying that trout effects do indeed extend beyond impacts on native fish, down to lower trophic levels. Total invertebrate density in streams containing trout was substantially higher than that in streams containing native fish only, and there were also consistent differences in taxonomic and functional composition of benthic invertebrate assemblages between these two types of streams. Compositional differences were driven largely by a greater abundance of epibenthic, herbivorous invertebrates in streams dominated by trout relative to streams with native fish only. On the other hand, the abundance of detritivores and predators did not differ significantly between streams with and without trout. Analyses of standing stocks of resources at the base of the food web revealed that chlorophyll *a* biomass, but not the biomass of fine (FPOM) or coarse (CPOM) particulate organic matter, in streams invaded by trout was significantly lower than that in streams with native fish only. Variation in environmental conditions among streams could not account for the differences in benthic community structure, implicating trout as the primary causal agent.

Taken together, the findings here indicate that the replacement of native fish by trout initiated a trophic cascade down the algae-based, but not the detritus-based, trophic pathway, mediated by differential top-down effects on benthic invertebrate assemblages. Specifically, the survey results imply that trout do not functionally compensate for the native fish that they have replaced, and rather, that trout are weaker regulators of benthic invertebrates than are native fish. In streams where trout have replaced native fish,

predation pressure was apparently relaxed, resulting in a proliferation of herbivorous, epibenthic invertebrates (particularly collector-gatherer taxa) on the stream bed. The associated increase in grazing pressure at invaded sites appears to have led to a decrease in benthic algal biomass relative to that at uninvaded sites supporting healthy native fish populations. The hypothesis that trout are weaker regulators of benthic community structure than are native fish was further examined through comparisons of trophic niche (Chapter 4), and top-down effects (Chapter 5), between trout and the native fish which they have replaced.

6.2.4 Behavioural observations, gut contents and stable isotopes reveal that the functional role performed by trout is not the same as that performed by native redfin

Differences in community structure between streams with and without trout suggest that native fish and trout have non-equivalent top-down effects on benthic invertebrate abundance and assemblage composition, and that trout do not regulate the abundance of aquatic invertebrates as strongly as do native fish. This may be due to the fact that trout invasions altered total predator biomass, or it could be a consequence of differences in the functional role performed by trout and native fish they have replaced. Since mean fish biomass in streams invaded by trout did not differ significantly from that in streams lacking trout, it was hypothesized that differences in the functional role performed by redfin (the dominant member of the predator assemblage in trout-free streams) and trout (the dominant predator at invaded sites) were responsible for the observed patterns in benthic community structure (Chapter 3).

This hypothesis was examined by characterizing and contrasting the trophic niches of redfin and trout using a combination of complementary approaches including focal animal watching (FAW), gut contents analysis (GCA) and stable isotope analysis (SIA). FAW was used to compare the foraging behaviours of trout and redfin, while GCA and SIA were used to characterize and compare the diets of these two species in a subset of the streams (trout: n = 3, redfin: n = 3) sampled during the broad-scale surveys described in Chapters 2 and 3. FAW offered insights into fish foraging modes, GCA provided detailed information about

foods recently ingested, and SIA was used to obtain a coarser-resolution, time-integrated measure of fish dietary habits (Gelwick & Matthews 2007).

The FAW study revealed clear differences in foraging behaviour: while redfin acquired the vast majority of their food items from the benthos, trout fed primarily from the drift. Gut contents and stable isotope analysis both showed that the diet of redfin was largely different to that of trout. Importantly, while aquatic invertebrates made up the majority of the diet of both species, they were found to contribute more strongly to the diet of redfin than to that of trout. The consumption of aquatic invertebrates by trout was apparently offset by their consumption of terrestrial invertebrates. Terrestrial invertebrates were found in the vast majority of trout guts and constituted approximately a third of their overall diet. On the other hand, terrestrial invertebrates were far less common in the guts of redfin, and only contributed ~10% to their overall diet.

Collectively, these findings support the hypothesis that non-native trout and the native redfin which they have replaced are not functionally equivalent predators in the study streams. Differences in the utilization of terrestrial invertebrates as a food source could potentially drive differences in predation pressure exerted by trout and redfin on benthic invertebrates. The relatively low density of benthic invertebrates in streams supporting healthy native fish populations (Chapter 3) is consistent with the finding that redfin are strongly reliant on aquatic invertebrates as prey. On the other hand, the relatively high density of benthic invertebrates in streams where trout have established, and replaced native fish, appears to be linked to the fact that the consumption of terrestrial invertebrates by trout reduces the predation pressure they exert on aquatic invertebrates.

6.2.5 Experimental evidence for differential top-down effects of benthic-feeding redfin and drift-feeding trout

Effects of fish predation on stream community structure are variable (Williams *et al.* 2003, Meissner & Muotka 2006, Winckler-Sosinski *et al.* 2008, Cheever & Simon 2009, Winkelmann *et al.* 2011). Developing an understanding about the factors driving this variation is important if we are to develop a capacity to predict how the modification of fish

assemblages will impact on other components of stream communities (Cheever & Simon 2009). Variation in fish effects among studies have been attributed to a range of environmental and biological factors, as well as to methodological differences among studies (Winkelmann *et al.* 2011). Dahl and Greenberg (1996) put forward the hypothesis that much of this variation may be related to differences in fish foraging mode. Specifically, they postulated that drift-feeding fish should have a weaker impact on benthic prey than do benthic-feeding fish, because the diet of drift feeders will be augmented by terrestrial invertebrates (Miyasaka & Nakano 1999, Nakano & Murakami 2001). However, subsequent studies evaluating this hypothesis have produced equivocal results.

The results of a broad-scale comparative study (Chapter 3) were consistent with Dahl & Greenberg's (1996) hypothesis because benthic invertebrate abundance in streams dominated by benthic-feeding redfin was substantially lower than that in streams dominated by drift-feeding trout. Furthermore, differences in the biomass of benthic algae between redfin- and trout-dominated streams suggested that differential fish effects on benthic invertebrate assemblages cascaded down to the base of the algae-based trophic pathway in these streams. Surveys, while useful for describing patterns at broad, realistic spatial scales, cannot be used to infer "cause-and-effect-type" relationships (Park 2004). Investigation of mechanisms behind survey patterns requires the use of controlled, manipulative experiments (Park 2004). In Chapter 5, a small-scale, manipulative field experiment was therefore conducted to test the hypothesis that benthic-feeding redfin regulate benthic invertebrate assemblages more strongly than do drift-feeding trout, and that differential fish effects on invertebrate assemblages would cascade down to the base of the food web and affect standing stocks of basal resources.

The experiment revealed that redfin were indeed stronger regulators of benthic invertebrates (particularly collector-gatherer and grazer-scraper taxa) than were trout, and thus differences in top-down fish effects is likely a mechanism behind the differences in invertebrate assemblage structure between streams with and without trout. This finding provides support for Dahl & Greenberg's (1996) "foraging mode" hypothesis, and is consistent with the view that differences in fish foraging mode can drive variations in top-down fish effects among systems. The strong suppression of herbivorous invertebrate abundance by redfin, but not trout, may have been expected to release benthic algae from

grazing pressure in the presence of redfin. Cascading effects on algae were, however, not recorded in my experiment, which contrasts with the patterns detected during the broad-scale survey (Chapter 3). Reasons for this discrepancy between survey and experimental results are not entirely clear, and are discussed in detail in Chapter 5. Finally, the lack of significant fish effects on standing stocks of organic matter is consistent with the survey patterns, and is perhaps not a surprising result given that shredder abundance in the experiment was low and was not significantly affected by fish treatment, and that organic matter biomass is strongly influenced by site-specific environmental conditions.

6.2.6 What can foraging mode tell us about potential consequences of a predator invasion?

The fact that differences in foraging behaviour can drive variation in top-down effects of predators has important implications for how we manage predator invasions, and highlights the need for a thorough understanding of the feeding biology of both native, and non-native predators if accurate predictions about the consequences of predator replacements are to be made. In situations where a native predator is replaced by a non-native predator, the degree to which impacts extend to other trophic levels will be influenced by how well the invader compensates for the trophic role previously performed by the native (Chalcraft & Resetarits 2003). If the non-native and native predators have a similar foraging mode, and if they consume a similar diet, then additional impacts on other components of the food web are likely to be minimal (Schmitz 2007, 2008). In such cases, although the identity of the predator is changed, its function in the food web is retained, and consequently management efforts may not be justified. On the other hand, if the invasive predator has a foraging mode that is clearly distinct from that of the native predator, the consequences of the invasion are likely to extend beyond impacts on native predator populations, down through lower trophic levels, and management interventions should be prioritized (Simon & Townsend 2003, Benjamin *et al.* 2011).

6.3 SUMMARY OF KEY FINDINGS

Figure 6.1 presents a graphical overview of the main findings emerging from the research described in this thesis.

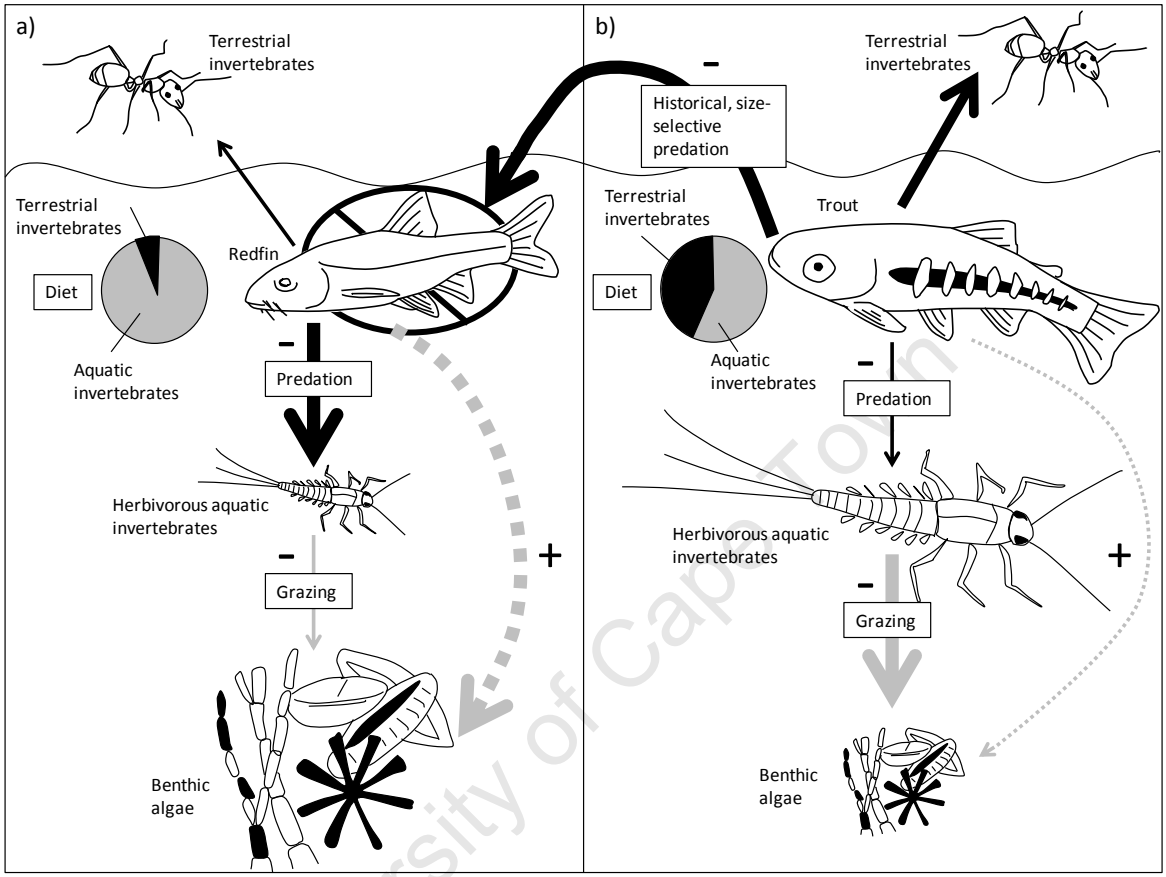

Figure 6.1 Headwater stream food webs (a) with trout, and (b) without trout. Where trout are absent native redfin dominate the predator assemblage. As benthic feeders, redfin rely heavily on aquatic invertebrates for food, and consequently suppress aquatic invertebrates (particularly herbivorous taxa), which decreases grazing pressure, allowing benthic algae to proliferate. Where trout have invaded, they replace native redfin as the dominant top predator through size-selective predation. As drift feeders, trout consume more terrestrial invertebrates than do redfin, offsetting the predation pressure that they exert on aquatic invertebrates. This allows aquatic invertebrates (particularly herbivorous taxa) to proliferate on the stream bed, which in turn graze down benthic algal biomass. Ultimately, by replacing a functionally different native predator, trout alter predation dynamics in streams with potential cascading consequences for lower trophic levels. Solid arrows represent direct, consumptive effects and dashed arrows indicate indirect effects. Relative effect strength is proportional to arrow thickness. Black lines represent agreement between survey and experimental results, while grey lines represent evidence from field surveys only. The sizes of invertebrate and alga vignettes indicate differences in density and/or biomass between the two food web states. Pie charts adjacent to fish indicate proportional dietary composition in terms of invertebrate prey based on the Index of Relative Importance *(% IRI*) from gut content analysis.

