Fish presence and the ecology of stream invertebrate predators

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Contents

Declaration

I hereby declare that I composed this thesis and that the work described therein is my own.

François K. Edwards.

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Abstract

Stream invertebrates are the top predators in fishless habitats, but when fish are present they are affected directly (predation) and indirectly (shared prey). Invertebrate predators must balance predation risk and foraging needs, and their responses to the presence of a predator may have to be flexible to exploit both types of habitat. This thesis describes the ecology of invertebrate predators (Plecoptera and Trichoptera) across streams, some without fish. Focusing primarily on stoneflies of the families Perlidae and Perlodidae, I studied the density and diversity of predators and the invertebrate prey, predator diet, size-mass relationships and microhabitat use. The abundance of invertebrate predators varied across streams and seasons but numbers of predatory invertebrates did not differ with fish presence. Only the smallest species of invertebrate predator was more abundant in substrate complexes in streams with fish. Overall biomass of invertebrate predators was lower in streams with fish, because the size-class distributions of some species were biased towards small individuals. The size-mass relationships and pre-emergent weights of all predators, except the Perlidae, varied across sites, but there was no clear relationship between condition and fish presence/absence. Invertebrate predators were mainly carnivorous in fishless sites, but in some species diet broadened with fish presence to include more algae. I hypothesise that the fixed nocturnal habit of Perlidae, permitted by their slower growth, accounted for the similarity in abundance, size, condition and diet across streams. Nocturnal activity and the coarse stony substrate, which provided abundant refugia, minimised any effect of fish. By contrast, Perlodidae and other predators have shorter life cycles, forage by day and night, and have greater growth

requirements, accounting for the greater variability in size and condition across streams. Though these species should incur greater exposure to fish, the coarse substrate may provide foraging space free from predation risk, thus minimising any effects of fish. For one daytime active species, feeding trials showed that they can facilitate the capture of prey by fish and this in itself may help them avoid predation. For stream invertebrate predators, direct predation effects of fish appear to be minimal and principal effects may be sublethal, indirect, and prey mediated.

1 Introduction

Fish are ubiquitous in many freshwater ecosystems and are an import part of the food webs of lakes, rivers and streams. Many fish are predatory and feed on aquatic invertebrates. When fish are absent, as in the upper reaches of some small streams, invertebrates, such as stonefly nymphs and caddisfly larvae, can often be the top predators (e.g. Harvey, 1993). These invertebrate predators, however, do usually coexist with fish, for example trout (e.g. Allan, 1975), which can affect them directly, through predation/consumption or behavioural alterations (e.g. Feltmate and Williams, 1989), and indirectly, as they also feed on the same prey resource as the fish (e.g. Peckarsky and McIntosh, 1998). Although there has been research on the overall effects of fish on stream invertebrate communities (reviews in Wooster, 1994; Wooster and Sih, 1995), their effects on intermediate predators are still poorly understood, despite evidence that these interactions can structure the whole community in some systems (e.g. Carpenter *et al*, 1987, Power, 1990).

Many invertebrate predators need to forage for prey, but incur the risk of predation when doing so, and thus face a trade-off (Lima and Dill, 1990) between long term fitness (resource acquisition) and short term survival (avoidance of fish). Traits that allow the avoidance of fish, such as reduced activity, may be a disadvantage when predation risk is low. In these situations, resource acquisition may be limited by competition for prey with other predatory invertebrates, including conspecifics. The abundance of fish, and therefore predation risk, varies across streams, and species which have flexible responses to the presence of fish may also exploit fishless and low fish density habitats.

This thesis describes changes in the ecological relationships of invertebrate predators across stream systems. I investigate whether these relationships vary, and whether this can be related to an effect of fish presence. Do fishless sites provide the best conditions for the invertebrate predators? Or are they better suited to habitats where the invertebrate community is shaped by the presence of fish. I use this stream example to better understand the multiple functional roles intermediate predators play in stream food webs as predators, competitors and prey.

1.1 Fish, invertebrate predators, and the trophic cascade

Cascading effects of fish in lake invertebrate communities

Fish have clear effects on the abundance of benthic and pelagic invertebrates of some lakes, and they are often used as model systems to explain the impacts of fish in aquatic food webs. The effects of fish on successive trophic levels in lentic systems were described by Carpenter *et al* (1987) in small lakes containing planktivorous and piscivorous fish. Increases in piscivore numbers reduced the density and foraging effort of planktivorous fish, larger zooplankton became more abundant and consequently phytoplankton densities were reduced. Inversely, reductions in piscivore numbers allowed planktivores to limit zooplankton abundance and phytoplankton densities thus increased. The organisation of these pelagic communities of invertebrates, vertebrates and algae into distinct trophic levels caused a predictable effect to spread from top predator (piscivorous fish) to intermediate predator (planktivorous fish) to grazer (zooplankton) to primary producer (phytoplankton), and the density of the top predator gave rise to distinct planktonic communities (Persson, 1997). This is a trophic 'cascade' (Paine, 1980; Pace *et al,* 1999), and is the essence of linear food chain theory (Hairston *et al,* 1960) which views communities as a succession of trophic levels, each inversely related by its abundance to the next (Chase, 2000), and the number of predatory levels determines whether basal resources are limited by primary consumers (Fretwell, 1987).

Effects of fish in lentic food webs do not always follow such a clear pattern, and sometimes invertebrate predators do not contribute to the cascade effect. Mancinelli *et al* (2002) observed an increase in the density of invertebrate detritivores in fish exclusions in the littoral zone of a lake and consequently an increase in litter consumption which limited fungal diversity. When fish were present, detritivores became less abundant, and fungal diversity increased. Invertebrate predators, mainly odonates, avoided fish through their size, burrowing habit and 'ambush' feeding mode, thus their density remained the same with and without fish. Vertebrate and invertebrate predators ate the same prey and behaved as a single trophic level but only fish affected the abundance of detritivores strongly enough to generate cascading effects on fungi. The interaction between fish and invertebrate predators can be important in transmitting the effects of fish through lake food webs, but this is dependant on the characteristics of the invertebrate predators, particularly their vulnerability to fish, and their ability to impact prey populations (Werner, 1992; Brett and Goldman, 1996; Polis *et al*, 2000).

The effects of fish on stream communities

Invertebrate predators can also influence the way in which fish affect stream invertebrate communities. Power (1990) reported that the fish of the Eel River, in California, fed predominantly on intermediate predators (damselfly nymphs), which themselves fed on algivorous Chironomidae larvae. Because fish could not capture the tuft-weaving Chironomidae, these proliferated and constrained the growth of algal mats. When fish were excluded, damselfly numbers increased and they significantly reduced chironomid abundance, allowing algae to proliferate. Damselflies were instrumental in 'channelling' the impact of fish through the food web and formed a clearly defined intermediary trophic level, like Carpenter's (1987) zooplanktivorous fish. However, such a clear trophic structure is not prevalent in all lotic systems, and fish rarely feed uniquely on invertebrate predators. In many cases, organisms are hard to assign to trophic levels and this can mitigate against cascade effects (Strong, 1992; Power *et al*, 1992). Indeed, many predators, including fish and stream invertebrates, are polyphagous and exploit each of their prey/food types to different extents, often in a density-dependant manner (Menge and Sutherland, 1976; Cooper *et al*, 1990), or according to body size (Polis *et al*, 1989; Persson, 1997).

Stream fish (particularly Salmonidae and Cottidae) often reduce the densities of large invertebrates (Bechara *et al*,1992; Rosenfeld, 2000), whether they are predators or herbivores, because they are preferentially selected as prey items by fish (Allan, 1981, 1984; Newman and Waters, 1984; Scrimgeour *et al*, 1994). This can have a cascading effect on small bodied herbivores, which, released from competition, increase in number and limit the growth of algae (Bechara *et al*, 1993; Rosenfeld, 1997). However, this does not always occur when these smaller invertebrates are less abundant, for example seasonally or locally, and algae benefit from the reduction in numbers of large grazers (Bechara *et al*, 1992; Flecker and Townsend, 1994; McIntosh and Townsend, 1996; Forrester *et al*, 1999). Thus, in most streams, like in Mancinelli *et al*'s (2002) lake, the role of intermediate predators in linking fish to the lower trophic levels may be minimal, and fish may control the abundance of grazers directly. These effects of fish on grazers and algae are widespread and have been reported in many fish/without fish field manipulations, but effects on invertebrate predators are less well understood (e.g. Bechara *et al*, 1993; Gilliam *et al*, 1989; Rosenfeld 2000).

The case of fishless streams

The trophic cascade model predicts that the abundance of large invertebrates should be greater in fishless streams than in streams with fish, and in such streams the abundance of algae should be limited by grazers (Fretwell, 1987). However, the few field surveys across streams with and without fish have consistently identified only small, if any, changes in invertebrate abundance (e.g. Allan, 1982a; Flecker and Allan, 1984; Bowlby and Roff, 1986; Culp, 1986; Harvey, 1993). If effects of fish are principally behavioural (e.g. habitat selection), then they may have little effect on the abundance of invertebrates. Alternatively, under field conditions, the effects of predation may be hard to detect in streams with fish. Rapid recolonisation rates may compensate for the local impacts of fish on invertebrate abundance (Cooper *et al*, 1990). Also, hydraulic disturbance can redistribute invertebrates in the benthos, and thus may mask the effects of predation too (McAuliffe, 1984; Hart and Finelli, 1999; Thomson *et al*, 2002).

Invertebrate abundance may be similar in streams with and without fish because invertebrate predators may compensate for their absence, and limit the abundance of grazers (Allan, 1982a; Harvey, 1993; Huhta *et al*, 1999; Rosenfeld, 2000). Invertebrate predators can indeed reduce the density of their prey. For example, predaceous stoneflies are known to impact populations of *Baetis* (Peckarsky and Penton, 1985, 1989) and Lancaster *et al* (1991) demonstrated that Polycentropodidae reduced local invertebrate density in a fishless stream. Invertebrate predators could compensate for fish, firstly, if their abundance increases in the absence of fish (Brooks and Dodson, 1965), and this may occur because fish tend to feed on large invertebrates, which include invertebrate predators (e.g. many Plecoptera, Trichoptera, Odonata and Megaloptera). Secondly, invertebrate predators can compensate for fish, if their behaviour, particularly foraging activity, changes with predation risk and the abundance of fish, to reflect the shift from an intermediate to a top predator (Lima and Dill, 1990).

1.2 How do predatory invertebrates respond to the presence of fish?

Abundance

Predation by fish can decrease the abundance of stream invertebrate predators under experimental conditions (Power, 1990), but effects are less pronounced under field conditions. Enclosure experiments indicate that fish can have a strong negative effect on overall invertebrate predator abundance (Walde and Davies, 1984a; Bechara *et al*, 1992, 1993; Rosenfeld, 1997, 1998, 2000). Nymphs of the predatory stonefly *Paragnetina media* decreased in density in rainbow trout (*Oncorhynchus mykiss*) enclosures (Feltmate and Williams, 1989, 1991) and bullheads (*Cottus gobio*) reduced densities of the predatory caddis-fly *Polycentropus* (Dahl, 1998a). However, most comparative surveys in streams with and without fish indicate no effect of fish on invertebrate predator abundance (e.g. Allan, 1982a; Flecker and Allan, 1984; Bowlby and Roff, 1986). Though Harvey (1993) observed that the density of some predatory stoneflies was lower in streams with fish than in fishless streams, not all species were affected by fish presence/absence. Thus, there appears to be a paradox between the results of field surveys and experimental manipulations.

Fish may have only a weak effect on invertebrate predator abundance *per se*, as most field data indicate, and their effects may be stronger in enclosure experiments because the effects of consumption and emigration are confounded on small spatial scales (Walde and Davies, 1984a). Consumption of invertebrate predators by fish is a direct, lethal effect, and the emigration of these invertebrates from enclosures with fish is a behavioural response, with only sub-lethal consequences. Both mechanisms reduce local abundance (i.e. abundance in the enclosures), but if emigration is high, then little actual predation by fish on invertebrate predators may occur. For example, Power (1990) could not determine whether damselflies were eaten by fish, or had left the enclosures, yet these two processes will have had very different consequences for the abundance of damselflies at the reach/stream level. It is unclear, under what circumstances fish impact invertebrate abundance, and under what circumstances only sublethal effects occur. In artificial systems where the emigration of invertebrate predators was prevented, Dahl and Greenberg (1997) and Soluk and Collins (1988a, 1988b) observed only occasional consumption of invertebrate predators (leeches and stonefly nymphs) by fish, but on the other hand, the foraging activity of the predatory invertebrates was greatly reduced. Differences in invertebrate predator density may not always occur in surveys across sites with and without fish, if the effect of fish on invertebrate predators is principally sublethal, and the net effect on their abundance may be weak (Harvey, 1993; Huhta *et al*, 1999).

Foraging activity

Fish often cause a reduction in the foraging activity of stream invertebrates (Lima and Dill, 1990; Forrester, 1994; Scrimgeour and Culp, 1994), indeed predators suppress the activity of their prey in many ecosystems and across taxonomic groups (Murdoch and Oaten, 1975; Abrams, 1987). Fish generate 'predator' cues visually and through hydrodynamics, and they also produce chemical cues. Several studies indicate invertebrates assess predation risk principally through these chemical cues (Bronmark, 2000). When predation risk by fish is high, many invertebrate predators spend less time foraging for food and more time in interstitial spaces (Rahel and Stein, 1988; Wooster, 1994; Dahl and Greenberg, 1999; Gido and Matthews, 2001). For predatory Rhyacophilidae, Huhta *et al* (1999) observed less foraging activity among larvae from streams with fish compared to larvae from fishless streams. Furthermore, the presence of fish reduced the time spent foraging by predatory leeches (Dahl and Greenberg, 1997), caddisflies (Otto, 1993) and stoneflies (Soluk, 1993). This behaviour reduces exposure to fish, and thus invertebrate predators may avoid being eaten, but at the cost of resource acquisition (Werner, 1992; Sih and Wooster, 1994).

Because predation risk varies across streams with the abundance of fish, invertebrate predators require a flexible foraging strategy to optimise the trade-off between fish avoidance and feeding (Lima and Bedneckoff, 1999). For example, in a stream with fish, Huhta *et al* (1999) found that the caddis fly *Rhyacophila nubila* foraged at night only, when predation risk was low, but foraged by day and night in the absence of fish, i.e. their foraging activity increased because they spent less time avoiding fish. For some species, night-time foraging appears to be a fixed response to the presence of fish. The stonefly *Diura bicaudata* maintained nocturnal foraging without fish (Huhta *et al* 1999), and as these nymphs rarely occur in fishless habitats (due to a preference for low altitude it is scarce in headwater streams), flexible diel foraging activity may not be advantageous. On the other hand, many strictly nocturnal predators commonly co-occur with fish, but do not increase daytime foraging activity when fish are absent (Feltmate *et al*, 1992; Elliott, 2000, 2003 a, 2003b) and may be at competitive disadvantage in fishless streams.

Food and prey types in the diet

Fish may affect the diet of invertebrate predators because the capture of different prey is associated with different levels of exposure to fish. When fish are present, invertebrate predators may feed on the prey which incurs the least risk of predation (Abrams and Matsuda, 1996). For example, stonefly nymphs rank prey preference according to their handling times rather than their energetic value, or abundance (Molles and Pietruszka, 1983; Peckarsky and Penton, 1989). This may be because stoneflies are more vulnerable to capture by fish when they are handling prey, e.g. because the increase in activity makes them more 'visible', and therefore prey with a short handling time reduce exposure to fish (Peckarsky *et al*, 1994). If fish are absent, prey which have long handling times may be more profitable, if they have a high nutritional value, i.e. the energy provided by the prey is greater than the energy required for capture and digestion (Abrahams and Dill, 1989). Furthermore, many invertebrate predators are omnivores (they eat algae, detritus and prey, Jones, 1950; Mackereth, 1957; Hynes, 1976), and the relative abundance of these food types in the diet may also vary with predation risk and the abundance of fish presence. A flexible diet may allow invertebrate predators to balance predation risk and resource acquisition across the wide range in fish abundance of different streams (Singer and Bernays, 2003).

Prey-mediated indirect effects

Fish can affect the foraging activity and diet of invertebrate predators, because they have overlapping diets, and fish can alter the distribution of the shared prey/resources (Flecker and Allan, 1984; Huhta *et al*, 1999; Soluk and Richardson, 1997; Peckarsky and McIntosh, 1998). Though stream fish can reduce the abundance of some invertebrate prey, such as large grazers (Scrimgeour *et al*, 1994), they can also cause an increase in the abundance of invertebrates less vulnerable to fish, through competitive release (e.g. Chironomidae, Rosenfeld, 1997; Power, 1990). The relative abundance of prey types may vary because of fish, and hence the occurrence of these prey in the diet of invertebrate predators may also vary. Predatory fish are also associated with higher algal growth, due to reduced grazing by invertebrates (Power, 1990; Bechara *et al*, 1993; Rosenfeld, 2000), and invertebrate predators may consume more algae in streams with fish, because the relative availability of prey/algal food types differs. Fish can thus have indirect effects on invertebrate predators, because they affect their encounter rates with their resources.

Fish also affect the behaviour of shared prey, and thus can facilitate or interfere with, prey capture by invertebrate predators (Dahl and Greenberg, 1996; Huhta *et al*, 1999). Fish induce drifting of some invertebrates, particularly baetid mayflies

(Malmqvist and Sjostrom, 1987; Flecker, 1992) and also reduce the activity of others, such as Plecoptera and Trichoptera (Forrester, 1994; Scrimgeour and Culp, 1994; Gido and Matthews, 2001), and therefore can affect the encounter rates between invertebrate predators and prey. For example, the reduced activity of mayfly prey in the presence of trout decreased the feeding rate of predatory stoneflies (Peckarsky and McIntosh, 1998) and leeches (Dahl and Greenberg, 1997). However, Rahel and Stein (1988) described how the increased use of crevices by prey as a shelter from fish benefited the prey capture rates of predatory crayfish. Hence, the response of prey to invertebrate predators and fish may conflict (Resetarits, 1991), and the avoidance of fish may either increase or decrease exposure of the prey to invertebrate predators, and thus prey capture rates. Because of these behavioural effects, the relative abundance of prey types in the diet of invertebrate predators may change in response to the abundance of fish (Dahl and Greenberg, 1996).

Fish mediated indirect effects

Complex effects of fish on invertebrate predator feeding can arise if the invertebrate predators affect encounter rates between shared prey and fish. Changes in prey behaviour or abundance because of invertebrate predators may eventually affect the availability of prey to fish (Wootton, 1994; Sih *et al*, 1998). Soluk (1993) described how stonefly facilitated the feeding of sculpin (Cottidae) when the prey were crawlers (*Ephemerella* sp. mayflies), but interfered with feeding (less prey are captured) when the prey were active swimmers (*Baetis* sp. mayflies). This was because the *Baetis* responded to stonefly with an escape response to the water column, which reduced their availability to sculpin. *Ephemerella*, on the other hand responded to the stonefly by posturing (scorpion stance) or crawling away, and this made them more conspicuous to sculpin. In contrast, trout are pelagic, and Soluk and Richardson (1997) found that stonefly nymphs increased *Baetis* capture by trout, because they induced a drift response (Malmqvist and Sjostrom, 1987). Though facilitation by invertebrate predators of prey capture by fish may reduce the availability of the shared prey, invertebrate predators may benefit if this makes them less susceptible to capture themselves. Invertebrate predators may be preferentially selected by fish because they are large (Scrimgeour *et al*, 1994), and therefore behaviour which increases the proportion of other prey types in the diet of the fish may be advantageous. This type of indirect effects, mediated by a shared predator, occur in host-parasite systems (Holt and Lawton, 1983; Bonsall and Hassell, 1997) and terrestrial invertebrates (Sih *et al*, 1985; Berdegue *et al*, 1996), and the interaction of fish, invertebrate predators, and their shared prey may give rise to similar effects in stream systems too.

1.3 Consequences for condition and fitness

Because fish can have direct and prey-mediated effects on the foraging behaviour and diet of invertebrate predators, they may have a strong sub-lethal impact on their growth, condition and reproductive fitness. For example, reduced foraging in the presence of fish had a negative effect on the growth predatory stoneflies (Feltmate and Williams, 1991) and caddisflies (Werner *et al*, 1983). The adult insect is non-growing and often non-feeding (e.g. stoneflies, Zwick, 1996), and therefore the final size and weight, or condition, of the larva/nymph is a good indicator of adult fecundity and reproductive success i.e. fatter is fitter (Gould, 1966; Peckarsky and Cowan, 1991; Peacor, 2002). If less prey, or less nutritious prey, is captured when fish are present, the condition of invertebrate predators may increase as fish abundance decreases (Peckarsky and McIntosh, 1998). For example, Bowlby and Roff (1986) and Harvey (1993) found that the individual biomass of invertebrate predators was higher in fishless streams. However, predators sometime have positive effects on the condition of their prey. Predatory dragonflies increased the condition of algivorous bullfrog tadpoles, because reduced tadpole abundance decreased the intraspecific competition for algae (Peacor, 2002). In streams with fish, invertebrate predators that escape capture by fish may be in better condition than those in fishless streams, if they can exploit the potential increase in the abundance of algae and small grazers. Furthermore, if the abundance of invertebrate predators is higher in fishless streams, competition for prey may increase, affecting condition also (Harvey, 1993).

Predatory stoneflies, for example, interfere with one another's feeding rates within (Elliott, 2003b) and between (Peckarsky, 1990) species. Taylor *et al* (1998) observed that nymphs of the predatory stonefly *Megarcys signata* where more abundant, but smaller, in fishless streams. The condition of predatory invertebrates may vary across different streams, reflecting the trade-off between feeding and predation risk.

1.4 Aims, hypotheses and thesis outline

The aim of this research was to understand how some aspects of the ecology of invertebrate predators change, if at all, across streams, particularly with respect to predation risk by fish (measured as the presence/absence of fish). Are some species of invertebrate predators better suited to fishless habitats or to habitats with fish, and do invertebrate predators display flexible ecological traits that allow them to exploit all habitats successfully? I studied the ecology of invertebrate predators in streams with fish, and in streams where fish were absent (the sites are described in Chapter 2). To simplify the interpretation of results I chose streams with only one main type of fish, salmonids. The effects of fish should be detectable across similar stream types with and without fish, if they have a significant effect on the structure of the invertebrate community.

In Chapter 3, I ask: How does the distribution of invertebrate predators change across similar streams, some with and some without fish? Do all species of predators display similar patterns of abundance? To answer these questions, I examined the seasonal abundance and diversity of predatory invertebrates and their prey across three streams with fish, and a stream without fish. I tried to link patterns in invertebrate predator abundance with patterns in the abundance of their invertebrate prey across the sites. I also tried to evaluate the relative magnitude of the effects of fish vs other habitat variables. I expected that if fish have strong effects on the invertebrate community, then the community in the fishless site would differ strongly from the other three streams. If fish have only weak effects, I expected the communities to be similar across sites.

In Chapter 4, I study the relationship between stable substrate structures and the abundance and biomass of invertebrate predators, in two streams with fish, and two streams without fish. Do the abundance, biomass and size of invertebrate predators in these structures change with the presence/absence of fish? I expected fish to reduce the abundance of larger invertebrate predators, and thus streams with fish to have more small individuals than fishless streams. I expected this to cause a lower biomass of invertebrate predators in streams with fish.

In Chapter 5, I ask whether the principal effects of fish on predatory invertebrates are sublethal, and reflected in the condition of the invertebrates from each stream, estimated by size-mass relationships. Does condition vary on small geographical scales across streams? Are invertebrate predators fitter in the absence of fish? The condition of species that display strong behavioural trade-offs between foraging and fish avoidance was expected to increase with reduced predation risk. The condition of species was expected to remain the same with and without fish if the trade-offs between foraging and fish avoidance are weak. In Appendix A, I examine some of the sources of error in the estimation of size-mass relationships, and suggest ways to improve them, so they can be compared across studies.

In Chapter 6, I study the diet of invertebrate predators and its variability across streams. Do fish affect the incidence of carnivory vs algivory among invertebrate predators? If invertebrate activity increases in the absence of fish, the incidence of carnivory may increase as encounter rates between invertebrate predator and prey increase. When fish are present omnivory may increase as prey are limited or less active. I expected species with flexible foraging strategies to have more variable diets, and species with fixed foraging strategies to show the least variation in diet across streams.

In Chapter 7, I use laboratory experiments to compare the foraging rates of an invertebrate predator, with and without fish present. Do fish and invertebrate predators facilitate or interfere with each other's feeding? Is the combined effect of the two types of predator greater than the sum of their separate effects? I expected fish to reduce the activity of invertebrate predators and thus their feeding rates. I manipulated prey density and availability of refugia, and compared prey capture by invertebrate predators with and without fish. I also studied how the invertebrate predators affect the foraging rates of the fish, and estimated the consequences for prey. From the results of other studies, I expected invertebrate predators to facilitate the feeding of the fish, due to the conflicting responses by the prey to the two types of predators.

Finally, in the conclusions chapter, I integrate patterns of abundance, condition, and diet across sites, and compare their variability with respect to the life history and foraging strategy of each species. I summarise any differences between streams with and without fish. I contrast the direct and indirect effects of fish on invertebrate predators, as well as the lethal and sublethal consequences of predation risk.

2 Site description

2.1 General information

Study streams were located in the Tweedsmuir Hills of south-east Scotland and were second order tributaries of St Mary's Loch, the Megget reservoir (both part of the Yarrow catchment) or the Talla reservoir (Tweed catchment). The streams were chosen because they are in low populated areas and were unmodified, natural, sites. Local annual rainfall was 10.5 cm in 1998 and 14.5 cm in 1999 at the Cappercleuch weather station (NT 152 200), within 4 km of all the sites (data from the British Atmospheric Data Centre).

The area is managed for sheep grazing with sparse habitation and forestation. The riparian vegetation along all streams was principally heather (*Calluna vulgaris* L.), bracken (*Pteridium aquilinum* L.) and grasses. The underlying geology is classified as sedimentary rock of Lower Palaeozoic 'Gala' group. Lithology is dominated by medium to thickly bedded (20 cm to 3 m) quartose greywacke, with interbedded units of laminated siltstone and thinly bedded greywacke (British Geological Survey, 1996). Soils along stream banks consisted of a peat layer of variable depth.

2.2 Study sites

The sites, 100 m delimited reaches, at the Chapelhope, Riskinhope, Megget and Talla burns were sampled for invertebrates every two months from January to October 2000 (Chapters 3, 4 & 5). The Chapelhope and Riskinhope Burn were sampled again in May 2001 along with the Linghope and Cramalt burns (Chapters 4 & 6). Figure 2.1 indicates location of the sampling sites, and Table 2.1 the exact location and altitude. Substrate at all sites was a poorly sorted mixture dominated by cobbles (64 – 256 mm) and pebbles (16 – 64 mm) with some boulders (> 256 mm) and gravel (2 – 16 mm), very little sand (< 2 mm) and occasional bedrock (Wentworth scale, see Bunte and Abt, 2001). Basic site characteristics are described in Table 2.2, highlighting the similarity in width, depth, water velocity and amount of detritus between sites.

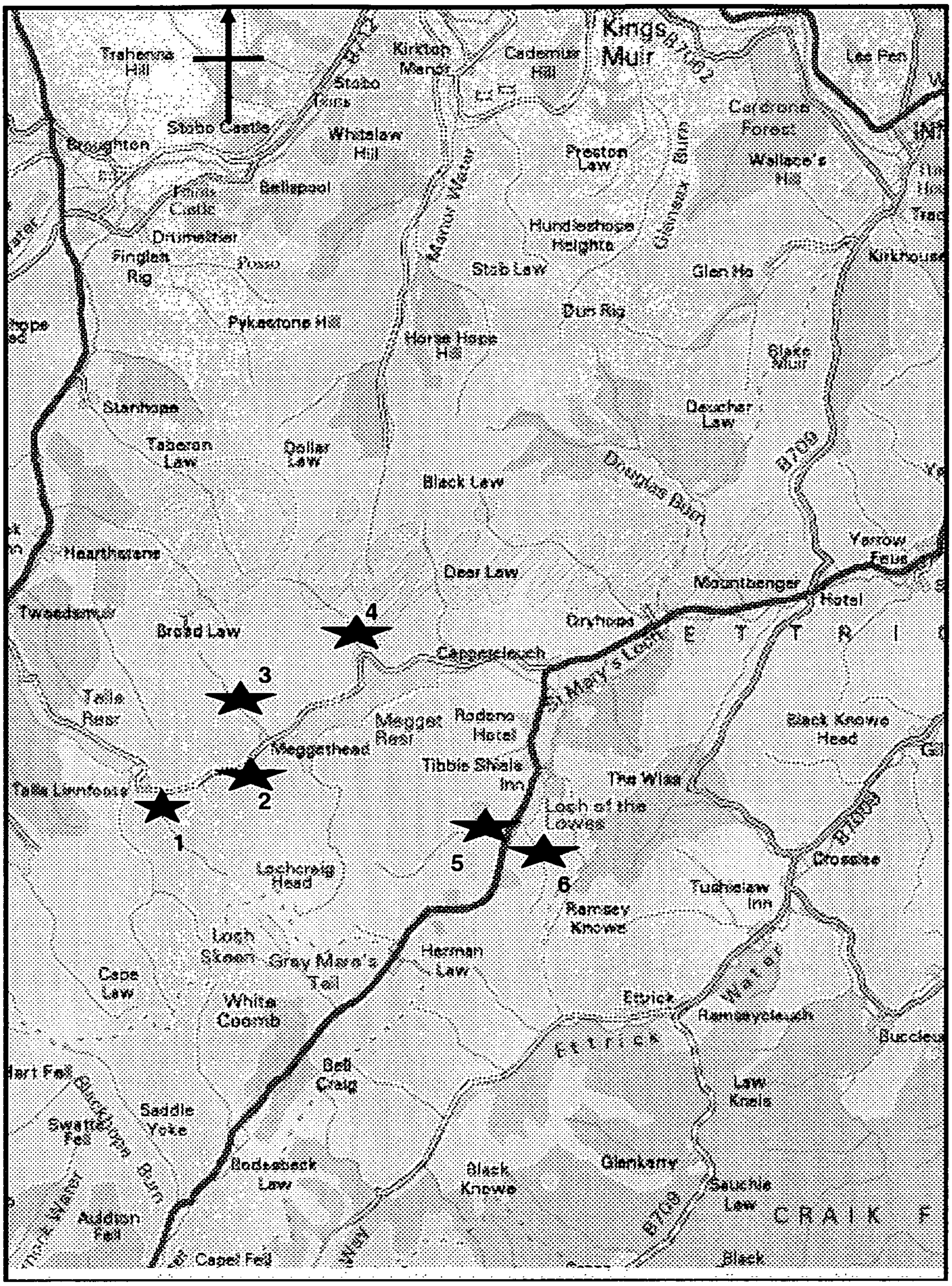

Figure 2.1: Location of sites in Scotland (inset) and ordnance survey 1:100 000 map (above) showing the sites as stars. Talla 1; Megget 2; Linghope 3; Cramalt 4; Chapelhope 5; Riskinhope 6. Original map from Digimap.

There were no macrophytes in any of the study reaches, and aquatic bryophytes (unidentified) were present, but sparse, in all streams. Conductivity, pH and temperature of stream water were measured every sample date in 2000 and once in May 2001 using one point reading (Whatman PHA 325 C combined meter), and were assumed homogeneous within the study reaches.

Mean pH was similar across the six sites, ranging from 6.9 to 7.4 units (Table 2.3). pH at the four sites sampled during 2000 varied by up to 2.5 units across sample dates, reflecting the variations in acidity caused by the run-off from the surrounding peat soil. Water conductivity was low in all sites, always less than 100 $\mu S \cdot cm^{-1}$. Conductivity varied between streams (Table 2.4), but mean values for the four streams sampled in 2000 were comparable, in the 50 to 60 $\mu S \cdot cm^{-1}$ range. Conductivity was generally highest in the Riskinhope Burn, lowest in the Talla Burn, and similar in all other streams. Water temperature (Table 2.5) tended to be lowest in the Talla Burn, consistent with its highest altitude.

Table 2-1: Study sites, location, altitude (m) and sampling dates.

Site	Catchment	NGR	Altitude	Dates
Chapelhope	St Mary's loch	NT 190 227	300	Jan-Oct 2000, May 2001
Riskinhope	St Mary's loch	NT 238 185	330	Jan-Oct 2000, May 2001
Megget	Megget reservoir	NT 166 210	360	Jan-Oct 2000
Talla	Talla reservoir	NT 147 199	420	Jan-Oct 2000
Linghope	Megget reservoir	NT 178 224	390	May 2001
Cramalt	Megget reservoir	NT 195 233	350	May 2001

Table 2-2: Basic stream characteristics: mean (± 1SE), max and min water velocity (m/s) at benthic surface, depth (cm) and amount of detritus (CPOM) (mg dry mass per 0.1 m^2). n = 72 samples per stream (January to October 2000) for Chapelhope. Megget, Talla and Riskinhope. n = 30 samples per streams (May 2001) for Cramalt and Linghope. Mean (± 1 SE), max and min stream width (m) for all sites. n = 10 measurements every 10 m in each 100 m reach (June 2002).

	Chapelhope	**Megget**	**Talla**	**Riskinhope**	**Cramalt**	**Linghope**
Mean flow	0.17 (0.02)	0.21 (0.03)	0.20 (0.02)	0.18 (0.02)	0.22 (0.04)	0.25 (0.04)
Max flow	0.65	0.87	0.89	0.74	0.97	0.82
Min flow	0.01	0.01	0.01	0.01	0.06	0.07
Mean depth	13.8 (0.6)	17.7 (1.0)	16.8 (0.9)	14.1 (1.1)	12.2 (0.8)	18.2 (1.8)
Max depth	31	41	43	45	37	45
Min depth	6	10	10	10	5	10
Mean width	3.0 (0.4)	2.6 (0.1)	2.5 (0.4)	2.1 (0.6)	2.9 (0.2)	2.3 (0.5)
Max width	3.8	3.5	4.0	2.9	3.3	3.1
Min width	2.1	2.0	1.8	1.0	2.5	1.2
Mean CPOM	437 (140)	243 (54)	323 (58)	375 (84)	393 (90)	304 (82)
Max CPOM	705	510	528	610	556	440
Min CPOM	119	131	82	87	93	63

Table 2-3: pH recorded Jan-Oct 2000 and May 2001 at the study sites.