In summary, the key findings of my work are (1) trout have largely replaced native fish as top predators in streams in the upper Breede River catchment, CFR; (2) predation by large-sized trout on small size-classes of native fish is a mechanism behind this replacement; (3) the abundance of herbivorous benthic invertebrates in streams with trout is higher than that in streams without trout; (4) the biomass of benthic algae, but not particulate organic matter, in streams with trout is lower than that in streams without trout; (5) because terrestrial invertebrates contribute importantly to their diet, trout are less reliant on aquatic invertebrate prey than are native redfin; and (6) differences in top-down effects of redfin and trout, as a result of differences in trophic niche, appear to be responsible for differences in invertebrate abundance and assemblage composition, but not necessarily differences in benthic algal biomass, between streams with and without trout.

6.4 CONSERVATION IMPLICATIONS

We are currently in the midst of a global biodiversity crisis (Singh 2002). The rate of human-induced extinctions has increased dramatically in recent times (Pimm *et al.* 1995, Singh 2002, Wilson *et al.* 2007), especially in freshwater systems (Dudgeon *et al.* 2006). This situation demands the identification of biologically import areas and the protection of species and ecosystems within such areas (Myers *et al.* 2000, Brooks *et al.* 2006). Many approaches exist for prioritizing areas for conservation, and the criteria most commonly incorporated include endemic species richness, total biodiversity and a measure of habitat modification (Wilson *et al.* 2007). Once identified, areas of biological importance can then be set aside as protected areas, and key species and processes within them can be conserved through the mitigation of major threats (Margules & Pressey 2000). Despite impressive advances in our understanding of the structure and function of the Earth's biological systems and the major threats with which they are faced, safeguarding biodiversity continues to present a formidable challenge for conservation biologists. The protection of biologically important areas often clashes with human interests and activities, adding an inescapable socio-economic dimension to the field of conservation biology (Margules & Pressey 2000).

One of the most celebrated frameworks for focusing global conservation efforts is the biodiversity hotspot concept developed by Myers *et al.* (2000). Biodiversity hotspots are areas that support exceptionally high concentrations of unique species, but that are also under serious threat from human-related activities. The CFR in South Africa is one of the 25 biodiversity hotspots identified by Myers *et al.* (2000), and is best known for its exceptionally high plant diversity (~9000 species) and endemicity (~70%) (Rouget *et al.* 2003, de Moor & Day 2013). Less well known is that the region's freshwater fauna also boasts unusually high levels of endemism, with ~64% of invertebrates (Wishart *et al.* 2006, de Moor & Day 2013) and ~89% of fish being endemic (Tweddle *et al.* 2009). Freshwater biodiversity in the CFR is under threat from many factors including habitat loss and fragmentation, hydrologic alteration, climate change, pollution and invasions by non-native species, and the middle and lower reaches of the majority of rivers in the region are in a degraded state (Tweddle *et al.* 2009).

Less severely degraded are the upper headwaters of CFR streams, which are largely free from the threats facing lower-lying reaches because they are situated in mountainous areas that are relatively inaccessible and unsuitable for agriculture, human settlements and reservoirs (Swartz *et al.* 2004, RHP 2011). Consequently, headwater streams function as ecological refugia within relatively disturbed riverscapes, and are thus critical habitats for conserving freshwater biodiversity in the CFR. The majority of fish species introduced to CFR streams cannot tolerate environmental conditions in headwater habitats (de Moor & Bruton 1988, Skelton 2001). Trout, on the other hand, are well adapted to headwater stream environments (Skelton 2001), and have established self-sustaining populations in many headwater tributaries across South Africa (de Moor & Bruton 1988, Cambray 2003). Although negative impacts of trout in headwater habitats have long been suspected, until now quantitative assessments of these impacts have been lacking. The findings of my research represent the first conclusive evidence that trout invasions pose a serious threat to the integrity of headwater stream communities in the CFR. Populations of endemic fish species have been greatly reduced by trout invasions, yet perhaps most concerning is that trout are capable of re-structuring entire stream communities, with potential consequences for other species with which stream biota interact.

Effective conservation of headwater stream environments will therefore rest, at least in part, on our ability to mitigate future impacts of non-native trout in headwater habitats. In the Breede River catchment (and other South African catchments), trout were originally stocked into larger tributaries, and from there spread into many of the smaller headwater tributaries that drain mountainous areas (de Moor & Bruton 1988). Fortunately, many headwater streams still remain trout-free, and these pristine (or near-pristine) habitats act as sanctuaries for native aquatic biodiversity. From a conservation perspective, it is therefore important to understand what stands between remaining trout-free headwater streams and new trout invasions. Essentially, what are the key factors regulating trout populations in headwater habitats in the CFR?

Addressing this question was not an objective of my thesis, but is clearly an import avenue for future research. Although not explicitly examined, some deductions about the factors influencing trout populations in the upper Breede River catchment based on data from my, and other, studies can be tentatively made. Elsewhere, a range of physical and chemical characteristics of aquatic environments, including temperature, pH and hydrological regime have been shown to limit trout invasions (Closs & Lake 1996, McIntosh 2000, Fausch *et al.* 2001, Olsson *et al.* 2006), and such factors probably influenced trout abundance in streams where they were present in my study. However, comparisons of a set of biologically-important physico-chemical variables (both separately and in combination, Chapter 2) between streams with and without trout revealed that there were no clear differences in environmental conditions between these two groups of streams. This result suggests that the streams in my study area that are presently trout-free trout are potentially capable of supporting trout populations, implying that some other factor must have prevented trout from invading. The majority of the trout-free reaches sampled in the present study were situated above waterfalls, weirs or dry/braided reaches (Figure 6.2), suggesting that such features function as physical barriers to trout dispersal. Indeed, physical barriers have been found to restrict trout invasions abroad (McDowall 2006, Fausch 2007, Herbst *et al.* 2009, McIntosh *et al.* 2010), and to restrict trout (Cambray & Meyer 1988, Karssing *et al.* 2012), and bass (*Micropterus* spp.) (Skelton *et al.* 1995, Impson & Swartz 2002, Woodford *et al.* 2005, Impson *et al.* 2007), invasions elsewhere in South Africa.

Given that many headwater streams are still trout-free, I propose that the primary goal of trout management in the CFR should be to prevent new trout introductions upstream of existing dispersal barriers; particularly in systems that harbor native species of special biological importance (i.e. species that are have very small distribution ranges, or face an especially high risk of extinction). A secondary objective would then be to eradicate trout populations from streams where their impacts are unacceptably high, for example, where trout populations directly threaten distinct native taxonomic lineages of native biota.

Figure 6.2 Examples of physical trout barriers in the study area including (a) a waterfall on Tierstel Stream, (b) a weir on the Titus tributary and (c) a drying braided reach on Tierkloof Stream.

New introductions above dispersal barriers could potentially occur for one of three main reasons: (1) deliberate introductions for angling, (2) accidental introductions via reservoirs that contain trout, or (3) accidental introductions resulting from aquaculture operations. As the angling and aquaculture industries in South Africa expand, the probability of new introductions via these pathways will no doubt increase. Anglers will look to create new fisheries, both in streams, and in reservoirs, and new aquaculture operations will surely be established. The expansion of the South African trout industry is inevitable, and important from a socio-economic perspective. The key to mitigating future trout impacts therefore lies not in constraining the development of the trout industry, but rather in guiding its expansion in a direction that minimizes damaging new trout introductions.

The objective of my research was to improve knowledge and understanding about how trout modify food web structure in headwater stream communities in the CFR, not develop a comprehensive framework for managing non-native trout. However, the findings of my research can be incorporated into management plans, and used to refine existing legislation regarding the conservation freshwater biodiversity in the CFR, and perhaps elsewhere in South Africa. The following management recommendations represent examples of how future trout impacts could be mitigated while concurrently catering for expansions in the trout angling and aquaculture industries.

1. *Trout should not be stocked into streams outside of their existing range*

How then can new angling locations be made available to satisfy an expanding trout angling community? New fisheries need to be created, and existing fisheries improved, in streams where trout already occur. This could be achieved through several approaches including negotiations with landowners to secure new fisheries (there are many streams, both small and large, in the CFR that contain trout but are not yet utilized by anglers); managing riparian vegetation to improve access to established fisheries (encroachment by riparian vegetation (often non-native) currently limits angling over notable stretches of stream within established fisheries); and supplementing existing populations by stocking to improve the quality of existing fisheries (some of the existing trout populations are not valued by anglers because of their low densities). Any proposals for stocking trout into CFR streams

will, however, need to be accompanied by thorough biological assessment of the target system before permission for the stocking is granted.

2. *Trout should not be stocked into reservoirs, nor new aquaculture facilities be established, in catchments where trout are not present, or at locations upstream of trout-free sections of stream in catchments where trout do occur*

Escapees from off-stream facilities are a notorious source of fish invasions (Fausch 2007). Any proposals to stock new reservoirs with trout, or open new trout aquaculture operations, therefore need to be carefully evaluated against information on the distribution of trout within that catchment. For this recommendation to be effectively implemented, additional surveys may be required to improve our knowledge about the present distribution range of trout in CFR river networks.

3. *Under certain circumstances, trout should be eradicated from sections of stream where populations are of no/little value to anglers and/or where native species/ecosystems of special concern are at risk*

Many of the headwater streams that presently support trout populations are of little, or no, value to anglers, because of their small size, because of dense riparian canopies, or because they occur in isolated areas that are difficult to access. Trout could potentially be removed from these streams with minimal repercussions for the trout angling community, but potentially great benefits for native stream biota, particularly if they contain species of special biological importance. Fish eradications are expensive, present a suite of ethical challenges, and have several negative side-effects that need to be weighed up against the benefits of the eradication (Marr 2011). Additionally, eradications often need to be coupled with the erection of artificial dispersal barriers to prevent future re-invasion, but such barriers can have serious negative isolation effects on populations of native species (Fausch *et al.* 2009). The outcome of a pilot fish eradication project presently underway, aimed at eliminating bass from a 5 km section of a small stream in the Olifants River catchment (CFR)

to increase habitat for highly threatened fish species, can be used assess the feasibility and effectiveness of future fish eradication schemes in CFR streams (Marr *et al.* 2012).

6.5 FUTURE DIRECTIONS

In the individual chapters of this thesis I have discussed the main strengths and limitations of my data, and made suggestions about how future studies could improve on the shortcomings of my research and explore new questions generated by my research findings. Here, I highlight and summarize some key avenues for future research on trout impacts in the CFR.

6.5.1 Spatial and temporal scale

A recurrent issue faced by ecological researchers is the challenge of finding the correct temporal and scale at which to investigate patterns and processes of interest (Levin 1992). In this thesis, both the surveys and experiments were conducted during summer because environmental conditions are most stable, and fish impacts therefore likely to be most pronounced. The influence of fish on stream communities can, however, vary greatly with season (Power *et al.* 2008), and top-down effects on invertebrates and basal resources may well be less pronounced during winter when environmental disturbances are frequent (Lancaster 1996). Future studies should therefore look to expand the temporal scale of this research by quantifying seasonal variations in trout impacts (e.g. Cheever & Simon 2009), perhaps at a subset of the sites sampled in the present study. From a spatial perspective, there is a need to extend the survey work conducted in my thesis to other catchments in the CFR, and also to other parts of South Africa, and thereby assess the generality of my findings. The results from manipulative experiments, such as those conducted in this thesis, are notorious for being highly sensitive to the size of the experimental unit. The experimental units used in both the predation experiment (Chapter 2), and the cage experiment (Chapter 3), were relatively small (<2 m^2), and this may have led to overestimation of trout impacts on native fish, and fish effects on lower trophic levels, respectively (Peckarsky *et al.* 1997). Future experimental studies of the types conducted

here need to give careful consideration to spatial scale, and should increase the size of experimental units where possible.

6.5.2 Factors limiting trout abundance and distribution

As evidence mounts for negative impacts of trout in CFR streams, there is a pressing need to understand the factors controlling trout abundance and distribution. Although relationships between trout populations and environmental factors were not explicitly examined in this thesis, my analyses of environmental variables suggest that trout could potentially inhabit the 12 streams from which they were absent. Furthermore, observations at these trout-free sites suggest that physical dispersal barriers (waterfalls, weirs and dry/braided reaches) may well be paramount in constraining trout invasions in headwater streams in the CFR, as is the case elsewhere (e.g. Resetarits 1991, Townsend & Crowl 1991, Lintermans 2000, Barr & Babbitt 2002, Herbst *et al.* 2009, Woodford & McIntosh 2010). Further study of the role of physical barriers in restricting trout distributions in CFR streams is needed, and future studies should look to assess the importance of such barriers in maintaining trout-free headwater refugia at the landscape scale (e.g. Townsend & Crowl 1991, Woodford 2009). Physical barriers may well be a principal determinant of whether or not trout are able to access a headwater stream reach, but the high variation in trout abundance among sites where they are present suggests that trout populations may also be strongly influenced by other site-specific factors. For example, stocking history within the stream itself, as well as in other parts of the catchment to which the stream is connected, could influence trout population density, and whether or not trout are able to establish at all (Eby *et al.* 2006). Biological interactions, such as competition, with other fish species, and predation by fish-eating predators, such as birds and otters, could also significantly affect trout density (Macneale *et al.* 2010, Wengeler *et al.* 2010). Additionally, variation in physico-chemical conditions could be an important source of variation in trout abundance among streams (McIntosh 2000, Olsson *et al.* 2006). Developing an understanding of how these types of factors influence trout populations in the CFR is important because trout impacts (at least on native fish populations) appear to be density dependant (Chapter 2). Also important will be to develop understanding about how trout interact with other invasive species (Nyström *et al.* 2001), and how trout populations respond to other disturbances such as water

abstraction, and predicted changes in river temperature and flow stemming from climate change (Dallas 2008). Ultimately, we need to look towards developing habitat suitability models for trout and using them predict possible scenarios for future trout invasions in the CFR, and indeed throughout South Africa.

6.5.3 Additional impacts on other biota

Although I adopted a multi-trophic-level approach to assessing trout impacts, the potential exists for additional effects on organisms other than those sampled in my study. Functionally important terrestrial consumers such as spiders, birds and bats can be affected by changes in benthic invertebrate abundance and the associated flux of aquatic invertebrates to the riparian zone (Nakano *et al.* 1999a, Nakano & Murakami 2001, Baxter *et al.* 2004, 2007, Benjamin *et al.* 2011), but such cross-ecosystem impacts have not yet been studied in the CFR, nor for that matter in Africa. Little is known about the impact of trout on native amphibians in the CFR, but recent work elsewhere in South Africa (Karssing *et al.* 2012), and a wealth of studies in other parts of the world (Gillespie 2001, Barr & Babbitt 2002, Kats & Ferrer 2003, Bosch *et al.* 2006) suggest that impacts may be negative and strong. This is a particularly worrying prospect, considering that South Africa's amphibian fauna includes many endemic species already under serious threat (du Preez & Carruthers 2009), and because amphibians can play important functional roles in stream food webs (Nyström *et al.* 2001). Native crabs within the genus *Potamonautes* also play functionally important roles in stream ecosystems (Hart *et al.* 2001), but whether or not crab populations have been affected by trout invasions has not yet been studied. Strong impacts of trout on native crayfish (which are functionally similar to crabs) populations in other parts of the world (e.g. Olsson *et al.* 2006) suggest that impacts may be severe, and far-reaching. Future work in this vein should begin by focusing on impacts on species that play important functional roles, and those that are rare or threatened strongly by extinction.

6.5.4 Kurper and Galaxias

My experimental work investigating size-selective predation by trout, and community-level fish effects, as well as my work on fish feeding mode and diet, focused on native redfin and

did not incorporate kurper and galaxias. Although redfin is generally the dominant member of the native fish assemblage, these other two species may have been differently preyed upon by trout, and no doubt perform functional roles that are different to that performed by redfin, since these species evolved in sympatry and niche diversification would therefore be expected. Future work in this avenue should therefore look to incorporate kurper and galaxias in experimental work and studies of fish feeding habits.

6.6 CONCLUSION

The research presented in this thesis represents the first quantitative assessment of the community-wide impact of trout in South Africa. My data have revealed that, not only do trout greatly reduce native fish populations, but also modify the functional structure of the benthic invertebrate assemblage, with potential consequences for resources at the base of the food web. These findings elucidate the severity of the threat posed by trout to native freshwater biodiversity and ecosystem functioning in the CFR, and contribute to the conceptual understanding of novel predator impacts in insular systems. My approach of combining broad-scale field surveys with small-scale controlled experiments to erect and evaluate specific hypotheses about invasive predator impacts is novel in the context of invasive fish research in South Africa, and holds promise for quantifying impacts of other invaders in other systems in the country. Although my research contributes to literature on non-native predator impacts, and represents an important advancement in our understanding of trout impacts in CFR headwater streams, perhaps its greatest value is to provide a platform on which to base further research on non-native fish invasions in South Africa.

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Appendices

Appendix 1a Forty sites visited during the pilot study that did not meet the site selection criteria. Site name and sub-catchment name were based on names on 1:250 000 topographic maps. GPS co-ordinates (in degrees and decimal minutes) were recorded on site with a hand-held GPS. An "X" indicates the reason(s) why each site did not meet the site selection criteria: "Canopy" indicates that the site had >50% canopy cover; "Bedrock" indicates that the site had >50% bedrock cover; "Abstraction" indicates that water was abstracted from the river upstream of the study site; "Alien vegetation" indicates that non-native plants were present in the riparian zone adjacent to the site; "Pollution" indicates that that there was a source of pollution upstream of, or adjacent to, the site; "No fish" indicates that fish were absent from the site. Non-native fish present at the sites are indicated by the symbols: "BT" = brown trout *Salmo trutta*, "RBT" = rainbow trout *Oncorhynchus mykiss*, and "SMB" = smallmouth bass *Micropterus dolomieui*. Native fish found at the sites are indicated by the symbols: "RE" = Breede River redfin *Pseudobarbus* sp. "Burchelli Breede"; "KU" = Cape kurper *Sandelia capensis* and "GZ" = Cape galaxias *Galaxias zebratus***.**

Site	Sub-catchment	GPS waypoint	Year	Native fish	Non-native fish	Canopy	Bedrock	Abstraction	Alien vegetation	Pollution	No fish
Bek van Boschkloof	Hex	S33 34.914 E19 33.998	2010			X		X			
Bier	Riviersonderend	S34 00.412 E19 13.322	2009	RE, KU				X	X		
De Braak	Nuy	S33 58.337 E19 11.522	2009	KU					X		
Doorn tributary	Doorn	S33 29.210 E19 31.764	2010			X					
Doorn tributary 2	Doorn	S33 27.954 E19 31.910	2010	RE	BT						
Drosterskloof	Titus	S33 48.702 E19 18.917	2009			X					
Elandskloof	Riviersonderend	S33 53.644 E19 16.756	2009		RBT			X		X	
Elandskloof tributary	Riviersonderend	S33 54.836 E19 15.132	2009								X
Elandspad	Molenaars	S33 44.197 E19 06.820	2009		SMB					X	
Elandspad (upper)	Molenaars	S33 33.521 E19 20.648	2009		SMB						
Groothoek (upper)	Hex	S33 25.370 E19 39.839	2009			X					X
Keurhoek	Hex	S33 31.355 E19 29.471	2010	KU, GZ				X			
Koekedouw	Koekedouw	S33 21.529 E19 17.042	2009	RE				X			
Kommisieskraal	Riviersonderend	S33 58.791 E19 16.743	2009				X			X	X
Malkopskloof	Hex	S33 33.476 E19 29.271	2010					X			
Milnerkloof	Hex	S33 29.637 E19 27.912	2009	GZ		X					
Molenaars (upper 1)	Molenaars	S33 43.870 E19 06.748	2009		RBT					X	

(continued overleaf)

Appendix 1a Continued

Site	Sub-catchment	GPS waypoint	Year	Native fish	Non-native fish	Canopy	Bedrock	Abstraction	Alien vegetation	Pollution	No fish
Molenaars (upper 2)	Molenaars	S33 42.890 E19 52.810	2010		RBT	X					
Molenaars tributary 2	Molenaars	S33 43.485 E19 74.510	2009	GZ		X					
Ratelkloof	Ratels	S33 55.208 E19 19.709	2009			X					X
Risjiespruit	Doorn	S33 39.120 E19 06.499	2010					X			X
Risjiespruit tributary	Doorn	S33 38.383 E19 06.603	2010					X			
Rooiels	Hex	S33 27.514 E19 36.813	2009			X					
Sandhoek	Hex	S33 27.634 E19 36.956	2010								X
Sandriftkloof	Hex	S33 29.210 E19 31.765	2009	RE, KU, GZ		X		X			
Sandriftkloof tributary	Hex	S33 27.861 E19 31.773	2009	GZ		X					
Sandspruit tributary	Sandspruit	S33 28.279 E19 08.017	2009	RE, KU					X		
Slanghoek	Slanghoek	S33 33.777 E19 20.396	2010	GZ				X			
Spek	Hex	S33 21.110 E19 37.507	2009								X
Steenboks	Witte	S33 32.168 E19 85.050	2010	RE, KU, GZ		X					
Stettynskloof	Holsloot	S33 49.638 E19 13.352	2009		SMB, RBT						
Tierstel (lower)	Molenaars	S33 43.911 E19 15.831	2009		SMB, RBT						
Titus (rooikloof)	Titus	S33 24.525 E19 27.172	2009			X		X			
Titus tributary 2	Titus	S33 25.103 E19 27.160	2009	KU			X				X
Vals	Titus	S33 26.167 E19 24.341	2009				X				X
Vals tributary	Titus	S33 23.547 E19 29.849	2009					X			
Waterkloof	Ratels	S33 56.159 E19 22 289	2009	RE				X			
Witte (upper)	Witte	S33 39.120 E19 06.498	2009		BT						
Witwater	Nuy	S33 58.285 E19 11.990	2009								X
Wolwenberg (lower)	Holsloot	S33 43.852 E19 17.958	2009		SMB						
Rooiels	Hex	S33 27.514 E19 36.813	2009			X					
Sandhoek	Hex	S33 27.634 E19 36.956	2010								X
Sandriftkloof	Hex	S33 29.210 E19 31.765	2009	RE, KU, GZ		X		X			
Sandriftkloof tributary	Hex	S33 27.861 E19 31.773	2009	GZ		X					
Sandspruit tributary	Sandspruit	S33 28.279 E19 08.017	2009	RE, KU					X		
Slanghoek	Slanghoek	S33 33.777 E19 20.396	2010	GZ				X			
Spek	Hex	S33 21.110 E19 37.507	2009								X
Steenboks	Witte	S33 32.168 E19 85.050	2010	RE, KU, GZ		X					
Stettynskloof	Holsloot	S33 49.638 E19 13.352	2009		SMB, RBT						
Tierstel (lower)	Molenaars	S33 43.911 E19 15.831	2009		SMB, RBT						
Titus (rooikloof)	Titus	S33 24.525 E19 27.172	2009			X		X			
Titus tributary 2	Titus	S33 25.103 E19 27.160	2009	KU			X				X

(continued overleaf)

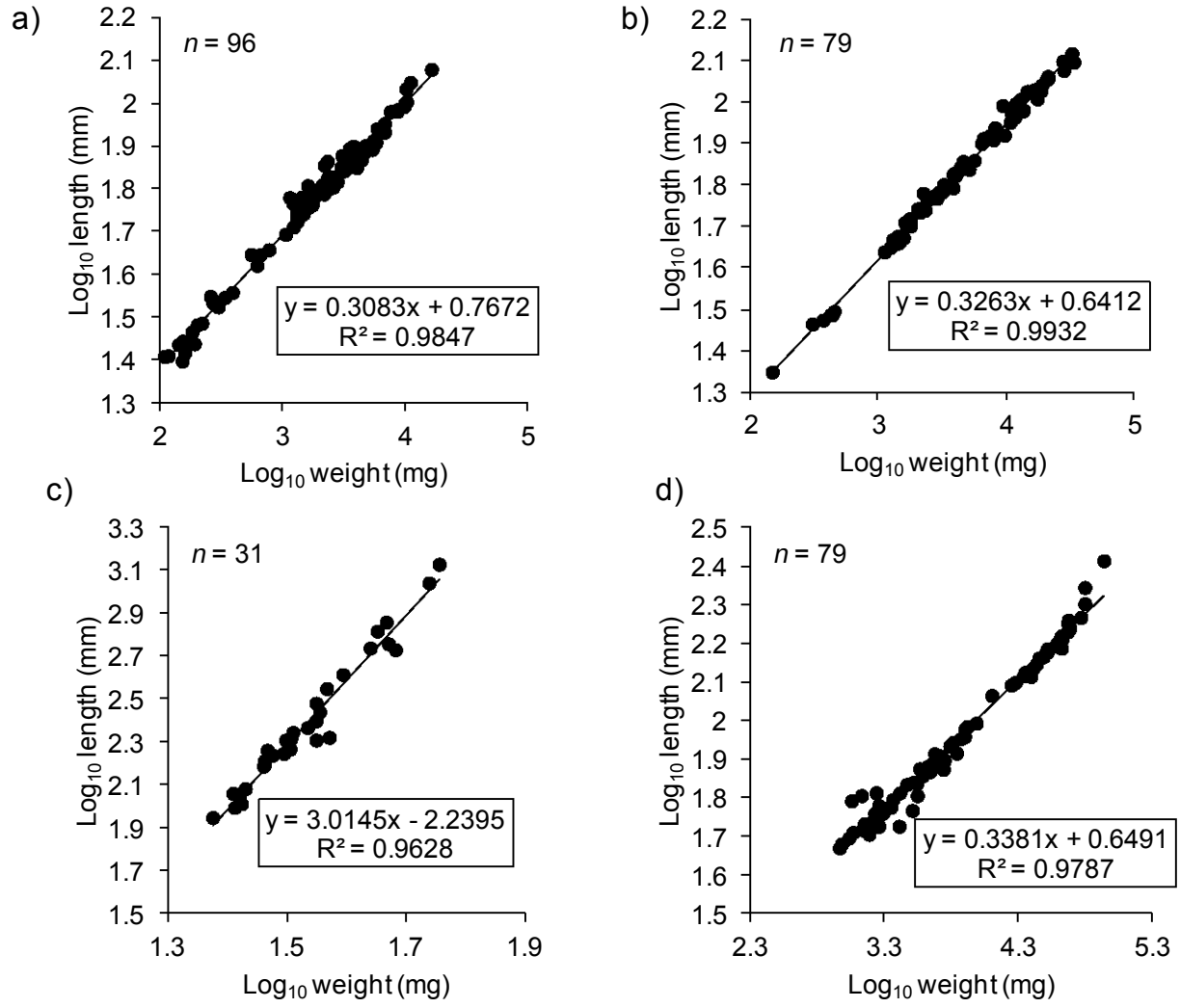
Appendix 1a Continued

Site	Sub-catchment	GPS waypoint	Year	Native fish	Non-native fish	Canopy	Bedrock	Abstraction	Alien vegetation	Pollution	No fish
Vals	Titus	S33 26.167 E19 24.341	2009				X				X
Vals tributary	Titus	S33 23.547 E19 29.849	2009					X			
Waterkloof	Ratels	S33 56.159 E19 22 289	2009	RE				X			
Witte (upper)	Witte	S33 39.120 E19 06.498	2009		BT						
Witwater	Nuy	S33 58.285 E19 11.990	2009								X
Wolwenberg (lower)	Holsloot	S33 43.852 E19 17.958	2009		SMB						