Site	Jan – Oct 2000 n = 6			May 2001
	Min.	**Max.**	**Mean ± 1 SE**	
Chapelhope	5.5	7.8	7.1 ± 0.3	7.4
Riskinhope	5.7	8.3	7.4 ± 0.3	7.3
Megget	5.5	7.4	6.9 ± 0.2	-
Talla	5.9	7.6	7.0 ± 0.2	-
Linghope	-	-	-	7.1
Cramalt	-	-	-	7.0

Table 2-4: Conductivity ($\mu S \cdot cm^{-1}$) recorded Jan-Oct 2000 and May 2001 at the study sites

Site	Jan – Oct 2000 n = 6			May 2001
	Min.	**Max.**	**Mean ± 1 SE**	
Chapelhope	39.4	91.5	53.7 ± 5.8	78.0
Riskinhope	54.4	104.5	69.1 ± 4.6	100.5
Megget	29.5	61.4	51.9 ± 3.2	-
Talla	34.1	47.3	41.7 ± 1.6	-
Linghope	-	-	-	57.2
Cramalt	-	-	-	58.9

Table 2-5: Water temperature (º C) recorded Jan-Oct 2000 and May 2001 at the study sites

Site	Jan – Oct 2000 n = 6			May 2001
	Min.	**Max.**	**Mean ± 1 SE**	
Chapelhope	3.4	15.8	9.2 ± 1.7	9.9
Riskinhope	2.8	14.7	8.8 ± 1.4	8.5
Megget	1.4	15.5	8.1 ± 2.1	-
Talla	1.5	13.8	6.7 ± 1.8	-
Linghope	-	-	-	9.8
Cramalt	-	-	-	8.3

2.3 Nutrients

To compare nutrient levels, stream water was sampled once in May 2001 at all six sites. Three 30 ml samples were collected at random along the study reach, from the centre of the streams and at a depth of approximately 5 cm. Samples were removed to the laboratory and frozen until analysis, within 48 hours, for ammonium, nitrate (available nitrogen) and total phosphate content.

Mean nitrogen and phosphate content varied widely, between replicates, in all the sites (Table 2.6). Nutrient levels were nonetheless very low. At these scales (ppb), differences between sites have little ecological consequence and none of these streams displayed other signs of enrichment, such as high algal colonisation biomass.

Table 2-6: Mean ± 1 SE ammonium (NH_4), nitrate (NO_3) and total Phosphate (P) in ppb (i.e. $\mu g \cdot l^{-1}$) in stream water, April 2001, n = 3.

Site	NH_4	NO_3	P
Chapelhope	13.8 ± 6.9	34.6 ± 18.0	0.4 ± 0.4
Riskinhope	21.9 ± 2.8	13.5 ± 13.5	0.0 ± 0.0
Megget	115.9 ± 42.3	55.3 ± 43.6	0.0 ± 0.0
Talla	22.8 ± 12.1	13.9 ± 13.9	0.0 ± 0.0
Linghope	10.7 ± 7.2	9.4 ± 9.3	35.6±35.6
Cramalt	18.0 ± 9.0	0.0 ± 0.0	0.0 ± 0.0

2.4 Fish survey

Sites were selected on the basis of data from the Tweed Foundation, who are responsible for fish stocks in the Tweed catchment (Campbell, 1992, 1995, 1998). Sites were further surveyed for the presence and abundance of fish in July 2000 and May 2001 by the Tweed Foundation, using their standard electrofishing method. Two, 3 minute sweeps were carried out along a 30 m reach by two operators and fish were collected and identified. Selected reaches were those previously used by the Tweed Foundation, but were within a few tens of metres from the study reaches. This undoubtedly underestimated fish abundance, compared to the depletive method of repeated sweeps in a netted-off reach, but the Tweed Foundation did not permit an independent electrofishing survey. The data was appropriate, however, to establish whether fish were present or absent at the sites.

At all sites the fish assemblage (Table 2.7) consisted almost exclusively of salmonids, especially brown trout (*Salmo trutta*). No fish were recorded in the Linghope Burn or Riskinhope Burn. Both have high waterfalls (more than 5 m) further downstream, providing a barrier to fish migration. Fish counts were intermediate in the Cramalt Burn and the Talla Burn. In the Cramalt, a shallow run over bare bedrock stretches several metres up from the mouth of the stream at the Megget Reservoir, possibly impeding fish migration. The Talla site is at high altitude and above a chain of waterfalls hence fish presence in this stream was a surprise, and probably due to introduction by local anglers. Only the Chapelhope burn had non-salmonid fish: stone loach and European minnow. There are no restrictions to fish migration between the study reach and the mouth of the stream into St Mary's Loch (Loch of the Lowes basin) several hundred metres downstream. The very low fish numbers recorded in the Megget Burn conflicted with the Tweed Foundation records, which indicated a much higher fish abundance. There are no restrictions to fish migration from the Megget reservoir further downstream, in which trout are abundant. When water levels in the reservoir are low however, a small cascade appears at the mouth of the stream and this may restrict fish. Indeed structural work was performed on the Megget dam from 1997 to early 1999 (information from East of Scotland Water), prior to the start of this study, necessitating low water levels. Successive years of impeded migration and poor recruitment may thus explain the change in fish counts between 1998 and 2000.

Because of the temporal variability in fish counts in streams with fish, and the unquantitative sampling method, no clear gradient in fish abundance could be established across sites. Sites were hence classified on the basis of fish presence or absence (Riskinhope and Linghope burns).

Table 2-7: Number, type and size class of fish captured at each site in July 2000 (Chapelhope, Megget, Talla, Riskinhope) or April 2001 (Cramalt, Linghope) and Tweed Foundation records (Campbell, 1992, 1995, 1998). Tt = trout, Sn = salmon, Lh = stone loach, Mw = minnow.

Site	Trout	Salmon	Total	Records		
				1992	**1995**	**1998**
Chapelhope	17	10	**28**	9 Tt, 14 Sn, 2 Mw	20 Tt, 8 Sn, 6 Mw	18 Tt, 4 Sn, 1 Lh, 1 Mw
Riskinhope	-	-	**0**	0	0	0
Megget	2	-	**2**	22 Tt	14 Tt	29 Tt
Talla	7	-	**7**	n/a	n/a	n/a
Linghope	-	-	**0**	0	0	0
Cramalt	12	-	**12**	16 Tt	4 Tt	8 Tt

3 The abundance of invertebrate predators across streams

3.1 Introduction

Invertebrate communities may differ across streams in their trophic structure, particularly if the presence of fish varies, affecting the abundance of invertebrate predators and their shared prey. Large-bodied invertebrates and active foragers may be easily detected by trout, which forage visually (Hynes, 1950; Elliott, 1976), and their abundance may be lower in streams with trout (Power, 1990; Harvey, 1993). Reductions in the abundance of large invertebrates can cause an increase in the abundance of small invertebrate grazers and algae (Bechara *et al*, 1992, 1993; Rosenfeld, 1997, 1998). Thus, invertebrate communities in streams with fish can have fewer invertebrate predators and more small grazers (Flecker and Allan, 1984; Power, 1990). In fishless streams invertebrate predator abundance should increase (Harvey, 1993), as well as the abundance of grazers (Rosenfeld, 2000), unless increased feeding by invertebrate predators can compensate for the absence of fish (Power, 1992; Flecker and Townsend, 1994). However, few studies have tested these predictions across natural stream systems. Can any effect of fish presence/absence on community structure be detected across streams? Can fish reduce the abundance of invertebrate predators, or can some species avoid predation by fish? Do fishless habitats necessarily provide the best conditions for invertebrate predators? How does the relative abundance of the main prey types change with fish presence and invertebrate predator abundance? The interaction between fish and invertebrate predators has a strong influence on the community structure in lakes of different fish abundance (Carpenter *et al*, 1987), and this may be the case for streams also. In this chapter, I report a survey of three similar with fish and a fishless stream, and describe the abundance and diversity of invertebrate predators and their prey in these systems. I contrast the relative impact of fish vs other variables on community structure.

Though fish often feed on invertebrate predators because they are large and easily detected, they do not necessarily impact their abundance in natural stream systems. In streams with fish, the abundance of invertebrate predators will depend on the vulnerability of these to fish (Lima and Dill, 1990; Lima and Bedneckoff, 1999). The foraging activity of many species allows a trade-off between feeding requirements and the avoidance of predation. Some invertebrate predators, e.g. stonefly nymphs forage at night only and this may reduce their encounters with daytime feeding salmonids (Huhta *et al*, 1999). This is a fixed trait in some species (Elliott, 2000), but other invertebrate predators are flexible, and decrease their foraging activity (Feltmate and Williams, 1991; Perlidae), or are nocturnal only when fish are present (e.g. Huhta *et al*, 1999; *Rhyacophila* sp.). Hence, in some cases, fish may only have limited effects on abundance, and their main effects may be sub-lethal.

Because fish predation influence competitive interactions among invertebrates, any effect of fish on invertebrate abundance may be hard to detect when streams with fish and fishless streams are compared. In fishless streams, the foraging activity of invertebrate predators may be higher due to the absence of their main predator, but this may lead to an increase in competition for resources (Gurevitch *et al*, 2000;

Grand, 2002). This may be because many invertebrate predators interfere with one another's feeding, as they cause the prey to disperse (Peckarsky, 1990; Elliott, 2003b). Invertebrate predators that possess fish avoidance traits may be poor resource competitors in fishless habitats (Lima and Dill, 1990), if they have fixed anti-predator traits such as nocturnal activity or low foraging activity (Huhta *et al*, 1999). Therefore, their abundance may not necessarily be higher in fishless streams, than it is in streams with fish. Invertebrate predators with flexible anti-predator traits, on the other hand, may be able to exploit habitats with and without fish successfully, because they can maximise resource acquisition according to predation risk (Peckarsky *et al*, 1994).

The abundance of grazers in fishless streams vs streams with fish may be determined by the relative impact invertebrate predators and fish have on their prey (Soluk, 1993; Dahl, 1998b). In streams with fish, salmonids can reduce the abundance of swimming invertebrates (Flecker, 1992; Forrester, 1994), particularly Baetidae mayflies (Tikkanen *et al*, 1994) because of their availability in the drift. However, the abundance of other grazers may be only weakly affected by fish predation, for example, some Chironomidae can avoid fish by weaving retreats from tufts of filamentous algae (Power, 1990). The impact of invertebrate predators on grazer abundance should be low in streams with fish, if the abundance and activity of invertebrate predators is reduced. In fishless streams, if invertebrate predator abundance and foraging activity is high, then the greater prey consumption may compensate for the absence of fish. Power (1992) showed experimentally that increased predation by odonates and megalopterans without trout present, had a similar impact on prey abundance as feeding by trout. Thus, there may be no net change in the abundance of some invertebrate grazers across streams with and without fish because they are always limited by predation (Allan, 1982a; Harvey, 1993). Understanding how the abundance of invertebrate predators changes with fish presence may provide valuable insight into how fish also affect grazer abundance across streams.

There are few data to test whether fish presence/absence has an impact on the trophic structure of natural stream communities. Strong effects of fish have been established under laboratory conditions (e.g. Soluk and Collins, 1988a, 1988b), and also in exclosure/enclosure experiments or artificial channels (e.g. Gilliam *et al*, 1989; Dahl and Greenberg, 1997; Gido and Mathews, 2001), but it is hard to extrapolate effects on small spatial scales to whole streams. In manipulative experiments, fish and invertebrates are often stocked above natural densities (Power, 1990; Bechara *et al*, 1993; Flecker and Townsend, 1994; Dahl, 1998a, 1998b) and encounter rates between predator and prey may be higher than in the natural stream benthos. Predator enclosure experiments suffer from 'cage' effects, as prey emigration from experimental units may overestimate the effects of fish predation on invertebrate abundance (Werner *et al*, 1983; Walde and Davies, 1984a; Lancaster *et al*, 1991; Woodward and Hildrew, 2002). Furthermore, field manipulations are often carried out in streams with fish, because ecologists are reluctant to risk introducing fish to naturally fishless systems (but see Rosenfeld, 2000), but there is evidence that invertebrates can detect their predators through chemical cues in the water (Martinez, 1987; Bronmark and Hansson, 2000; Dicke and Grostal, 2001). Hence, the invertebrates in fish exclusions, in streams with fish, may still detect predator cues and behave as if fish are present. Field surveys across streams with fish and fishless streams, are necessary to estimate the impacts of fish on community structure, yet such comparisons are rare.

Several environmental factors may mask the effects of fish predation on invertebrate abundance across streams. Firstly, effects of fish may be confounded with nutrient status of the stream. Naturally fishless habitats are often the upper reaches of high gradient upland streams, above waterfalls, and low productivity may compensate for the absence of fish, yielding invertebrate and algal density patterns similar to the more productive lowland reaches that have fish (Rosenfeld, 1998). Secondly, strong effects of fish are often associated with low flow environments (Power, 1990; Resetarits, 1991; Bechara *et al*, 1993), as these are more practical for experiments, and tests in high flow environments are difficult. In fast flowing streams, invertebrates and resources are redistributed among patches of streambed, and the effects of fish and entrainment on local abundance may be hard to separate (Cooper *et al*, 1990). Thirdly, strong effects of fish are also often associated with fine substrate (e.g. Gilliam *et al*, 1989; Dudgeon, 1991; Bechara *et* al, 1992; Harvey, 1993). Coarse stony substrate has a greater three-dimensional complexity and may provide invertebrates with more hiding places (refugia) from fish (Fuller and Rand, 1990; Power, 1992; Hart and Merz, 1998). Indeed, several studies with coarse substrate were associated with weak effects of fish on invertebrate abundance (e.g. Allan, 1982a; Culp, 1986; Dahl and Greenberg, 1997). Hence, the effects of fish on the invertebrate community may be hard to identify from invertebrate abundance only. However, if the abundance of invertebrate predators and prey in the benthos does not vary between streams with fish and fishless streams, or cannot be separated from natural cross-stream variation, then fish predation may have little influence on the trophic structure of natural stream invertebrate communities.

Though few in number, benthic surveys across streams with and without fish indicate that changes in invertebrate abundance, community structure and the invertebrate predator assemblage can occur. Allan (1975, 1982a) observed an increase in overall invertebrate abundance in the fishless upper reaches of small Colorado streams, but the fauna was identical to the trout-bearing reaches. Invertebrate predators (the chloroperlid stonefly *Alloperla* sp. and the caseless caddisflies *Rhyacophila coloradensis* and *R. alberta*) also increased in number, but were not proportionally more abundant in fishless reaches. Other invertebrate predators were less abundant (*R. angelita* and *R. acropedes*) in the fishless reaches. Bowlby and Roff (1986) found that total invertebrate abundance and invertebrate predator abundance was lower with fish, across 30 Ontario streams. Across streams, fish had little effect on invertebrate species richness and invertebrate predator/prey abundance ratios. Nonetheless, fish affected the species evenness, and the dominance of some taxa, particularly Chironomidae, increased with fish abundance. Harvey (1993) surveyed a series of Utah streams with fishless headwaters and found large stonefly predators (*Hesperoperla* sp., Perlidae) were more abundant in fishless sites, but smaller bodied predatory stoneflies (*Cultis* sp. and *Skwala* sp., Perlodidae) were more abundant with trout, consistent with size-dependant predation by fish. Harvey did not find a significant effect of fish presence on grazers, which were principally mayflies, and speculated that increased density of large invertebrate predators in the fishless streams compensated for the absence of trout. He also suggested that fish had strong effects on some invertebrate predators because the gravel substrate provided few refugia for large invertebrates. Hence, across streams, patterns in the presence of fish, and the abundance of invertebrate predators and prey may be context-specific, and generalisations may be hard to make.

I surveyed, over a period of ten months, the invertebrate community in three streams with fish and a fishless stream, and described the richness, density and diversity of the invertebrate predators. I predicted that, overall benthic densities would be lower with fish, but invertebrate species richness and identity would be maintained. I expected the relative abundance of Chironomidae (Chironominae and Orthocladinae only) vs. Baetidae to be biased towards Baetidae in the fishless site. I tested the null hypothesis, for each predator species, that their density did not vary across streams. I expected that the abundance of invertebrate predators would be higher in the fishless site if fish can significantly reduce their abundance through predation. I also constructed a multivariate model to compare the relative influence of seasonality, fish presence and a number of abiotic variables in determining differences in community structure across sites.

3.2 Methods

3.2.1 Field survey

The reaches of the Chapelhope, Megget, Talla and Riskinhope (no fish) burns (described in Chapter 2) were sampled at approximately two months interval from January to October 2000 (6 occasions). The Megget and Talla Burn were sampled on the same dates and the Riskinhope and Chapelhope Burn were sampled within 2 days of this, also on the same date. A 100 m stretch was delimited and all samples were taken from this section. To sample the range of microhabitats present, Surber samples (0.1 m^2, 220 μm mesh) were collected at random from three pools, three pool margins, three riffles and three riffle margins on each sampling run (i.e. 12 samples per stream and date). Pools and riffles were determined visually, at base flow, on the basis of relative depth and water velocity. Margins were defined as the section of the stream within a Surber sampler's width of the banks (approximately 30 cm). Contents of the Surber samples were washed out *in situ* into polypropylene sample bags, preserved in 70% alcohol and removed to the laboratory for analysis.

At each Surber sample point, the following habitat variables were measured before collecting samples: depth (meter rule), water velocity at 2.5 cm from the substrate surface (mini bucket wheel meter, 5 cm diameter, fitted with photofibre optic sensor to minimise friction and maximise accuracy at low flow), water temperature, pH and conductivity (Whatman PHA 325 C combined meter). Dominant substrate size category at each sample point was assessed visually as fine, medium or coarse.

3.2.2 Sample processing

Contents of the sample bags were sieved through 2 mm, 1 mm, and 220 μm sieves. Samples were examined in white plastic trays (using a x10 magnifying lens with lamp for contents of the 220 μm sieve) and invertebrates removed and placed in glass vials containing 70% alcohol. Coarse particulate organic matter, CPOM, (organic matter retained by two and one mm sieves) was removed and dried at 80°C for 72 hours, and then weighed on an electronic balance (Mettler PS 360 Deltarange) to the nearest 10^{-1} mg. Invertebrate samples were examined at a minimum of x10 magnification (Leica MZ 6 binocular dissection microscope), counted and identified to the lowest taxonomic level. All Plecoptera, Trichoptera, Ephemeroptera, Hemiptera and Coleoptera were identified to species. All Diptera were identified to sub-families. Other organisms were identified to order or family.

3.2.3 Response variables

Community level response variables consisted of the total invertebrate abundance (N) and total species richness (S). For the most abundant predators, samples were grouped by season (spring, summer and fall) and mean densities calculated in each stream. The predator/prey abundance ratio was determined for each stream and sample date. For the purpose of this chapter, all carnivores in the community constituted the predators (using information from the literature), and all other invertebrates constituted prey. The mean density of the two most abundant primary consumer taxa (Baetidae and Chironominae/Orthocladinae) was also calculated by stream and sample date.

3.2.4 Taxon accumulation curves

To estimate how well species richness had been described for each site, taxon accumulation curves were developed. For this purpose all samples from a stream (n = 72) were considered to be replicates and were arranged in a random order, eliminating seasonal trends. Cumulative taxon richness was calculated with increasing number of samples. With each successive sample, the number of so far unrecorded species are added to the species richness of all preceding samples. The process was repeated 3 times, and means (± 1 SE) calculated. If the community is well described, then the number of new species found in each sample should eventually decrease, and the taxon accumulation curve will reach a plateau.

3.2.5 Diversity indices

Two indices were used to examine the richness and evenness components of diversity. The Margalef index (D_{mg}, Equation 3.1) is a simple measure of species richness, standardised for total abundance. Simpson's index (D_s, Equation 3.2) is a common measure of evenness which uses proportions of individuals in each taxon. Simpson's index was chosen over Shannon's index because it is the least sensitive to rare species and is biased towards patterns in the more common species. Simpson's index was expressed in its reciprocal form ($1/D_s$) and can be most easily interpreted as the number of equally common species required to generate the observed heterogeneity of the sample (Krebs, 1984, pp 357-360). Mean values of S, D_{mg} and D_s were calculated for each stream, pooling all sample dates.

$$D_{mg} = \frac{S - 1}{Ln\ (N)} \qquad \text{Equation 3.1}$$

$$D_s = \sum_{i=1}^{s} \left(\frac{n_i\ (n_i - 1)}{N\ (N - 1)} \right) \text{ where } n_i = \text{number of individuals in the } i\text{th species} \qquad \text{Equation 3.2}$$

3.2.6 Rarefaction

Species richness of samples generally increases with the total number of individuals collected. Comparison of species richness of samples with different abundances requires that the samples be standardised to a common abundance. Rarefaction (Hurlbert, 1971) is a statistical method for estimating the number of species expected ($E(S_n)$, Equation 3.3) in a random sample of *n* individuals i.e. rarefaction accounts for the passive increase in species number with abundance. The standardisation applied (i.e. value of n) was 10, 20, 50, 100 and 200 individuals. These were used to produce 'rarefaction' curves, representing the expected number of species in the four sites for the range of sample sizes. For each value of n, samples with less than n individuals were not included in the analysis as equation 3 requires that $N < S_n$. This method assumes that all individuals of a species are randomly dispersed with respect to conspecifics and heterospecifics. In practice, most species distributions are clumped (Hurlbert, 1990) and furthermore positive or negative associations often exist between species (e.g. between a predator and a prey, or two competitors). The rarefaction method thus tends to overestimate the expected number of species (Fager, 1972). Replicates within and across sites were taken using the same method, from similar habitats and were of similar taxonomic composition. The deviation from the assumption of random dispersal was thus similar across sites, allowing meaningful comparisons. To avoid calculation problems associated with large factorial values (see equation 3.4), an Ln (x+1) transformation was applied and factorials were approximated to a logarithmic gamma function (Squires, 1968).

$$E(S_n) = \sum_{i=1}^{s} \left\{ 1 - \frac{\binom{N-n_i}{n}}{\binom{N}{n}} \right\}$$ Equation 3.3

where $\binom{N}{n}$ is the number of combinations of n individuals chosen from a set of N individuals, i.e.,

$$\binom{N}{n} = \frac{N!}{n!(N-n)!}$$ Equation 3.4

3.2.7 Univariate statistical analyses

Three-way ANOVA was used to analyse differences in the total number of individuals per sample (N) with respect to site, microhabitats and date. Data was Log_{10} transformed to satisfy requirements of normality (Anderson-Darling test) and homoscedasticity (Levene test). Data were fitted to a fully factorial model using MINITAB® (as for all ANOVA in this thesis). Terms of the model were date (6 levels), stream (4 levels) and habitat (4 levels) as well as all interactions (three 2-way and one 3-way). All terms were fixed. Differences between the level means of significant terms and interactions ($\alpha = 0.05$) were tested *post hoc* with Tukey's pair-wise comparisons as detailed in Zar (1996, p302), corrected for number of comparisons using the sequential Bonferroni procedure (Holm, 1979). Magnitude of effects were calculated as (ω^2) using the method in Howell (1989, pp 260-261) and expressed as a percentage of total variance, and were used to assess the relative importance of significant effects in the model.

The mean number of species per sample (S, Log_{10} transformed for normality and homoscedasticity) was compared across sites using one-way ANOVA and Tukey's comparisons). D_s and D_{mg} (also Log_{10} transformed) were analysed using a fully factorial two-way ANOVA and Tukey's comparisons with stream and date as fixed factors. The relative magnitude of effects was compared using ω^2, as described above.

The mean abundance of each invertebrate predator was analysed with a fully factorial two-way ANOVA (fixed factors: stream and season) and Tukey's *post hoc* comparisons. Similar analyses were carried out on the $\log_{10}$ transformed abundance of Chironomidae and Baetidae, as well as the predator-prey abundance ratio.

3.2.8 Multivariate analyses

Community abundance data were Log_{10} (x+1) transformed and analysed using multivariate ordination techniques, available in the CANOCO® software package (ter Braak and Smilauer, 1998). These noise reduction methods simplify the variance in community composition to a set of scores (co-ordinates) for species, samples and environmental variables which can be plotted along arbitrary orthogonal axes (an ordination). The placement of the samples (usually along 2 or 3 dimensions i.e. along two or three axes) in the ordination plot reflects the similarity of their biological communities. The choice of ordination method depends on the distribution of species among samples. The response in the abundance of species may be unimodal, where species are represented in only some samples, and abundance reaches a maximum at some point on a long environmental gradient; or linear, where species are ubiquitous, and abundance changes through short sections of environmental gradients (ter Braak and Prentice, 1988).

Ordination methods were used to construct a model relating species distribution to the environment, defined by the abiotic variables and fish presence/absence.

Community data contained 61 species, 288 samples from four sites, 4 linear environmental variables (Altitude (m), flow ($m \cdot s^{-1}$), depth (cm) and amount of CPOM (mg)) and 4 nominal variables (fish: two categories, date: six categories, habitat: 4 categories, substrate: 3 categories) entered as dummy variables. With the aim of establishing which ordination method best suited the data, invertebrate abundance was initially analysed using detrended correspondence analysis (DCA) with detrending by segments. This indirect method calculates an ordination based on species variation only, unconstrained by the environmental variables. Gradients in species abundance and environmental gradients are examined for comparable trends *post hoc*. The resulting segment lengths generated by the analysis were used to determine unimodality in the species data. Segment lengths describe the number of units covered by the data on each theoretical ordination axis. In DCA segment lengths are in units proportional to standard deviation and segment lengths greater than 4 units indicate a strong unimodal response, and the use of weighted averaging methods (e.g. CCA) is recommended (ter Braak and Prentice, 1998). In this case, however, segment lengths generally indicated short gradients and thus the use of linear methods was necessary.

Abundance data were further analysed with redundancy analysis (RDA), a linear multiple regression method. The underlying effect of seasonality was partialled out by fitting sample date as a covariable. The species scores on the ordination axes are obtained by regression of the species abundance data on the sample scores. The species scores hence represent the slopes of the regression and all sample scores are linear combination of environmental variables. This technique is a direct gradient analysis and the resulting ordination axes are aggregates of environmental variables that best explain the species data. The ordination (spread of species points) shows only patterns that can be explained by the environmental variables (constrained ordination). Environmental variables to be included in this model were determined by forward selection using CANOCO, a method which determines how much each variable contributes to the model. Environmental variables were ranked by their importance in determining the species scores. Statistical significance of the contribution of each variable to the model was determined by Monte Carlo permutation tests (null hypothesis: species data are unrelated to the variable). These tests generate new data sets all equally likely under the null hypothesis and determine the probability of obtaining the real data set by these random permutations (α = 0.05). Permutations were restricted to blocks set by the date covariable. Linear environmental variables and the categories of nominal variables that did not contribute significantly to the variance in the species data were excluded.

The resulting model was used to carry out the partial RDA and obtain an ordination biplot. Overall significance of the RDA was determined using global permutation tests. These tests determine if the inferred species-environment correlation is significant, for the first axis and for the whole ordination i.e. all axes (null hypothesis: combination of variables represented by axis/axes has no effect on species variance, $\alpha = 0.05$).

3.3 Results

3.3.1 The invertebrate assemblage

The invertebrate assemblages were dominated by Ephemeroptera, Plecoptera, Trichoptera and Diptera (Table 3.1). Predators (including omnivores) were mainly stoneflies (*Perla bipunctata*, *Dinocras cephalotes*, *Perlodes microcephala*, *Isoperla grammatica*, *Diura bicaudata*, *Siphonoperla torrentium*) or caseless caddisflies (*Rhyacophila dorsalis*, *Plectrocnemia* sp., *Hydropsyche* sp.). Low numbers of carnivorous Tanypodinae (Diptera, Chironomidae), Ceratopogonidae (Diptera), Limoniinae (Diptera, Tipulidae) and Dugesidae (Turbellaria) were also present. All these predators were present in the four streams with the exception of the Perlidae *Perla bipunctata* which occurred in the fishless Riskinhope Burn only and *Dinocras cephalotes* which occurred in the Riskinhope Burn, the Megget Burn (where it was very rare) and the Chapelhope Burn. Nonetheless, the four assemblages were generally similar with 60.7 % of taxa common to all streams. Baetidae (predominantly *B. rhodani*) and Chironomidae (Orthocladinae and Chironominae) were the most abundant invertebrates in the 4 streams. The cumulative number of taxa vs number of samples displayed an asymptotic response curve (Figure 3.1) with 90 % of total taxa detected after approximately 45 samples in all streams over the year.

Table 3 - 1: List of taxa identified in the four streams, organised by Order, Family, Genus and Species where possible, January to October 2000, '+' denotes presence. Plecoptera & Ephemeroptera.

Taxon			Chapelhope	Megget	Talla	Riskinhope
Ephemeroptera						
Heptageniidae	*Ecdyonurus*	*venosus*	+	+	+	+
	Ecdyonurus	*torrentis*	+	+	+	+
	Rhithrogena	*semicolorata*	+	+	+	+
	Heptagenia	*sulfurea*	+	+	+	+
Baetidae	*Baetis*	*rhodani*	+	+	+	+
	Baetis	*niger*	+	+	+	+
Ephemerellidae	*Ephemerella*	*ignita*	+	+	+	+
Siphlonuridae	*Siphlonurus*	*lacustris*		+	+	
Leptophlebiidae	*Paraleptophlebia*	*submarginata*	+	+	+	+
Caenidae	*Caenis*	*rivulorum*	+			+
Plecoptera						
Leuctridae	*Leuctra*	*inermis*	+	+	+	+
	Leuctra	*hippopus*	+	+	+	+
Nemouridae	*Amphinemura*	*sulciollis*	+	+	+	+
	Nemoura	*erratica*	+	+	+	+
	Nemoura	*cambrica*	+			+
	Nemoura	*cinerea*	+			
	Protonemoura	*praecox*	+	+	+	+
	Protonemoura	*meyeri*	+			+
Perlodidae	*Isoperla*	*grammatica*	+	+	+	+
	Perlodes	*microcephala*	+	+	+	+
	Diura	*bicaudata*	+	+		
Chloroperlidae	*Siphonoperla*	*torrentium*	+	+	+	+
Perlidae	*Perla*	*bipunctata*				+
	Dinocras	*cephalotes*	+	+		+
Capniidae	*Capnia*	*bifrons*			+	
Taeniopterygidae	*Brachyptera*	*risi*		+		+

Table 3 -1 continued. Trichoptera & more common Diptera.

Taxon		Site	Chapelhope	Megget	Talla	Riskinhope
		Fish density	high	Medium	Low	None
Trichoptera						
Philopotamidae	*Philopotamus*	*montanus*	+	+	+	+
Odontoceridae	*Odontocerum*	*albicorne*	+	+	+	+
Sericostomatidae	*Sericostoma*	*personatum*	+		+	+
Rhyacophilidae	*Rhyacophila*	*dorsalis*	+	+	+	+
Polycentropodidae	*Plectrocnemia*	*conspersa*	+	+	+	+
Hydropsychidae	*Hydropsyche*	*pellucidula*	+			+
	Hydropsyche	*angustipennis*	+			+
	Hydropsyche	*siltalai*	+	+	+	+
Limnephilidae	*Drusus*	*annulatus*	+	+	+	
	Potamophylax	*latipennis*	+	+		
	Halesus	*radiatus*	+		+	+
Hydroptilidae	*Oxyethira*	sp.		+		
Goeridae	*Goera*	*pilosa*				+
Glossosomatidae	*Glossosoma*	*boltoni*				+
Diptera						
Tipulidae						
Limoniinae	*Dicranota*	sp.	+	+	+	+
	Other sp.		+	+	+	+
Tipulinae	*Tipula*	sp.	+	+	+	+
Simuliidae	*Simulium*	sp.	+	+	+	+
Chironomidae						
Podonominae	sp.		+	+	+	+
Tanypodinae	sp.		+	+	+	+
Chironominae	sp.		+	+	+	+
Orthocladinae	sp.		+	+	+	+

Table 3 - 1 continued. Scarcer Diptera, Coleoptera and other classes and orders.