Appendix 1b The 24 sites that met the site-selection criteria. Site name and sub-catchment name were based on names on 1:250 000 topographic maps. GPS co-ordinates (in degrees and decimal minutes) were recorded on site with a hand-held GPS, and the year of sampling indicated. "RBT" = rainbow trout *Oncorhynchus mykiss*, "RE" = Breede River redfin *Pseudobarbus* sp. "Burchelli Breede"; "KU" = Cape kurper *Sandelia capensis* and "GZ" = Cape galaxias *Galaxias zebratus*.

Site	Sub-catchment	GPS waypoint	Year	Native fish	Non-native fish
Kaaimansgat	Holsloot	S33 25.103 E19 27.160	2010	GZ	RBT
Wolwenberg	Holsloot	S33 25.323 E19 26.633	2010	RE, KU	
Molenaars tributary	Molenaars	S33 41.618 E19 11.300	2010		RBT
Tierkloof	Molenaars	S33 29.151 E19 31.755	2010	RE, KU	
Klip	Molenaars	S33 52.138 E19 20.819	2010		RBT
Waaihoek	Jan du Toit	S33 50.215 E19 15.483	2010	RE, GZ	
Jan du Toit	Jan du Toit	S33 25.647 E19 39.774	2010	RE, KU	RBT
Morainekloof	Hex	S33 43.478 E19 06.746	2010	RE, KU	RBT
Hartmanskloof	Holsloot	S33 24.525 E19 27.172	2010		RBT
Wolwekloof	Witte	S33 24.738 E19 29.885	2010	RE, KU, GZ	
Bobbejaans	Witte	S33 25.783 E19 17.000	2010	RE	
Tierhok	Tierhok	S33 48.702 E19 18.917	2010	RE	
Kraal	Molenaars	S33 46.243 E19 09.199	2010		RBT
Krom	Molenaars	S33 46.124 E19 08.955	2010	RE	RBT
Tierstel	Molenaars	S33 56.362 E19 10.112	2010	RE, KU, GZ	
Groothoek	Hex	S33 43.028 E19 09.667	2010		RBT
Titus tributary	Titus	S33 55.210 E19 19.758	2010	RE, KU	
Sandspruit	Sandspruit	S33 42.987 E19 09.683	2010	RE, KU. GZ	
Du Toits	Riviersonderend	S33 43.485 E19 06.747	2010	RE	
Amandel	Riviersonderend	S33 44.197 E19 06.820	2010	RE, KU, GZ	
Stettynskloof	Ratel	S33 56.374 E19 10.129	2010		RBT
Houtboskloof	Holsloot	S34 00.412 E19 13.322	2010		RBT
Raaswater	Nuy	S33 58.791 E19 16.743	2010		RBT
Buffelshoek	Hex	S33 27.514 E19 36.813	2010	RE, KU	RBT

Appendix 2 Length-weight regression relationships for (a) redfin, (b) kurper, (c) galaxias and (d) trout. Sample size breakdown for redfin was: Tierstel *n* = 27, Waaihoek *n* = 33, Tierkloof *n* = 36. Sample size breakdown for kurper was: Tierstel *n* = 35, Waaihoek *n* = 15, Tierkloof *n* = 28. Sample size breakdown for galaxias was: Tierstel *n* = 3, Waaihoek *n* = 16, Tierkloof *n* = 14. Sample size breakdown for trout was: Kraalstroom *n* = 28, Klip *n* = 22, Groothoek *n* = 29.



Appendix 3 Mean ± SE density (number of individuals/m^2) of invertebrate taxa recorded in the main survey of 24 headwater streams (12 with trout, 12 without trout) in the upper Breede River catchment. Functional feeding groups (FFGs) are CG = collector-gatherers, GS = grazer-scrapers, FF = filter-feeders, SH = shredders and P = predators. "Abund." = mean abundance (number of individuals/m^2) of the taxon when averaged across all sites. "# streams" = the number of stream in which each taxon was present.

Class	Order	Family	Taxon	FFG	Abund.	No trout (*n* = 12)			Trout (*n* = 12)		
						# streams	Mean	SE	# streams	Mean	SE
Arachnida	Acarina		Acarina	P	1.06	10	22.21	6.70	12	39.58	13.56
Crustacea	Amphipoda	Paramelitidae	*Paramelita*	SH	0.01	1	0.17	0.17	3	0.32	0.21
Crustacea	Decapoda	Potamonautidae	*Potamonautes*	CG	0.01	1	0.56	0.56	2	0.30	0.21
Hydrozoa	Hydroida	Hydridae	*Hydra*		0.00	1	0.03	0.03	0	0.00	0.00
Insecta	Coleoptera	Curculionidae	Curculionidae	GS	0.08	4	0.88	0.56	3	3.94	3.35
Insecta	Coleoptera	Dryopidae	Dryopidae	GS	0.64	8	10.61	6.06	11	26.53	20.38
Insecta	Coleoptera	Elmidae	Elmidae	GS	11.96	12	317.19	49.48	12	377.03	72.78
Insecta	Coleoptera	Gyrinidae	Gyrinidae	P	0.08	9	4.29	2.18	5	0.27	0.10
Insecta	Coleoptera	Haliplidae	Haliplidae	GS	0.00	0	0.00	0.00	1	0.10	0.10
Insecta	Coleoptera	Hydraenidae	Hydraenidae	GS	3.08	12	75.48	20.29	12	103.14	27.88
Insecta	Coleoptera	Hydrophilidae	Hydrophilidae	GS	0.02	0	0.00	0.00	2	1.21	1.13
Insecta	Coleoptera	Ptilodactylidae	Ptilodactylidae	GS	0.70	10	28.69	12.48	10	12.08	4.44
Insecta	Coleoptera	Scirtidae	Scirtidae	GS	3.46	11	68.37	17.32	12	132.44	36.17
Insecta	Diptera	Athericidae	Athericidae	P	1.94	12	46.99	12.95	12	65.38	39.52
Insecta	Diptera	Blephariceridae	*Elporia*	GS	0.13	5	5.67	3.29	6	1.94	0.74
Insecta	Diptera	Ceratopogonidae	Bezzia	GS	0.01	3	0.29	0.19	3	0.22	0.13
Insecta	Diptera	Ceratopogonidae	*Forcipomyia*	GS	0.01	3	0.72	0.47	0	0.00	0.00
Insecta	Diptera	Chironomidae	Chironomidae		0.52	6	20.52	10.08	5	9.40	4.24
Insecta	Diptera	Chironomidae	Chironominae	GS	5.41	12	141.59	35.11	12	172.75	48.14
Insecta	Diptera	Chironomidae	Orthocladiinae	GS	8.17	12	214.55	50.81	12	259.84	61.61
Insecta	Diptera	Chironomidae	Tanypodinae	P	2.01	12	48.52	9.73	12	68.40	10.13

(continued overleaf)

Appendix 3 Continued

Class	Order	Family	Taxon	FFG	Abund.	No trout (*n* = 12)			Trout (*n* = 12)		
						# streams	Mean	SE	# streams	Mean	SE
Insecta	Diptera	Culicidae	*Anopheles*	FF	0.00	0	0.00	0.00	1	0.12	0.12
Insecta	Diptera	Dixidae	*Dixa*	FF	0.03	2	1.24	0.87	3	0.35	0.18
Insecta	Diptera	Dixidae	*Ptychoptera*	FF	0.02	2	1.13	0.80	2	0.28	0.19
Insecta	Diptera	Empididae	*Clinocera*	P	0.01	2	0.20	0.13	2	0.51	0.40
Insecta	Diptera	Empididae	*Hemerodromia*	P	0.16	9	3.73	1.22	9	5.65	3.05
Insecta	Diptera	Psychodidae	*Pericoma*	CG	0.01	2	0.20	0.13	3	0.20	0.14
Insecta	Diptera	Simuliidae	*Simulium*	FF	4.02	12	38.54	14.55	12	194.91	125.12
Insecta	Diptera	Tabanidae	Tabanidae	P	0.07	4	3.69	2.69	3	0.34	0.22
Insecta	Diptera	Tipulidae	*Limnophila*	P	0.02	5	0.83	0.46	4	0.34	0.16
Insecta	Ephemeroptera	Baetidae	*Acanthiops erepens*	SH	0.14	2	1.19	0.92	3	7.04	4.46
Insecta	Ephemeroptera	Baetidae	*Afroptilum*	CG	0.08	2	0.70	0.56	5	4.11	3.07
Insecta	Ephemeroptera	Baetidae	*Baetis*	CG	14.01	12	165.39	76.54	12	648.02	143.50
Insecta	Ephemeroptera	Baetidae	*Bugilliesia*	CG	0.03	3	0.67	0.38	3	1.07	0.65
Insecta	Ephemeroptera	Baetidae	*Cheleocloeon mirandei*	CG	0.24	8	8.96	3.81	4	4.99	3.92
Insecta	Ephemeroptera	Baetidae	*Cloeodes*	CG	0.59	7	3.01	1.07	8	31.51	13.41
Insecta	Ephemeroptera	Baetidae	*Demoreptus capensis*	CG	4.09	10	34.96	13.73	12	202.36	77.65
Insecta	Ephemeroptera	Baetidae	*Labiobaetis/Pseudocloeon*	CG	2.98	12	99.13	27.27	12	73.83	33.42
Insecta	Ephemeroptera	Caenidae	*Caenis*	CG	0.35	8	14.43	11.61	6	5.65	3.24
Insecta	Ephemeroptera	Heptageniidae	*Afronurus*	GS	1.60	10	41.90	17.71	9	50.84	25.77
Insecta	Ephemeroptera	Leptophlebiidae	*Adenophlebia*	CG	0.10	5	1.47	0.62	8	4.61	1.96
Insecta	Ephemeroptera	Leptophlebiidae	*Aprionyx peterseni*	CG	2.31	12	43.38	9.20	12	90.68	15.21
Insecta	Ephemeroptera	Leptophlebiidae	*Aprionyx rubicundus*	CG	0.38	6	21.74	18.79	1	0.23	0.23
Insecta	Ephemeroptera	Leptophlebiidae	*Aprionyx tabularis*	GS	0.04	2	0.23	0.16	5	2.01	1.12
Insecta	Ephemeroptera	Leptophlebiidae	*Castanophlebia*	CG	2.82	12	80.07	16.16	12	83.81	21.59
Insecta	Ephemeroptera	Leptophlebiidae	*Choroterpes*	CG	0.15	4	1.56	0.75	5	7.40	6.90
Insecta	Ephemeroptera	Teloganodidae	*Ephemerellina*	CG	0.01	3	0.64	0.34	1	0.12	0.12

(continued overleaf)

Appendix 3 Continued

Class	Order	Family	Taxon	FFG	Abund.	No trout (*n* = 12)			Trout (*n* = 12)		
						# streams	Mean	SE	# streams	Mean	SE
Insecta	Ephemeroptera	Teloganodidae	*Lestagella penicillata*	CG	7.27	11	139.61	48.30	12	282.16	87.13
Insecta	Ephemeroptera	Teloganodidae	*Lithogloea harrisoni*	CG	1.44	11	47.88	20.55	11	35.49	16.43
Insecta	Ephemeroptera	Teloganodidae	*Nadinetella*	CG	0.00	1	0.13	0.13	1	0.02	0.02
Insecta	Hemiptera	Corixidae	*Micronecta*	P	0.01	2	0.23	0.16	1	0.16	0.16
Insecta	Hemiptera	Naucoridae	*Laccocoris*	P	0.07	2	4.14	4.07	2	0.20	0.15
Insecta	Hemiptera	Naucoridae	*Naucoris*	P	0.01	2	0.22	0.18	1	0.11	0.11
Insecta	Hemiptera	Notonectidae	*Anisops*	P	0.02	0	0.00	0.00	1	0.91	0.91
Insecta	Hemiptera	Veliidae	*Microvelia*	P	0.03	3	0.47	0.29	6	1.46	0.82
Insecta	Lepidoptera	Crambidae	Crambidae	GS	0.02	3	0.48	0.28	5	0.72	0.41
Insecta	Megaloptera	Corydalidae	Corydalidae	P	0.42	12	12.33	3.57	11	11.96	2.56
Insecta	Odonata	Aeshnidae	*Aeshna*	P	0.07	7	1.00	0.38	12	3.14	0.90
Insecta	Odonata	Aeshnidae	*Anax*	P	0.03	4	0.99	0.48	3	0.64	0.36
Insecta	Odonata	Coenagrionidae	*Pseudagrion*	P	0.02	3	0.52	0.35	6	0.69	0.32
Insecta	Odonata	Gomphidae	*Ceratogomphus*	P	0.04	6	1.92	0.73	1	0.14	0.14
Insecta	Odonata	Gomphidae	*Notogomphus*	P	0.07	7	2.73	2.00	8	1.18	0.39
Insecta	Odonata	Lestidae	*Lestes*	P	0.00	0	0.00	0.00	1	0.27	0.27
Insecta	Odonata	Libellulidae	*Orthetrum*	P	0.00	1	0.07	0.07	2	0.13	0.10
Insecta	Odonata	Libellulidae	*Trithemis*	P	0.01	3	0.40	0.26	4	0.41	0.22
Insecta	Odonata	Synlestidae	*Chlorolestes*	P	0.01	1	0.04	0.04	3	0.26	0.19
Insecta	Plecoptera	Notonemouridae	*Aphanicerca*	SH	1.73	12	31.59	6.81	12	69.02	16.40
Insecta	Plecoptera	Notonemouridae	*Aphanicercella*	SH	3.54	12	70.62	22.88	12	134.86	37.45
Insecta	Plecoptera	Notonemouridae	*Aphanicercopsis*	SH	0.22	8	6.18	3.20	7	6.87	3.44
Insecta	Plecoptera	Notonemouridae	*Desmonemoura*	SH	0.06	3	0.54	0.36	6	3.18	1.62
Insecta	Trichoptera	Barbarochthonidae	*Barbarochthon brunneum*	GS	0.70	6	38.49	32.63	6	2.15	1.15
Insecta	Trichoptera	Ecnomidae	*Ecnomus*	P	0.02	4	0.70	0.30	5	0.73	0.27
Insecta	Trichoptera	Ecnomidae	*Parecnomina resima*	P	0.68	11	19.43	11.10	12	19.95	7.05

(continued overleaf)

Appendix 3 Continued

Class	Order	Family	Taxon	FFG	Abund.	No trout (*n* = 12)			Trout (*n* = 12)		
						# streams	Mean	SE	# streams	Mean	SE
Insecta	Trichoptera	Glossosomatidae	*Agapetus*	GS	1.40	7	8.41	3.34	11	72.74	24.95
Insecta	Trichoptera	Hydropsychidae	*Cheumatopsyche*	P	1.65	11	37.02	10.97	12	58.89	27.78
Insecta	Trichoptera	Hydroptilidae	*Hydroptila*	GS	0.04	3	0.98	0.64	4	1.09	0.50
Insecta	Trichoptera	Hydroptilidae	Hydroptilidae sp.	GS	0.07	3	1.83	1.22	7	2.02	0.73
Insecta	Trichoptera	Hydroptilidae	*Orthotrichia*	GS	0.12	6	3.01	1.18	7	3.82	1.80
Insecta	Trichoptera	Hydrosalpingidae	*Hydrosalpinx*	GS	0.01	2	0.57	0.55	0	0.00	0.00
Insecta	Trichoptera	Leptoceridae	*Athripsodes*	CG	3.73	12	140.04	39.44	12	76.24	21.89
Insecta	Trichoptera	Leptoceridae	*Leptecho*	SH	0.00	2	0.21	0.18	0	0.00	0.00
Insecta	Trichoptera	Leptoceridae	*Leptocerus*	SH	0.32	6	17.07	13.31	4	1.35	0.92
Insecta	Trichoptera	Leptoceridae	*Oecetis*	P	0.02	3	0.72	0.39	2	0.30	0.20
Insecta	Trichoptera	Petrothrincidae	*Petrothrincus*	GS	0.05	3	1.13	0.79	2	2.05	1.45
Insecta	Trichoptera	Philopotamidae	*Chimarra*	FF	0.37	7	2.74	1.11	9	19.02	9.23
Insecta	Trichoptera	Philopotamidae	*Dolophilodes*	FF	0.14	4	1.22	0.68	9	6.88	3.66
Insecta	Trichoptera	Pisuliidae	*Dyschimus*	SH	0.08	3	1.10	0.69	4	3.49	1.75
Insecta	Trichoptera	Polycentropodidae	Polycentropodidae	P	0.01	2	0.65	0.47	0	0.00	0.00
Insecta	Trichoptera	Sericostomatidae	*Petroplax*	SH	0.94	10	24.41	14.60	7	30.00	28.47
Oligochaeta			Oligochaeta	CG	0.07	4	3.54	2.44	3	0.24	0.14
			Nematoda	P	0.89	12	35.43	24.83	9	16.40	7.05
			Nematomorpha		0.01	1	0.31	0.31	1	0.11	0.11
			Nemertea	P	0.01	2	0.61	0.52	2	0.20	0.16

Appendix 4 Mean ± SE abundance (number per sample) of invertebrate taxa recorded in the preliminary survey of 16 headwater streams (9 containing trout, 7 lacking trout) in the upper Breede River catchment. "Abund." = mean abundance (number per sample) of the taxon when averaged across all sites. "# streams" = the number of stream in which each taxon was present.

				No trout			Trout		
Class	Order	Family	Abund.	# streams	Mean	SE	# streams	Mean	SE
Arachnida	Acarina		0.06	0	0.00	0.00	1	0.22	0.22
Insecta	Coleoptera	Dryopidae	0.45	3	0.43	0.20	5	1.44	0.85
Insecta	Coleoptera	Dyticidae	0.03	0	0.00	0.00	1	0.11	0.11
Insecta	Coleoptera	Elmidae	7.23	7	16.00	5.12	9	16.00	3.64
Insecta	Coleoptera	Gyrinidae	0.11	2	0.57	0.43	0	0.00	0.00
Insecta	Coleoptera	Hydraenidae	3.08	5	4.43	3.45	7	8.67	3.55
Insecta	Coleoptera	Scirtidae	0.93	1	0.29	0.29	6	3.44	1.94
Insecta	Diptera	Athericidae	2.15	5	5.57	4.27	8	4.11	1.10
Insecta	Diptera	Blephariceridae	0.06	1	0.14	0.14	1	0.11	0.11
Insecta	Diptera	Chironomidae	4.63	6	7.14	2.01	9	12.67	3.66
Insecta	Diptera	Culicidae	0.03	1	0.14	0.14	0	0.00	0.00
Insecta	Diptera	Simuliidae	0.56	3	0.86	0.46	5	1.56	0.80
Insecta	Ephemeroptera	Baetidae	7.59	7	6.86	1.82	9	24.56	9.76
Insecta	Ephemeroptera	Caenidae	0.14	0	0.00	0.00	2	0.56	0.44
Insecta	Ephemeroptera	Heptageniidae	3.61	3	5.14	4.50	8	10.22	6.26
Insecta	Ephemeroptera	Leptophlebiidae	14.96	7	14.57	6.58	9	47.56	10.76
Insecta	Ephemeroptera	Teloganodidae	45.84	7	14.43	4.08	9	169.22	40.26
Insecta	Hemiptera	Naucoridae	0.14	2	0.71	0.57	0	0.00	0.00
Insecta	Hemiptera	Veliidae	0.14	1	0.29	0.29	1	0.33	0.33
Insecta	Odonata	Aeshnidae	0.25	3	0.43	0.20	3	0.67	0.37
Insecta	Odonata	Coenagrionidae	0.03	0	0.00	0.00	1	0.11	0.11
Insecta	Odonata	Corduliidae	0.08	1	0.29	0.29	1	0.11	0.11
Insecta	Odonata	Corydalidae	0.62	6	1.29	0.47	5	1.44	0.67
Insecta	Odonata	Gomphidae	0.23	1	0.29	0.29	2	0.67	0.55

(continued overleaf)

Appendix 4 Continued

				No trout			Trout		
Class	Order	Family	Abund.	# streams	Mean	SE	# streams	Mean	SE
Insecta	Odonata	Libellulidae	0.25	3	0.71	0.36	2	0.44	0.34
Insecta	Odonata	Synlestidae	0.06	0	0.00	0.00	2	0.22	0.15
Insecta	Plecoptera	Notonemouridae	2.00	2	0.86	0.55	9	7.22	2.26
Insecta	Trichoptera	Barbarochthonidae	0.71	3	2.43	1.45	1	0.89	0.89
Insecta	Trichoptera	Ecnomidae	0.06	0	0.00	0.00	2	0.22	0.15
Insecta	Trichoptera	Glossomatidae	0.54	0	0.00	0.00	3	2.11	1.65
Insecta	Trichoptera	Hydropsychidae	0.65	4	1.71	0.84	4	1.22	0.60
Insecta	Trichoptera	Hydroptilidae	0.06	0	0.00	0.00	1	0.22	0.22
Insecta	Trichoptera	Leptoceridae	2.06	5	6.29	3.32	6	3.22	1.70
Insecta	Trichoptera	Philopotamidae	0.11	2	0.29	0.18	1	0.22	0.22
Insecta	Trichoptera	Sericosomatidae	0.51	4	0.71	0.29	4	1.44	0.87
Oligochaeta			0.06	0	0.00	0.00	2	0.22	0.15

Appendix 5 Length (TL, mm) and weight (g) of trout and redfin that were sampled for gut content and stable isotope analysis (codes correspond to Appendix 4.4). Sample sizes for trout were Groothoek: *n* = 29, Klip: *n* = 23, Kraal: *n* = 28. Sample sizes for redfin were Tierkloof: *n* = 36, Tierstel: *n* = 27, Waaihoek: *n* = 33.

Trout				Redfin			
Site	Isotope code	Total length (mm)	Weight (g)	Site	Isotope code	Total length (mm)	Weight (g)
Groothoek	grt1	122.9	18.13	Tierkloof	tkr1	58.0	1.79
Groothoek	grt2	81.9	7.10	Tierkloof	tkr2	66.6	2.38
Groothoek	grt3	63.6	3.61	Tierkloof	tkr3	69.0	3.18
Groothoek	grt4	69.0	3.42	Tierkloof	tkr4	52.5	1.33
Groothoek	grt5	135.8	26.41	Tierkloof	tkr5	30.3	0.21
Groothoek	grt6	58.2	3.32	Tierkloof	tkr6	36.0	0.40
Groothoek	grt7	74.4	5.64	Tierkloof	tkr7	45.3	0.79
Groothoek	grt8	162.8	42.27	Tierkloof	tkr8	119.0	16.70
Groothoek	grt9	59.3	2.28	Tierkloof	tkr9	97.5	9.96
Groothoek	grt10	77.2	4.67	Tierkloof	tkr10	41.6	0.63
Groothoek	grt11	77.8	5.56	Tierkloof	tkr11	44.0	0.67
Groothoek	grt12	153.1	43.06	Tierkloof	tkr12	30.5	0.22
Groothoek	grt13	172.5	49.66	Tierkloof	tkr13	49.2	1.08
Groothoek	grt14	219.9	64.30	Tierkloof	tkr14	65.3	2.44
Groothoek	grt15	199.4	64.33	Tierkloof	tkr15	95.0	8.81
Groothoek	grt16	177.9	47.97	Tierkloof	tkr16	58.3	1.74
Groothoek		136.9	28.82	Tierkloof		61.0	2.21
Groothoek		129.4	25.43	Tierkloof		63.8	2.41
Groothoek		64.7	1.78	Tierkloof		74.9	4.00
Groothoek		63.6	1.39	Tierkloof		54.9	1.41
Groothoek		61.7	1.17	Tierkloof		60.3	1.86
Groothoek		87.3	6.66	Tierkloof		66.9	2.62
Groothoek		158.0	40.31	Tierkloof		74.7	4.24
Groothoek		78.4	5.73	Tierkloof		27.2	0.15
Groothoek		132.6	22.96	Tierkloof		33.3	0.31
Groothoek		148.5	32.68	Tierkloof		64.0	2.14
Groothoek		129.9	22.38	Tierkloof		35.2	0.26
Groothoek		145.5	31.50	Tierkloof		27.7	0.16
Groothoek		138.8	27.86	Tierkloof		29.2	0.19
Klip	klt1	115.5	13.00	Tierkloof		53.6	1.37
Klip	klt2	164.8	42.92	Tierkloof		71.1	3.38

(continued overleaf)