Taxon		Site	Chapelhope	Megget	Talla	Riskinhope
		Fish density	high	Medium	Low	None
Diptera (cont.)						
Psychodidae	*Pericoma*	*trivialis*	+	+	+	
Muscidae	*Limnophora*	sp.	+	+	+	+
Dixidae	*Dixa*	sp.			+	+
Ceratopogonidae	sp.		+	+		+
Coleoptera						
Elmidae	*Elmis*	*aenea*	+	+	+	+
	Limnius	*volckmari*	+	+	+	+
	Oulimnius	*troglodytes*	+	+	+	+
	Esolus	*parallelepipedus*	+	+	+	+
Hydrophilidae	*Anacaena*	*globulus*		+		+
Dystiscidae	*Oreodytes*	*sanmarkii*	+	+	+	+
Hemiptera						
Veliidae	*Velia*	*saulii*		+		+
COLLEMBOLA						
Isomotidae	*Isotonurus*	*palustris*	+	+	+	+
	Sminthurides	*aquaticus*	+			
GASTROPODA						
Planorbidae	*Planorbus*	*contortus*				+
Ancylidae	*Ancylus*	*fluviatilis*	+	+	+	+
CRUSTACEA	*Gammarus*	*pulex*	+	+	+	+
HYDRACARINA			+	+	+	+
OLIGOCHAETA			+	+	+	+
TURBELLARIA	*Dugesia*	sp.	+	+	+	+

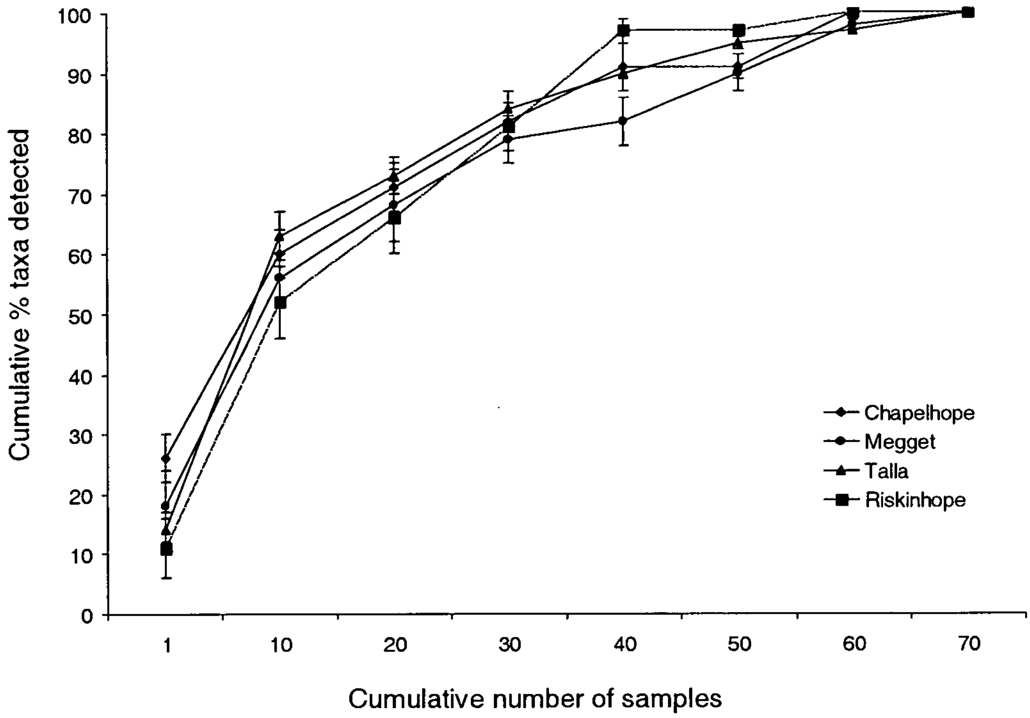

Figure 3 - 1: Taxon accumulation curves: mean percentage of total taxon richness detected with increasing sample size (± 1 SE) for the four streams. 72 samples per stream. Samples arranged in a random order, n = 3 runs.

3.3.2 Overall invertebrate abundance

The number of individuals per sample (N) was highest in the fishless stream in January only. Otherwise, there was no clear pattern in the mean invertebrate density across streams over the study period (Figure 3.2), with no stream having consistently more individuals per sample than the other three. In October, for example, when numbers of small instar insect larvae were high, there was no difference in total abundance between the four streams. The most variable period was during the late spring and summer (May – August). ANOVA indicated that stream, date and habitat were all significant factors in explaining the differences in log N (Table 3.2). Date and its interaction with site accounted for nearly 50 % of the total variance. The dominant effect was thus seasonal, and seasonal patterns varied with site (stream × date interaction). However, magnitude of effects indicated that, although stream and habitat factors were statistically significant in the model, they accounted for little overall variance, and thus were not ecologically significant factors. Multiple comparisons indicated higher invertebrate densities in the Chapelhope Burn and fishless Riskinhope Burns than in the Megget and Talla Burns. This pattern might reflect a negative effect of altitude, but not fish presence. The weak effect of habitat was mainly driven by lower invertebrate density in pools. The interactions of habitat with date and habitat with stream were not significant, (the Bonferroni corrected critical p value was 0.016 for the 95 % CI).

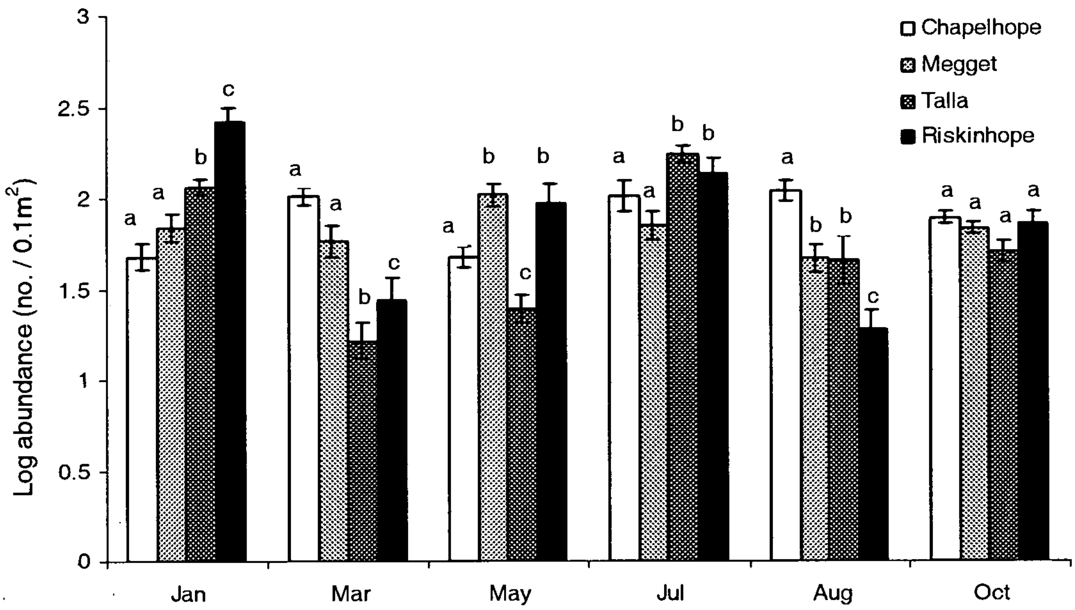

Figure 3 - 2: Mean log number of individuals per sample ± 1 SE. 12 samples per stream/date combination. Bars with the same letter are not significantly different (within date levels), α = 0.05, and Tukey post hoc tests. See Table 3.2 for summary of ANOVA

Table 3 - 2: Analysis of variance comparing $\log_{10}$ N across streams, sample dates and microhabitats. Magnitude of effects (ω^2) are expressed as a percentage of total variance.

Source	DF	SS	MS	F	P	ω^2
Stream	3	1.23	0.41	6.49	< 0.001	2.8
Date	5	7.47	1.49	23.63	< 0.001	17.2
Habitat	3	2.49	0.83	13.17	< 0.001	5.7
Stream × date	15	13.97	0.93	14.73	< 0.001	32.2
Stream × habitat	9	1.25	0.13	2.20	0.024	2.9
Date × habitat	15	1.21	0.08	1.28	0.216	2.8
Three-way	45	3.64	0.08	1.28	0.129	8.4
Error	192	12.14	0.06			28.0
Total	287	43.43				

3.3.3 Overall invertebrate diversity

The total number of species identified in each site over the sample period (Table 3.3) was similar, but the mean number of species per sample (all dates) differed (Table 3.4), and was greater in the Chapelhope/Riskinhope Burns than it was in the Megget/Talla Burns (Table 3.4). Analysis of variance indicated that both site and date, and their interaction, contributed to the variance in Simpson's diversity and Margalef richness across samples (Tables 3.5 and 3.6). Magnitude of effects indicated that date contributed little to overall variance in Simpson's index and the main ecologically significant effect was across streams. Simpson's diversity index was lowest in the fishless site, and highest in the Chapelhope Burn. However, for the Margalef richness, also highest in the Chapelhope Burn, date contributed as much to overall variance as the stream factor. Hence, diversity and richness varied across streams, mainly driven by higher indices in the Chapelhope Burn. Richness varied seasonally, reflecting patterns in hatching and emergence, but diversity did not. No one stream had consistently either the highest or lowest diversity over the 6 sample dates. For samples with few invertebrates, the expected number of species per sample (ES_{10}) was significantly higher in the Chapelhope Burn (ANOVA, df = 274, MS = 19.8, F = 16.9, p = 0.001) than the other three streams, which did not differ. As number of individuals in the samples increased, patterns for each site converged (Figure 3.5), and expected invertebrate species richness (ES_{100}) did not differ for samples with many invertebrates (ANOVA, df = 100, MS = 27.3, F = 1.64, p = 0.184).

Table 3 - 3: Total number of taxa recorded January to October 2000 (S_{tot}), mean number of species per sample (S), mean Margalef richness (D_{mg}) and Simpson's diversity index (D_{sp}). All means are ± 1 SE and are derived from 72 samples per stream, 6 sample dates pooled.

	S_{tot}	S		D_{mg}		D_{sp}	
Chapelhope	55	16.6	± 0.5	3.4	± 0.08	7.3	± 0.34
Megget	51	10.0	± 0.6	2.4	± 0.10	5.0	± 0.47
Talla	47	11.1	± 0.5	2.5	± 0.08	4.9	± 0.34
Riskinhope	57	15.0	± 0.4	3.0	± 0.09	4.7	± 0.23

Table 3 - 4: Results of a one-way analysis of variance comparing mean log S across the four streams (all six sample dates). 72 samples for each stream.

Source	**DF**	**SS**	**MS**	**F**	**p**
Stream	3	2.35	0.78	29.47	< 0.001
Error	284	7.57	0.02		
Total	287	9.93			

Table 3 - 5: Results of a two-way analysis of variance comparing mean log D_{sp} across four streams and six sample dates. 12 samples for each stream/date combination. Magnitude of effects is expressed as a percentage of total variance.

Source	DF	SS	MS	F	p	ω^2
Stream	3	1.07	0.36	16.48	< 0.001	12.4
Date	5	0.34	0.06	3.07	0.014	4.0
Stream × date	15	1.40	0.09	4.24	< 0.001	16.3
Error	261	5.77	0.02			67.1
Total	287	8.60				

Table 3 - 6: Results of a two-way analysis of variance comparing mean log D_{mg} across four streams and six sample dates. 12 samples for each stream/date combination. Magnitude of effects is expressed as a percentage of total variance.

Source	DF	SS	MS	F	p	ω^2
Stream	3	0.70	0.23	47.07	< 0.001	22.3
Date	5	0.69	0.13	27.64	< 0.001	22.0
Stream × date	15	0.43	0.03	5.74	< 0.001	13.7
Error	264	1.31	0.01			41.7
Total	287	3.14				

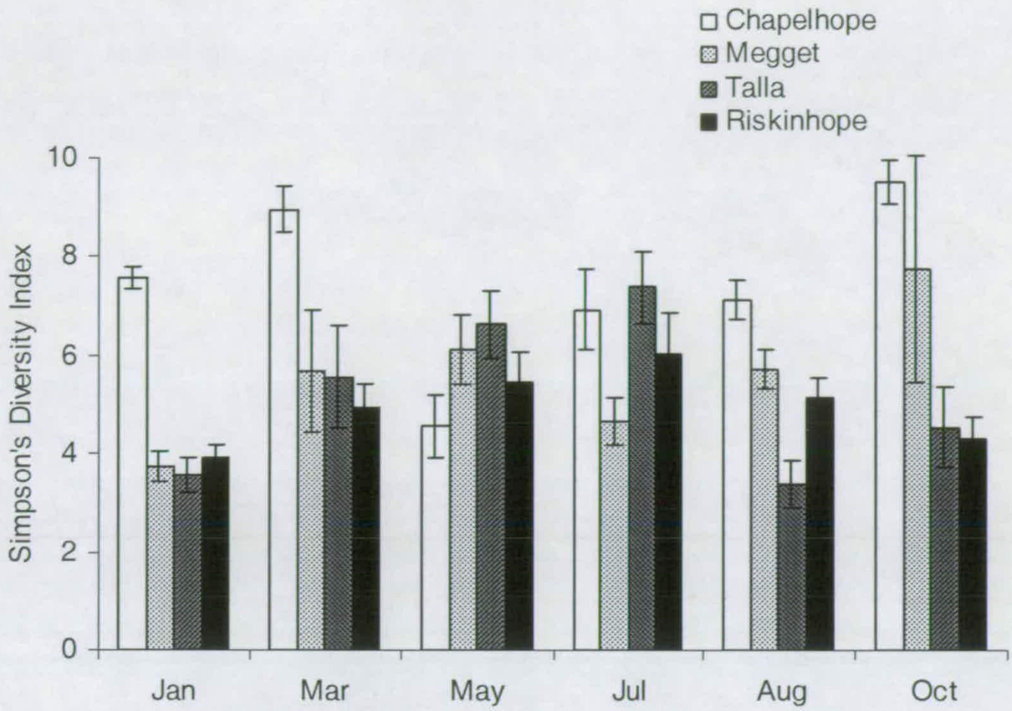

Figure 3 - 3: Mean Simpson's diversity index per stream/date ± 1 SE (n = 12).

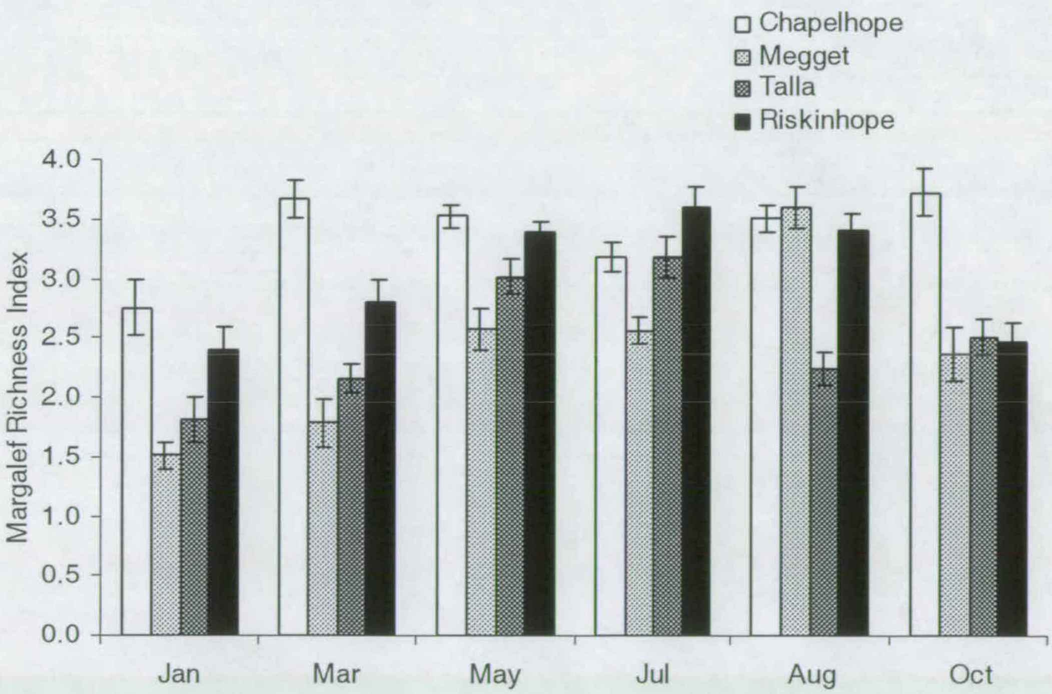

Figure 3 - 4: Mean Margalef Richness index per stream/date ± 1 SE (n = 12).

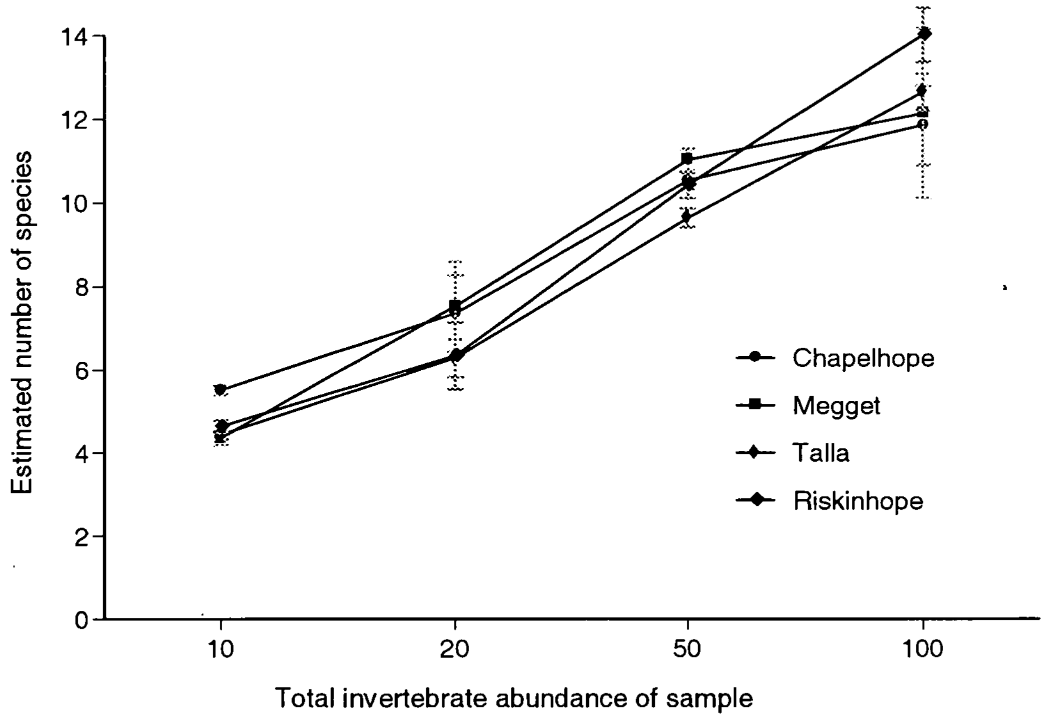

Figure 3 - 5: Rarefaction curves for the four sites. Mean expected number of species in a sample ± 1 SE versus total invertebrate abundance of the sample.

3.3.4 Density of invertebrate predators

Perlodidae (Plecoptera)

Isoperla grammatica was present at all four sites. Density patterns varied across streams and season, but no consistent patterns arose with respect to fish presence. In winter, when numbers in samples are not affected by emergence or hatching, density was lowest in the Megget Burn, but densities in other streams did not differ (Figure 3.6). Densities were not significantly different across sites in late spring/early summer, when this species emerges, but in late summer/fall, when egg hatching occurs, the density in the Megget burn was significantly higher than in the other streams.

Perlodes microcephala was virtually absent from the fishless Riskinhope Burn (Figure 3.7), no individuals were captured in this survey although 3 individuals were captured in this stream over January to October 2000, using kick nets. In late summer/autumn samples, densities (small instars) were four times greater in the Talla Burn than in the Chapelhope and the Megget. In August, *P. microcephala* were found in the Talla Burn only, and had already emerged from the other streams. During the winter period, *P. microcephala* were only present in significant numbers in the Chapelhope Burn, though a few individuals were captured in the Megget Burn.

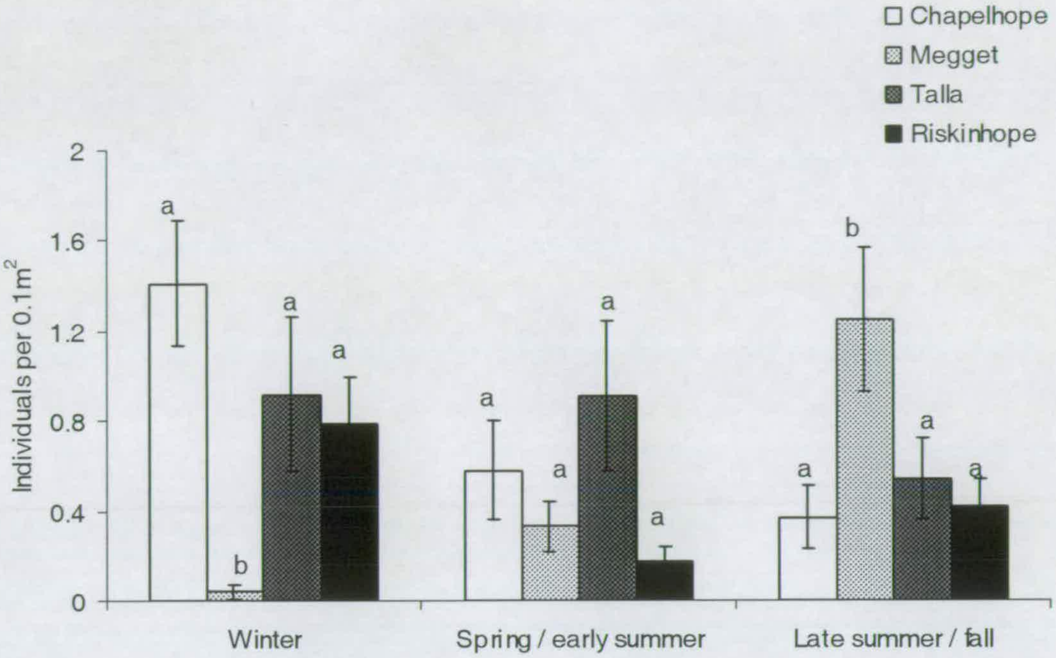

Figure 3 - 6: Mean density (individuals per 0.1 m^2) ± SE of *Isoperla grammatica* in 4 streams. Winter (Jan-Mar), Spring/summer (May-Jul) and Summer/fall (Aug-Oct) 2000. Differences between streams and season were assessed with two way ANOVA and Tukey's multiple comparisons. n = 24 samples per stream and season. Bars with the same letter are not significantly different within season. Results of two way ANOVA: df = 3,5, 287, stream factor MS = 1.0, F = 0.98, p = 0.41; date factor MS = 3.5, F = 3.48, p = 0.005; Interaction MS = 5.68, F = 5.52, p < 0.001).

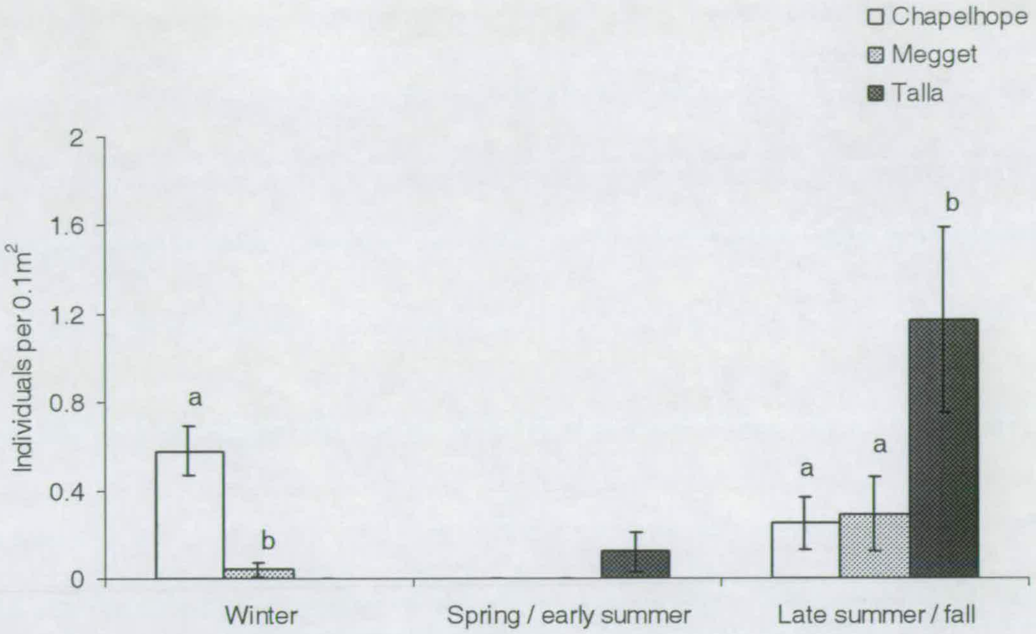

Figure 3 - 7: Mean density (individuals per 0.1 m^2) ± SE of *Perlodes microcephala* in 3 streams. Winter (Jan-Mar), Spring/summer (May-Jul) and Summer/fall (Aug-Oct) 2000. Differences between streams and season were assessed with two way ANOVA and Tukey's multiple comparisons. n = 24 samples per stream and season. Bars with the same letter are not significantly different within season. Results of two way ANOVA: df = 3,5, 287, stream factor MS = 2.83, F = 7.41, $p < 0.001$; date factor MS = 2.66, F = 6.95, $p < 0.001$; Interaction MS = 3.14, F = 8.21, $p < 0.001$).

Perlidae (Plecoptera)

Dinocras cephalotes were absent from the Talla Burn and were rare in the Megget Burn (a few specimens were recovered from kick samples over the year). Densities of *D. cephalotes* were not significantly different between the Chapelhope and Riskinhope burns (Figure 3.8) within any of the seasons. Nonetheless, it was clear winter densities were lowest, which is surprising as at this time of year 3 different cohorts should be present. Kick samples taken at the same time yielded many individuals in both sites, from several cohorts, and thus this species may have been poorly sampled by the Surber net, e.g. large stones that do not fit in the frame. *Perla bipunctata* was present in the Riskinhope Burn only, and was never recorded at any other site.

Chloroperlidae (Plecoptera)

Density of *Siphonoperla torrentium*, present in all four streams, differed across sites in the winter only (Figure 3.9). Densities in the Chapelhope and Riskinhope Burn were significantly greater than in the Megget and Talla Burn, and there was no clear pattern linking abundance to either altitude or fish presence.

Rhyacophilidae (Trichoptera)

Patterns in densities of *Rhyacophila dorsalis* did not differ across streams (Figure 3.10) in winter and spring. Only in late summer/autumn did a significant difference between streams occur, with densities in the Megget Burn over 4 times higher than in the other three streams.

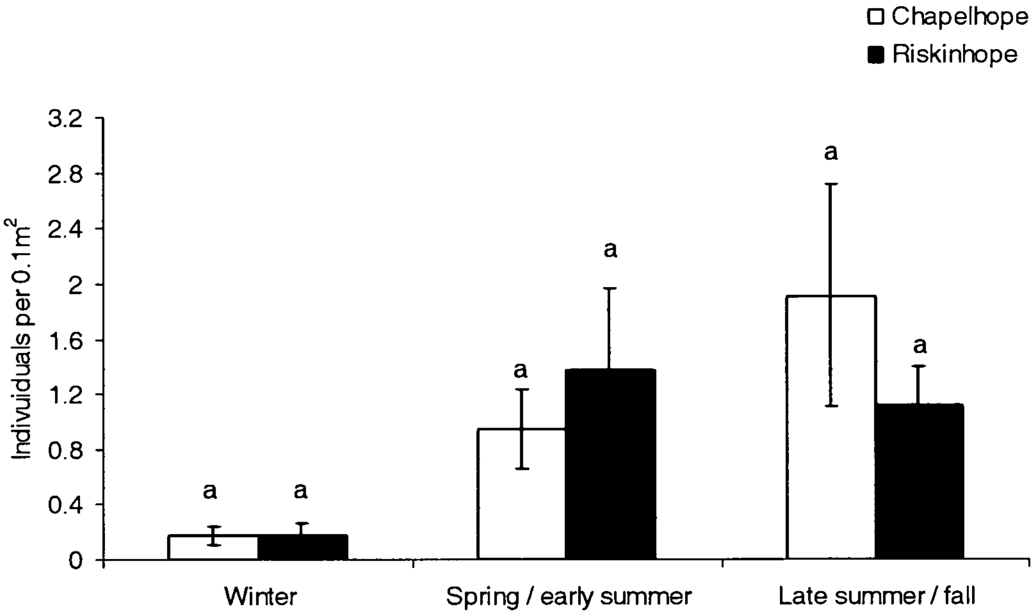

Figure 3 - 8: Mean density (individuals per 0.1 m^2) ± SE of *Dinocras cephalotes* in a stream with fish (Chapelhope Burn) and a fishless stream (Riskinhope Burn). Winter (Jan-Mar), Spring/summer (May-Jul) and Summer/fall (Aug-Oct) 2000. Differences between streams and season were assessed with two way ANOVA and Tukey's multiple comparisons. n = 24 samples per stream and season. Bars with the same letter are not significantly different within season. Results of two way ANOVA: df = 3,5, 287, stream factor MS = 2.3, F = 0.41, p = 0.74; date factor MS = 11.5, F = 2.19, p = 0.007; Interaction MS = 5.02, F = 0.90, p = 0.48).

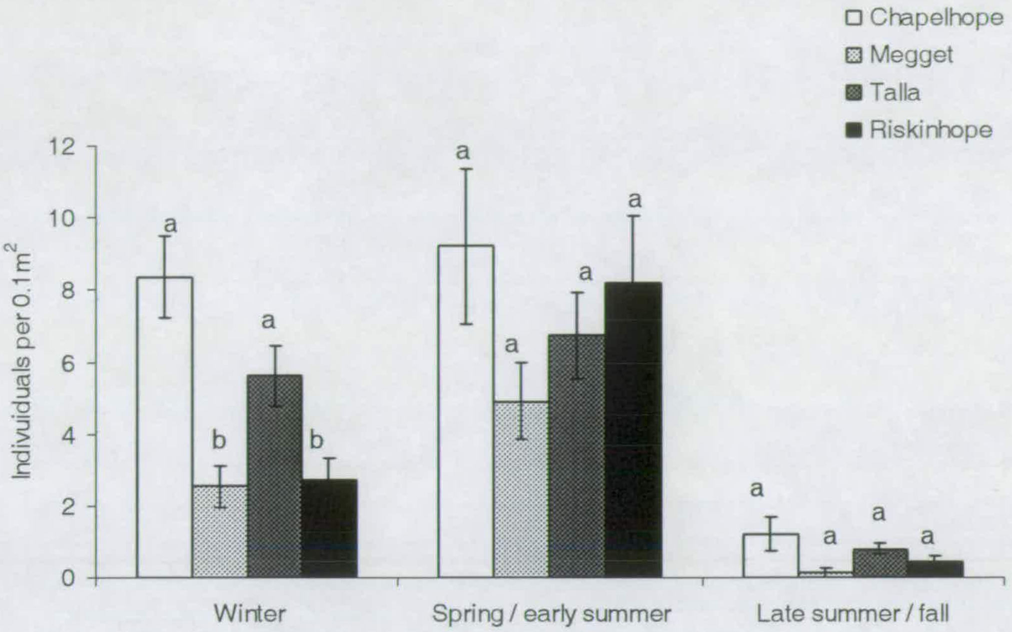

Figure 3 - 9: Mean density (individuals per 0.1 m^2) ± SE of *Siphonoperla torrentium* in 4 streams. Winter (Jan-Mar), Spring/summer (May-Jul) and Summer/fall (Aug-Oct) 2000. Differences between streams and season were assessed with two way ANOVA and Tukey's multiple comparisons. n = 24 samples per stream and season. Bars with the same letter are not significantly different within season. Results of two way ANOVA: df = 3,5, 287, stream factor MS = 472.05, F = 42.12, $p < 0.001$; date factor MS = 374.11, F = 33.38, $p < 0.001$; Interaction MS = 287.12, F = 25.62, $p < 0.001$).

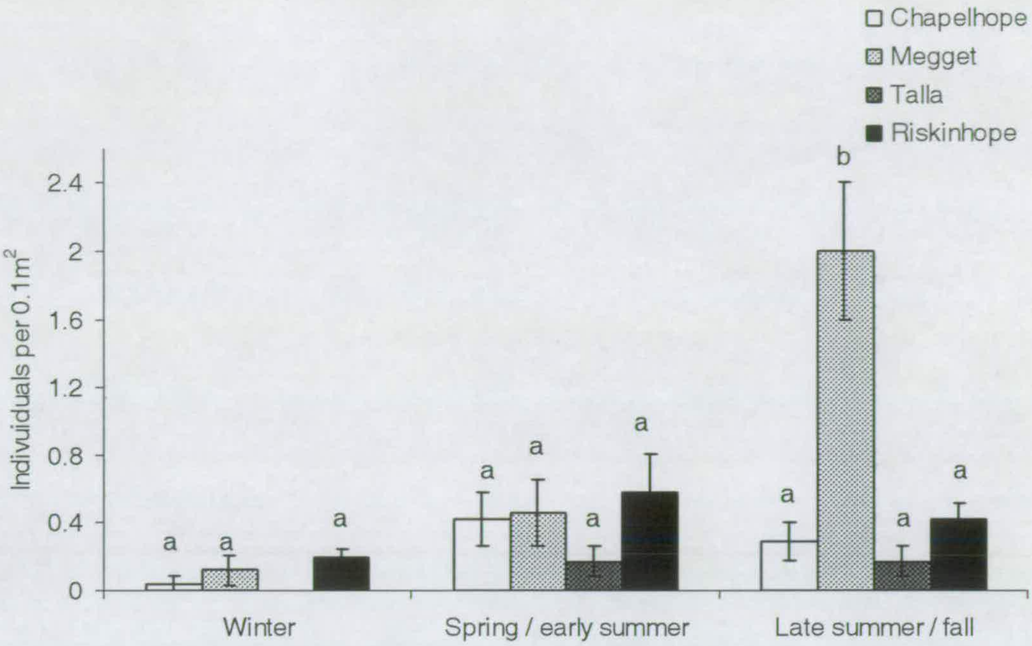

Figure 3 - 10: Mean density (individuals per 0.1 m^2) ± SE of *Rhyacophila dorsalis* in 4 streams. Winter (Jan-Mar), Spring/summer (May-Jul) and Summer/fall (Aug-Oct) 2000. Differences between streams and season were assessed with two way ANOVA and Tukey's multiple comparisons. n = 24 samples per stream and season. Bars with the same letter are not significantly different within season. Results of two way ANOVA: df = 3,5, 287, stream factor MS = 6.21, F = 0.45, p = 0.72; date factor MS = 3.78, F = 0.27, p = 0.93; Interaction MS = 32.65, F = 2.35, p = 0.004).

3.3.5 Invertebrate predator-prey ratio and prey densities

The predator-prey abundance ratio differed across sites and sample dates (Table 3.7), and was the same across the four streams only in March and October. The Talla and Megget showed a strong peak in the predator-prey ratio in May (Figure 3.11), but for the other sample dates (January, July, August) the ratio was highest in the Chapelhope Burn. The predator-prey ratio never differed between the fishless Riskinhope Burn and the Talla Burn.

The abundance of Chironomidae (Chironominae and Orthocladinae only, Table 3.8) differed with stream and sample date. Chironomidae were rare in all streams in October, and in the Chapelhope Burn in January (Figure 3.12), when the Megget Burn had the highest Chironomidae abundance. Chironomidae were more abundant in the Chapelhope Burn by an order of magnitude in March and August, but abundance in the other three streams did not differ. The same pattern occurred with the Talla Burn in July. In May both the Riskinhope and Chapelhope Burns had a higher abundance of Chironomidae than the other two streams, but abundance in the Chapelhope Burn was higher than in the Riskinhope Burn.

The abundance of Baetidae (Table 3.9) also differed with site and date, but was the same across streams in March and October (Figure 3.13). Abundance was significantly higher in January and July in the fishless Riskinhope Burn than in the other three streams, in which the abundance of Baetidae did not differ. In August, abundance was the same in the Chapelhope and Riskinhope Burns, but was higher in these two streams than in the other streams.

Table 3 - 7: Results of a two-way analysis of variance comparing the mean predator/prey abundance ratio across the four streams and six sample dates. 12 samples for each stream/date combination.

Source	DF	SS	MS	F	p
Stream	3	0.62	0.21	7.17	< 0.001
Date	5	1.78	0.35	12.24	< 0.001
Stream × date	15	1.66	0.11	3.81	< 0.001
Error	264	7.70	0.03		
Total	287	11.77			

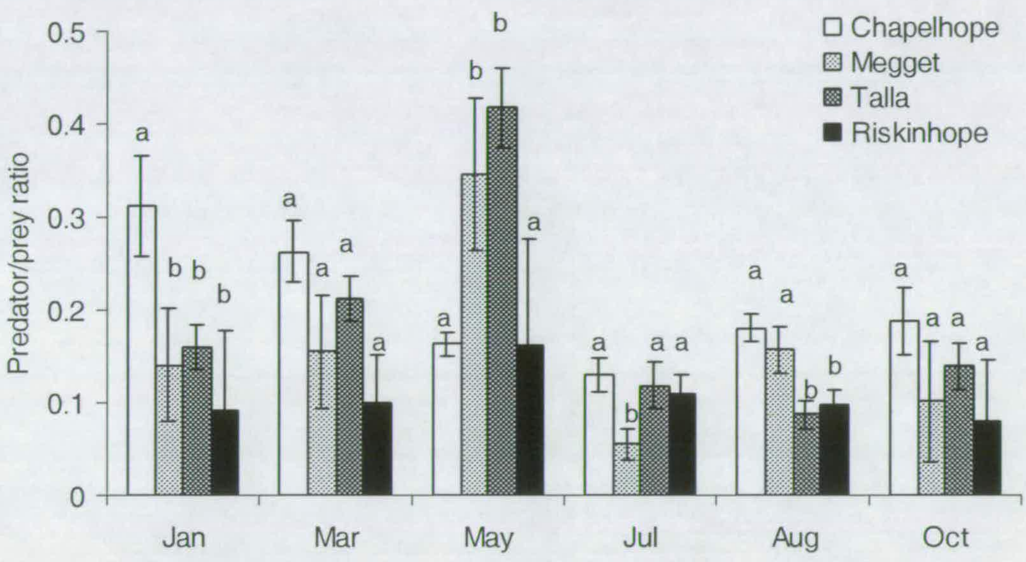

Figure 3 - 11: Mean (± 1 SE) invertebrate predator to prey abundance ratio January – October 2000. 12 samples per stream/date. Bars with the same letter are not significantly different within date.

Table 3 - 8: Results of a two-way analysis of variance comparing the $\log_{10}$ abundance of Chironomidae across the four streams and six sample dates. 12 samples for each stream/date combination.

Source	DF	SS	MS	F	p
Stream	3	21.53	4.31	69.2	< 0.001
Date	5	4.03	1.34	21.6	< 0.001
Stream × date	15	105.10	7.00	112.6	< 0.001
Error	264	16.42	0.06		
Total	287	147.09			

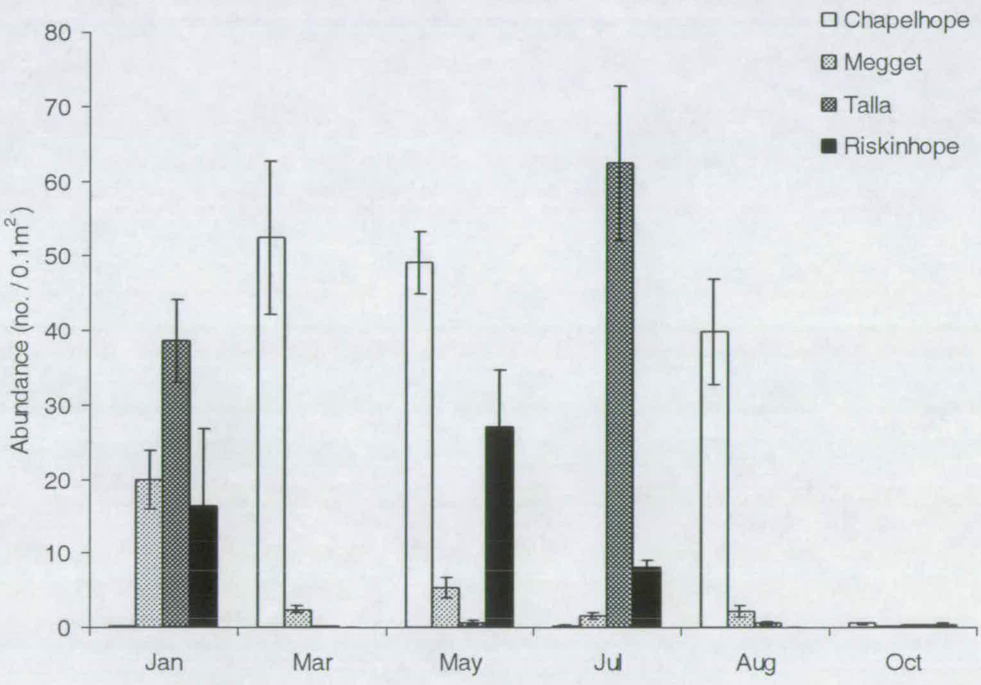

Figure 3 - 12: Mean density (± 1 Se) of Chironomidae for each stream, Jan – Oct 2000.

Table 3 - 9: Results of a two-way analysis of variance comparing the $\log_{10}$ abundance of Baetidae across the four streams and six sample dates. 12 samples for each stream/date combination.

Source	DF	SS	MS	F	p
Stream	3	20.48	1.60	10.6	< 0.001
Date	5	25.52	5.10	32.1	< 0.001
Stream × date	15	15.26	1.01	6.40	< 0.001
Error	264	21.94	0.15		
Total	287	83.21			

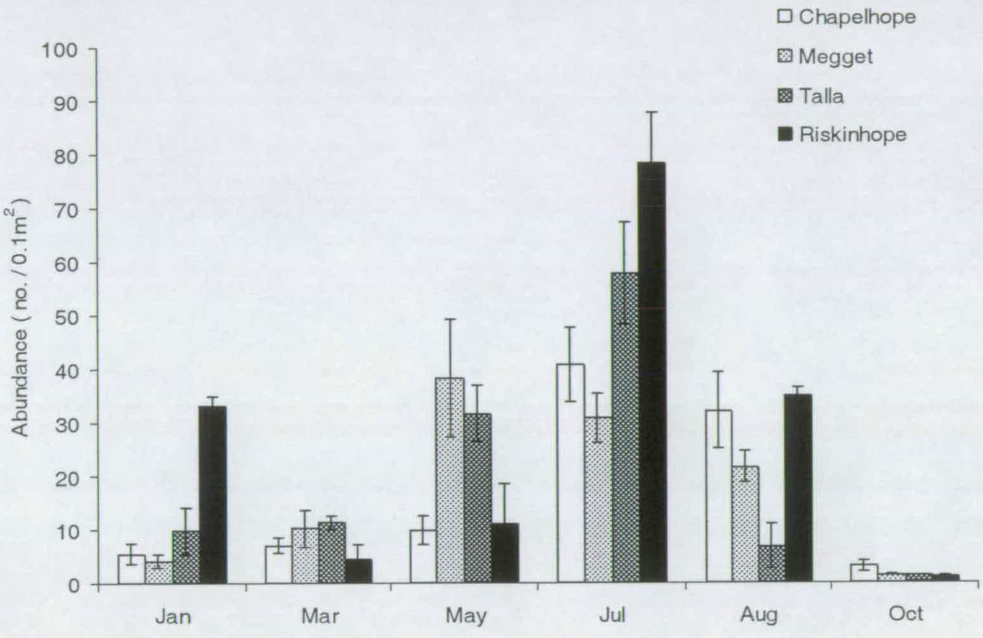

Figure 3 - 13: Mean density (± 1 SE) of Baetidae in each stream, Jan – Oct 2000.

3.3.6 Multivariate analyses

The environmental model

The detrended correspondence analysis model had gradient lengths of less than three units of standard deviations, indicating that a species response based on linear gradients rather than environmental optima was appropriate, i.e. RDA.

In the RDA, forward selection of variables indicated that date accounted up to 31 % of overall variance in species data. Once the effect of date was partialled out, forward selection (Table 3.10) retained 2 linear and 3 nominal variables. Altitude and fish presence accounted for 3.5 and 2.9 % of overall variance in species data respectively. All other variables accounted for less than 1.2 % of variance. Of the linear variables, only water velocity did not contribute significantly to the model. Dominant substrate types did not contribute to the model and of the habitat types, only riffles and riffle margins were significant, but weak, contributors.

Results of the RDA (Table 3.11) indicated the species-environment model was well described by the first two axes and accounted for 23.2 % (100×0.16 / 0.69) of variance not explained by the date covariable. Overall 53 % ($0.69 - 0.16 \times 100$) of total variance remained unexplained by the covariable and the fitted variables. Eigenvalues (a measure of the importance of each axis) indicated the first two axes carried most of the species-environment relation and correlation between environment and species data was high, yet each axis itself explained only a limited amount of variance, hence some underlying gradients had not been quantified. Thus, overall, the model successfully integrated species data to the recorded environmental variables, but these variables could only account for a small percentage of species variance in the samples.

Interpreting the ordination

The ordination plot was limited to the first two axes (Figure 3.14) and this type of plot is interpreted as follows. The position of species points with respect to the origin represents the change of species composition along each axis and thus species are represented by vectors. The vectors point in the direction in which species abundance increases at the highest rate and their relative lengths indicate the strength of the abundance response. Dominant gradients in linear environmental variables are also vectors of steepest increase and nominal variables are points (centroids). Correlation between linear variables, or between linear variables and species vectors, can be inferred by the angle that they form. Species and variables with long vectors are the most important in the analysis, the longer the vectors, the greater the confidence in the inferred correlation. Nominal variable centroids represent individual levels of the factors fitted as dummy variables (in this case riffle and riffle margin habitats) and the distance between the centroids indicates the dissimilarity in species composition between these two factor levels (habitats). Individual samples are usually represented by points and polygonal envelopes drawn around points belonging to the same sites. The degree of overlap of the envelopes represents the degree of similarity in species composition between these groups of samples (sites).

The four invertebrate communities differed in terms of the distribution of invertebrate abundance among species (Figure 3.14), although 60 % of species were common to all sites (Section 3.3.1). The first axis principally represented the effects of fish presence (Table 3.12). The Riskinhope Burn samples were distinct from the other sites along this first axis. The second axis represented mainly altitude (Table 3.12), the 3 streams with fish were separated along this axis. CPOM and habitat were spread equally on both axes, but their correlation to the axes, and the amount of variance represented, was much lower than fish presence and altitude (Tables 3.10 and 3.12) The central position of the Riffle and Riffle Margins habitat centroids indicated that the species assemblage in these habitats varied little across sites. The small distance between the two centroids indicated the two habitats were grossly similar in terms of their species assemblage.

The correlation between abundance and the presence of fish varied between species of invertebrate predators. Altitude had a negative effect on the abundance of invertebrate predators. Among the large stoneflies, Perlidae were poorly correlated with fish presence. *Perla bipunctata* was present only in the fishless site and thus the species vector was directed towards the Riskinhope polygon. *Dinocras cephalotes* was negatively correlated with altitude, and positively associated with CPOM. The response of Perlodidae differed between the large and small species. *Perlodes microcephala* was uncorrelated with altitude and was positively correlated, though weakly, to fish presence. For *I. grammatica* the very short vector indicated little variation in abundance across sites. The other small stonefly predator, the Chloroperlidae *Siphonoperla torrentium* was correlated positively with fish, but negatively with altitude. The two predatory Trichoptera were negatively associated with fish, but their short vectors indicated only a minor response to the model variables. Indeed *R. dorsalis* was abundant in all four sites and *Plectrocnemia* sp. was rare in all four sites. Of the prey, Baetidae showed a negative response to both fish and altitude. All chironomid types were positively associated with fish, but the Orthocladinae only weakly. Chironominae and the predatory Tanypodinae displayed a stronger correlation to fish presence.

Table 3 - 10: Forward selection of variables: environmental variables in order of inclusion to the partial RDA model, additional variance explained by the variable when added to the model (λ), and significance of the variable (F ratio and p value) determined Monte Carlo permutation tests. Non significant variables in italics.

Variable	λ	F	P
Fish	0.06	25.13	0.002
Altitude	0.05	23.91	0.002
Riffle margins	0.02	7.76	0.002
Riffles	0.01	7.73	0.002
CPOM	0.01	4.71	0.004
Coarse substrate	*0.01*	*1.55*	*0.08*
Depth	*0.00*	*1.50*	*0.08*
Flow	*0.00*	*1.43*	*0.116*
Pools	*0.00*	*1.04*	*0.388*
Fine substrate	*0.00*	*0.64*	*0.868*
Medium substrate	*0.00*	*0.58*	*0.925*
Pool margins	*0.00*	*0.54*	*0.905*

Table 3 - 11: Summary of partial RDA and global permutation tests on invertebrate abundance data.

Axes	1	2	3	4	Total variance
Eigenvalues	0.069	0.055	0.016	0.007	1.00
Species-environment correlations	0.75	0.76	0.52	0.44	
Cumulative percentage variance					
of species data	10.0	18.0	20.3	21.3	
of species-environment relation	44.0	79.4	89.5	93.8	
Sum of all eigenvalues					0.69
Sum of all canonical eigenvalues					0.16
	F	p			
Significance first axis	30.4	0.002			
Significance ordination	12.5	0.002			

Table 3 - 12: Correlation of environmental variables with first two axes of ordination of the partial RDA.

Variable	Axis 1	Axis 2
Fish	-0.73	0.46
Altitude	-0.36	-0.92
CPOM	0.10	0.15
Riffle habitats	0.38	-0.39
Riffle margin habitats	0.33	-0.21

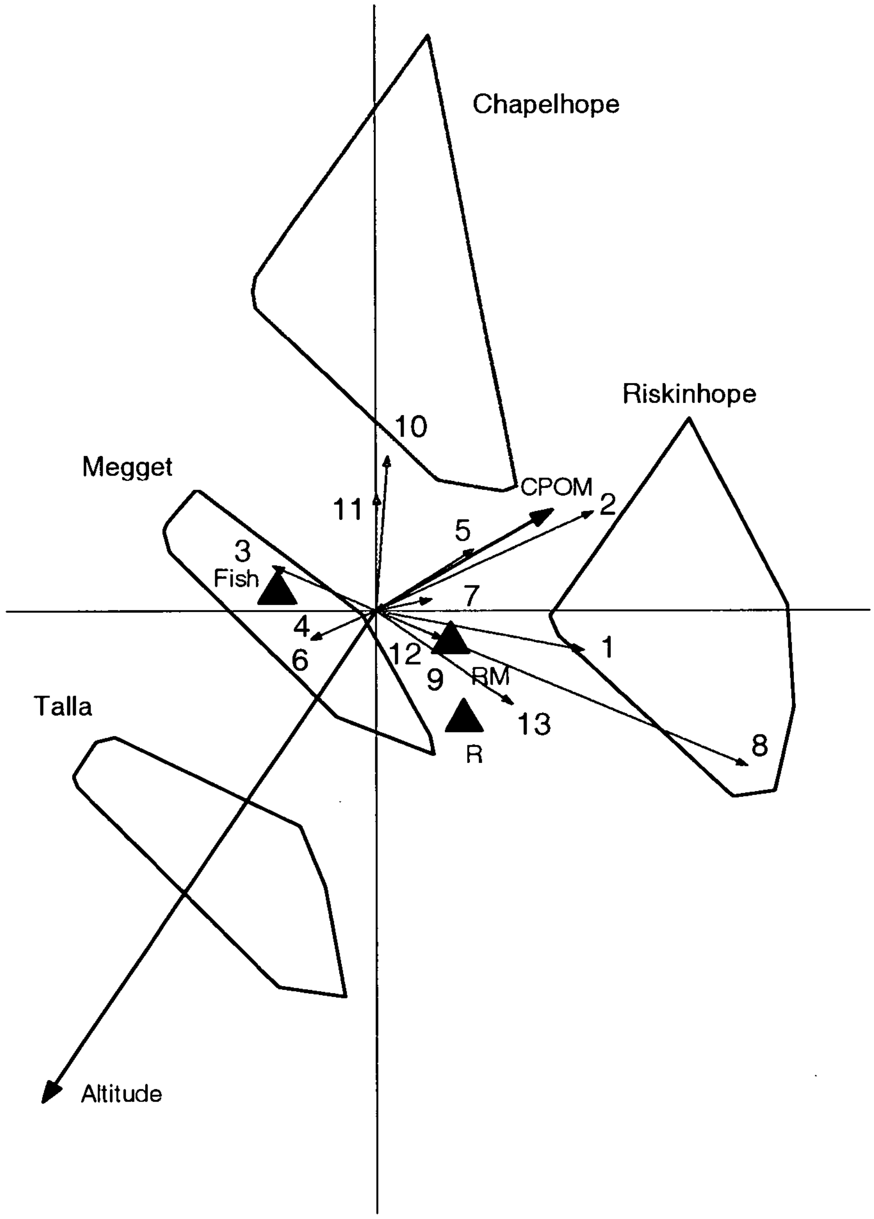

Figure 3 - 14: Ordination plot of first two axes of partial RDA on log (x + 1) invertebrate abundance data. Site groups are represented by polygons (Sample points not included). Triangles are nominal variables (fish presence, RM = riffle margins, R = riffles). Linear variables are represented by thick arrows. Only 14 of the 61 species vectors in the analysis were plotted on the graph. Species were selected on their relevance to the study (i.e. the main predators and their most abundant prey types). Species are represented by grey arrows. 1 = *P. bipunctata*, 2 = *D. cephalotes*, 3 = *P. microcephala*, 4 = *I. grammatica*, 5 = *S. torrentium*, 6 = *R. dorsalis*, 7 = *P. conspersa*, 8 = *Baetis* sp., 9 = *Leuctra* sp., 10 = Tanypodinae, 11 = Chironominae, 12 = Orthocladinae, 13 = Simuliidae.

3.4 Discussion

Overall invertebrate abundance and diversity

Total benthic invertebrate abundance was not highest in the fishless site, contrary to expectations and some other surveys (e.g. Allan, 1975, 1982a; Bowlby and Roff, 1986). Though invertebrate abundance varied across sites, the main effect was seasonal, and fish presence and abiotic variables accounted for only a small amount of variation in invertebrate abundance. Dominant substrate types did not contribute to community structure but this may have been due to the subjective nature of the coarse/medium/fine classification, as all samples consisted of a mixture of the 3 types. The study streams had a poorly sorted stony substrate, and the effects of fish on invertebrate abundance may be weak in these types of habitats because benthic refugia are abundant, limiting predation by fish (Power, 1992; Williams *et al*, 1993; Rader and McArthur, 1995). The lack of clear effects of fish on total invertebrate abundance in this survey was consistent with the hypotheses that, across stream types, the influence of predation on community structure decreases with increasing substrate complexity (Fuller and Rand, 1990; O'Connor, 1991). Species richness, evenness and diversity were greatest in one of the streams with fish, which was also the site of lowest altitude, perhaps reflecting the effects of seasonality. However, there was no difference in diversity and richness across the other sites, including the fishless site. In similar surveys, Allan (1975; 1982a), Bowlby and Roff (1986), and Harvey (1993) also observed little effect of fish presence/absence on invertebrate diversity and species richness.

Relative abundance of prey

The relative abundance of Chironomidae and Baetidae differed in the fishless site compared to a stream with fish of similar altitude. Baetidae were the dominant grazer in the fishless site, however these differences did not occur on all sample dates. An increase in chironomid abundance, and decrease in baetid abundance, is characteristic of many fish/no fish manipulations (Flecker and Allan, 1984; Bechara *et al*, 1993; Rosenfeld, 1997) and is due to the low vulnerability of Chironomidae (Power, 1990; Power *et al*, 1992) and high vulnerability of Baetidae (Dahl and Greenberg, 1996; Huhta *et al*, 2003), to predation by fish. If the relative abundance of these prey changes, then their encounter rates with invertebrate predators should change too, an indirect effect of fish (Sih and Wooster, 1994). This study indicated the potential for this to may occur in these streams at some times of year.

Diversity of invertebrate predators

There was no evidence that fish affected the diversity and richness of the invertebrate predator assemblage, as most predators were present in the fishless Riskinhope Burn and in the 3 streams with fish, similar to Allan (1982a) and Harvey's (1993) surveys. One predator, *Perla bipunctata*, was present only at the fishless site, but this species is commonly recorded in sites with fish (Bird, 1983).

Abundance of invertebrate predators

Weak effects of fish on abundance were expected for species with fixed nocturnal foraging, and the abundance of the perlid *D. cephalotes* did not differ between the fishless stream and the Chapelhope Burn. This species is strictly nocturnal, even in the absence of fish (Elliot, 2000, 2003a, 2003b). Indeed, *D. cephalotes* ambush their prey at very low light levels (i.e. dawn and dusk) and only forage actively in complete darkness (Elliott, 2000). Therefore, the activity patterns of *D. cephalotes* and salmonids have little overlap, as salmonids forage in the benthos during the day, and feed on the drift at dawn/dusk. Encounters in the field may be rare, the stoneflies foraging with minimal risk of predation by fish, and this may be why abundance did not differ across sites. On the other hand, Harvey (1993), found that the large Perlidae were less abundant in steams with fish, on gravel substrate. This suggests that the availability of daytime hiding places may determine whether fish affect their abundance, but these were not limited in the coarse stony substrate of my study streams. For this species, there is a permanent trade-off between time spent foraging and avoidance of fish and this may be why they develop slowly (over three years, Hynes, 1976), whether fish are present or not.

The abundance of several other invertebrate predators (*Isoperla grammatica*, Chloroperlidae and Rhyacophilidae) varied across sites but did not appear to be affected by fish presence/absence, although they are active during the day (Otto, 1993; Elliott, 2000) and should incur more risk of predation by trout than a strictly nocturnal species. Two factors could have contributed to this lack of effect of fish. 1)

The coarse substrate may allow these predators to forage actively during the daytime in interstitial spaces, where they are protected from trout (Feltmate *et al*, 1992). 2) Their foraging activity may be flexible and may be very low at high fish densities, reducing encounter rates with fish (Lima and Dill, 1990), thus predation may not lower their abundance compared to the fishless stream. Some Rhyacophilidae, for example, forage at night only when fish are present (Huhta *et al*, 1999). This kind of flexibility could be reflected in their condition, which should be highest in fishless streams.

The large Perlodidae *P. microcephala* was virtually absent from the fishless site, and winter abundance, which isn't affected by emergence/egg-hatching, increased with decreasing altitude. Hence, environmental factors seemed to influence the abundance of this species more than fish presence/absence. *Perlodes microcephala* were the largest of the daytime-active invertebrate predators and predation risk by fish was expected to be highest for this species than any other, so its very low abundance in the fishless Riskinhope Burn was hard to explain. Because the other predatory stoneflies were not less abundant with fish, there is no reason to suspect competitive release increased the abundance of *P. microcephala* in streams with fish. Nonetheless, a competitive effect caused by the presence of *P. bipunctata* could not be discounted in the fishless stream. Prey-mediated effects of fish were also possible, because the relative abundance of prey types was different with/without fish at some times of year (Flecker and Allan, 1984; Wootton, 1994; Sih *et al*, 1998). This species attains the same size as the Perlidae, but in one year only (Hynes 1976), thus energetic requirement is high, and a trade-off between foraging activity and avoidance of fish may strongly compromise condition and fitness. Hence, to maintain high foraging activity, *P. microcephala* may consume food/prey types that are more often encountered (Peckarsky and McIntosh, 1998), or incur less risk of predation (Huhta *et al*, 1999), when fish are present, such as Chironomidae or algae (Power, 1990). In fishless sites, the abundance of *P. microcephala* may be limited by the abundance of these food types, if diet is a fixed rather than a flexible trait.

Conclusions

The invertebrate communities were generally similar across all four sites. It appeared that in these stream systems, any effect of fish on invertebrate abundance was minor compared to environmental factors such as seasonality, habitat and altitude. Fish presence/absence had no clear effects on the abundance of invertebrate predators. These included invertebrate predators with strict nocturnal foraging (*D. cephalotes*) and flexible foraging behaviour (*R. dorsalis*). It was unclear whether the absence of *P. microcephala* from the fishless site was due to the absence of fish or other factors. The principal difference between the fishless site and the other streams was the greater abundance of Baetidae vs. Chironomidae but this was only apparent on some sampling occasions. Fish may therefore have indirect and sublethal effects on invertebrate predators, mediated by their effects on the abundance of shared prey, but have little impact on invertebrate predator density. Because, the activity and behaviour of invertebrate predators may change with predation risk from fish, the differences across streams with and without fish may be reflected in aspects of their ecology such as condition (studied in Chapter 5) and diet (studied in Chapter 6).

Densities of invertebrate predators were low in all streams, and this limited comparisons across sites. However, I observed in the field that the abundance of invertebrate predators may be higher in large stable stone complexes, which could not be sampled easily using Surber samplers. If invertebrate predators are associated with these microhabitats, then their abundance was underestimated by this survey. In Chapter 4, I compare the abundance, size and biomass patterns of invertebrate predators associated with large stone complexes across streams with and without fish.

4 The abundance, size and biomass of invertebrate predators in substrate complexes in streams with and without fish

4.1 Introduction

There appears to be an association between invertebrate predators and the stony substrate of streams, and this may affect comparisons across streams with and without fish. Benthic densities of invertebrate predators were low in all streams in the randomised survey (Chapter 3), however, substrate complexes formed around large stones could not be sampled using the Surber sampler. When these complexes were kick sampled, they consistently yielded more invertebrate predators than other samples/habitats, and provided specimens for the data used in Chapters 5 and 6. The abundance of invertebrate predators in these microhabitats may provide a better basis for comparisons across streams with and without fish than randomised surveys. The abundance of invertebrate predators should increase passively with increasing substrate complex size (Downes *et al*, 1998), but this increase may be greater in streams without fish. The size class distribution and overall biomass of invertebrate predators in substrate complexes may also vary with the presence of fish if, for example, larger individuals are selected as prey by the fish (Allan, 1981; Newman and Waters, 1984; Scrimgeour *et al*, 1994). In this chapter, I report on a survey of the abundance, size and biomass of invertebrate predators in substrate complexes across two streams with fish and two streams without fish. Do these metrics differ with the presence of fish? Do fish affect the relationship between invertebrate predator abundance and the size of substrate complexes?

Invertebrate predators may be abundant in substrate complexes because they are more stable than the surrounding substrate, and benthic invertebrate density often increases with substrate stability (Death, 2002). Substrate complexes form around large stones, which are immovable due to their size. Brayshaw (1984) described substrate complexes as "cluster bedforms" and defined them as "an accumulation of bed particles, typically formed around an exceptionally large clast, above the level of an otherwise planar bed". They are generally aligned with the flow, and consist of a large stone or "obstacle clast", preceded by a "stoss side" and followed by a "wake". Constituent substrate particles in the stoss and wake are protected from entrainment, and the complex can only be disturbed by flows sufficient to dislodge the nucleus stone (Brayshaw, 1984). Increased stability means that detritus accumulates between the stones, and the epilithic biofilm is well developed. Substrate complexes may attract invertebrate detritivores and grazers and, in turn, their invertebrate predators too. A stable stone complex could form a high quality resource patch, which supports more predators per unit area than the surrounding substrate.

Predators may be more abundant in substrate complexes because these habitats provide refuge from hydraulic disturbance. Because of the inherent resistance to bedload movement, they provide protection to invertebrates from spate events, and the consequent risks of displacement, crushing and stranding. Use of stone complexes as hydraulic refugia, may be a permanent habitat choice, or may be a short-term behavioural response to hydrodynamic cues (Hart and Merz, 1998; Muotka *et al*, 1999), and lead to transient patterns in local invertebrate abundance.

Alternatively, substrate complexes may provide a refuge from fish predators, but allow invertebrate predators to forage freely in interstitial spaces within the complex. These microhabitats provide a strong element of structural complexity due to the accumulation and stacking of different sized stones, and habitat complexity and availability of refugia are often positively correlated (Fuller and Rand, 1990; Power, 1992; Hart and Merz, 1998). Use of these refugia by invertebrates can mitigate the effects of predation by fish, (e.g. Feltmate *et al*, 1986; Feltmate and Williams, 1991; Dahl and Greenberg, 1997). In gravel streams, Harvey (1993) observed a negative effect of trout on predatory stonefly density and biomass in headwaters where large substrate complexes did not occur. Substrate complexes may be well suited to the habit of nocturnal predators, which require daytime hiding places, and of predators with diurnal foraging, which are active at the same time as the fish.

In Chapter 3, fish presence had little effect on invertebrate predator abundance, which was variable across all 4 sites. If predators are associated with substrate complexes, and these were under-sampled, the impacts of fish on invertebrate predator abundance may have been underestimated. Furthermore, if fish affect the relative abundance of different sizes of invertebrate predators (Scrimgeour *et al*, 1994), then they may have no net effect on overall abundance, but differences in size-class distributions may occur, within species, with the presence of fish. This may lead to a different biomass of invertebrate predators associated with the substrate complexes in streams with and without fish. The distribution of invertebrate biomass among trophic levels and pathways has important consequences for the flow of organic matter through freshwater food webs (Benke and Wallace, 1997), and fish may affect this.

The aims of this survey were to compare the abundance, biomass and size class distributions of invertebrate predators in substrate complexes across streams with and without fish. I expected fish to decrease the total abundance of invertebrate predators across streams and to reduce the abundance of the larger size classes of each species, thus reducing overall invertebrate predator biomass in complexes. In keeping with the predictions of Chapter 3, I expected the species with a fixed foraging habit, strict nocturnal activity, to show the least difference across sites. I expected daytime active species, which may incur a greater exposure to fish, to be represented by smaller individuals in streams with fish. I expected the abundance of invertebrate predators to increase passively with increasing complex size (number of stones) in all streams.

4.2 Methods

4.2.1 Field survey

Sampling took place during the first week of June 2001 in the fishless Riskinhope and Linghope Burns, and in the Chapelhope and Cramalt Burns which have fish populations (details of these sites in Chapter 2). In each stream, a 100 m reach was delimited and sampling was semi-randomised within these reaches. Patches of bedrock and fine sediment were excluded from the sampling so that samples were all taken from stony habitats, and within these, all samples contained at least one non-emergent rock >128 mm. Complexes were thus defined, for the purpose of this survey, as an accumulation of stones around one or more large stones, surrounded by smaller sized and better sorted substrate.

Using a kick net, I assessed the stone size composition and invertebrate assemblage in these complexes. This introduced the problem of variable sample size, as it was impossible to standardise samples on an areal basis. However, in stony streams, particularly when the substrate is a heterogeneous mix of various sized stones, samples standardised by benthic area (i.e. Surbers) would still differ in the 'amount' of habitat actually sampled. Stone complexes were the sampling 'unit' and although inherently of different size, the practicality of kick nets superseded any advantage of areal standardisation. The quantity of habitat sampled was dependant on the number of stones and stone size, and I measured these two variables to estimate sample size. I removed every single stone, and all the detritus and invertebrates, associated with each stone complex.

Rock complexes (n = 30) were sampled in an upstream direction, by carefully washing the pre-selected large rock into a kick net (mesh size 310 μm) as well as all stones in contact with this rock. Numbers of stones in a sample were recorded in one of 4 size classes: Size 1- less than 64 mm, size 2 - 64 to 91 mm, size 3 - 91 to 128 mm and size 4 - greater than 128 mm. The size classes are consistent with the Wentworth scale of sediment gradation (Bunte and Abt, 2001) and correspond to the categories: Size 1 – all up to and including very coarse gravel, size 2 - small cobbles, size 3 - medium cobbles and size 4 - large cobbles. At each sample point water depth (cm) and water velocity ($m \cdot s^{-1}$) were recorded. Contents of the net were preserved in the field in 70% alcohol. Samples were sorted and all invertebrates were identified, counted, and their head capsule widths were measured to the nearest 0.1 mm. CPOM (> 1 mm particle size) was separated from the samples, dried for 48 hours at 80°C and weighed to the nearest 0.1 mg.

4.2.2 Substrate characteristics

Total number of stones (T) was calculated for each sample and the total number of stones in all 30 samples was used to produce size class frequencies for the substrate complexes in each site. These frequency distributions were compared using a χ^2 test across the four streams. For each sample, substrate heterogeneity was estimated using Simpson's diversity index (see Chapter 3) D_{sub}, for which each stone size class constituted a 'species'). Substrate evenness (the distribution of T among the size classes) was estimated using the Berger-Parker index Bp_{sub} (Equation 4.1), where T_{max} is the number of stones in the most abundant size class (Death, 2002).

$$Bp_{sub} = T_{max} / T \qquad \text{Equation 4.1}$$

Mean values of T and D_{sub} were calculated for each stream and compared using one way ANOVA and Tukey's *post-hoc* comparison tests ($\alpha = 0.05$). Normality and homoscedasticity were always tested with Anderson-Darling and Bartlett's test respectively (Zar, 1996).