Appendix 5 Continued

Trout				Redfin			
Site	Isotope code	Total length (mm)	Weight (g)	Site	Isotope code	Total length (mm)	Weight (g)
Klip	klt3	48.1	33.87	Tierkloof		62.5	2.37
Klip	klt4	90.5	8.14	Tierkloof		62.7	2.19
Klip	klt5	125.0	19.54	Tierkloof		57.7	1.75
Klip	klt6	81.4	4.93	Tierkloof		57.5	1.78
Klip	klt7	181.0	48.41	Tierkloof		77.2	4.23
Klip	klt8	98.0	9.96	Tierstel	tsr1	75.4	4.20
Klip	klt9	74.7	3.76	Tierstel	tsr2	110.9	11.26
Klip	klt10	94.4	8.19	Tierstel	tsr3	94.9	7.75
Klip	klt11	80.8	5.22	Tierstel	tsr4	77.3	4.67
Klip	klt12	58.8	2.11	Tierstel	tsr5	62.5	1.70
Klip	klt13	52.0	1.41	Tierstel	tsr6	35.0	0.35
Klip	klt14	53.5	1.50	Tierstel	tsr7	27.3	0.72
Klip	klt15	64.7	2.67	Tierstel	tsr8	60.0	1.46
Klip	klt16	75.5	4.26	Tierstel	tsr9	59.8	1.17
Klip		81.6	4.87	Tierstel	tsr10	50.0	0.07
Klip		76.7	4.61	Tierstel	tsr11	89.0	6.91
Klip		60.0	1.86	Tierstel	tsr12	70.5	3.06
Klip		54.9	0.08	Tierstel	tsr13	72.7	4.12
Klip		89.4	7.66	Tierstel	tsr14	34.0	0.28
Klip		68.0	3.02	Tierstel	tsr15	25.6	0.12
Klip		79.2	5.65	Tierstel	tsr16	77.5	5.49
Kraal	krt1	169.0	47.82	Tierstel		72.7	2.37
Kraal	krt2	162.2	43.62	Tierstel		63.8	1.64
Kraal	krt3	149.4	33.55	Tierstel		58.0	1.24
Kraal	krt4	144.7	29.39	Tierstel		71.1	2.24
Kraal	krt5	258.0	89.27	Tierstel		61.5	0.07
Kraal	krt6	155.0	40.14	Tierstel		44.1	0.57
Kraal	krt7	150.0	33.88	Tierstel		77.7	3.56
Kraal	krt8	184.0	60.12	Tierstel		78.8	3.84
Kraal	krt9	85.5	6.31	Tierstel		64.9	2.51
Kraal	krt10	62.0	2.36	Tierstel		61.9	1.90
Kraal	krt11	96.1	8.55	Tierstel		69.2	3.28
Kraal	krt12	57.2	1.74	Waaihoek	war1	73.0	3.45
Kraal	krt13	68.3	3.56	Waaihoek	war2	70.3	4.09
Kraal	krt14	55.0	1.70	Waaihoek	war3	27.3	0.20
Kraal	krt15	53.8	1.45	Waaihoek	war4	100.0	10.48
Kraal	krt16	51.1	1.20	Waaihoek	war5	74.2	3.79

(continued overleaf)

Appendix 5 Continued

Trout				Redfin			
Site	Isotope code	Total length (mm)	Weight (g)	Site	Isotope code	Total length (mm)	Weight (g)
Kraal		53.0	1.86	Waaihoek	war6	85.0	9.37
Kraal		53.0	2.63	Waaihoek	war7	85.0	6.92
Kraal		57.2	1.99	Waaihoek	war8	107.2	10.37
Kraal		49.4	1.11	Waaihoek	war9	55.0	1.51
Kraal		46.6	0.95	Waaihoek	war10	56.1	1.45
Kraal		73.3	4.47	Waaihoek	war11	58.3	1.50
Kraal		58.9	2.02	Waaihoek	war12	55.5	1.34
Kraal		50.5	1.57	Waaihoek	war13	54.0	1.34
Kraal		47.7	0.98	Waaihoek	war14	48.0	0.09
Kraal		52.7	1.67	Waaihoek	war15	51.1	1.25
Kraal		71.6	3.94	Waaihoek	war16	54.4	1.41
Kraal		51.6	1.47	Waaihoek		25.5	0.11
				Waaihoek		26.0	0.16
				Waaihoek		24.9	0.16
				Waaihoek		78.3	5.09
				Waaihoek		22.2	0.01
				Waaihoek		86.6	6.01
				Waaihoek		78.0	4.97
				Waaihoek		56.1	1.56
				Waaihoek		96.0	8.89
				Waaihoek		76.4	4.75
				Waaihoek		69.0	3.12
				Waaihoek		73.3	4.48
				Waaihoek		56.9	1.67
				Waaihoek		79.2	4.87
				Waaihoek		75.0	4.14
				Waaihoek		63.3	2.63
				Waaihoek		54.9	1.51

Appendix 6 Frequency of occurrence (*% O*), mean ± SE proportional weight (*% W*), mean ± SE proportional abundance (*% N*) and the index of relative importance (*% IRI*) for all food items (totals included for total aquatic invertebrates, aquatic invertebrate functional feeding groups, terrestrial invertebrates and "other") recorded in the guts of redfin and trout at the finest taxonomic resolution.

Food source	Trout						Redfin					
	% O	*% W*		*% N*		*% IRI*	*% O*	*% W*		*% N*		*% IRI*
		Mean	SE	Mean	SE			Mean	SE	Mean	SE	
Total aquatic invertebrates	**95.00**	**43.78**	**19.32**	**69.46**	**19.73**	**55.83**	**88.54**	**54.63**	**18.94**	**87.37**	**23.49**	**93.48**
Collector-gatherers	**88.75**	**16.13**	**5.79**	**42.33**	**9.90**	**42.10**	**83.33**	**32.05**	**8.69**	**63.06**	**14.11**	**81.29**
Aprionyx peterseni	18.75	4.02	1.69	4.85	1.66	3.01	3.13	2.02	1.19	2.75	1.59	0.40
Athripsodes	5.00	0.07	0.05	0.49	0.30	0.05	15.63	3.99	1.28	5.35	1.64	3.96
Baetidae	43.75	3.30	0.75	9.57	1.66	10.19	29.17	10.83	2.31	12.88	2.65	18.76
Baetis	32.50	3.26	1.19	5.62	1.36	5.22	4.17	0.20	0.13	0.64	0.41	0.09
Castanophlebia	2.50	0.44	0.33	0.26	0.19	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Cheleocloeon mirandei	1.25	0.02	0.02	0.16	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chironomidae (non-Tanypodinae)	3.75	0.01	0.01	0.53	0.43	0.04	21.88	6.39	1.69	14.09	2.79	12.16
Chironominae	16.25	0.16	0.08	1.75	0.60	0.56	3.13	0.15	0.09	1.33	1.08	0.13
Cloeodes	2.50	0.07	0.07	0.14	0.11	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Demoreptus capensis	17.50	1.18	0.50	1.81	0.56	0.95	0.00	0.00	0.00	0.31	0.31	0.00
Labiobaetis/Pseudocloeon	3.75	0.27	0.17	0.34	0.20	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Lestagella penicillata	1.25	0.00	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Orthocladiinae	51.25	3.32	0.93	16.77	2.62	18.63	40.63	8.47	2.00	25.72	3.65	37.67
Filter-feeders	**41.25**	**5.40**	**1.49**	**5.56**	**1.51**	**3.67**	**17.71**	**8.25**	**3.21**	**8.56**	**3.47**	**3.05**
Anopheles	2.50	0.14	0.11	0.25	0.20	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Chimarra	2.50	0.05	0.04	0.10	0.07	0.01	3.13	0.56	0.40	0.46	0.29	0.09
Dixa	3.75	0.04	0.02	0.16	0.10	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Dolophilodes	3.75	0.31	0.20	0.33	0.22	0.04	2.08	1.18	0.90	1.47	1.12	0.15
Simulium	36.25	4.86	1.12	4.71	0.92	6.28	13.54	6.51	1.91	6.64	2.05	4.83

(continued overleaf)

Appendix 6 Continued

Food source	Trout						Redfin					
	% O	*% W*		*% N*		*% IRI*	*% O*	*% W*		*% N*		*% IRI*
		Mean	SE	Mean	SE			Mean	SE	Mean	SE	
Grazer-scrapers	**43.75**	**2.97**	**1.79**	**1.73**	**0.80**	**1.67**	**33.33**	**8.59**	**3.14**	**7.90**	**2.40**	**5.64**
Afronurus	8.75	1.73	1.07	0.66	0.30	0.38	3.13	1.39	0.89	0.89	0.58	0.19
Agapetus	1.25	0.05	0.05	0.05	0.05	0.00	2.08	0.04	0.03	0.11	0.07	0.01
Barbarochthon brunneum	1.25	0.08	0.08	0.06	0.06	0.00	2.08	0.66	0.49	0.29	0.20	0.05
Bezzia	1.25	0.02	0.02	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Elmidae	12.50	0.91	0.45	0.72	0.26	0.37	27.08	6.51	1.73	6.61	1.55	9.64
Elporia capensis	5.00	0.19	0.12	0.21	0.11	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Forcipomyia	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.04	0.04	0.16	0.15	0.01
Hydraenidae	2.50	0.05	0.04	0.08	0.06	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Hydroptila	18.75	7.99	2.41	6.98	1.96	5.08	2.08	0.56	0.43	0.13	0.09	0.04
Hydroptilidae sp.(undiscribed genus)	1.25	0.57	0.57	0.20	0.20	0.02	1.04	0.39	0.39	0.14	0.13	0.01
Orthotrichia	7.50	0.24	0.11	0.46	0.22	0.10	1.04	0.00	0.00	0.01	0.01	0.00
Oxyethira	3.75	0.25	0.16	0.31	0.19	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Petrothrincus	1.25	0.03	0.03	0.08	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Scirtidae	7.50	0.13	0.07	0.34	0.16	0.06	2.08	0.22	0.18	0.12	0.08	0.02
Shredders	**5.00**	**1.13**	**1.08**	**0.23**	**0.19**	**0.06**	**1.04**	**0.18**	**0.18**	**0.37**	**0.36**	**0.01**
Aphanicerca	2.50	0.06	0.05	0.09	0.07	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Paramelita	2.50	1.08	1.03	0.14	0.13	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Petroplax	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.18	0.18	0.37	0.36	0.02
Predators	**51.25**	**8.90**	**5.78**	**11.16**	**4.46**	**8.34**	**30.21**	**4.33**	**2.68**	**6.93**	**2.69**	**3.49**
Acarina	21.25	0.24	0.10	5.21	1.41	2.10	17.71	1.78	0.55	4.21	1.15	2.88
Aeshna	6.25	0.61	0.35	0.27	0.13	0.10	0.00	0.00	0.00	0.00	0.00	0.00
Aeshnidae	1.25	0.01	0.01	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Athericidae	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.43	0.43	0.16	0.15	0.02
Cheumatopsyche	8.75	1.69	0.92	0.87	0.36	0.41	1.04	0.07	0.07	0.33	0.32	0.01
Corydalidae	1.25	0.30	0.30	0.12	0.11	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Ecnomus	10.00	1.93	0.94	1.83	0.76	0.68	0.00	0.00	0.00	0.00	0.00	0.00
Gyrinidae	1.25	1.12	1.12	0.63	0.63	0.04	1.04	0.51	0.51	0.27	0.27	0.02
Laccocoris	6.25	1.43	0.89	0.73	0.34	0.24	0.00	0.00	0.00	0.00	0.00	0.00
Microvelia	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.29	0.29	0.04	0.04	0.01

(continued overleaf)

Appendix 6 Continued

Food source	Trout						Redfin					
	% O	*% W*		*% N*		*% IRI*	*% O*	*% W*		*% N*		*% IRI*
		Mean	SE	Mean	SE			Mean	SE	Mean	SE	
Naucoris	1.25	0.06	0.06	0.07	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Notogomphus	1.25	0.17	0.17	0.09	0.09	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Oecetis	1.25	0.04	0.04	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Parecnomina resima	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.48	0.48	0.08	0.08	0.02
Tanypodinae	13.75	1.28	0.86	1.23	0.45	0.62	12.50	0.79	0.36	1.84	0.69	0.89
Terrestrial invertebrates	**78.75**	**38.57**	**11.46**	**30.54**	**8.07**	**44.17**	**26.04**	**11.80**	**5.31**	**12.63**	**5.74**	**6.52**
Adult Ephemeroptera	16.25	4.79	1.81	2.16	0.87	2.04	1.04	0.69	0.69	1.10	1.07	0.05
Adult Plecoptera	1.25	0.01	0.01	0.09	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Adult Trichoptera	8.75	0.88	0.42	0.59	0.22	0.23	0.00	0.00	0.00	0.00	0.00	0.00
Adult/terrestrial Diptera	55.00	11.84	2.20	11.16	1.84	22.90	7.29	2.38	1.13	2.49	1.27	0.96
Araneae (spiders)	10.00	1.57	0.94	0.94	0.42	0.45	0.00	0.00	0.00	0.00	0.00	0.00
Diplopoda (millipedes)	2.50	1.06	1.01	0.30	0.21	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Hymenoptera	21.25	3.05	1.17	3.29	1.03	2.44	2.08	1.56	1.10	1.65	1.19	0.18
Orthoptera	1.25	0.31	0.31	0.09	0.09	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Terrestrial Coleoptera	36.25	5.77	1.31	6.85	1.74	8.27	1.04	0.38	0.38	0.18	0.18	0.02
Terrestrial Hemiptera	31.25	9.29	2.29	5.08	1.57	8.12	17.71	6.80	2.01	7.21	2.03	6.73
Invertebrate remains	**68.75**	**11.16**	**1.51**	**-**	**-**	**-**	**25.00**	**11.50**	**2.42**	**-**	**-**	**-**
Algae	**6.25**	**1.71**	**1.25**	**-**	**-**	**-**	**60.42**	**7.42**	**2.06**	**-**	**-**	**-**
Detritus	**16.25**	**3.74**	**1.52**	**-**	**-**	**-**	**60.42**	**9.72**	**1.92**	**-**	**-**	**-**
Sand	**3.75**	**0.24**	**0.17**	**-**	**-**	**-**	**34.38**	**4.76**	**1.23**	**-**	**-**	**-**
Other	**16.25**	**0.79**	**0.37**	**-**	**-**	**-**	**3.13**	**0.18**	**0.11**	**-**	**-**	**-**
Diptera pupae	17.50	0.70	0.29	-	-	-	0.00	0.00	0.00	-	-	-
Invertebrate eggs	0.00	0.00	0.00	-	-	-	3.13	0.18	0.11	-	-	-
Trichoptera pupae	1.25	0.09	0.09	-	-	-	0.00	0.00	0.00	-	-	-

Appendix 7 Proportional abundance of aquatic and terrestrial invertebrates in the drift, and of aquatic invertebrate functional feeding groups in the benthos, at each sampling site. See methods for details of calculation of proportional abundance.