4.2.3 Sample size correction

To standardize invertebrate abundance data for sample size, i.e. the number and size of stones, a correction factor was calculated based on the number of stones of each size class for each substrate complex (i.e. each sample). The correction factor, C is described in Equation 4.2, where N_1, N_2, N_3, N_4 are the number of stones in each size classes 1, 2, 3 and 4 respectively.

$$1/C = (N_1/4) + (N_2/3) + (N_3/2) + N_4 \qquad \text{Equation 4.2}$$

4.2.4 Predator density and biomass

Predator abundance was log (x + 1) transformed and corrected by multiplying by the factor C (Equation 4.2). Mean abundance was compared across sites using one-way ANOVA and Tukey's comparisons ($\alpha = 0.05$). The fixed treatment factor was the presence of fish (2 levels, fish or no fish). Using length-mass regressions from Appendix A, C-corrected predator abundance was converted to biomass (mg dry mass), and this was also compared across streams using the same one-way ANOVA model.

4.2.5 Size frequencies and fish presence

The size class frequencies of the predators were compared across streams with and without fish using a χ^2 test for each species of predator ($\alpha = 0.05$, 60 samples per fish/no fish category). The number of size classes was determined using predator head width:

- *Rhyacophila dorsalis:* Individual head width ranged from 0.1 to 1.4 mm. To obtain size classes of similar increment, necessary for the χ^2 analyses (Zar, 1996), seven size classes were created in increments of 0.2 mm head width. Hence, size classes did not reflect the number of instars of *R. dorsalis*, as the size range (i.e. increment of head width) increases from the first to the fifth instar (Edington and Hildrew, 1995).

- *Dinocras cephalotes:* Individual head widths ranged from 0.7 to 6 mm and 3 cohorts were present in all streams. Six size classes were formed in increments of 1 mm, corresponding to two size classes for each cohort.

- *Isoperla grammatica:* Individual head widths ranged from 1.5 to 3 mm. Three size classes in increments of 0.5 mm were created.

- *Siphonoperla torrentium:* Individual head widths ranged from 0.3 to 1.0 mm. Four size classes in increments of 0.2 mm were used.

4.2.6 Multivariate analysis

To examine species trends, I fitted the invertebrate predator abundance data (corrected using C, Equation 4.2) to a partial RDA model (see Chapter 3 for details of method). The corrected abundance was ($\log_{10}$ x+1) transformed, and only the following 5 species were included: *D. cephalotes*, *P. bipunctata*, *I. grammatica*, *S. torrentium* and *R. dorsalis*. Several sample variables were partialled out (i.e. their effect accounted for) by fitting them to the RDA model as covariates. These consisted of the linear variables: amount of CPOM in samples (mg dry mass per sample), water velocity at the sample point ($m \cdot s^{-1}$) and water depth at the sample point (cm), and the nominal variable: altitude (entered as a dummy variable, low altitude or high altitude). The environmental variables fitted to the model were: the presence of fish (nominal variable: fish or no fish) and the number of stones in size classes one to 4 (i.e. 4 linear variables). Four interaction terms were also fitted to the model, one for each interaction of fish presence and number of stones in a size class (i.e. fish $\times$ size 1, etc.). The interaction terms were fit to test whether a difference in the number of stones in each size class existed between the streams with fish and fishless streams. Significance of variables and interaction terms to the model was determined using forward selection and Monte Carlo permutations ($\alpha = 0.05$; see Chapter 3 for explanation) and only significant variables were retained in the model. The model was used to produce an ordination plot of the invertebrate predator abundance data and the significant variables.

4.3 Results

4.3.1 Substrate complex characteristics

The stone size class frequencies of the complexes (Figure 4.1) were similar across the four sites, all equally dominated by size 1 stones and with roughly the same proportions of each stone size. A chi-square test indicated no significant difference in substrate size class frequencies between the four streams (df = 9, $\chi^2 = 6.93$, $p = 0.64$). ANOVA indicated that the number of stones in samples (T, Table 4.1) did not differ across the four sites (df = 3; 116, $F = 1.07$, $p = 0.36$). Likewise the D_{sub} index (Table 4.1), representing substrate complexity, did not differ between sites (df = 3; 116, $F = 2.31$, $p = 0.08$). The BP_{sub} dominance index was the same for all sites (Table 4.1). The range of number of stones in each size class was comparable across the four sites (Table 4.1).

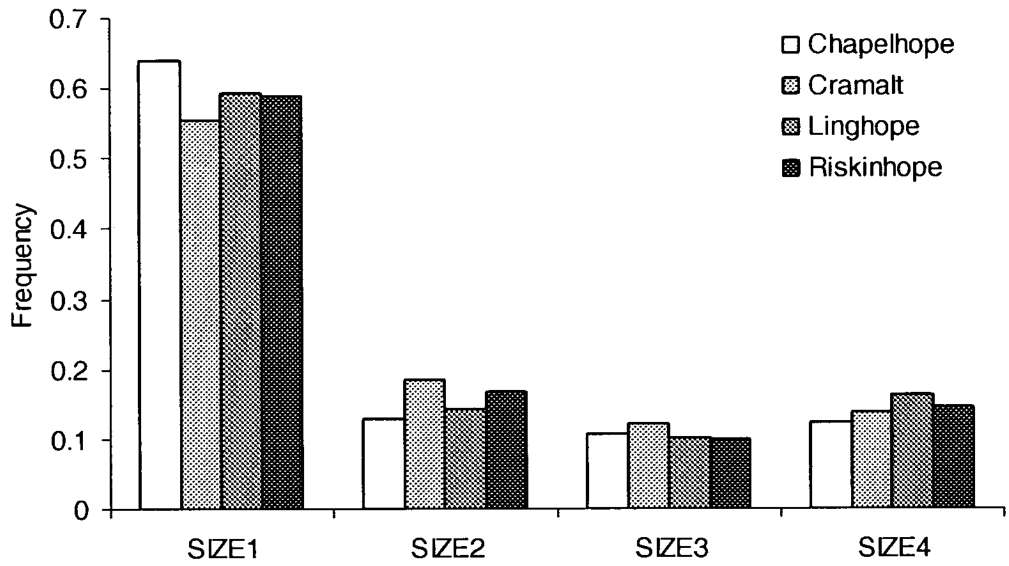

Figure 4 - 1: Size class frequency of the stony substrate in the four streams (30 samples pooled). Wentworth sediment gradation scale equivalents are size 1, coarse gravel , size 2,small cobbles , size 3, medium cobbles and size 4, large cobbles.

Table 4 - 1: Mean (± 1 SE) total number of stones (T) in samples (n = 30) for the four streams and mean (± 1 SE) Simpson's diversity index (D_{sub}) and Berger-Parker dominance index (BP_{sub}) for the substrate. Range of number of stones in each size class per stream (Min – Max), 30 samples per stream.

	Chapelhope	Cramalt	Linghope	Riskinhope
T	**9.1** (0.62)	**8.8** (0.49)	**8.4** (0.40)	**9.8** (0.62)
D_{sub}	**2.7** (0.21)	**3.6** (0.35)	**2.9** (0.21)	**3.1** (0.22)
BP_{sub}	**0.6** (0.03)	**0.6** (0.03)	**0.6** (0.03)	**0.6** (0.02)
Size 1	0 - 15	1 - 10	2 - 8	2 – 13
Size 2	0 – 4	0 – 4	0 – 4	0 – 4
Size 3	0 – 3	0 – 3	0 – 3	0 – 4
Size 4	1 - 3	1 - 3	1 - 3	1 - 3

4.3.2 Predator abundance and biomass

Predators consisted of Perlidae, Perlodidae, Chloroperlidae, and Rhyacophilidae, and were present in all four streams. *Perlodes microcephala* (Perlodidae) was absent as this species emerges earlier has a shorter flight period than the other predators. The warm month of May 2001 probably contributed to their total absence from the benthos by the time of sampling. *Plectrocnemia* sp. and *Diura bicaudata* were excluded from the analyses as they were rare.

Mean predator abundance in complexes, corrected for sample size, was greater in the Cramalt Burn than any other burn (Figure 4.2), but mean abundance was not significantly different between streams with and without fish (Table 4.2). Predator biomass, also corrected for sample size, was clearly highest in fishless sites (Figure 4.3), and this difference was significant (Table 4.3). There was no correlation between biomass of predators and number of stones of each size class, nor the substrate diversity index D_{sub} (Table 4.4), in either streams with fish or fishless streams.

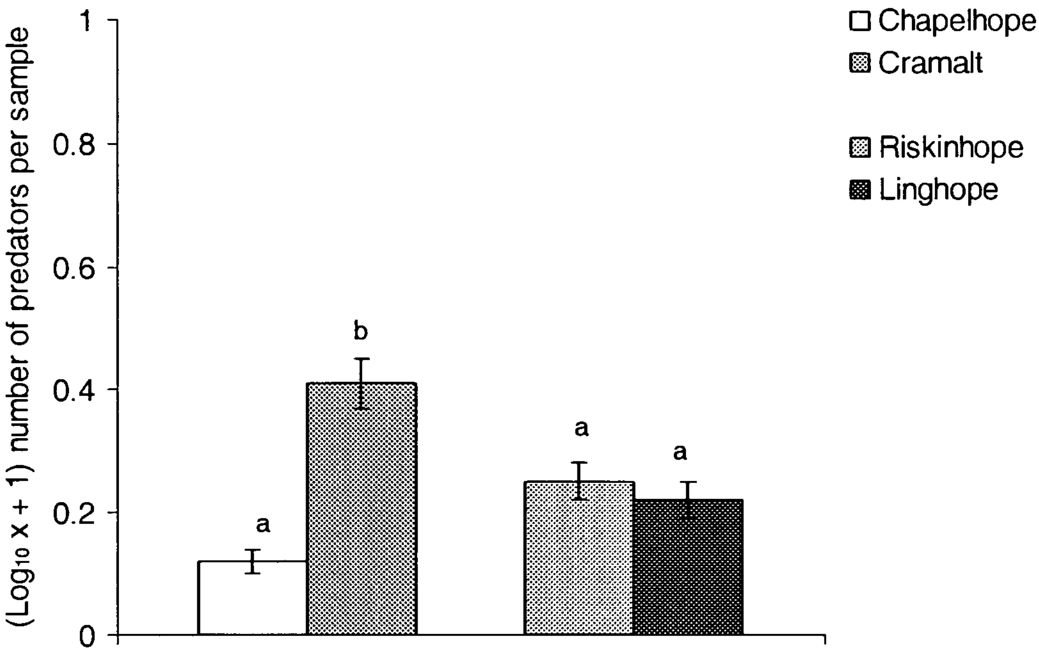

Figure 4 - 2: Predator abundance per sample (mean ± 1SE) in each stream (n = 30 stone complexes per stream). Bars with the same letter are not significantly different (ANOVA and Tukey's test).

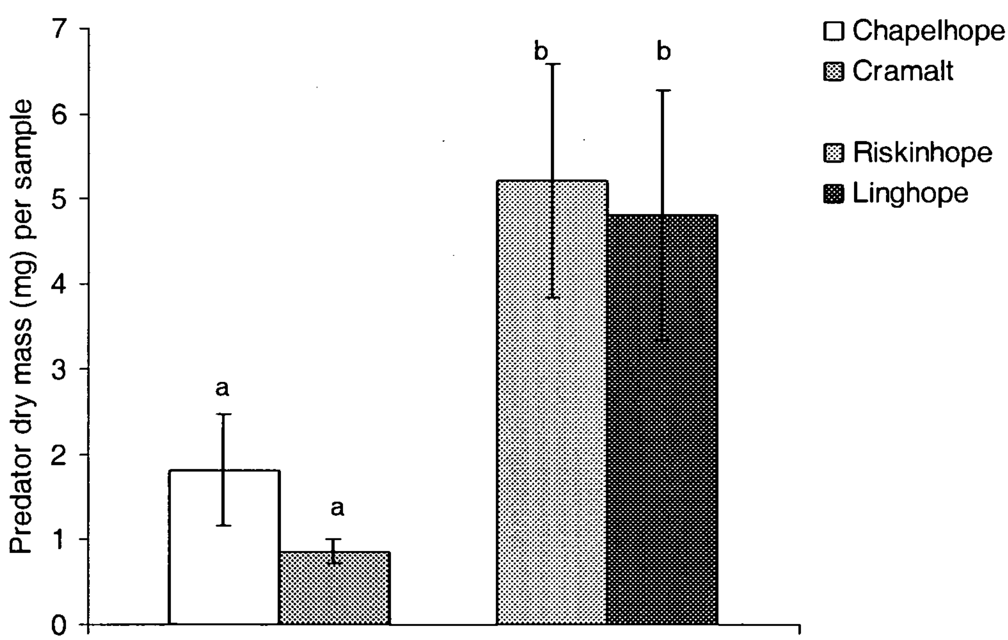

Figure 4 - 3: Predator biomass (mean ± 1 SE) in each stream (n = 30 samples). Bars with the same letter are not significantly different (ANOVA and Tukey's test).

Table 4 - 2: Results of a one-way analysis of variance comparing mean (log x + 1) total abundance of invertebrate predators in substrate complexes across streams with and without fish ('fish', fixed factor). 30 samples per stream, 2 streams per treatment.

Source	DF	SS	MS	F	p
Fish	1	0.02	0.02	0.44	0.510
Error	116	5.16	0.04		
Total	119	5.18			

Table 4 - 3: Results of a one-way analysis of variance comparing mean total biomass of invertebrate predators in substrate complexes across streams with and without fish ('fish', fixed factor). 30 samples per stream, 2 streams per treatment.

Source	DF	SS	MS	F	p
Fish	1	403.5	403.5	12.15	0.001
Error	118	3917.6	33.2		
Total	119	4321.1			

Table 4 - 4: Correlation coefficients of predator biomass with numbers of stones in each size class with and without fish (n = 30 samples per stream, 2 streams per category). There are no significant correlations (adjusted for multiple comparisons, α = 0.05, df = 29, r_{crit} = $|0.44|$).

Fish		No fish	
D_{sub}	0.00	D_{sub}	-0.28
SIZE1	-0.01	SIZE1	0.22
SIZE2	-0.10	SIZE2	-0.03
SIZE3	0.03	SIZE3	-0.26
SIZE4	0.10	SIZE4	0.03

4.3.3 Predator size class frequencies

The size class frequencies of *D. cephalotes* and *I. grammatica* did not differ between streams with and without fish (Table 4.5). *Rhyacophila dorsalis* and *S. torrentium* showed a significantly different pattern between sites with and without fish (Table 4.5). Examination of the size class frequencies (Figures 4.4 and 4.5) indicated more small individuals in sites with fish, and more large individuals in sites without fish.

Table 4 - 5: Results of chi square analyses testing for differences in the size class frequencies of each predator between streams with and without fish. Significant differences in size class frequencies are highlighted in bold type. n = 30 samples per stream, 2 streams per category.

		df	χ^2	p
R. dorsalis	7 size classes	6	8.04	0.046
D. cephalotes	6 size classes	5	9.01	0.110
I. grammatica	3 size classes	2	3.45	0.179
S. torrentium	4 size classes	3	30.92	**0.001**

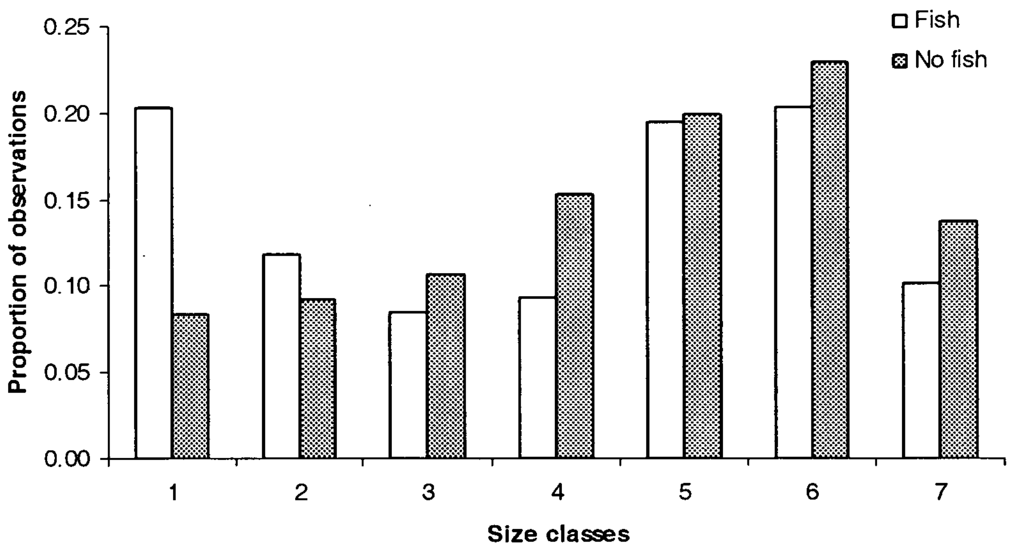

Figure 4 - 4: Size class frequency of *Rhyacophila dorsalis* in streams with (2 streams pooled, n = 60) and without fish (2 streams pooled, n = 60). N = 109 larvae.

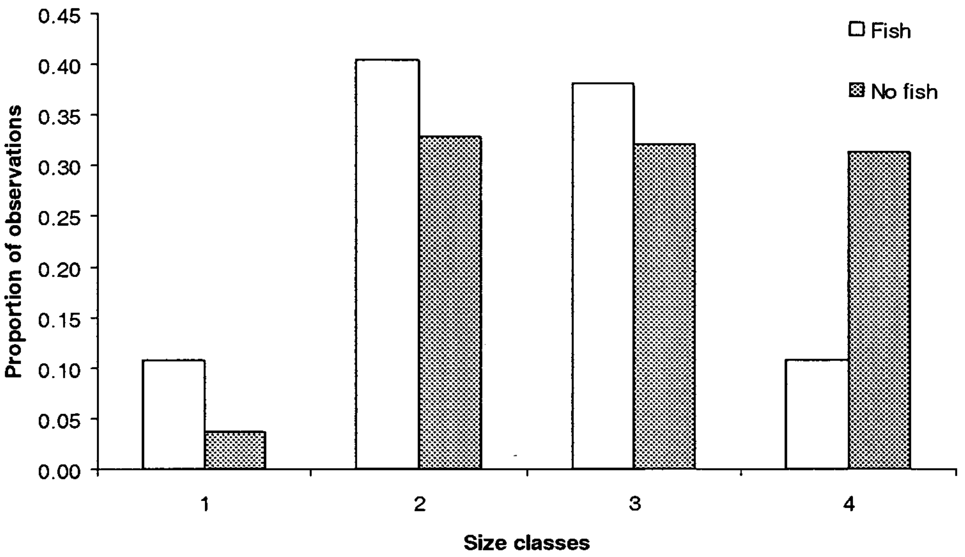

Figure 4 - 5: Size class frequency of *Siphonoperla torrentium* in the 4 streams (n = 30). N = 439 nymphs.

4.3.4 Multivariate analyses

Forward selection of variables indicated that only fish presence, number of size 4 stones and number of size 2 stones contributed significantly to the RDA model (Table 4.6). Of these, fish and number of size 4 stones accounted for most variance, and number of size 2 stones was a weak factor. Interactions of number of stones of each size class with presence/absence of fish were not significant, indicating no bias in substrate complex size across streams. Covariables explained 19.6 % of total variance in predator abundance, and the fitted variables 17.9 % (Table 4.7). Monte Carlo permutations indicated the overall model was significant ($n = 999$, $F = 18.03$, $p = 0.005$). The model was well summarised on two ordination axes, with most variation in predator abundance represented on the first axis (highest eigenvalue), and thus residual (i.e. unexplained) variance in predator abundance represented on the second axis.

Only the abundance of *S. torrentium* was positively correlated to fish presence along the first axis. The short vector of *R. dorsalis* was poorly correlated to fish presence/absence and indicated a weak response. *Perla bipunctata* was present in the only fishless stream and thus its vector was biased towards the fishless sites. The vectors for *D. cephalotes* and *I. grammatica* were also strongly associated with the fishless sites, and negatively correlated to fish presence/absence. Vectors representing numbers of stones of size 2 and size 4 were poorly correlated to the species vectors, with the exception of *S. torrentium*, which showed a negative relationship to the number of these sizes of stones.

Table 4 - 6: Forward selection of variables: λ represents the amount of inertia (variance) in predator biomass which can be explained by each variable using a partial RDA, and variables are ranked according to this from top to bottom. F and p values refer to Monte-Carlo permutation tests, $\alpha = 0.05$, significant values denoted by an asterisk.

Variable	**λ**	**F**	**p**
Fish presence	0.09	15.22	0.005*
Size 4	0.04	5.22	0.005*
Size 2	0.02	4.11	0.01*
Size 3	0.01	1.76	0.125
Fish presence * Size 4	0.01	1.13	0.355
Fish presence * Size 3	0.01	0.84	0.440
Fish presence * Size 2	0.00	0.64	0.615
Fish presence * Size 1	0.01	0.47	0.755
Size 1	0.00	0.21	0.91

Table 4 - 7: Results of the partial RDA on the predator biomass in substrate complexes based on 5 species in 120 samples.

Axes	**1**	**2**	**3**	**4**	**Total inertia**
Eigenvalues	0.117	0.045	0.013	0.004	1.000
Species-environment correlations	0.58	0.45	0.35	0.21	
Cumulative percentage variance					
of species data	14.5	20.1	21.7	22.1	
of species-environment relation	65.2	90.1	97.3	99.3	
Sum of all eigenvalues					0.804
Sum of all canonical eigenvalues					0.179

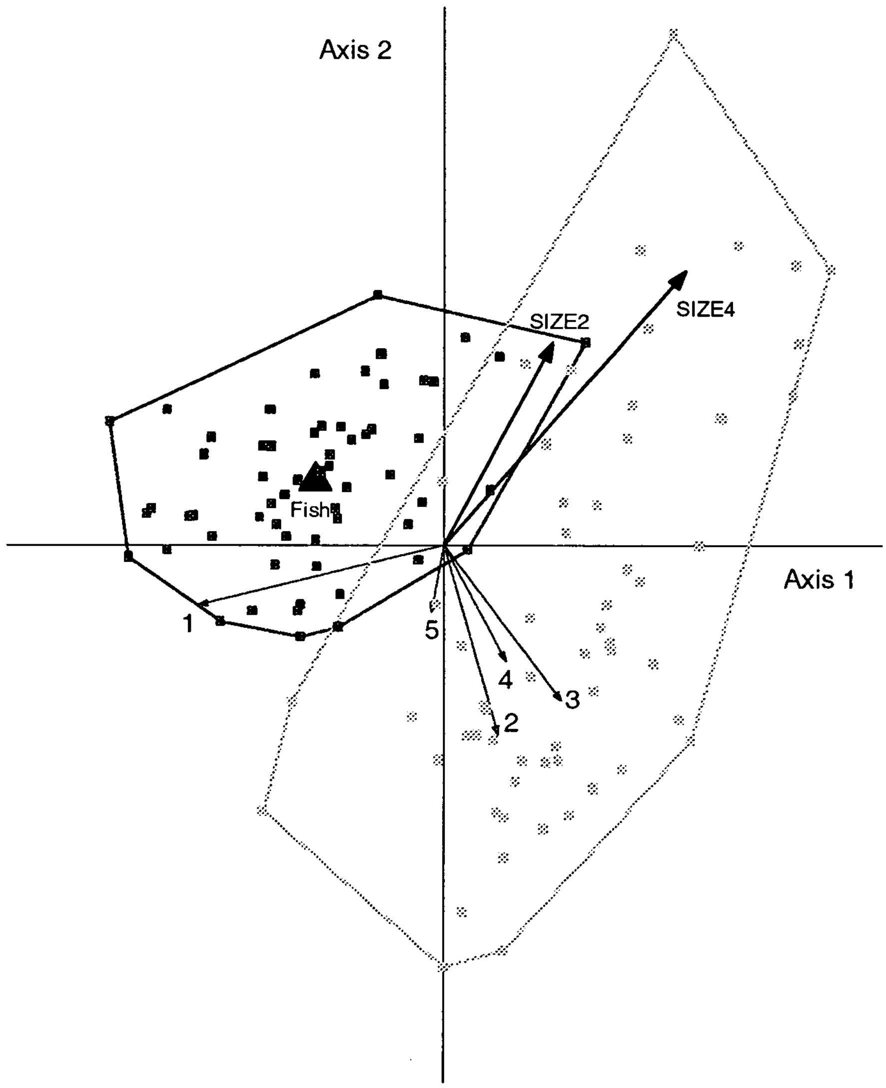

Figure 4 - 6: Partial RDA triplot of predator abundance in substrate complexes. Black squares are samples from sites with fish and grey squares are samples from fishless sites. Vectors with numbers represent species: 1 = *S. torrentium*, 2 = *I. grammatica*, 3 = *D. cephalotes*, 4 = *P. bipunctata*, 5 = *R. dorsalis*. Environment variables are fish presence (black triangle) and number of size 2 and size 4 stones (vectors).

4.4 Discussion

The abundance of predatory invertebrates did not differ between streams with and without fish. This is in contrast to many experimental manipulations (Feltmate and Williams, 1989; Power, 1990; Feltmate and Williams, 1991; Bechara *et al*, 1992, 1993; Rosenfeld, 1998) and some field surveys (Bowlby and Roff, 1986; Harvey, 1993). Because substrate complexes provide habitable space free of fish, the abundance of invertebrate predators may be similar across streams with and without fish when such substrate structures are abundant, explaining weak effects of fish on invertebrate abundance in some surveys (e.g. Allan, 1982).

The biomass of predatory invertebrates was lower in substrate complexes in sites with fish. Harvey (1993) also observed a lower biomass of invertebrate predators across sites with and without fish, but this was due to a strong difference in abundance. In this survey, however, overall invertebrate predator biomass was lower in streams with fish but their overall abundance wasn't. Two mechanisms could have accounted for this: 1) Larger species were more abundant in fishless sites and smaller species were more abundant in streams with fish, thus obscuring overall invertebrate predator abundance patterns. 2) Within species, individuals were larger in fishless sites and smaller in streams with fish, but overall abundance was similar.

Differences in size class frequencies occurred for the two smaller predators only, *S. torrentium* and *R. dorsalis*, for which smaller individuals occurred in streams with fish and larger individuals in fishless streams. These two species hence contributed to the higher biomass pattern in fishless sites through larger individuals. A difference in biomass patterns in Harvey's (1993) survey was also due to smaller body sizes of invertebrate predators in streams with fish. These differences in size with and without fish may occur for three reasons:

1) Fish feed selectively on the larger individuals of invertebrate predators (Scrimgeour *et al*, 1994). However, this process doesn't explain why smaller predators are more abundant in streams with fish vs fishless streams, unless they are preyed upon significantly by larger invertebrates in fishless streams (Culp, 1986).

2) Earlier emergence could have occurred in streams with fish, an effect observed in mayflies by Peckarsky *et al* (1991) in streams with trout, which consequently had a smaller adult body-size, but reduced the time spent exposed to fish. A study of emergence patterns would be necessary to establish if this effect occurred in my sites too.

3) Invertebrate predators are smaller in streams with fish because foraging activity is limited by the presence of fish (Feltmate and Williams, 1991), and the condition of the invertebrates (of which body size is a determinant, Peckarsky and Cowan, 1991) is affected. To determine whether this may have been the case, I examine patterns in condition and body-size in Chapter 5.

The presence of fish did not affect the size class distribution of *D. cephalotes* and *I. grammatica*. For the perlid, this supported the prediction that species with strict nocturnal foraging activity are little exposed to predation by fish, and this may be why there was no difference in either their abundance (Chapter 3) or size class distribution. In contrast, Harvey (1993) observed differences in size class distribution of Perlidae, larger individuals were more abundant in fishless streams. The gravel substrate in Harvey's streams provided little or no daytime hiding places from trout, in comparison to the substrate complexes of my survey, and this may be why he observed strong effects of trout on perlid stoneflies. For the perlodid *I. grammatica,* the substrate complexes provide interstitial spaces in which this daytime-active species can forage. Hence, for both these species, the nature of the substrate complexes provides refugia from fish, whether they are active during the night or day, or both. In my study streams, and both surveys, trout appeared to have no direct effect on the Perlidae and Perlodidae, but indirect effects mediated by invertebrate prey abundance or behaviour may nonetheless occur, and the main effects of fish may be sublethal and reflected in the diet and condition of these invertebrate predators. This is examined in Chapters 5 and 6.

Multivariate analyses indicated that the abundance of *S. torrentium* in complexes was higher in the streams with fish. Other species showed weak trends for a greater abundance in the fishless sites, and one species was present in one of the fishless sites only. This helped to explain overall patterns in invertebrate predator abundance and biomass. Indeed, the higher abundance of *S. torrentium* with fish, particularly in the Cramalt Burn, overwhelmed the patterns for other species (in Chapter 3, densities of *S. torrentium* were always the highest of all predators) and overall invertebrate abundance did not differ across sites. *Siphonoperla torrentium* is the smallest species, and furthermore individuals were smaller in the streams with fish, thus their higher abundance accounted for little biomass. The fishless streams were characterised by larger individuals of both *S. torrentium* and *R. dorsalis,* and the presence of the large perlid *P. bipunctata* in one of the streams, thus accounting for the higher overall invertebrate predator biomass in these streams.

The substrate complexes were similar across streams, in terms of the number of stones of each size class. This illustrated the basic geomorphology precept that similar stream types, in similar geological areas, will produce similar types of substrate structures e.g. bedform clusters, transverse bars (Bunte and Abt, 2001). On the other hand, the Wentworth scale of sediment gradation may have been too coarse to detect differences between similar stream types, and a greater number of size classes may have highlighted site-specific patterns in stone size. The relevance of such fine-scale differences across streams in substrate composition is unclear for invertebrate predators such as Plecoptera and Trichoptera, but may be important for e.g. invertebrates that attach to the substrate (Walton, 1978). There is always a conflict in ecology between the scale of observation, the scale at which natural processes operate and the scale at which different patterns are detectable (Levin, 1992; Ray and Hastings, 1996; Li *et al*, 2001).

The survey was not designed to test whether proportionally more predators were associated with substrate complexes than with the rest of the stream bed, however invertebrate predators were present in 96 % of samples and predatory Plecoptera in

73% of samples, despite the early emergence of *P. microcephala*. In contrast, in the Chapter 3 survey, at the equivalent time of year in May 2000, predators occurred in 80 % of samples, and predatory Plecoptera in 50 % of samples. In stony streams, the distribution of substrate into discrete patches or microhabitats may cause strong differences in invertebrate abundance on small spatial scales (Minshall, 1984). For example, Hassage and Stewart (1991) observed higher abundances of *Isoperla* sp. in patches of mixed, poorly sorted substrate vs more homogeneous patches. More invertebrate predators may be associated with substrate complexes simply because more stones are sampled per unit area of benthos than in other types of substrate patches. This is a direct result of the three-dimensional nature of the complexes, where substrate units accumulate at a greater rate than the surrounding benthos (Brayshaw, 1984), and create a greater 'depth' of stones. The relationship between invertebrate abundance and biomass and the number, size and stacking of substrate units is poorly understood. The number of stones of each size does not reflect the imbrication and stacking of the substrate, and these two factors govern the quantity of habitable space available to the invertebrates. This space consists of the exposed are of the stones on which the invertebrates can crawl, and the void space between stones in which they can move (Hynes, 1974). Though the random sampling of streams is designed to avoid biases in the selection of sample points (Davis *et al*, 2001), this method may provide inadequate estimates of abundance if the 'target' organisms have an association with the substrate which is not accounted for by the sampling protocol.

5 A comparison of the length mass relationships of predatory invertebrates

5.1 Introduction

The presence of predatory fish may have sub-lethal effects on stream invertebrate predators, which are reflected in the condition of individuals (e.g. size, weight), rather than their abundance. Invertebrates must minimise the possibility of capture by fish, despite the need to acquire resources, and there is a potential trade-off between survival and feeding (Abrams, 1987; Lima and Dill, 1990). Behavioural traits that decrease predation risk, such as lower foraging activity in the presence of fish, often also reduce encounter rates with food/prey, and impair feeding and growth (Werner *et al*, 1983; Feltmate and Williams, 1991; Peacor, 2002). Furthermore, fish may also influence the feeding rates of invertebrate predators because they affect the availability of resources, e.g. the abundance and activity of the shared prey (e.g. Baetidae, Peckarsky and McIntosh, 1998). Hence, because of these effects on food intake, invertebrate predators may be in different condition across streams with and without fish. The fecundity of the mature, non-growing, adult female are correlated with its condition (Peckarsky *et al*, 2001, 2002) and therefore, predation risk by fish in the immature, growing stage may have long-term consequences for the fitness of stream invertebrate predators. This chapter explores the variations in the condition (size-mass relationships, pre-emergent weight) of invertebrate predators across similar stream systems, one fishless. Is condition similar across all streams and do species show the same cross-site patterns? Are invertebrate predators fitter in the streams with fish or the fishless stream?

Many invertebrates reduce their foraging activity when fish are present (Otto, 1993; Dahl, 1998a) and this can have a negative effect on their condition. For example, the poorer condition (mass to head width ratio) of nymphs and adults of *Paragnetina media* (Plecoptera: Perlodidae) in the presence of trout was due to a reduction in time spent foraging (Feltmate and Williams, 1991). Exposure to fish over developmental time can reduce the condition (individual biomass) of Rhyacophilidae caddisflies (Otto, 1993), and Huhta *et al* (1999) showed this could be due to reduced daytime foraging when fish are present. If invertebrate predators have a flexible foraging activity, and food intake is lower when predation risk by fish is high, then their condition should be lower in streams with fish than fishless streams. Fish may also reduce the abundance or activity of the prey, further limiting encounter rates with invertebrate predators. For example, lower activity of mayflies when fish were present reduced their encounters with predatory stoneflies, and had a negative effect on the prey capture rate of the stoneflies (Peckarsky and McIntosh, 1998).