Prey type	Trout			Redfin		
	Groothoek	Kraalstroom	Klip	Tierstel	Waaihoek	Tierkloof
Drift						
Aquatic	50.43	67.22	68.83	84.49	7.37	74.68
Terrestrial	49.57	32.78	31.17	15.51	92.63	25.32
Benthos						
Collector-gatherers	42.43	50.97	38.69	21.67	43.49	34.68
Filter-feeders	1.89	2.42	0.47	4.19	1.25	0.30
Grazer-scrapers	34.51	33.12	49.01	63.42	35.26	45.67
Predators	9.26	7.24	11.12	5.92	11.09	17.74
Shredders	11.90	6.24	0.71	4.80	8.91	1.61

Appendix 8 Invertebrate taxa subjected to stable isotope analysis representing FFGs of aquatic invertebrates, as well as terrestrial invertebrates, at the study sites.

Invertebrates	Trout			Redfin		
	Groothoek	Klip	Kraalstroom	Tierstel	Waaihoek	Tierkloof
Aquatic invertebrates						
Collector-gatherer						
Baetis	3	4	2	3	2	-
Demoreptus capensis	2	-	-	-	2	-
Aprionyx peterseni	1	-	3	1	1	3
Castanophlebia	-	1	-	1	-	2
Filter-feeder						
Simulium	5	5	5	5	5	5
Grazer-scraper						
Elmide	2	3	1	2	3	5
Scirtidae	-	3	-	2	-	-
Afronurus	3	-	4	1	2	-
Predator						
Corydalidae	3	1	3	2	3	5
Aeshna	2	4	2	3	-	-
Anax	-	-	-	-	2	-
Shredder						
Aphanicerca	1	3	4	3	5	-
Aphanicercella	4	2	1	2	-	5
Terrestrial invertebrates						
Hymenoptera	4	3	6	4	1	3
Orthoptera	2	3	-	2	1	4
Coleoptera	1	1	2	-	3	-
Diptera	1	-	1	-	2	1
Araneae	2	3	1	4	3	2

Appendix 9 $\delta^{15}N$ and $\delta^{13}C$ signatures of individual redfin and trout samples collected from the six sampling sites.

Redfin				Trout			
Site	Code	$\delta^{15}N$	$\delta^{13}C$	Site	Code	$\delta^{15}N$	$\delta^{13}C$
Tierkloof	tkr1	4.63	-20.38	Groothoek	grt1	7.68	-21.64
Tierkloof	tkr2	5.18	-21.26	Groothoek	grt2	6.48	-20.23
Tierkloof	tkr3	4.71	-20.32	Groothoek	grt3	6.55	-20.18
Tierkloof	tkr4	5.29	-21.60	Groothoek	grt4	6.69	-20.07
Tierkloof	tkr5	3.75	-21.74	Groothoek	grt5	7.48	-20.87
Tierkloof	tkr6	3.06	-26.39	Groothoek	grt6	6.23	-22.74
Tierkloof	tkr7	3.34	-27.58	Groothoek	grt7	7.13	-19.94
Tierkloof	tkr8	5.23	-24.64	Groothoek	grt8	7.59	-20.75
Tierkloof	tkr9	4.85	-25.67	Groothoek	grt9	6.47	-20.17
Tierkloof	tkr10	2.89	-19.95	Groothoek	grt10	6.76	-20.07
Tierkloof	tkr11	3.13	-25.36	Groothoek	grt11	6.80	-19.94
Tierkloof	tkr12	3.74	-20.89	Groothoek	grt12	7.89	-22.07
Tierkloof	tkr13	4.18	-19.54	Groothoek	grt13	7.29	-21.57
Tierkloof	tkr14	4.19	-20.37	Groothoek	grt14	8.43	-21.06
Tierkloof	tkr15	4.54	-23.18	Groothoek	grt15	8.92	-21.75
Tierkloof	tkr16	3.96	-26.37	Groothoek	grt16	7.83	-21.38
Tierstel	tsr1	4.90	-22.77	Klip	tkr1	8.19	-19.59
Tierstel	tsr2	6.10	-24.24	Klip	tkl2	7.25	-22.14
Tierstel	tsr3	5.58	-22.51	Klip	tkl3	8.02	-20.27
Tierstel	tsr4	5.06	-23.81	Klip	tkl4	6.52	-22.92
Tierstel	tsr5	4.92	-21.45	Klip	tkl5	8.08	-20.83
Tierstel	tsr6	4.23	-21.57	Klip	tkl6	7.76	-22.98
Tierstel	tsr7	4.57	-21.51	Klip	tkl7	9.37	-19.78
Tierstel	tsr8	4.87	-21.70	Klip	tkl8	7.60	-19.30
Tierstel	tsr9	5.02	-21.82	Klip	tkl9	7.98	-22.10
Tierstel	tsr10	4.80	-21.05	Klip	tkr10	7.67	-20.77
Tierstel	tsr11	5.95	-23.49	Klip	tkr11	7.68	-20.69
Tierstel	tsr12	6.10	-23.04	Klip	tkr12	7.55	-20.61
Tierstel	tsr13	5.45	-23.34	Klip	tkr13	7.53	-19.70
Tierstel	tsr14	4.29	-22.18	Klip	tkr14	7.52	-21.90
Tierstel	tsr15	4.48	-21.95	Klip	tkr15	8.11	-21.44
Tierstel	tsr16	5.83	-22.99	Klip	tkr16	7.55	-20.94
Waaihoek	war1	5.74	-23.12	Kraal	tkr1	6.77	-20.42

(continued overleaf)

Appendix 9 Continued

Redfin				Trout			
Site	Code	$\delta^{15}N$	$\delta^{13}C$	Site	Code	$\delta^{15}N$	$\delta^{13}C$
Waaihoek	war2	5.92	-22.37	Kraal	tkr2	5.84	-20.94
Waaihoek	war3	5.62	-24.11	Kraal	tkr3	6.21	-21.31
Waaihoek	war4	5.84	-23.30	Kraal	tkr4	6.85	-19.55
Waaihoek	war5	4.81	-22.24	Kraal	tkr5	6.70	-19.26
Waaihoek	war6	6.13	-24.16	Kraal	tkr6	5.65	-23.37
Waaihoek	war7	6.40	-23.18	Kraal	tkr7	5.97	-24.53
Waaihoek	war8	5.77	-23.69	Kraal	tkr8	6.64	-23.29
Waaihoek	war9	5.03	-23.61	Kraal	tkr9	5.73	-18.89
Waaihoek	war10	4.49	-22.16	Kraal	tkr10	5.64	-20.75
Waaihoek	war11	4.61	-22.41	Kraal	tkr11	5.55	-22.74
Waaihoek	war12	4.55	-22.28	Kraal	tkr12	5.63	-20.11
Waaihoek	war13	4.50	-23.26	Kraal	tkr13	6.15	-23.84
Waaihoek	war14	5.05	-23.26	Kraal	tkr14	6.04	-22.04
Waaihoek	war15	4.35	-22.34	Kraal	tkr15	5.81	-21.39
Waaihoek	war16	4.77	-23.11	Kraal	tkr16	5.90	-23.35

Appendix 10a Mean ± SE $\delta^{13}C$ and $\delta^{15}N$ for basal resources (algae and detritus), aquatic invertebrates (collector-gatherers, filter-feeders, grazer-scrapers, predators and shredders), terrestrial invertebrates and fish (trout), at sites where the trophic niche of trout was investigated.

Site	Food source	$\delta^{15}N$		$\delta^{13}C$		*n*
		Mean	SE	Mean	SE	
Groothoek	Algae	0.74	0.01	-22.89	0.18	5
	Detritus	-0.06	0.19	-26.56	0.15	5
	Collector-gatherers	1.83	0.13	-25.67	0.47	5
	Filter-feeders	2.26	0.05	-22.40	0.05	5
	Grazer-scrapers	2.19	0.18	-23.32	0.17	5
	Predators	3.50	0.11	-24.17	0.19	5
	Shredders	3.16	0.07	-24.28	0.13	5
	Terrestrial invertebrates	4.08	0.22	-23.76	0.15	5
	Trout	7.26	0.19	-20.90	0.22	16
Klip	Algae	1.58	0.02	-22.44	0.35	5
	Detritus	-0.02	0.06	-25.87	0.23	5
	Collector-gatherers	2.38	0.11	-21.92	0.48	5
	Filter-feeders	2.83	0.02	-21.37	0.34	5
	Grazer-scrapers	2.12	0.25	-18.49	0.45	5
	Predators	4.59	0.13	-18.59	0.04	5
	Shredders	3.00	0.14	-21.07	0.13	5
	Terrestrial invertebrates	3.84	0.15	-21.50	0.44	5
	Trout	7.77	0.15	-21.00	0.29	16
Kraal	Algae	0.78	0.09	-23.50	0.80	5
	Detritus	-1.27	0.09	-29.23	0.08	5
	Collector-gatherers	1.14	0.09	-27.98	1.26	5
	Filter-feeders	1.53	0.03	-25.71	0.21	5
	Grazer-scrapers	1.55	0.15	-26.04	0.31	5
	Predators	2.91	0.07	-26.05	0.05	5
	Shredders	1.61	0.05	-25.09	0.21	5
	Terrestrial invertebrates	2.26	0.08	-25.88	0.11	5
	Trout	6.07	0.11	-21.61	0.44	16

Appendix 10b Mean ± SE $\delta^{13}C$ and $\delta^{15}N$ for basal resources (algae and detritus), aquatic invertebrates (collector-gatherers, filter-feeders, grazer-scrapers, predators and shredders), terrestrial invertebrates and fish (redfin), at sites where the trophic niche of redfin was investigated.

Site	Food source	$\delta^{15}N$		$\delta^{13}C$		*n*
		Mean	SE	Mean	SE	
Tierstel	Algae	-1.70	0.01	-23.13	0.14	5
	Detritus	-1.45	0.11	-26.90	0.17	5
	Collector-gatherers	0.19	0.09	-24.18	0.26	5
	Filter-feeders	0.60	0.14	-24.59	0.10	5
	Grazer-scrapers	0.70	0.19	-24.94	0.12	5
	Predators	1.90	0.06	-24.00	0.04	5
	Shredders	1.90	0.21	-25.38	0.34	5
	Terrestrial invertebrates	3.04	0.22	-23.71	0.19	5
	Redfin	5.17	0.16	-22.53	0.24	16
Waaihoek	Algae	-2.22	0.00	-22.69	0.06	5
	Detritus	-1.58	0.18	-26.38	0.22	5
	Collector-gatherers	-0.71	0.30	-24.03	0.30	5
	Filter-feeders	0.12	0.04	-23.51	0.08	5
	Grazer-scrapers	-0.35	0.05	-23.19	0.28	5
	Predators	2.36	0.15	-24.12	0.13	5
	Shredders	1.32	0.14	-25.30	0.20	5
	Terrestrial invertebrates	3.00	0.18	-21.70	0.22	5
	Redfin	5.27	0.17	-23.09	0.16	16
Tierkloof	Algae	-1.84	0.02	-23.20	0.19	5
	Detritus	-2.20	0.10	-25.82	0.14	5
	Collector-gatherers	-0.78	0.04	-26.05	0.08	5
	Filter-feeders	0.41	0.03	-23.45	0.04	5
	Grazer-scrapers	-1.33	0.10	-24.07	0.29	5
	Predators	0.29	0.16	-24.91	0.13	5
	Shredders	1.15	0.05	-25.45	0.19	5
	Terrestrial invertebrates	2.05	0.04	-23.40	0.11	5
	Redfin	4.17	0.20	-22.83	0.68	16

Appendix 11 Mean ± SE density (number/m^2) of invertebrate taxa and functional feeding groups in treatments containing no fish, trout and redfin. "Abundance (%)" is the mean percentage abundance of each taxon/FFG based on all samples from all treatments combined.