Fish may also have a positive effect on condition, because they reduce the overall abundance of invertebrate predators (Harvey, 1993), and may reduce competition for resources. For example, the prey capture rates of predatory stoneflies decrease with their abundance (Elliott, 2003b) because they cause the prey to disperse, a form of 'interference' competition (Walde and Davies, 1984b; Peckarsky, 1990). This can reduce their condition, and for example, Taylor *et al* (1998) observed that the body size at emergence and adult fecundity of the predatory stonefly *Megarcys signata* decreased with its abundance. In streams with fish, invertebrate predators that escape capture by fish may be in better condition than those in fishless streams, because the *per capita* amounts of resources are higher. The condition of invertebrate predators across streams may reflect the balance between competition and predation on foraging and prey capture rates (Sih *et al*, 1985; Grand, 2002).

Size-mass relationships may provide the best indicator of the condition of invertebrate predators across streams (Benke *et al*, 1999). The size and weight of freshwater invertebrates is positively correlated to their reproductive potential (Taylor *et al*, 1998; Peckarsky *et al*, 2001, 2002) and these measures are often used to assess the condition of individuals (e.g. Feltmate and Williams, 1991; Werner, 1991; McPeek *et al*, 2001). If invertebrate predators are smaller in streams with fish, this could be due to either lower condition of individuals with fish present, or the consumption of larger individuals by fish. Hence, patterns in simple body size or weight of individuals across streams do not allow to separate the lethal and sublethal effects of fish. However, the biomass of insects has a direct relationship to body size (Gould, 1966), which can be summarised using a linear regression model (Smock, 1980). These relationships are often used in a predictive way, to estimate weight when body size is known (e.g. Meyer, 1989). Size-mass relationships can also be used to compare the relative accrual of weight with increasing body size across taxonomic groups or sites, when both body size and weight are known (Short *et al*, 1987; Gee, 1988; Wenzel *et al*, 1990; Griffith *et al*, 1993; Eggert and Burton, 1994; Basset and Glazier, 1995; Gonzalez *et al*, 2002). If fish affect the feeding rates of invertebrate predators, the rate at which their weight increases with size should be affected too. The size-mass relationships of invertebrate predators may therefore be different in streams with and without fish.

I summarised the condition of predatory invertebrates, as the slope of their length-mass relationships. I tested the hypothesis that these slopes do not vary across 3 streams with fish and a fishless stream. Because these slopes are a representation of condition over developmental time, I also derived a 'point' estimate of condition across the sites by comparing the mean weight of pre-emergent nymphs, another correlate of adult fitness. I expected the species with a fixed foraging trait, nocturnal foraging, to show the least difference, if any, in condition across sites. I expected the condition of daytime-active invertebrate predators to be higher in the fishless site, as their foraging rates may be greater, if condition is mainly determined by predation risk.

5.2 Methods

Sampling and processing of specimens

Predatory invertebrates were captured downstream of the reaches of the Talla, Megget, Chapelhope and Riskinhope Burns. Two people performed repeated kick samples for one hour. Samples were taken from the four streams over two days, in January, March, May, July, August and October 2000. Contents of the nets (500 μm mesh) were washed into plastic trays and the desired specimens were removed with forceps and preserved in 70% alcohol. Perlidae, Perlodidae and Rhyacophilidae were sufficiently abundant for numerical analyses.

Invertebrates were processed between three and five weeks after sampling. Specimens were kept away from sunlight, which increases losses to the preservative medium (Leuven *et al*, 1985). Invertebrates were examined under a minimum of x10 magnification (Leica MZ 6 binocular dissection microscope), cleared of attached detritus and identified to species. Individuals were measured to the nearest 0.1 mm for head capsule width (at the widest point) and overall body length (excluding antennae, palps, cerci and anal claws) using an eyepiece graticule and microscope (Leica MZ 6). A ventral incision was made from the front of the thorax to the rear of the abdomen; the gut was severed at the mouth and malphigian complex and removed, taking care not to remove any other tissue. The carcasses were placed in foil trays and dried in an oven for 48 hours at 80 °C. After removal from the oven, the trays were cooled completely and the invertebrates weighed individually to the nearest 10^{-2} mg on a microbalance (Sartorius MC 5).

In appendix B, I quantify the errors in weight estimates that arise from this method of processing specimens. I estimate the underestimate in dry mass caused by preservation of specimens. I also test whether the gutting procedure has an effect on weight lost on drying by invertebrates.

Length-mass equations

In most animals different body parts grow at different rates, but maintain their relative proportions (Huxley, 1932). The majority of freshwater invertebrates are associated with exoskeletons, shells or tubes, within which the growth of soft tissue is constrained. Basic relationships hence exist between the size and the biomass of the whole organism (Gould, 1966). A power function (Smock, 1980; Johnston and Cunjak, 1999; Meyer, 1989; Towers *et al* 1994; Burgherr and Meyer 1997; Benke *et al*, 1999; Cressa, 1999a; Gonzalez *et al*, 2002) best describes the weight (W) of aquatic insects according to a linear measurement (X) so that:

$W = b \cdot X^a$ (a and b constants) Equation 4.1

Although other equations exist, such as quadratic models, the log-linear form of the above is used most widely (Equation 4.2) and these equations are often generically referred to as 'length-mass regressions', though overall length of the animal is not always used as the predictor variable.

$$\text{Log W} = \text{Log b} + \text{a Log X} \qquad \text{Equation 4.2}$$

The log-linear equation does not represent growth rate as such, as there is no time parameter, but greater slopes (b) indicate greater accumulation of weight with size.

Data analysis

Both the relationships between dry mass (DM) and head capsule width (HW), and DM and body length (BL) were investigated using regression analysis. Assumptions of normality and homoscedasticity required by least squares regression were tested with Anderson–Darling and Bartlett's test respectively. Fit of the models was assessed by the coefficient of determination (R^2) and the level of significance of the analysis.

In scaling analysis the values of the predictor variables are not set *a priori* by the investigator, and for example head width and body length (the predictors) are subject to measurement error, in the same way as weight (the response) is. Model II regression analysis is therefore more suitable (Niklas, 1994). Reduced major axis (RMA) regression was used as the variables are standardised and hence comparisons are scale independent. Data were log transformed. Regression equations were determined using an ordinary least squares (OLS) model to yield log-linear equations as Equation 4.2. The exponent and coefficient were then corrected to the RMA model using the method detailed by Niklas (1994).

Site specific regressions were determined for each predator, and compared, using an equivalent to a t-test for comparing two regressions and ANCOVA for more than two regressions (Zar , 1996; Sokal and Rohlf, 1995). Differences in slopes were first compared. If no difference was found between the slopes of the regressions, then elevation (intercept) was also compared. Small or absent sample sizes for all species in some months prevented an analysis of temporal patterns.

<u>Size prior to emergence</u>

The mean wet weight of pre-emergent predators was determined for all sites. For *I. grammatica* and *S. torrentium* this consisted of nymphs captured in May 2000. For *P. microcephala*, nymphs captured in March were used as very few nymphs were present in May and none in July. For *D. cephalotes*, which has a longer emergence period, all nymphs with a head width greater than 4 mm from May to July were deemed to belong to the emergent cohort, as all nymphs from August had a head width smaller than 3.5 mm. For *R. dorsalis*, which has a very long emergence period, all larvae in their fifth instar captured in May, July and August were included. Indeed, in October, only first and third instar larvae were present, the latter belonging to the 'resting' subsection of the cohort which does not emerge and over-winters in the larval stage. Difference in final wet weights between streams were analysed using

ANOVA for the Chloroperlidae, Perlodidae and *R. dorsalis*, and with a t-test for *D. cephalotes* as data was available for two streams only.

5.3 Results

5.3.1 Site specific length-mass regressions

Equations were derived over a wide range of head widths and body lengths for all Perlidae and Perlodidae, though there was little data on very small size classes (Table 5.1). For Rhyacophilidae, only head width was used as a predictor of mass, as preliminary investigations indicated that body length fitted the log linear model poorly. Data for head width and body length of Chloroperlidae fitted the log linear model poorly and they were not included in this analysis.

Perlidae

The length-mass relationships of *D. cephalotes* were similar between the fishless stream and the Chapelhope Burn for both head width (HW, Figure 5.1) and body length (BL, Figure 5.2), and indeed the slopes and intercepts did not differ across sites for either the HW or BL regressions (Table 5.2). Comparison of the length-mass relationships across the two Perlidae species in the Riskinhope Burn indicated differences in the slopes of the BL regression only (Table 5.2). *Perla bipunctata* accrued more weight with increasing body length than *D. cephalotes*.

Perlodidae

The relationship between mass and size varied across sites for the Perlodidae for both HW (Figure 5.1) and BL (Figure 5.2). The slopes of the dry mass (DM) to HW regressions of *Perlodes microcephala* in the three sites with fish did not differ (Table 5.3). However, a similar trend in the DM to BL regressions was significantly different (Table 5.3), and slopes were greater in the Chapelhope Burn than the other two streams with fish. In contrast, the slopes of both HW and BL regressions differed for *I. grammatica* between the four sites (Table 5.4). In both relationships, individuals in the Chapelhope Burn showed a greater weight gain with size than at the other three sites which did not differ from one another.

Rhyacophilidae

The slope of the HW regression was significantly steeper in the Chapelhope Burn for *R. dorsalis* than in the three other streams (Figure 5.1 and Table 5.5).

Table 5 - 1: Size ranges (mm) of specimens used to derive length mass regressions.

Family	Species	n	HW range (mm)	BL range (mm)
Perlodidae	*P. microcephala*	157	0.7 - 4.8	2.6 - 19.4
	D. bicaudata	10	1.4 - 3.5	4.1 - 17.4
	I. grammatica	136	0.5 - 2.3	1.9 - 13.9
Perlidae	*D. cephalotes*	256	0.4 - 6.0	2.0 - 29.1
	P. bipunctata	57	1.8 - 4.9	5.7 - 25.0
Rhyacophilidae	*R. dorsalis*	141	0.4 - 1.8	na

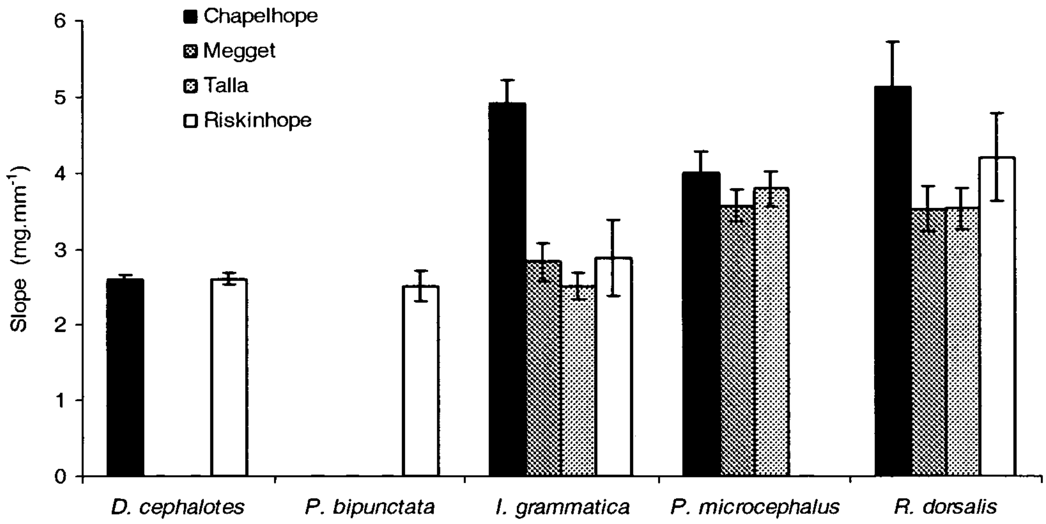

Figure 5 - 1: Slopes of dry weight to head width regressions for five invertebrate predators, bars represent ± 1 SE.

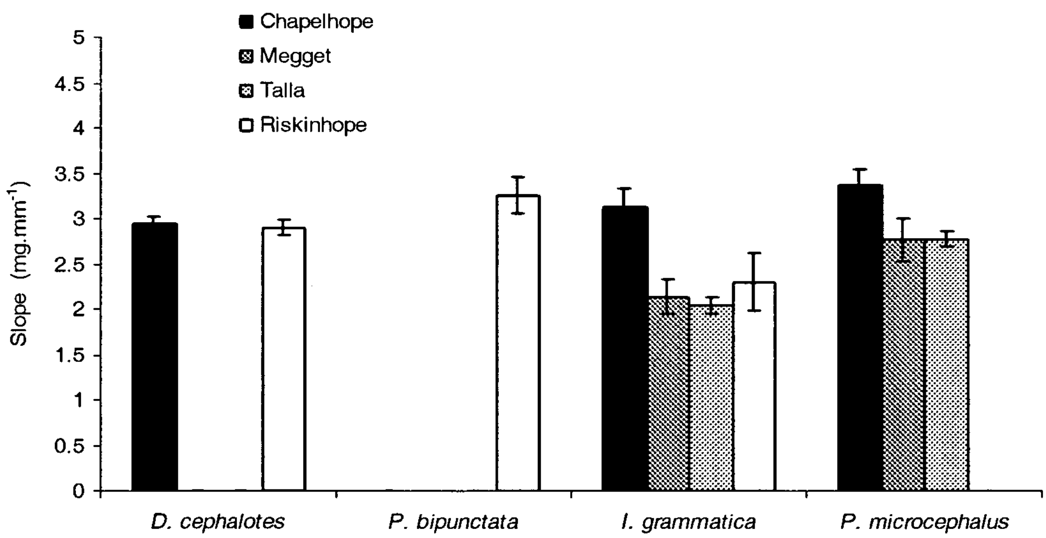

Figure 5 - 2: Slopes of dry weight to body length regressions for four invertebrate predators, bars represent ± 1 SE.

Table 5 - 2: T tests comparing slope and elevations of the length mass regressions of Perlidae in the Riskinhope and Chapelhope Burns.

		DW to HW			DW to BL		
		df	t	p	df	t	p
D. cephalotes:	Slope	186	-0.64	ns	186	-1.07	ns
Chapelhope vs.							
Riskinhope	Elevation	186	0.12	ns	186	1.62	ns
D. cephalotes vs.	Slope	151	-0.45	ns	151	-3.65	0.004
P. bipunctata							
Riskinhope only	Elevation	151	1.55	ns	-	-	-

Table 5 - 3: Summary of ANCOVA (df = 2, 141) and Tukey's multiple comparisons tests for slopes and elevations of length-mass and head width-mass regressions of *P. microcephala* in 3 streams.

		DW to HW			DW to BL		
Sites		F	p	Tukey	F	p	Tukey
Chapelhope (C)	Slopes	1.84	ns	-	3.36	p < 0.05	C >M, T
Megget (M)							
Talla (T)	Elevations	2.61	ns	-	-	-	-

Table 5 - 4: Summary of ANCOVA (df = 3, 126) and Tukey's multiple comparisons tests for slopes and elevations of length-mass and head width-mass regressions of *I. grammatica* in 4 streams.

		DW to HW			DW to BL		
Sites		F	p	Tukey	F	p	Tukey
Chapelhope (C)	Slopes	2.88	P < 0.05	C > M,R,T	6.64	P < 0.01	C > M,R,T
Megget (M)							
Talla (T)	Elevations	-	-	-	-	-	-
Riskinhope (R)							

Table 5 - 5: Summary of ANCOVA (df = 3, 127) and Tukey's multiple comparisons tests for slopes and elevations of length-mass and head width-mass regressions of *R. dorsalis* in 4 streams.

		DW to HW		
Sites		F	p	Tukey
Chapelhope (C)	Slopes	6.68	P < 0.01	C > M, T, R
Megget (M)				
Talla (T)	Elevations	-	-	-
Riskinhope (R)				

Weight of pre-emergent invertebrates

The weight of pre-emergent nymphs of *D. cephalotes* did not differ between the fishless site and the Chapelhope Burn over May to July 2000 (Table 5.6) consistent with the pattern of similar length-mass relationships. The pre-emergent weight of the other large Plecoptera, *P. microcephala* did not differ across the three sites with fish (Table 5.7) despite the significantly steeper length-mass relationships in the Chapelhope Burn. Both *I. grammatica* and *R. dorsalis* (Table 5.7) showed a two fold increase in final weight in the Chapelhope Burn, compared to the other three sites which did not differ from one another. This pattern was consistent with the significantly steeper length-mass relationships for these species in the Chapelhope Burn. The mean pre-emergent weight of *S. torrentium* was higher in the Chapelhope Burn and did not differ between the other streams (Table 5.7).

Table 5 - 6: Mean weight of pre-emergent nymphs of *D. cephalotes* (± 1 SE) in two streams and results of a test for difference between these means.

Site	n	Mean weight (mg)	± SE	df	t	p
Chapelhope	16	44.3	9.2	31	-0.20	0.84
Riskinhope	18	46.8	8.2			

Table 5 - 7: Mean pre-emergent weight of 3 Plecoptera and 1 Trichoptera at four sites and summary results of ANOVA testing for differences in weight between sites ($\alpha = 0.05$).

Species	Site	n	Mean	±1SE	df	F	p
P. microcephala	Chapelhope	17	13.25	1.54	2, 37	0.06	0.941
	Megget	10	14.14	1.73			
	Talla	11	13.93	2.67			
I. grammatica	Chapelhope	15	3.63	0.40	3, 56	9.90	0.006
	Megget	9	1.81	0.29			
	Talla	22	1.72	0.22			
	Riskinhope	11	1.53	0.32			
R. dorsalis	Chapelhope	16	5.71	0.39	3, 54	3.18	0.032
	Megget	15	3.68	0.55			
	Talla	14	3.71	0.62			
	Riskinhope	10	3.69	0.90			
S. torrentium	Chapelhope	14	0.71	0.04	3, 58	2.81	0.050
	Megget	10	0.65	0.05			
	Talla	17	0.64	0.04			
	Riskinhope	18	0.54	0.03			

5.4 Discussion

The presence of fish did not reduce the condition of invertebrate predators, measured by their size-mass relationships and pre-emergent weights. This lack of a negative, sublethal, effect on fitness contradicts the predictions of experiments and manipulations, in which fish reduce the condition of invertebrate predators *via* a decrease in foraging activity (e.g. Soluk and Collins, 1988a, 1988b; Feltmate and Williams, 1991), or prey encounter rates (e.g. Peckarsky and McIntosh, 1998; Soluk, 1993). The consequence of these behavioural effects of fish on invertebrate predator condition may be too weak to detect under field conditions. In manipulations, fish present a permanent risk of predation, on a small spatial scale. Because fish are highly mobile, invertebrate predators in streams may only be intermittently exposed to predation risk, and reductions in foraging activity may only be occasional, and short-term (Lima and Bedneckoff, 1999).

Patterns in the condition of Perlodidae stoneflies and Rhyacophilidae differed in one stream from all others, but this was a stream with fish, not the fishless stream. Perlodidae and Rhyacophilidae forage during the day, when they are exposed to salmonids, and can reduce their foraging activity in response to predation risk (e.g. Huhta *et al*, 1999), thus it was surprising that condition was highest in a stream with fish. Several indirect mechanisms have been suggested, which may mitigate the effect of fish on foraging activity. There was no clear effect of lower elevation on condition, because the fishless site was at the same altitude as this stream.

1) Fish can reduce the overall abundance of invertebrate predators (Power, 1990) and may have increased *per capita* amounts of resources/prey. However there was no clear evidence in Chapters 3 and 4 that fish impacted invertebrate predator abundance.

2) Fish affected the relative abundance of prey types at some times of year (Chapter 3), and can increase algal abundance (Bechara *et al*, 1992; Rosenfeld, 1997, 1998), and invertebrate predators may have benefited from an increase in an abundant food/prey type. However, it isn't clear why this occurred in one stream with fish and not the others. In Chapter 6, I examine how the diet of invertebrate predators varies across streams.

3) Fish may have facilitated the feeding of invertebrate predators through the behaviour of their shared invertebrate prey. Usually the presence of fish is associated with a reduction in activity of prey (e.g. Feltmate and Williams, 1989). However, fish may also drive the prey into benthic refugia (e.g. Resetarits, 1991), such as interstitial spaces, and if invertebrate predators occur in these refugia too, their encounter rates with the prey may be higher when fish are present. In Chapter 7, I test whether fish can facilitate prey capture by invertebrate predators, and how this is affected by the availability of a refuge.

In streams with fish, if the individuals that avoid predation and emerge from the stream are in good condition, their reproductive potential may be high (Taylor *et al*, 1998). For predatory stoneflies, for example, only a few gravid females are necessary to repopulate a whole reach, because of the large number of eggs they produce, and the high 'survivorship' of the eggs (Elliott, 1995; Frutiger, 1996; Zwick, 1996). If the fecundity of surviving adults is high, then this may balance the losses of individuals to predation during the developmental life stages. If predation by fish reduced both survivorship and fecundity, as some studies suggest (e.g. Soluk and Collins, 1988a, 1988b; Feltmate and Williams, 1991; Dahl and Greenberg, 1999), then invertebrate predators would be unlikely to persist in streams with fish. This is not the case, even in streams which have no fishless headwaters, nearby fishless streams, or other source of colonists (Bowlby and Roff, 1986).

The condition of the species with a strict nocturnal foraging activity, *D. cephalotes* did not vary across a stream with fish and a stream without fish. This is perhaps because they incur little exposure to salmonid fish when they are active. This may be why their abundance (Chapter 3), size class distribution (Chapter 4), condition and pre-emergent weight did not differ with and without fish. The size-mass coefficients of this species were lower than for other invertebrate predators, consistent with their longer life cycle (Hynes, 1976), and this may reflect their restricted foraging activity patterns, and a lower prey intake rate than other predators (Elliott, 2000). *Dinocras cephalotes* was the only predator that did not show greater pre-emergent weights and/or steeper length-mass slopes in the Chapelhope Burn. If fish facilitated the feeding of invertebrate predators by driving prey into benthic refugia (point 3 above), this effect would have been strongest during the daytime, when fish are active but *D. cephalotes* aren't. Therefore, a positive effect of fish on all predators, but not *D. cephalotes*, was consistent with the facilitation hypothesis. However, if fish affect the relative abundance of food/prey types (point 2 above), *D. cephalotes* may have achieved the same condition on the basis of different diets, or they may have maintained the same diet across streams. Differences in the feeding strategy of *D. cephalotes* vs the other predators, may explain why it showed different patterns to other species in the Chapelhope Burn. This is investigated in Chapter 6.

For some species of invertebrate predators, size-mass relationships differed across streams, and provided a better means of comparison than size alone. The results of this study, like several others, contradicted speculation that the size-mass relationships of stream invertebrates only vary across large geographical scales, due to changes in geology and water chemistry (Smock, 1980; Wenzel *et al*, 1990; Eggert and Burton, 1994). Though differences in size-mass relationships across streams have occurred in other studies, they have only been interpreted in terms of the abiotic environment. The slope of length-mass relationships decreased with increasing acidity for a capnid stonefly (Griffith *et al*, 1993), increased with temperature for a megalopteran (Short *et al*, 1987), and increased with detritus for an amphipod (Gee, 1988; Basset and Glazier, 1995). In contrast, Gonzalez *et al* (2002) obtained different relationships for several species from streams within the same basin, and could not explain the patterns in terms of the abiotic environment. The size-mass relationships of invertebrates reflect biotic factors too (Benke *et al*, 1999), and they may be a valuable tool with which the impacts of fundamental processes on condition, such as predation, can be assessed.

6 The diet of predatory invertebrates across streams

6.1 Introduction

The diet of invertebrate predators, *i.e.* the different food and prey types consumed, may vary across streams with and without fish. Fish can influence the diet of invertebrate predators because, firstly, they can affect the abundance of prey and algae, and thus the availability of these food resources may change in response to fish density. Secondly, behaviour such as increased drifting by prey, inactivity and use of refugia by predators, in response to fish, can alter the encounter rates between invertebrate predators and different prey types, and also affect diet. The quantity and quality of resources acquired by invertebrates are fundamental to their growth and condition. If their diet differs across streams, then nutritional status may be affected too. However, for invertebrate predators with a generalist feeding strategy, the effect of fish, or other factors, on the community may have only minor consequences for nutritional status. For example, in Chapter 5, *Dinocras cephalotes* may have achieved a similar condition across streams on the basis of different diets. In this chapter, I compare the diet of invertebrate predators across 3 streams with fish and a fishless stream. Do fish influence the proportion of different food types, such as prey or algae, in the diet of predatory invertebrates? Do invertebrate predators consume the same invertebrate prey across streams, and does the presence of fish influence this?

Invertebrate predators as omnivores: carnivory vs algivory

Many stream invertebrate predators are omnivores and hence consume a mixture of plant and animal foods (Jones, 1950; Mackereth, 1957; Hynes, 1976; Edington and Hildrew, 1995). Some predatory Plecoptera and Trichoptera predominantly feed on algae and plant-derived detritus in their early instars (e.g. Siegfried and Knight, 1976; Cereghino, 2002), and some consume substantial amounts of algae throughout their life cycle (Martin and Mackay, 1982; Lancaster *et al*, 2005). Nonetheless, the majority of these invertebrates, such as Perlidae, are predominantly carnivorous, and their morphology (large mandibles, wide gape, etc) and foraging behaviour are consistent with a mainly predatory habit (Feminella and Stewart, 1986). It is unclear if they possess the physiological adaptations necessary to process algae, and how much of the algal component of their diet is assimilated into their body tissues. Omnivorous predatory invertebrates derive nutrition from the plant material in their diet in terrestrial ecosystems (Coll and Guershon, 2002) and marine systems (Cruz-Rivera and Hay, 2000), but the nutritional ecology of freshwater invertebrate predators is less well understood. However, a recent study by Lancaster *et al* (2005) shows that some stream invertebrate predators can indeed derive a large part of their nutrition from algae, for example up to 50 % of body nitrogen was of algal origin for the Perlodidae *Isoperla grammatica* and *Perlodes microcephala*. The intake of plant material by invertebrate predators varies temporally (Hynes, 1941; Winterbourn, 1974; Lancaster *et al*, 2005) and across streams (e.g. Allan, 1982b; Malmqvist *et al*, 1991), and if algae are a true food, then these differences in diet indicate invertebrate predators may be able to adapt their feeding strategy according to factors in their environment, such as the presence of fish.

Fish can have a strong positive effect on standing stocks of filamentous algae (Power, 1990; Bechara *et al*, 1993; Rosenfeld, 2000), and invertebrate predators may feed on algae when they are an abundant resource. For example, Siegfried and Knight (1976) observed that periods of high carnivory alternated with periods of high algivory in a predatory stonefly's diet, which may correspond to changes in the relative availability of prey and algae in the benthos. Invertebrate predators may also feed on algae when animal prey is scarce, rather than simply when algae are abundant. If such density-dependant effects occur in streams with fish, then a shift from carnivory in fishless streams to omnivory in streams with fish should occur. The long-term consequences for the growth and fitness of predatory invertebrates are dependant on whether algae constitute a sub-optimal food type, or provide the same level of nutrition as animal prey, for which there is little empirical evidence (Lancaster *et al*, 2005).

Invertebrate predators as carnivores: the relative abundance of prey types in diet

Many predators, across aquatic and terrestrial habitats, are polyphagous with respect to animal prey, and consume different prey types, often as a response to prey density (Menge and Sutherland, 1976). Though stream fish can limit the abundance of some invertebrates, particularly large bodied grazers (Scrimgeour *et al*, 1994), they sometimes also cause an increase in the abundance of other invertebrates, less vulnerable to fish (e.g. Chironomidae, Rosenfeld, 1997; Power, 1990). The relative abundance of potential prey types in the benthos may vary with fish presence/absence, and hence the occurrence and abundance of these prey in the diet of invertebrate predators may also vary.

In some systems, the diet of invertebrate predators does 'track' benthic densities of prey. In a fishless acid stream, the diet of *Plectrocnemia conspersa* (Polycentropodidae: Trichoptera) followed the seasonal abundance of prey (Hildrew and Townsend, 1982). In stony streams with fish, Muotka (1993) observed that the relative abundance of prey in the diet of two Rhyacophilidae (Trichoptera) was similar to the relative abundance of prey in the benthos. In other systems, however, there is no evidence of a direct relationship between the diet of invertebrate predators and the abundance of their prey. The diet of predatory stoneflies did not reflect seasonal prey abundance in fishless headwater streams (Allan, 1982b). Likewise Lucy *et al* (1990) found only weak evidence of a link between prey density in the benthos and in the diet of *Dinocras cephalotes* and *Perla bipunctata* (Plecoptera: Perlidae) in streams with fish. The proportion of a type of prey in a predator's diet may be higher than in the surrounding environment (selectivity, Murdoch, 1969), for example, Perlidae have a clear preference for Baetidae prey (Fuller and Stewart, 1977; Malmqvist and Sjostrom, 1980; Allan, 1982b; Peckarsky and Penton, 1989; Lancaster *et al*, 2005), though the abundance of these prey is often reduced by fish (e.g. trout: Soluk and Richardson, 1997; Peckarsky and McIntosh, 1998; Huhta *et al*, 1999). Hence, some invertebrate predators may select for a prey type irrespective of its benthic density, and thus their diet may be similar across streams with fish and fishless streams.

Invertebrate predators as prey: the effects of predation risk on diet

The availability of prey is dependant on encounter rates between predator and prey (Murdoch and Bence, 1987; Cooper *et al*, 1990), and thus the activity patterns of predators and prey influence diet. Many invertebrate predators reduce the amount of time spent foraging for prey when predation risk by fish is high, e.g. leeches (Dahl and Greenberg, 1997), caddisflies (Otto, 1993) and stoneflies (Soluk, 1993). In streams with fish, Huhta *et al* (1999) observed a reduction of foraging activity in predatory Rhyacophilidae compared to fishless streams, and this led to a lower prey capture rate. For a predatory Plecoptera, Feltmate and Williams (1991) also observed that reduced activity in enclosures with fish was accompanied by a lower prey intake. Furthermore, fish also reduce the activity of the invertebrate prey (Forrester, 1994; Scrimgeour and Culp, 1994; Gido and Matthews, 2001) or can induce drifting (Soluk and Richardson, 1997), and this too affects their encounter rates with invertebrate predators. For example, behavioural changes by mayfly prey in the presence of fish had a strong negative effect on the feeding rate of a predatory stonefly (Peckarsky and McIntosh, 1998). Fish may affect the behaviour of different prey types to different extents (Dahl and Greenberg, 1996): Trout often induce a stronger escape response in swimming prey than crawling prey (Dahl and Greenberg, 1999) and sculpin have a greater impact on the behaviour of crawling prey (Soluk, 1993).

Because of these different behaviour, encounter rates with each potential prey type, and their relative abundance in the diet of invertebrate predators, may differ in streams with fish and fishless streams.

Different prey/food types have different search and handling times, and their capture may be associated with different levels of exposure to fish (Abrams and Matsuda, 1996). When fish are present, invertebrate predators may feed on the prey types whose capture incurs the least risk. These prey, for example, could share the same benthic refugia as the predators (Rahel and Stein, 1988), have a high capture probability, or a low handling time (Peckarsky and Penton, 1989). Several North American predatory stoneflies rank prey preference according to the shortest handling time, rather than their energetic value or abundance, and this may be a fixed response to the presence of fish (Molles and Pietruszka, 1983). The diet of invertebrate predators when fish are present could be independent of the "nutritional reward" of the food they consume (Singer and Bernays, 2003). Hence, for omnivorous predators, feeding on static algae may incur less predation risk than foraging for mobile prey. The increased survival of individuals may mitigate the consequences for condition and fitness of differences in food quality, if algae are a sub-optimal food type. Changes in the diversity of food types and prey types in the diet, in response to the abundance of fish, may allow invertebrate predators to balance the avoidance of fish and their own feeding requirements, and diet may be the underlying mechanism for the trade-off between survival and fitness (Lima and Dill, 1990).

Aims and hypotheses

I studied the diet of predatory invertebrates across 3 streams with fish and a fishless stream using gut content analysis. I characterised dietary diversity of the six most abundant predators (five Plecoptera and one Trichoptera) in terms of pure carnivory, omnivory and pure algivory. I hypothesised that predators would be mainly carnivorous in the fishless site, but omnivory would be higher in streams with fish, as prey may be less abundant, or less active, and predator foraging rates may decrease. I compared the relative abundance of prey types in diet, particularly Baetidae and Chironomidae, as the relative density of these two prey changed across the gradient in fish abundance (Chapter 3). The species of invertebrate predators which showed the greatest variability in their condition (Chapter 5) across streams would display the strongest differences in diet, and species which had similar condition would have similar diets across streams. In particular, I expected a strong difference in the Chapelhope Burn vs other streams because the pre-emergent weights and length-mass slopes were higher in this stream for some predators (Chapter 5). I also constructed a multivariate model to summarise the diet of the species of Perlidae and Perlodidae, and determine whether diet differed more across species or across sites.

6.2 Background: the recorded diet of some predatory invertebrates

Perlidae and Perlodidae

Nymphs of both families are predominantly carnivorous and many benthic taxa have been reported as prey, but diet is invariantly dominated by Chironomidae and Baetidae (Hynes, 1941; Sheldon, 1969; Allan, 1982b; Feminella and Stewart, 1986; Stewart and Stark, 1988; Lucy *et al*, 1990), and Hydropsychidae may be an important prey in some situations (Sheldon, 1969; Johnson, 1983). Plant and mineral matter is also a constituent of gut contents of Perlidae (Hynes, 1941, *P. bipunctata*; Lucy *et al*, 1990, *D. cephalotes*) and Perlodidae (Mackereth, 1957; Sheldon, 1972; Allan, 1982b; Lancaster *et al*, 2005, *Isoperla grammatica*, *Perlodes microcephala*). Several studies indicate a shift from algal and detrital feeding to carnivory over nymphal development (Mackereth, 1957; Siegfried and Knight, 1976; Fuller and Stewart, 1977, 1979; Allan, 1982b). For the Perlidae (but not the Perlodidae), cannibalism by large nymphs on small nymphs is possible due to overlapping multivoltine cohorts and has been reported, particularly for *D. cephalotes* (Lillehammer, 1985; Sjostrom, 1985). A shift in diet from Chironomidae in the smaller instars to Baetidae in the larger instars is also common in both Perlidae (Allan, 1982b; Lucy *et al*, 1990) and Perlodidae (Fuller and Stewart, 1977; Allan, 1982b; Johnson, 1983; Walde and Davies, 1987). Perlidae and Perlodidae engulf prey (Siegfried and Knight, 1976; Hynes, 1976; 1977; Malmqvist and Sjostrom, 1980; Allan, 1982b) but partial prey consumption has been observed in small instars (Brink, 1949, *D. cephalotes*; Minshall and Minshall, 1966, *Isoperla* sp.). Nymphs with empty guts are common in surveys of Perlodidae (Allan, 1982b; Feminella and Stewart, 1986) and Perlidae (Siegfried and Knight, 1976; Allan, 1982b; Lucy *et al*, 1990), accounting for up to 30% of the sample population. This is due partly to periods of no feeding during moulting, as the inner gut lining is also shed (Allan, 1982b). Mature nymphs also cease feeding just prior to emergence, as salivary glands and foreguts start to atrophy (Chisholm, 1962).

Siphonoperla torrentium

This species is generally considered to be detritivorous (Hynes, 1941), but diet does include prey (Mackereth, 1957; Hynes, 1977). Woodward and Hildrew (2001) reported that the diet of *S. torrentium* in an acid forest stream contained fine particulate matter, microcrustaceans, Leuctridae (Plecoptera), and Diptera (Chironomidae and Tipulidae). Similar diets are reported in the North American Chloroperlidae, but some taxa are wholly detritivorous or herbivorous (Stewart and Stark, 1988).

Rhyacophila dorsalis

Diet of Rhyacophilidae varies between species and among individuals, and can consist of detritus, algae or prey, and most are omnivorous (Williams and Williams, 1979). Preferred prey types are Chironomidae, Simuliidae and Baetidae (Thut, 1969; Scott, 1958, *Rhyacophila dorsalis*). In a detailed study of the feeding habits of *R. fuscula*, Martin and Mackay (1982) observed partial prey consumption, the larvae often 'excavating' dipteran pupae and mayfly nymphs, consuming only soft tissues. This makes gut contents hard to identify, often classified as 'detritus' or 'amorphous material' and thus underestimating the true extent of carnivory. Satija (1964, 1974) suggested that selection of soft prey tissue in *R. dorsalis* is a consequence of the absence of proventricules and limited muscularisation of gut tissue, which decreases the ability to digest large prey fragments and sclerites. Nonetheless Rhyacophilidae do engulf small prey items (e.g. *R. dorsalis*, Jones, 1950).

6.3 Methods

6.3.1 Analysis of gut contents

Gut contents were examined for all predators collected during the 2000 benthic survey (Chapter 3), and additional predators collected on the same sampling dates using kick nets. These specimens included those used for the calculation of length mass regressions in Chapter 5. Specimens were preserved in 70% alcohol and removed to the laboratory. The thorax and abdomen of each predator was opened and the foregut dissected from the buccal cavity to the malphigian complex. Gut contents were mounted using Aquamount® and examined at x40 magnification. The entire slide was scanned and prey items were counted and identified on the basis of sclerotised parts, to the lowest taxonomic level possible. The presence of algae, large organic detritus and amorphous fine detritus was recorded.

Individual nymphs and larvae were classified on the basis of gut content as either empty, carnivorous (prey only), omnivorous (prey and non-prey matter) and non-carnivorous (detritus and algae only). The proportion of carnivorous and omnivorous individuals were compared across sites using a χ^2 test for each species of predator. The test was performed in an hierarchical way, firstly testing between all the streams in which the predator occurred. If a difference between streams was detected then gut content frequencies were compared pair-wise between sites. For each species, the critical p-value of the χ^2 test at $\alpha = 0.05$ was adjusted for multiple comparisons by dividing it by the number of test results compared (Bonferroni procedure, Underwood, 1997). For each predator/site the percentage of all prey items belonging to the categories of Baetidae, Chironomidae and Other Taxa was determined. The relative frequency of the three categories in the diet was compared across sites using a χ^2 test for each species of predator.

Total diversity (TD) was determined as the number of different prey types found at least once in the gut contents of a species at a site, all sample dates combined. Mean number of prey types per gut (ID, all sample dates) was calculated as well as the mean number of prey items per gut (ND, all sample dates). The population feeding diversity (PD, the number of different types of prey occuring in the sample population on each sampling occasion meaned over the 6 dates) was also calculated.

6.3.2 Discriminant analysis of diet

Multivariate discriminant analysis was used to separate the species of predatory Plecoptera on the basis of dietary composition. All species of Perlidae and Perlodidae were included, bar *Diura bicaudata* as only 8 individuals were available. Chloroperlidae were only considered as prey in this analysis despite an element of carnivory in their diet, as they were regularly found in the guts of the larger Plecoptera. Each sample consisted of an individual nymph and data consisted of the presence/absence of prey taxa, detritus and algae in the gut contents.

Presence/absence was used as most guts contained one prey item only. The discriminant analysis was based on a canonical correspondence analysis model (Pappas and Stoermer, 1997) using the CANOCO program. Predatory Plecoptera nymphs were grouped and coded according to species and site. The 'species' data set consisted of a binary coded matrix, each sample (row) belonging to only one species/site grouping (columns). The presence/absence matrix of food types in each sample (i.e. each nymph) formed the 'variables' data set, each food or prey type corresponding to a variable. Body measurements (head width, body length, dry mass) were also fitted to the model as linear variables. Preliminary analysis determined that these three variables were collinear (because they are interrelated), and thus only head with was retained and fitted to the model, as it represented the most variation and also is least sensitive to male/female dimorphism in body length (personal observation). Sexual dimorphism is pronounced in the Perlidae, but less so in the Perlodidae (Hynes, 1976). Predation between Perlidae and Perlodidae was ignored because exceedingly rare, thus no species of these two families were included as prey types. Seasonal effects were partialled out by fitting date as a covariable, 'dummy coded' for each sample date. Contribution of each food/prey type to the model was determined by forward selection and tested using Monte-Carlo permutations (α = 0.05, see Chapter 3) and only variables which discriminated between the predator/site groups were retained. Overall significance of the model (α = 0.05) was tested with Monte-Carlo permutations (see Chapter 3). Scores for predators and prey/food types were plotted in ordination space. The axes of this ordination represented linear combinations of prey types that best discriminated between species of predators on the basis of their occurrence in diet.

6.4 Results

6.4.1 Description of diet

Perlidae

Both species were omnivorous, taking prey, filamentous algae and fine organic detritus. Large detritus was never recovered from the foreguts of Perlidae. *Perla bipunctata* was present in the Riskinhope Burn only, where less than 1 % of individuals were wholly herbivorous. Many specimens were purely carnivorous (Figure 6.1), though 30 % had empty guts. Baetidae were the main prey item, accounting for 46 % of all prey, Chironomidae 22 % and other taxa 32 %.

The proportion of omnivorous and carnivorous individuals of *D. cephalotes* differed across the Chapelhope and Riskinhope Burns (Table 6.1), and carnivory was highest in the fishless site and omnivory highest with fish (Figure 6.1). Purely herbivorous individuals were uncommon (< 10 % of guts), and in both sites approximately 30 % of specimens had empty guts. The proportions of Baetidae, Chironomidae and other taxa in the diet (Figure 6.2) did not differ between the two streams (df = 2, $\chi^2 = 2.01$, $p = 0.365$). This species rejected Chironomidae and selected for Baetidae above their benthic density in the Chapelhope Burn, but rejected Baetidae in the Riskinhope Burn (Table 6.2).

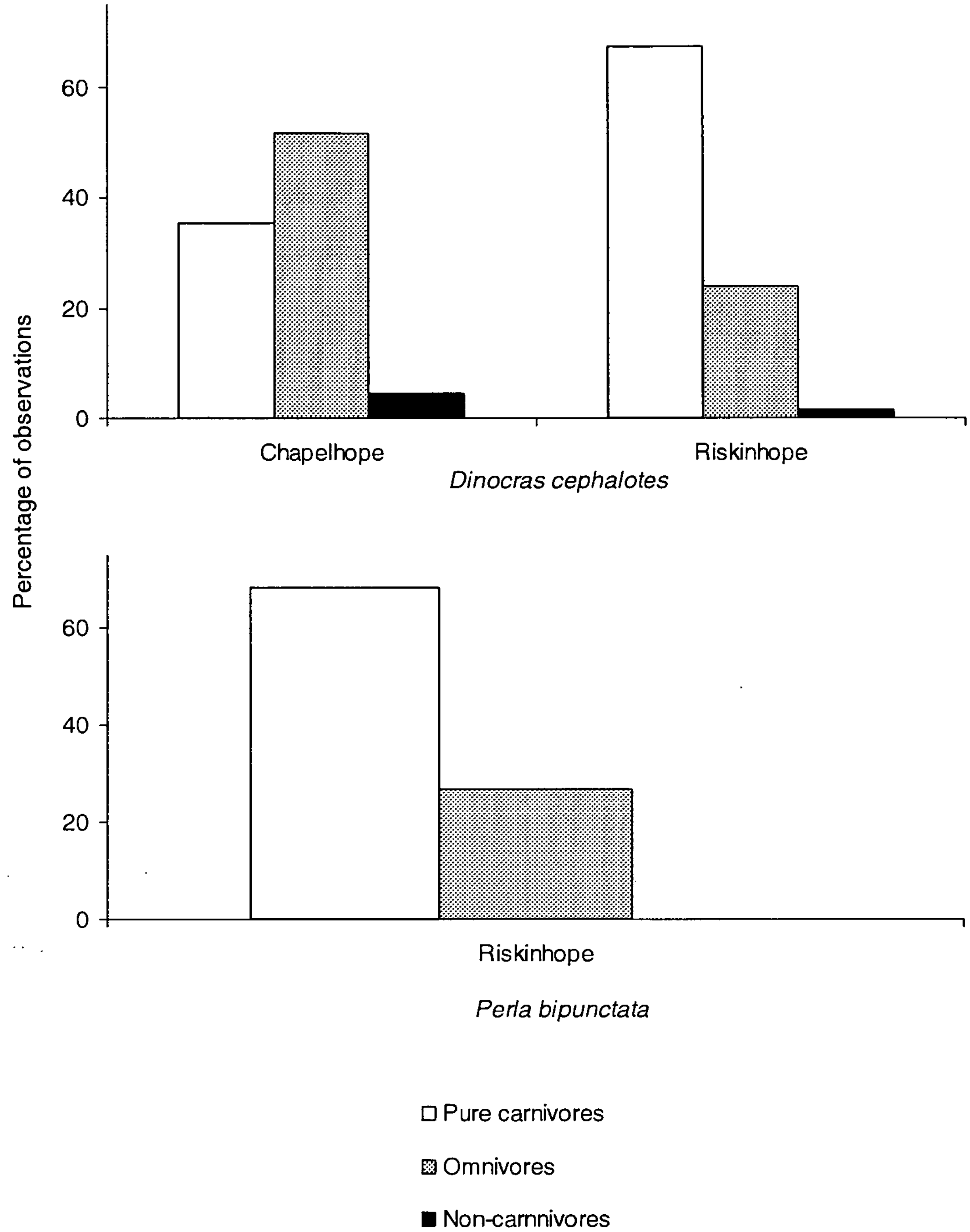

Figure 6 - 1: Dietary composition of two species of Perlidae stoneflies January to October 2000. Bars represent percentage of individuals falling in one of three mutually exclusive categories: omnivores (prey and other material), pure carnivores (prey only) and non-carnivores (algae and organic detritus only). Sample sizes: *D. cephalotes*: Chapelhope, 94, Riskinhope, 97; *P. bipunctata*: Riskinhope, 56.

Table 6 - 1: Results of chi square analyses testing for differences in the frequencies of omnivorous and carnivorous gut contents between streams for 4 predatory Plecoptera and one Trichoptera. Critical p values (α = 0.05) for each predator were adjusted for number of comparisons. Significant differences are highlighted in bold type. Sample sizes are the same as in Figures 6.1, 6.3 and 6.5

		df	χ^2	p
D. cephalotes	Chapelhope v Riskinhope	2	14.47	**0.008**
I. grammatica	All four streams	6	17.34	**0.002**
	Chapelhope v Megget	2	0.70	0.705
	Chapelhope v Talla	2	4.88	0.251
	Chapelhope v Riskinhope	2	13.85	**0.001**
	Megget v Riskinhope	2	11.70	**0.003**
	Talla V Riskinhope	2	6.02	**0.050**
	Megget v Talla	2	2.77	0.251
P. microcephala	Three streams with fish	4	6.45	0.169
S. torrentium	All four streams	6	17.34	**0.002**
	Chapelhope v Megget	2	4.16	0.126
	Chapelhope v Talla	2	0.34	0.841
	Chapelhope v Riskinhope	2	6.63	0.037
	Megget v Riskinhope	2	6.07	0.049
	Talla V Riskinhope	2	6.39	0.041
	Megget v Talla	2	4.98	0.084
R. dorsalis	All four streams	6	5.10	0.532

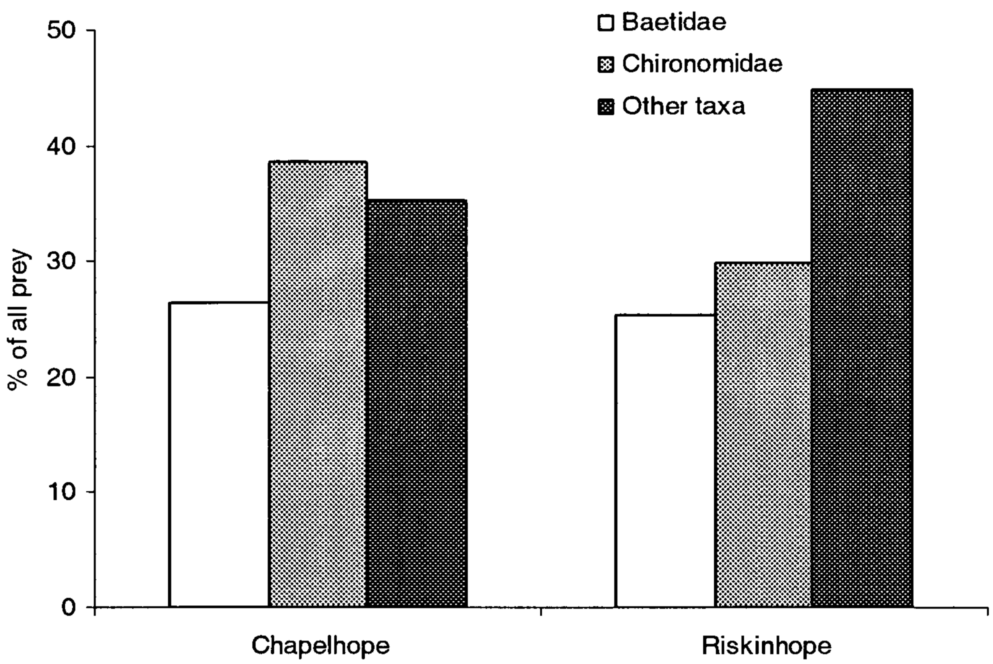

Figure 6 - 2: Percentage of Baetidae, Chironomidae and other taxa in gut contents of *Dinocras cephalotes*. January to October 2000. Total number of prey recovered from guts were 87 in the Chapelhope and 91 in the Riskinhope.

Perlodidae

Isoperla grammatica were predominantly carnivorous in the Riskinhope Burn (Figure 6.3). The relative proportion of carnivorous and omnivorous nymphs differed between the three sites with fish and the Riskinhope only, and the three sites with fish did not differ from one another (Table 6.1). Purely herbivorous nymphs were common, in excess of 20 % of specimens in the sites with fish, but there were no herbivorous specimens in the fishless site. Empty guts accounted for 20 to 30 % of specimens. Diet was principally Chironomidae in sites with fish, and Baetidae and other taxa in the fishless site (Figure 6.4). Proportions of Baetidae in the diet were significantly greater in the Riskinhope Burn than in the Chapelhope or Talla Burns (Table 6.2).

Perlodes microcephala did not occur in the fishless site, and the relative proportion of omnivory and carnivory did not differ across the 3 sites with fish (Figure 6.3, Table 6.1). Purely herbivorous individuals accounted for 20 to 25 % of specimens across the three sites and 15 % of all nymphs had empty guts. There was a clear difference across sites in the proportion of Baetidae, Chironomidae and other taxa in the diet (Figure 6.5), the relative proportion of Baetidae v Chironomidae differed in all three streams (Table 6.2).

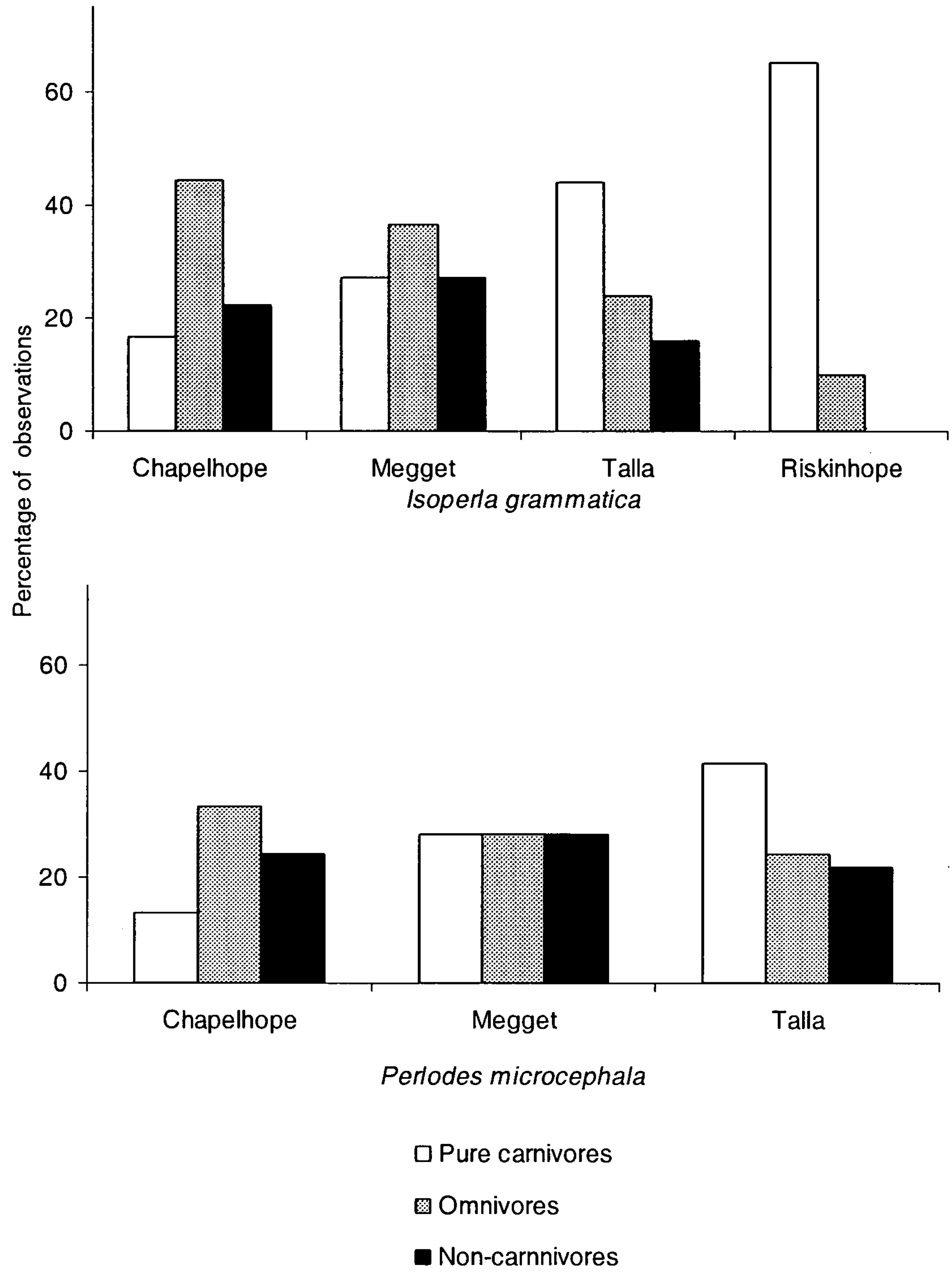

Figure 6 - 3: Dietary composition of two species of Perlodidae stoneflies January to October 2000. Bars represent percentage of individuals falling in one of three mutually exclusive categories: omnivores (prey and other material), pure carnivores (prey only) and non-carnivores (algae and organic detritus only). Sample sizes: *I. grammatica*: Chapelhope, 25, Megget, 25, Talla, 55, Riskinhope, 27; *P. microcephala*: Chapelhope, 52, Megget, 28, Talla, 49.

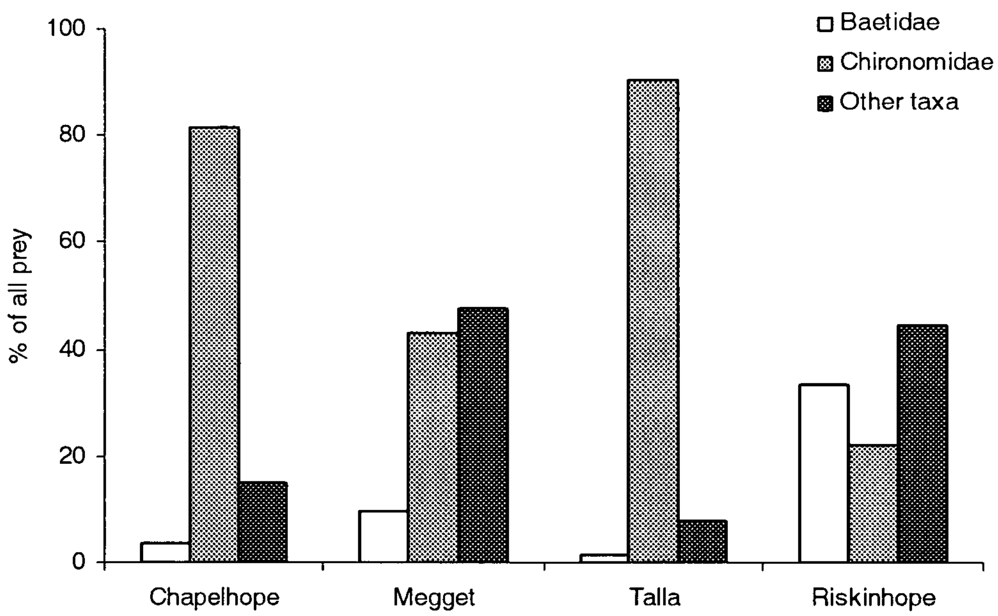

Figure 6 - 4: Percentage of Baetidae, Chironomidae and other taxa in gut contents of *Isoperla grammatica*. January to October 2000. Total number of prey recovered from gut contents were 27 in the Chapelhope, 21 in the Megget, 37 in the Talla and 18 in the Riskinhope.

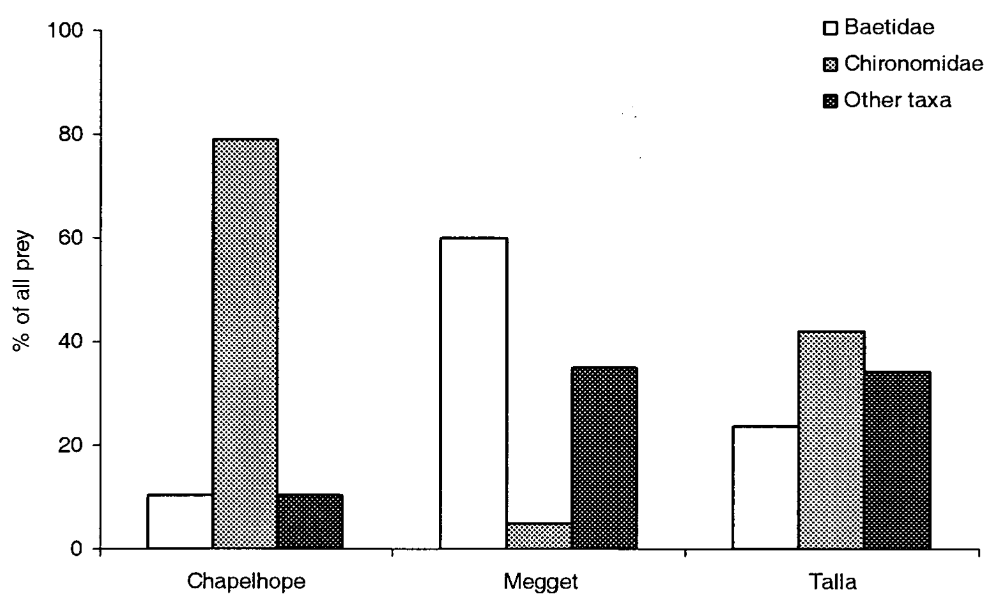

Figure 6 - 5: Percentage of Baetidae, Chironomidae and other taxa in gut contents of *Perlodes microcephala*. January to October 2000. Total number of prey recovered from gut contents were 38 in the Chapelhope, 20 in the Megget, and 38 in the Talla.

Table 6 - 2: Results of chi square analyses testing for differences in the proportion of Baetidae and Chironomidae in gut contents of two Perlodidae between streams. Critical p values ($\alpha = 0.05$) for each predator were adjusted for number of comparisons. Significant differences are highlighted in bold type.

		df	χ^2	**p**
I. grammatica	Chapelhope v Megget	1	1.86	0.173
	Chapelhope v Talla	1	0.49	0.457
	Chapelhope v Riskinhope	1	12.10	**0.001**
	Megget v Riskinhope	1	3.48	0.062
	Talla V Riskinhope	1	26.68	**<0.001**
	Megget v Talla	1	4.46	0.035
P. microcephala	Chapelhope v Megget	1	27.17	**<0.001**
	Chapelhope v Talla	1	4.91	0.027
	Megget v Talla	1	10.97	**0.001**

Other taxa

For *S. torrentium* (Figure 6.6), differences in the prevalence of carnivory vs omnivory were not significant (Table 6.1). Pure herbivory was more common for this species than the other predatory Plecoptera. The number of nymphs with empty guts was variable across sites, ranging from 5 % and 10 % of specimens in the Megget and Talla burns, to over 50 % of specimens in the Riskinhope and Chapelhope Burns.

For *Rhyacophila dorsalis* (Figure 6.6), the relative proportions of carnivory and omnivory were not significantly different (Table 6.1). There were no herbivorous larvae in the Riskinhope Burn. Empty guts were common, accounting approximately for 10 to 25 % of specimens examined. *Rhyacophila dorsalis* consumed principally Chironomidae (Figure 6.7). The relative proportion of Chironomidae vs Baetidae in the diet did not differ across the four sites (df = 2, $\chi^2 = 5.25$, p = 0.073).

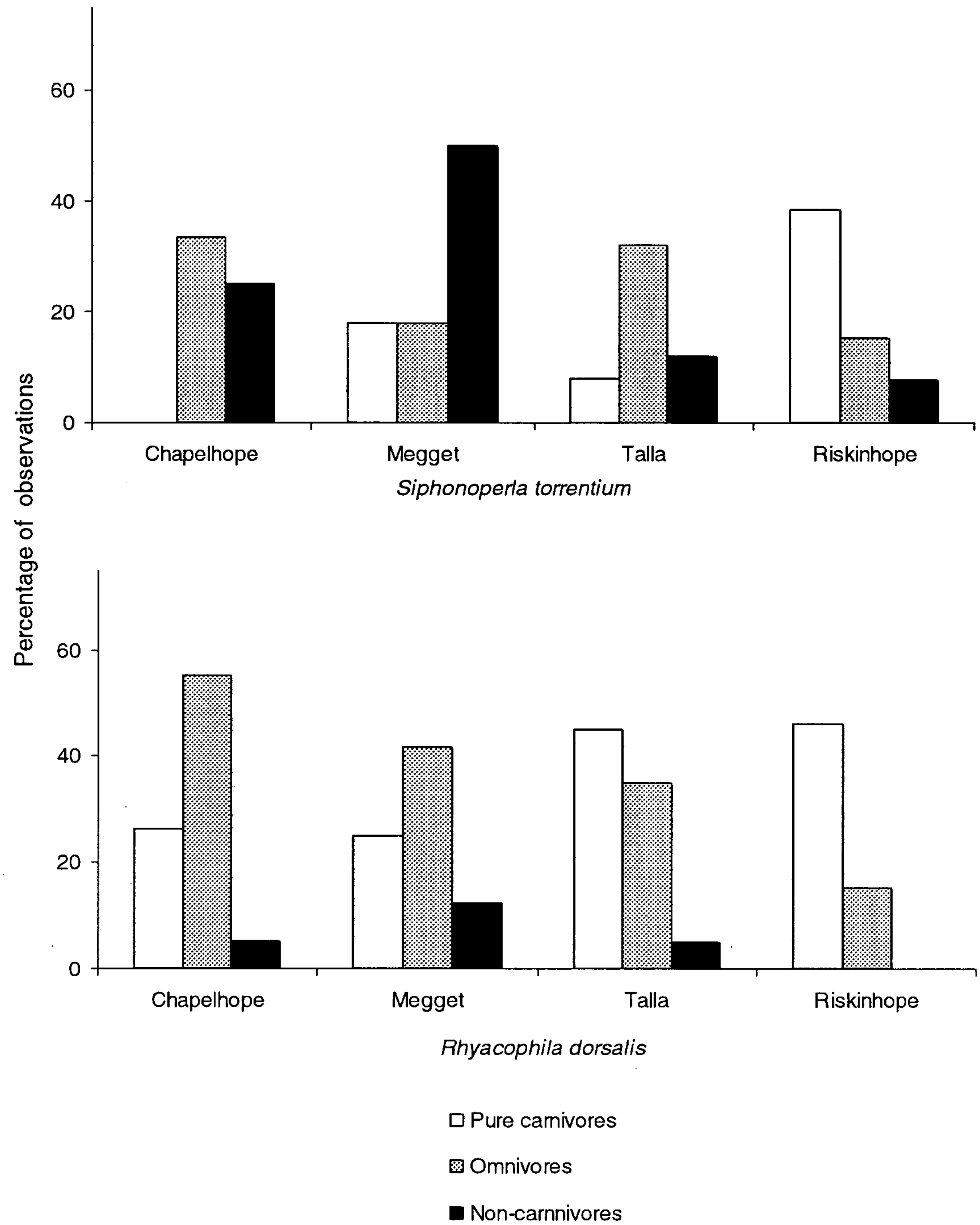

Figure 6 - 6: Dietary composition of, top: *S. torrentium* (Plecoptera, Chloroperlidae), and bottom: *R. dorsalis* (Trichoptera, Rhyacophilidae) January to October 2000. Bars represent percentage of individuals falling in one of three mutually exclusive categories: omnivores (prey and other material), pure carnivores (prey only) and non-carnivores (algae and organic detritus only). Sample sizes: *S. torrentium*: Chapelhope, 24, Megget, 30, Talla, 32, Riskinhope, 28; *R. dorsalis*: Chapelhope, 42, Megget, 31, Talla, 27, Riskinhope, 18.

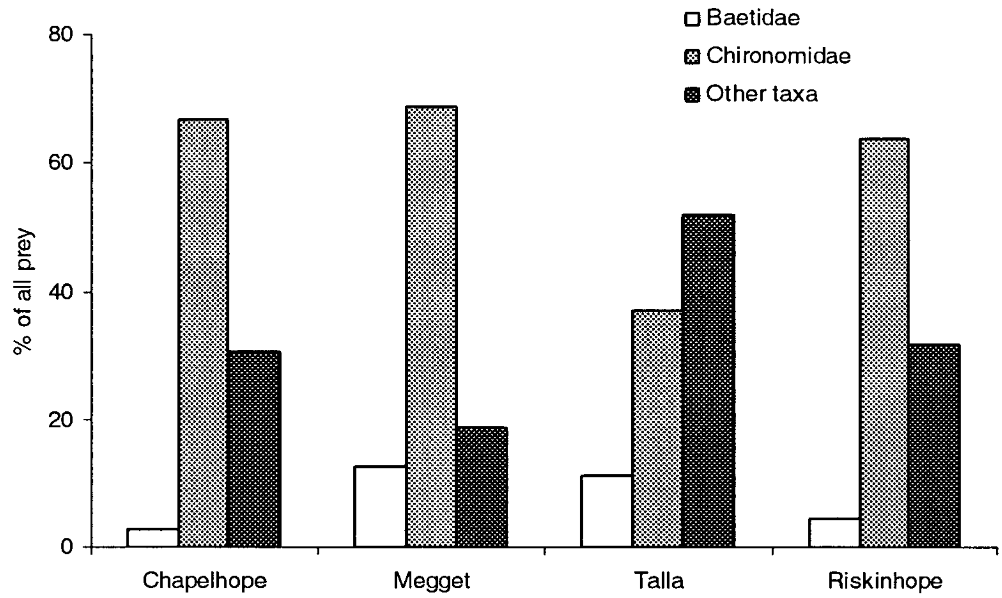

Figure 6 - 7: Percentage of Baetidae, Chironomidae and other taxa in gut contents of *Rhyacophila dorsalis*. January to October 2000. Total number of prey recovered from gut contents were 72 in the Chapelhope, 32 in the Megget, and 27 in the Talla and 22 in the Riskinhope.