Invertebrate taxon/FFG	Abundance (%)	No fish		Trout		Redfin	
		Mean	SE	Mean	SE	Mean	SE
Total invertebrates	**100.00**	**6866.67**	**288.15**	**6644.44**	**564.59**	**3188.89**	**98.97**
Collector-gatherers	**82.24**	**5647.22**	**235.81**	**5530.56**	**448.84**	**2555.56**	**130.32**
Afroptilum	2.05	169.44	47.53	166.67	47.21	5.56	3.21
Aprionyx peterseni	1.33	88.89	17.57	91.67	26.37	41.67	6.61
Athripsodes	1.60	113.89	9.58	86.11	20.52	66.67	15.04
Baetis	3.23	130.56	36.42	333.33	42.63	75.00	12.39
Bugilliesia	0.07	0.00	0.00	11.11	4.54	0.00	0.00
Caenis	0.07	8.33	4.81	0.00	0.00	2.78	1.60
Castanophlebia	0.32	16.67	6.14	30.56	13.70	5.56	3.21
Chironominae	43.36	2952.78	242.27	3047.22	237.53	1241.67	147.79
Choroterpes	0.02	0.00	0.00	2.78	1.60	0.00	0.00
Cloeodes	0.07	0.00	0.00	5.56	1.85	5.56	3.21
Demoreptus capensis	0.02	2.78	1.60	0.00	0.00	0.00	0.00
Labiobaetis/Pseudocloeon	13.27	980.56	127.94	894.44	32.55	341.67	34.88
Lestagella penicillata	5.52	377.78	58.15	241.67	40.69	302.78	28.37
Lithogloea harrisoni	0.15	2.78	1.60	2.78	1.60	19.44	3.07
Oligochaeta	0.07	11.11	6.42	0.00	0.00	0.00	0.00
Orthocladiinae	10.83	769.44	86.24	600.00	116.03	438.89	51.95
Parecnomia resima	0.28	22.22	8.69	16.67	9.62	8.33	3.07
Filter-feeders	**0.30**	**16.67**	**4.14**	**27.78**	**8.49**	**5.56**	**1.85**
Simulium	0.30	16.67	4.14	27.78	8.49	5.56	1.85

(continued overleaf)

Appendix 11 Continued

Invertebrate taxon/FFG	Abundance (%)	No fish		Trout		Redfin	
		Mean	SE	Mean	SE	Mean	SE
Grazer-scrapers	**6.64**	**436.11**	**22.59**	**422.22**	**33.74**	**250.00**	**17.67**
Afronurus	1.08	72.22	18.24	50.00	10.64	58.33	12.39
Atrichopogon	0.02	0.00	0.00	2.78	1.60	0.00	0.00
Barbarochthon brunneum	0.10	2.78	1.60	8.33	1.60	5.56	1.85
Bezzia	0.02	2.78	1.60	0.00	0.00	0.00	0.00
Crambidae	0.38	11.11	2.62	41.67	4.81	11.11	2.62
Elmidae	3.08	200.00	34.25	177.78	25.26	136.11	14.90
Elporia capensis	0.05	5.56	3.21	2.78	1.60	0.00	0.00
Forcipomyia	0.03	0.00	0.00	0.00	0.00	5.56	1.85
Haliplidae	0.02	0.00	0.00	2.78	1.60	0.00	0.00
Hydraenidae	0.03	2.78	1.60	2.78	1.60	0.00	0.00
Hydroptila	0.73	55.56	20.12	44.44	6.42	22.22	7.41
Orthotrichia	1.00	80.56	33.27	75.00	26.76	11.11	3.70
Petrothrincus	0.03	0.00	0.00	5.56	3.21	0.00	0.00
Scirtidae	0.07	2.78	1.60	8.33	3.07	0.00	0.00
Predators	**9.08**	**688.89**	**70.61**	**483.33**	**106.01**	**344.44**	**75.95**
Acarina	0.07	5.56	1.85	5.56	1.85	0.00	0.00
Aeshna	0.12	11.11	2.62	0.00	0.00	8.33	3.07
Anax	0.02	2.78	1.60	0.00	0.00	0.00	0.00
Atherix	0.37	25.00	6.61	30.56	6.61	5.56	3.21
Cheumatopsyche	0.67	69.44	40.09	30.56	8.02	11.11	4.54
Corydalidae	0.02	0.00	0.00	2.78	1.60	0.00	0.00
Gyrinidae	0.08	5.56	1.85	5.56	3.21	2.78	1.60
Mesovelia	0.32	44.44	7.86	5.56	3.21	2.78	1.60

(continued overleaf)

Appendix 11 Continued

Invertebrate taxon/FFG	Abundance (%)	No fish		Trout		Redfin	
		Mean	SE	Mean	SE	Mean	SE
Micronecta	0.10	8.33	1.60	8.33	4.81	0.00	0.00
Nematoda	0.02	2.78	1.60	0.00	0.00	0.00	0.00
Oecetis	1.31	147.22	9.21	44.44	18.14	27.78	6.14
Pseudagrion	2.43	122.22	11.42	75.00	18.95	208.33	69.11
Tanypodinae	3.56	241.67	52.07	275.00	69.60	77.78	14.10
Trithemis	0.02	2.78	1.60	0.00	0.00	0.00	0.00
Shredders	**1.75**	**77.78**	**17.17**	**180.56**	**40.86**	**33.33**	**5.86**
Aphanicerca	0.10	2.78	1.60	13.89	4.81	0.00	0.00
Aphanicercella	1.61	75.00	15.80	163.89	39.23	30.56	4.81
Dyschimus	0.03	0.00	0.00	2.78	1.60	2.78	1.60

Appendix 12 Mean ± SE dry mass (mg/m^2) of invertebrate taxa and functional feeding groups in treatments containing no fish, trout and redfin. "Abundance (%)" is the mean percentage abundance of each taxon/FFG based on all samples from all treatments combined.

Invertebrate taxon/FFG	Abundance (%)	No fish		Trout		Redfin	
		Mean	SE	Mean	SE	Mean	SE
Total invertebrates	**100.00**	**487.13**	**47.77**	**451.22**	**39.25**	**252.21**	**17.00**
Collector-gatherers	**57.85**	**275.66**	**19.53**	**282.46**	**22.88**	**130.62**	**9.18**
Afroptilum	2.10	12.43	3.49	12.22	3.46	0.41	0.24
Aprionyx peterseni	14.87	70.79	13.99	73.01	21.00	33.18	5.27
Athripsodes	0.41	2.07	0.17	1.57	0.37	1.21	0.27
Baetis	3.52	10.15	2.83	25.93	3.32	5.83	0.96
Bugilliesia	0.05	0.00	0.00	0.56	0.23	0.00	0.00
Caenis	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Castanophlebia	0.13	0.50	0.18	0.92	0.41	0.17	0.10
Chironominae	14.18	68.86	5.65	71.06	5.54	28.96	3.45
Choroterpes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cloeodes	0.00	0.00	0.00	0.01	0.00	0.01	0.00
Demoreptus capensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Labiobaetis/Pseudocloeon	15.55	81.92	10.69	74.73	2.72	28.54	2.91
Lestagella penicillata	0.80	3.91	0.60	2.50	0.42	3.13	0.29
Lithogloea harrisoni	1.78	2.36	1.36	2.36	1.36	16.53	2.61
Oligochaeta	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Orthocladiinae	4.20	21.25	2.38	16.57	3.20	12.12	1.43
Parecnomia resima	0.25	1.39	0.54	1.04	0.60	0.52	0.19
Filter-feeders	**0.17**	**0.69**	**0.17**	**1.16**	**0.35**	**0.23**	**0.08**
Simulium	0.17	0.69	0.17	1.16	0.35	0.23	0.08

(Continued overleaf)

Appendix 12 Continued

Invertebrate taxon/FFG	Abundance (%)	No fish		Trout		Redfin	
		Mean	SE	Mean	SE	Mean	SE
Grazer-scrapers	**21.55**	**92.31**	**16.04**	**93.68**	**8.02**	**70.51**	**9.93**
Afronurus	11.28	53.70	13.56	37.17	7.91	43.37	9.21
Atrichopogon	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Barbarochthon brunneum	0.09	0.19	0.11	0.56	0.11	0.37	0.12
Bezzia	0.02	0.28	0.16	0.00	0.00	0.00	0.00
Crambidae	3.69	7.64	1.80	28.65	3.31	7.64	1.80
Elmidae	5.46	25.29	4.33	22.48	3.19	17.21	1.88
Elporia capensis	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Forcipomyia	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Haliplidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hydraenidae	0.02	0.09	0.05	0.09	0.05	0.00	0.00
Hydroptila	0.88	4.76	1.72	3.81	0.55	1.90	0.63
Orthotrichia	0.01	0.08	0.03	0.08	0.03	0.01	0.00
Petrothrincus	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Scirtidae	0.09	0.28	0.16	0.83	0.31	0.00	0.00
Predators	**20.21**	**117.97**	**33.26**	**71.91**	**12.05**	**50.77**	**11.94**
Acarina	0.00	0.01	0.00	0.01	0.00	0.00	0.00
Aeshna	0.53	3.61	0.85	0.00	0.00	2.71	1.00
Anax	0.05	0.56	0.32	0.00	0.00	0.00	0.00
Atherix	0.70	3.41	0.90	4.17	0.90	0.76	0.44
Cheumatopsyche	7.32	54.49	31.46	23.97	6.29	8.72	3.56
Corydalidae	0.44	0.00	0.00	5.28	3.05	0.00	0.00
Gyrinidae	1.08	5.14	1.71	5.14	2.97	2.57	1.48
Mesovelia	0.48	4.85	0.86	0.61	0.35	0.30	0.17
Micronecta	0.00	0.01	0.00	0.01	0.00	0.00	0.00
Nematoda	0.00	0.00	0.00	0.00	0.00	0.00	0.00

(continued overleaf)

Appendix 12 Continued

Invertebrate taxon/FFG	Abundance (%)	No fish		Trout		Redfin	
		Mean	SE	Mean	SE	Mean	SE
Oecetis	1.57	12.51	0.78	3.78	1.54	2.36	0.52
Pseudagrion	4.58	16.44	1.54	10.09	2.55	28.02	9.29
Tanypodinae	3.43	16.58	3.57	18.87	4.78	5.34	0.97
Trithemis	0.03	0.37	0.21	0.00	0.00	0.00	0.00
Shredders	**0.22**	**0.50**	**0.22**	**2.00**	**0.60**	**0.07**	**0.01**
Aphanicerca	0.16	0.32	0.19	1.62	0.56	0.00	0.00
Aphanicercella	0.05	0.17	0.04	0.38	0.09	0.07	0.01
Dyschimus	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 13 Dry mass estimates of invertebrate taxa recorded during the cage experiment.

FFG	Taxon	*n*	Mean dry mass/individual (mg)
Collector-gatherers			
	Afroptilum	45	0.073
	Aprionyx peterseni	28	0.796
	Athripsodes	22	0.018
	Baetis	63	0.078
	Bugilliesia	5	0.050
	Caenis	5	0.010
	Castanophlebia	10	0.030
	Chironominae	5	0.300
	Choroterpes	253	0.023
	Cloeodes	5	0.010
	Demoreptus capensis	16	0.687
	Labiobaetis/*Pseudocloeon*	14	0.086
	Lestagella penicillata	158	0.084
	Lithogloea harrisoni	58	0.010
	Oligochaeta	20	0.085
	Orthocladiinae	5	0.010
	Parecnomia resima	20	0.010
Filter-feeders			
	Simulium	5	0.100
Grazer-scrapers			
	Afronurus	23	0.743
	Atrichopogon	5	0.010
	Barbarochthon brunneum	3	0.067
	Bezzia	5	0.100
	Crambidae	5	1.900
	Elmidae	5	0.010
	Elporia capensis	34	0.126
	Forcipomyia	5	0.010
	Haliplidae	5	0.925
	Hydraenidae	5	0.010
	Hydroptila	5	0.033
	Orthotrichia	105	0.028
	Petrothrincus	8	0.063
	Scirtidae	5	0.050
Predators			
	Acarina	5	0.010
	Aeshna	8	0.325
	Anax	5	0.200
	Athericidae	11	0.136
	Cheumatopsyche	13	0.785

(continued overleaf)

Appendix 13 Continued

FFG	Taxon	*n*	Mean dry mass/individual (mg)
	Corydalidae	5	0.010
	Gyrinidae	5	0.010
	Mesovelia	8	0.850
	Micronecta	11	0.109
	Nematoda	5	0.010
	Oecetis	5	0.010
	Pseudagrion	29	0.134
	Tanypodinae	5	0.010
	Trithemis	51	0.069
Shredders			
	Aphanicerca	6	0.117
	Aphanicercella	43	0.002
	Dyschimus	5	0.133