6.4.2 Prey diversity in the diet

The total number of prey taxa that occurred in the diet (TD) of the Perlodidae (Table 6.3) was always lower than the TD of Perlidae. TD was similar for Perlidae and *R. dorsalis*, and varied little across the sites. The number of prey items in foreguts (ND) was similar for *P. microcephala* and the Perlidae, ranging from 1 to 2. *Isoperla grammatica* was the only Plecoptera with ND values above 2 in the Chapelhope and Talla, due to nymphs with > 10 Simuliidae in foreguts. ND values for *R. dorsalis* were close to 2, the standard errors overlap indicating no true differences across sites. The number of prey types per foregut (ID) reflected the low ND values and ranged from 1.0 to 1.5 for all taxa. Sample population prey diversity (PD) over the six sample dates (sample populations) were lowest for *R. dorsalis* but ranged from 3 to 5 for all Plecoptera, but error terms obscured patterns across streams.

Table 6 - 3: Total prey diversity over all sampling occasions (TD), mean number of prey per foregut all dates combined (ND ± 1 SE), mean prey diversity per gut (ID ± 1 SE) all dates combined and mean sample population prey diversity (PD ± 1 SE, n = 6 sampling occasions) for four Plecoptera and one Trichoptera.

Species	**Stream**	**TD**	**ND** (± 1 SE)	**ID** (± 1 SE)	**PD** (± 1 SE)
Isoperla grammatica	Chapelhope	**4**	2.9 *(0.74)*	1.1 *(0.09)*	3.0 *(0.00)*
	Megget	**5**	1.9 *(0.52)*	1.2 *(0.10)*	4.0 *(1.00)*
	Talla	**7**	4.6 *(0.86)*	1.0 *(0.03)*	4.5 *(1.50)*
	Riskinhope	**7**	1.2 *(0.12)*	1.1 *(0.07)*	5.0 *(2.00)*
Perlodes microcephala	Chapelhope	**5**	1.8 *(0.27)*	1.1 *(0.06)*	3.5 *(1.50)*
	Megget	**5**	1.1 *(0.08)*	1.1 *(0.08)*	3.5 *(0.50)*
	Talla	**7**	1.8 *(0.25)*	1.6 *(0.24)*	5.0 *(1.00)*
Dinocras cephalotes	Chapelhope	**10**	1.7 *(0.11)*	1.4 *(0.06)*	4.0 *(0.51)*
	Riskinhope	**9**	1.5 *(0.12)*	1.3 *(0.07)*	4.0 *(0.73)*
Perla bipunctata	Riskinhope	**8**	1.3 *(0.08)*	1.2 *(0.07)*	3.5 *(0.56)*
Rhyacophila dorsalis	Chapelhope	**8**	2.3 *(0.52)*	1.2 *(0.07)*	3.3 *(0.33)*
	Megget	**7**	1.9 *(0.38)*	1.1 *(0.09)*	2.0 *(1.00)*
	Talla	**8**	1.8 *(0.56)*	1.1 *(0.07)*	2.3 *(1.45)*
	Riskinhope	**8**	2.5 *(0.50)*	1.5 *(0.18)*	3.0 *(1.15)*

6.4.3 Intraguild predation

There was no predation between the two Perlidae and the large Perlodidae. In each of these, the smaller *I. grammatica* occurred very occasionally in the diet (1 to 3 specimens recovered for each species across streams and sample dates). *Rhyacophila dorsalis* occurred in the diet of *P. bipunctata* only. The chloroperlid *Siphonoperla torrentium* occurred in the diet of all other Plecoptera/Trichoptera predators, and was found in their gut contents in every stream and at every sample date, though it accounted for only a small proportion of the consumed prey (< 5 %). *Siphonoperla torrentium* did not consume any of the other Plecoptera or Trichoptera predators. All predators consumed Tanypodinae midge larvae, which are predatory.

6.4.4 Multivariate discriminant of diet

The multivariate model was significant (Table 6.4), and the date covariate accounted for 7.7 % of total inertia ($0.69 \times 100 / 9.00$). Permutation tests indicated predator head width was a significant variable and retained 8 significant prey/food types (Table 6.5). Head width was better correlated to the second axis than the first (Figure 6.8). Centroids for the food/prey types Baetidae, Chironomidae, Leuctridae and filamentous algae were close to the origin, indicating they were common in the diet of all predators. Three predator groups were distinct in the ordination space: one formed by *Perla bipunctata*, one formed by the *Isoperla grammatica* centroids from the four streams and another formed by *Perlodes microcephala* and *Dinocras cephalotes*.

Present in only the fishless stream *Perla bipunctata*, was separated from the other three predators along the first axis, due to the presence of prey such as Rhyacophilidae, Tipulidae and Hydropsychidae in its diet. The centroids of *Isoperla grammatica* were negatively related to head width on both axes, reflecting the size difference between this species and the three large Plecoptera. The position of the Chironomidae centroid with respect to the *I. grammatica* centroids indicated the prevalence of these in the diet. *Perlodes microcephala* and *Dinocras cephalotes* were closest in the ordination space indicating similar diets and were correlated to the second axis. The spread of centroids for these two species indicated a shift in prey with increasing body size from Chironomidae to include Baetidae, Leuctridae and Chloroperlidae, and this why they were distinct from the *I. grammatica* group. The centroid for *P. microcephala* in the Chapelhope Burn was the most distant from the other centroids in the *D. cephalotes* and *P. microcephala* group, and this was the only stream in which the two species actually co-occurred. The diet of *P. microcephala* in this stream appeared to contain more algae and Chloroperlidae than the other predator/site combinations.

Table 6 - 4: Results of CCA ordination used to discriminate between predators on basis of diet, and results of permutation tests for significance of first two axes.

Axes	**1**	**2**	**3**	**4**	**Overall**
Eigenvalues	0.580	0.258	0.179	0.036	
Species-environment correlation	0.77	0.58	0.44	0.20	
Cumulative percentage variance:					
Of species data	7.0	10.1	12.2	13.2	
Of species-environment relation	48.6	70.7	85.1	91.8	
Total inertia					9.00
Covariate inertia					0.69
Sum of unconstrained eigenvalues					8.31
Sum of canonical eigenvalues					1.19
Significance of axes test:					
F-ratio	36.03	6.20			
p-value	0.01	0.01			

Table 6 - 5: Results of forward selection of variables in order of inclusion to the discriminant analysis model: additional variance explained by the variable when added to the model (λ), and significance of the variable (F ratio and p value) determined Monte Carlo permutation tests.

Variables	λ	F	P
Predator head width	0.29	18.09	0.01
Tipulidae	0.21	12.74	0.01
Filamentous algae	0.17	11.09	0.01
Hydropsychidae	0.12	7.94	0.01
Rhyacophilidae	0.12	7.79	0.01
Baetidae	0.07	4.33	0.01
Chironomidae	0.05	3.67	0.01
Leuctridae	0.03	2.07	0.04
Detritus	0.03	2.03	0.02

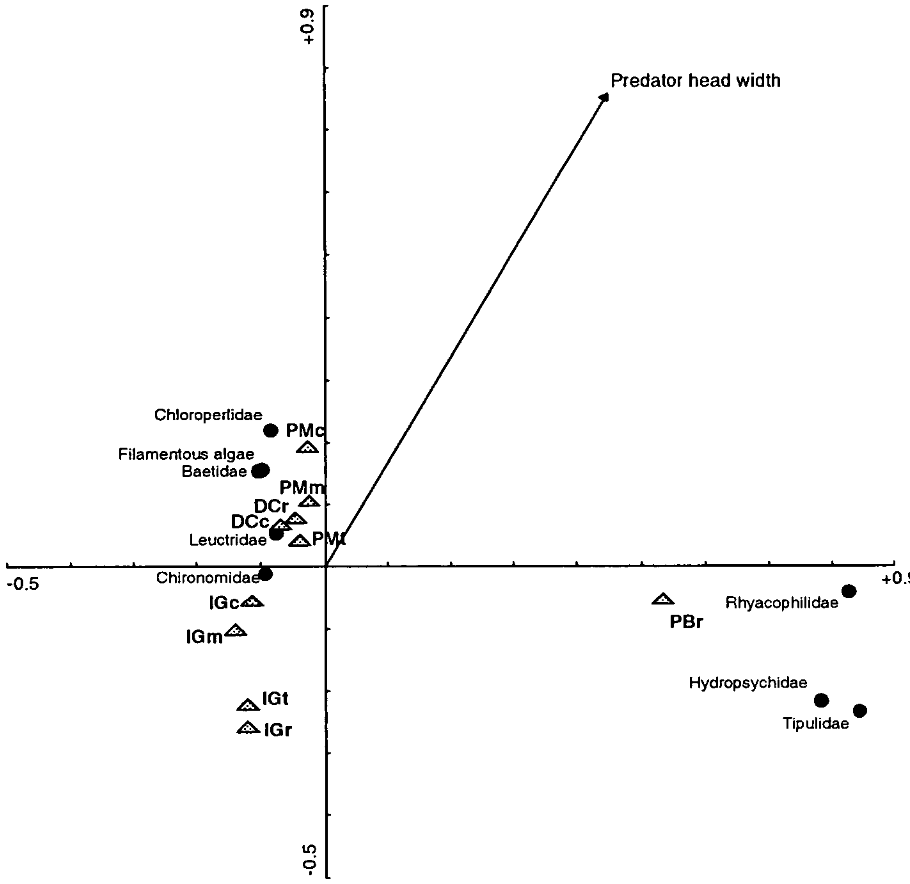

Figure 6 - 8: Ordination based on CCA used for discriminant analysis between species of predatory Plecoptera based on their diet. Circles represent food/prey type centroids, Triangles represent predator/site group centroids. Key: c = Chapelhope Burn, m = Megget Burn, t = Talla Burn, r = Riskinhope Burn, DC = *Dinocras cephalotes*, PB = *Perla bipunctata*, PM = *Perlodes microcephala*, IG = *Isoperla grammatica*.

6.5 Discussion

The presence or absence of Fish was accompanied by a shift in diet from carnivory to omnivory for two species of predators only. The presence of algae in diet was higher in streams with fish for *I. grammatica* and *D. cephalotes*, and guts with prey only were less frequent. For all other predators the relative occurrence of pure carnivores and omnivores did not change with and without fish, or across streams with fish. Some species were more likely to ingest algae than others. In particular, algivory was considerable less frequent for Perlidae than for Perlodidae and Rhyacophilidae, and this is consistent with the observations of Lancaster *et al* (2005) for the same assemblage of species, albeit at a different location.

Differences in the consumption of algae vs prey could not be linked to the patterns in condition observed in Chapter 5. Though some of the length-mass relationships of *Perlodes microcephala* and *Rhyacophila dorsalis* varied across sites, their diet did not differ. In contrast *D. cephalotes* showed no difference in condition across sites, despite a more carnivorous diet in the fishless Riskinhope Burn. Only *I. grammatica* showed differences in both diet and condition, but indicated increased carnivory in the fishless site vs other sites, and better condition in the site with most fish vs other sites. The hypothesis that differences in condition were due to differences in diet was rejected, but this lack of effect has important implications for the feeding ecology of invertebrate predators.

Algae cannot be a sub-optimal food type for Perlodidae and Rhyacophilidae, considering they were a major component of their diet, and changes in the proportion of prey and algae in the diet did not reduce condition. In terrestrial and marine systems, food quality (C:N ratio) often increases along the food chain, and animal tissue often provides better nutrition to an omnivore than plant tissue does (Hastings and Conrad, 1979; Hairston and Hairston, 1993; Elser *et al*, 2000; Diehl, 2003). However, freshwater algae have little structural tissue, and their C:N ratio can be similar to that of animal tissue (Adams and Sterner, 2000). It is clear that Perlodidae and Rhyacophilidae do assimilate the algae they eat (Lancaster *et al*, 2005), and thus they may be able to switch between prey and algal food according to availability (Menge and Sutherland, 1976) or encounter rate (Murdoch and Bence, 1987), if both are optimal food types.

The flexibility of an omnivorous diet is an advantage if the abundance of algae and prey changes with the abundance of fish, as it sometimes does (Power, 1990; Rosenfeld, 2000). However, the true cost of carnivory vs algivory in diet, in terms of predation risk by fish, is dependant on the exposure incurred during foraging. The assimilation efficiency of algae by invertebrate predators is assumed to be considerably lower than that of animal prey (Benke and Wallace, 1997; Hall *et al*, 2000; Benke *et al*, 2001) and larger quantities, and thus longer foraging, may be required to derive the same nutrition. For the Perlodidae, Feminella and Stewart (1986) suggest that handling times for algae may be longer than for prey, which are quickly engulfed (Peckarsky and Penton, 1989; Elliott, 2000). On the other hand, prey are not always successfully captured (Peckarsky, 1980), and may be encountered less often when fish are present (Huhta *et al*, 1999). If predation risk by fish increases with foraging time, the selection of prey or algal food by some predators may reflect the basic need to avoid fish and survive, rather than the need to optimise long-term fitness (Lima and Dill, 1990). Identifying the costs and benefits of omnivory for these invertebrate predators is impossible until the relative nutritional value of algae and prey are known, and foraging rates on algae are quantified and compared to prey capture rates.

The consumption of algae may not be a true source of food for all predators. Though *D. cephalotes* do not assimilate algae (Lancaster *et al*, 2005), they were omnivorous, as other surveys indicate (Hynes, 1941; Lucy *et al*, 1990). And though their condition did not differ with and without fish (Chapter 5), there were more pure carnivores in the fishless site. Why should this species consume algae at all, and why should they consume more when fish are present? In some terrestrial invertebrates, mixing of food types is often necessary for complete nutrition (Gillespie and McGregor, 2000), and perhaps *D. cephalotes* derive trace nutrients, such as metals or minerals, from algae, that are essential for development but are not found in their animal prey. Also, some invertebrate predators may consume algae to increase gut fullness (and thus the surface area available for digestion) and facilitate clearance of indigestible prey sclerites from the gut, in the same way that many terrestrial omnivores consume large quantities of roughage but do not assimilate it. Neither of these hypotheses can be supported from field data, and furthermore they do not explain why *D. cephalotes* consumed less algae in the fishless site. The intake of algae may be 'accidental' and occur when prey, themselves feeding on algae, are captured (Sheldon, 1980; Allan, 1982b; Power *et al*, 1992). The quantity of algae ingested will depend on the extent to which the prey is associated with the algae, for example, Baetidae graze 'on' algae, whereas Chironominae/Orthocladinae often live 'inside' tufts of algal filaments (Power, 1990). The proportion of Baetidae and Chironominae/Orthocladinae in diet did not vary with the presence of fish for *D. cephalotes*, and thus the proportion of algae in diet could not be explained by this hypothesis either.

The presence/absence of fish did not affect the diet of species with either fixed or a flexible foraging strategies. Perlidae may avoid fish through a strictly nocturnal habit (Elliott, 2000), and thus foraging rates may be the same with and without fish. They have a two to three year life cycle (Hynes, 1976), and thus they may tolerate periods of low food intake, and grow more slowly, like some odonates (Macan, 1977). By contrast the Perlodidae *P. microcephala* attains the same size as the Perlidae but in one year only (Hynes, 1976), and their energetic requirements for growth are greater. They feed by day and night (Elliott, 2000), and therefore have considerably longer foraging times than Perlidae. Exposure to fish may increase with foraging time, and a generalist diet, which includes algae and prey according to their abundance, may be the most efficient feeding strategy (i.e. less search and handling time) to satisfy the high energetic requirement, yet limit the risk of predation (Abrahams and Dill, 1989).

The predominant effect of fish on the diet of invertebrate predators may be indirect, sublethal, and mediated by the behaviour of shared prey in response to predation risk.

Soluk and Collins (1988a, 1988b), Dahl and Greenberg (1997, 1999) and Peckarsky and McIntosh (1998) identified indirect effects of fish on the feeding of invertebrate predators *via* their prey. However, these effects had a negative impact on invertebrate predator fitness. In my survey, there was no evidence the presence (or absence) of fish limited foraging rates, compromised nutrition or was detrimental to condition. Complex behavioural interactions between top and intermediate predators that share the same prey have been reported from marine (Paine, 1980) and terrestrial (Holt and Lawton, 1994; Polis *et al*, 1989) systems, and they may be widespread in stream systems too (Wooster, 1994; Sih *et al*, 1998). In Chapter 7, I examine how the behaviour of prey, invertebrate predators and fish affect their respective feeding rates.

7 Feeding interactions between vertebrate and invertebrate benthic predators

7.1 Introduction

Fish may have a positive effect on the condition of invertebrate predators, if they increase the encounter rates between invertebrate predators and their prey. *Perlodes microcephala* may incur the strongest reductions in foraging activity when fish are present. They are active during the day (Hynes, 1976), when fish are active, and have higher foraging rates than other predators (Elliott, 2000), due to a high energetic requirement (large size, one year life-cycle), therefore their exposure to fish may be high. However, previous chapters did not identify any effects of fish presence on their abundance, condition and diet. Fish can affect the behaviour of the shared prey (Peckarsky and McIntosh, 1998; Dahl, 1998a, 1998b), and in stony streams, may drive prey into interstitial spaces, where they encounter invertebrate predators such as *P. microcephala* (Rahel and Stein, 1988; Soluk and Richardson, 1997). Hence, in streams with fish, the decrease in daytime activity by *P. microcephala* may be minor, because they can forage in interstitial spaces, and they may benefit from increased encounter rates with prey in these refugia (Peacor, 2002). Furthermore, feeding by the stonefly may also affect the prey capture rates of the fish, through changes in prey abundance or behaviour (Soluk, 1993), and the interactions between the two predators may have strong impacts on the abundance of prey across streams. In this chapter, I test whether fish facilitate the prey capture by *P. microcephala*, and how this is affected by refugia and prey density. I also examine the consequences for the feeding rates of the fish, and the abundance of the prey.

Facilitation, or interference, can take place between two predators that share a prey, i.e. prey capture rates can respectively increase, or decrease, when the predators occur together. This is often mediated by changes in the behaviour of the prey in response to the different sources of predation risk (Resetarits, 1991). The diet of stream fish and invertebrate predators overlap, and the behaviour of prey reflects the avoidance of both types of predator. Invertebrate predators sometimes facilitate prey capture by fish because they induce an escape response in the prey, which increases their detection by fish (Soluk and Richardson, 1997). On the other hand, fish reduce the daytime activity of many stream invertebrates, (Culp *et al*, 1991; Flecker, 1992; McIntosh and Townsend, 1996), and this can reduce their availability to invertebrate predators (Dahl and Greenberg, 1997; Peckarsky and McIntosh, 1998). The prey capture rates of invertebrate predators are hence dependant on their own behavioural response to fish, the behavioural response they induce their prey, and the behavioural response of prey to the fish (Sih *et al*, 1998). It is unclear how these effects combine, and what the net effect is for invertebrate feeding rates, but in some situations, fish can increase the prey capture rates of invertebrate predators.

The stony substrate in my study streams may have allowed a facilitation effect of fish on the feeding rates of *P. microcephala* to occur, because it provided refugia from fish for both invertebrate predators and their prey. The availability of refugia can contribute to feeding interactions between predators. Rahel and Stein (1988) described how small stream fish (the prey) spent more time in crevices when larger piscivorous fish were present. However, this behaviour increased their encounters with crayfish (the other predator) which also used crevices as a refugium from large fish. Benthic refugia, such as interstitial spaces and crevices provide a foraging space free from fish, and this may be advantageous to a daytime active species such *P. microcephala*. Elliott (2000), in laboratory experiments without fish, observed that this species foraged within the substrate during the day, and only occupied the surface of stones at night. Because fish reduce prey activity and increase their use of refugia, the abundance of prey in interstitial spaces may be higher in streams with fish. Hence, if *P. microcephala* forage in these spaces during the daytime, they may encounter more prey when fish are present.

The use of interstitial spaces by invertebrate predators may also affect the feeding rates of the fish. To use the same example, Rahel and Stein (1988) observed that the presence of crayfish in crevices drove small fish out of these refugia, into the open water, which benefited the feeding rates of large piscivorous fish. Likewise, *P. microcephala* may have the same effect on prey, forcing them out of interstitials spaces onto the top of stones, where they are more vulnerable to fish. Soluk and Richardson (1997) showed that predatory stonefly nymphs could facilitate the prey capture rates of trout, because they caused Baetidae mayflies to leave the stony substrate, and thus increased their encounters with trout. If the behavioural responses of the prey to the two types of predator conflict, then prey behaviour is a compromise between avoiding fish and avoiding invertebrate predators (Lima and Dill, 1990), and they are never free from the risk of predation, i.e. avoidance of one predator increases exposure to the other predator. Hence, mutual facilitation between fish and invertebrate predators may occur, and this may help both predators maintain their food intake, for example when prey abundance is low.

Cottidae may be better suited than salmonids to manipulative experiments. Trout forage on the benthos, in the pelagium and at the water surface (Elliott, 1976, 1978). They are highly mobile, and thus, present a diffuse risk of predation in streams, which is concentrated into a small area in experiments. Cottidae, on the other hand, reside and feed in the benthos. They are daytime active, though they tolerate lower light intensities than salmonids (Welton *et al*, 1991). They ambush invertebrate prey, including predatory stoneflies, at the sides of large stones (Dahl, 1998a), with which their distribution is associated (Wheeler, 1977), like the distribution of invertebrate predators may also be (Chapter 4). As Cottidae are virtually sedentary (Smyly, 1957), they present a localised source of predation risk in streams, and so they may be better suited to the small scale of experimental manipulations. If a facilitation of feeding rates occurs between fish and invertebrate predators, because of prey migration in and out of benthic refugia, then this effect should be stronger when the fish are Cottidae (e.g. bullheads, *Cottus gobio*), than when the fish are Salmonidae. If bullheads cause no such facilitation, or none can be detected, then it is unlikely that any would be detected using trout *i.e.* If bullheads have no effect on the feeding of *P. microcephala*, then it is unlikely that salmonids will. Bullheads are easier to keep and handle than trout for experiments, and they have been used in laboratory studies to predict the impacts of salmonids on natural stream communities successfully (e.g. Soluk, 1993; Soluk and Richardson, 1997).

Feeding interactions such as facilitation, or interference, may contribute to the patterns in the abundance of invertebrate prey across streams with fish. If facilitation occurs between fish and predatory stoneflies, their effects on prey are synergistic i.e. the impact of the two predators on prey abundance will be greater than the sum of their separate effects. If interference occurs, the impact on prey abundance will be less. Hence, the impact of fish on the stream invertebrate community may be dependant on their interaction with invertebrate predators, as it is in other freshwater systems (e.g. Carpenter *et al*, 1987; Power, 1990), and may be mediated by the behavioural response of invertebrate prey.

I carried out experiments to examine whether fish can facilitate the feeding of invertebrate predators. I used *P. microcephala* because they were expected to be the most sensitive species to fish, because they are active during the day and have high foraging rates (Elliott, 2000). Their condition, like several other species, varied across the streams with fish and this may indicate effects of fish on feeding rates. I used bullheads because they were practical and they were more likely than trout to generate feeding interactions with invertebrate predators, due to their benthic habit.

The null hypothesis was that feeding rates of the predators separately and combined do not differ. Facilitation occurred if the prey capture rate was greater when in the presence of the other predator (facilitation). Interference occurred when prey capture rate was lower (interference). In terms of prey populations, a null hypothesis of simple additivity in the effects of the predators was tested. If additivity occurred, the number of prey captured in combined predator treatments was the same as the number of prey captured in the two separate predator treatments. Refugia were constrained to a small standard area, sufficient to provide shelter for the stonefly nymphs. This eliminated variation in available safe foraging area between treatments and replicates, an unknown quantity in natural cobble substrata. Prey were thus faced with a choice between encountering the stonefly in the refugium or the fish in the open area, and did not have alternative refugia.

The hypotheses were tested at two different prey densities and in the presence and absence of the refugium. The results of other studies suggested that increased prey density would increase prey capture rates of the stonefly (Elliot, 2003a), and promote facilitation (Soluk and Collins, 1988a; Soluk, 1993). The absence of refugia was expected to raise predation by sculpins on stonefly nymphs. If prey preferentially avoided the stonefly and the refugium, a weak effect of refugium presence/absence on prey survival was expected. If prey used the refugium to avoid the fish, the absence of the refugium was expected to increase prey capture by the fish, and lower prey capture by the stonefly.

7.2 Methods

7.2.1 Experiments

Three separate experiments were carried out consecutively over a period of approximately one month during April 2003 and monitored the prey capture rates of bullheads and *P. microcephala* in experimental arenas. The response variable was the number of prey eaten by each predator. All three experiments had three predator treatments (one fish only, one stonefly only, one of each). Prey densities were 10 individuals per arena (equivalent to 120 individuals per m^2) in Experiment 1, and 20 individuals (240 per m^2) in Experiments 2 and 3. These densities are within the range observed in the field (Chapter 3), and hence are "realistic". A refugium was provided in Experiments 1 and 2, but not in Experiment 3.

Experiments took place in an unheated outdoor facility, with a translucent roof that allowed a natural day-night light cycle. Feeding trials were carried out in plastic trays with a bottom area of ~ 0.085 m^2 (32 $\times$ 26 cm) filled with dechlorinated tap water, circulation was provided by a pump and air stone. Trays were lined with a thin layer of washed gravel (particle size 2 to 3 mm), providing a semi-natural substrate but devoid of hiding places for invertebrates. Refugia consisted of a brown, unglazed, clay tile (10 $\times$ 5 cm) placed in the middle of the tray. The refugia were raised 1 to 2 mm above the gravel substrate by piling gravel at the four corners of the tiles. Trays, tiles or gravel used in trials with fish were never used for treatments without fish, avoiding contamination with chemical cues, a possible confounding factor in this type of experiment (e.g. Soluk, 1988a, 1988b, 1993).

Predatory fish were bullheads (*Cottus gobio*), of which thirty specimens, ranging from 64 to 86 mm body length were collected from the Braid Burn, Midlothian, in March 2003 using kick sampling. Fish were kept in an aerated holding tank containing a mixture of gravel and cobbles and fed regularly with freshly collected Diptera larvae and Ephemeroptera nymphs. Fish were selected at random from the available stock a day before the trials and placed in trays, set up as experimental arenas, but devoid of invertebrates. This allowed the fish to acclimatise to the trays and avoided the effect of prior satiation on feeding rates during the trials.

Nymphs of the perlodid stonefly *P. microcephala* were collected as required from the Braid Burn during April 2003 using kick sampling. Stoneflies were kept in groups of ten in holding trays, similar to experimental trays (see below), with natural substrate and an excess of Baetidae larvae (> 200 individuals per tray). Before each trial stoneflies were selected at random from the available stock and kept in similar trays, but starved for 3 days prior to the start of the trial, allowing the gut to clear (see Chapter 4). Mean head width of nymphs did not differ between Experiments 1 and 2 (3.8 and 3.9 mm respectively, one tailed t-test, $\alpha = 0.05$, $t = -1.16$, $p = 0.130$) or between Experiments 2 and 3 (3.9 and 4.0 mm respectively, $t = 1.45$, $p = 0.081$). Head width nonetheless differed between Experiments 1 and 3 ($t = -2.08$, $p = 0.025$).

The prey species were Baetidae nymphs (> 90% *B. rhodani*) collected as required from the Braid Burn during April 2003. These were selected as prey because they were abundant at the study sites (Chapters 3), and were an important part of the diet of *P. microcephala* nymphs (Chapter 6). Baetidae nymphs were all of the same size class (1 - 2 mm head width). Mayflies for a trial were collected the day prior to the trial and immediately assigned to an experimental tray. Prey thus had a day to acclimatise to experimental arenas.

Each experiment consisted of two replicated 24 hour trials, carried out 2 days apart. Each trial consisted of 5 replicates of each predator treatment, randomly assigned to a bank of 15 experimental trays. The total number of replicates for each treatment was thus 10 in all three experiments. Separate experiments took place approximately 1 week apart. Five predator-free controls (no predators, 20 prey) were carried out prior to experiments to identify errors due to miscounts in recovering prey. Recovery was very high, 97% of *Baetis* nymphs were recovered, so treatments with no predators were not included in the experiments.

At the start of the trial, at mid-day, the pre-selected predators were placed in the experimental trays. The stonefly nymph was placed first, on top of the tile refugium and allowed to crawl beneath it, which occurred in 100% of cases. The fish was then added, always in the top left corner of the tray. Predators were left to feed undisturbed for 24 hours then removed. Bullheads were returned to their holding tank for subsequent return to the stream; stonefly nymphs were killed and preserved in

70% alcohol. Trays were carefully searched for remaining prey both alive or dead. Surviving prey were not re-used in trials to avoid effects of conditioning. Stonefly nymphs were measured (head width to 0.1 mm) and gut contents were examined in the laboratory and number of prey items counted. The number of prey consumed by bullheads was derived from the number of prey remaining and the number of prey consumed by the stoneflies.

7.2.2 Data analysis

The prey consumption rate of each predator type was compared across Experiments 1 and 2 (fixed factors: presence of other predator, prey density), and Experiments 2 and 3 (presence of other predator, presence of refugium) by two-way ANOVA. Data were $\sqrt{(x + 0.5)}$ transformed to satisfy assumptions of normality and homoscedasticity. The absence of an experiment with prey density of 10 and no refugium precluded 3-way analysis. This was because experiments 1 and 2, and 2 and 3, were one week apart, but experiments 1 and 3 were separated by two weeks, and thus two 2-way ANOVA reduced the potential confounding influence of developmental stage of the stoneflies.

The prey consumption of each predator separately were used to calculate expected combined consumption, if the impact of predators is purely additive, using the model:

$$C_{fs} = N_p P_{fs} \qquad \text{Equation 7.1}$$

And:

$$P_{fs} = P_f + P_s - P_f P_s \qquad \text{Equation 7.2}$$

Where C_{fs} is the expected prey consumption of predators combined for a prey density of N_p assuming no interference/facilitation occurs between the predators. P_f and P_s are the proportion of prey consumed by the fish or the stonefly respectively in the single predator trials. P_{fs} is the expected proportion of prey consumed by fish and stonefly when together. This model assumes a lack of independence in prey capture probabilities, i.e., the expected combined consumption must reflect the fact that the same prey cannot be eaten twice (Wilbur and Fauth, 1990; Soluk, 1993). The model was calculated using observed mean values of P_f and P_s. The error associated with P_{fs} was calculated from the standard error of these observed means using the propagation of errors method described by Parratt (1961, p. 37) and Squires (1968, pp. 116-117) and given by Equation 7.3 and. Equation 7.4 where Δ represents standard errors:

$$\Delta P_f P_s = P_f P_s \bullet \sqrt{\left(\frac{\Delta P_f}{P_f}\right)^2 + \left(\frac{\Delta P_s}{P_s}\right)^2} \qquad \text{Equation 7.3}$$

$$\Delta P_{fs} = \sqrt{(\Delta P_f)^2 + (\Delta P_s)^2 + (\Delta P_f P_s)^2} \qquad \text{Equation 7.4}$$

One sample *t*-tests for each experiment (two tailed, $\alpha = 0.05$) were used to compare the derived value of C_{fs} to the mean observed consumption in the combined predator trials n = (10). Facilitation occurred when observed > expected, interference occurred if expected > observed. No difference between observed and expected (null hypothesis) corresponded to simple additivity in predator impacts, i.e., no or neutral fish/stonefly interaction. Probability values were adjusted for multiple comparisons using the non-sequential Bonferroni procedure, i.e. critical $p = \alpha / 3$, which is better suited to the comparison of a small number of means (Zar, 1996).

7.3 Results

Stonefly prey capture rates

The presence of bullheads decreased the number of prey captured by *P. microcephala* in all three experiments (Figure 7.1). This was a highly significant effect across low and high prey densities (Table 7.1) and with or without a refugium (Table 7.2). The null hypothesis of neutral interaction was thus rejected for the stonefly. At the low prey density, stoneflies captured virtually no prey when fish were present. As expected, higher prey density increased prey capture rates by stoneflies, whether fish were present or absent (Table 7.1). The reduction of capture rates with fish was less pronounced (Table 7.1) at the higher prey density. Absence of a refugium did not affect the prey capture rates of the nymphs whether bullheads were present or absent (Table 7.2), and did not affect the scale of the interference effect at this higher prey density. Correlation analyses for each experiment did not indicate a significant effect of small variations in stonefly size (head capsule width) on number of prey captured during a trial (Experiment 1, $r = 0.150$; Experiment 2, $r = 0.024$; Experiment 3, $r = -0.185$). Not a single stonefly was consumed by the bullheads in all three experiments, even without refugia. When refugia were present, stoneflies were never observed in the open water during daytime. When refugia were absent, stoneflies were always inactive when observed and did not select for any part of the arenas, such as corners. Night time observations were not carried out.

Bullhead prey capture rates

The prey capture rate of bullheads was higher in the presence of stonefly in all three experiments, i.e., stoneflies facilitated the feeding of bullheads. This effect was significant across prey density (Table 7.3) and refugium presence or absence (Table 7.4) and the null hypothesis of neutral interaction was thus rejected for the bullheads also. Capture rates increased with prey density with or without stoneflies (Table 7.3), and the extent of the facilitation was reduced, but not significantly, at the higher prey density (Figure 7.2). Absence of a refugium did not affect prey capture rates in either the presence or absence of stoneflies (Table 7.4). Bullheads were never observed either on top of, or under the refugia.

Overall effect on prey numbers

Observed combined prey consumption was always higher than expected by the null hypothesis of simple additivity, which was therefore rejected in all three experiments (Table 7.5). A synergistic effect thus occurred overall, combined consumption exceeding the separate consumption of the predators. This effect was highly significant at both prey densities and also without a refugium, even after the strict Bonferroni correction was applied. However, deviation from expected decreased in successive experiments and was strongest at the low prey density.

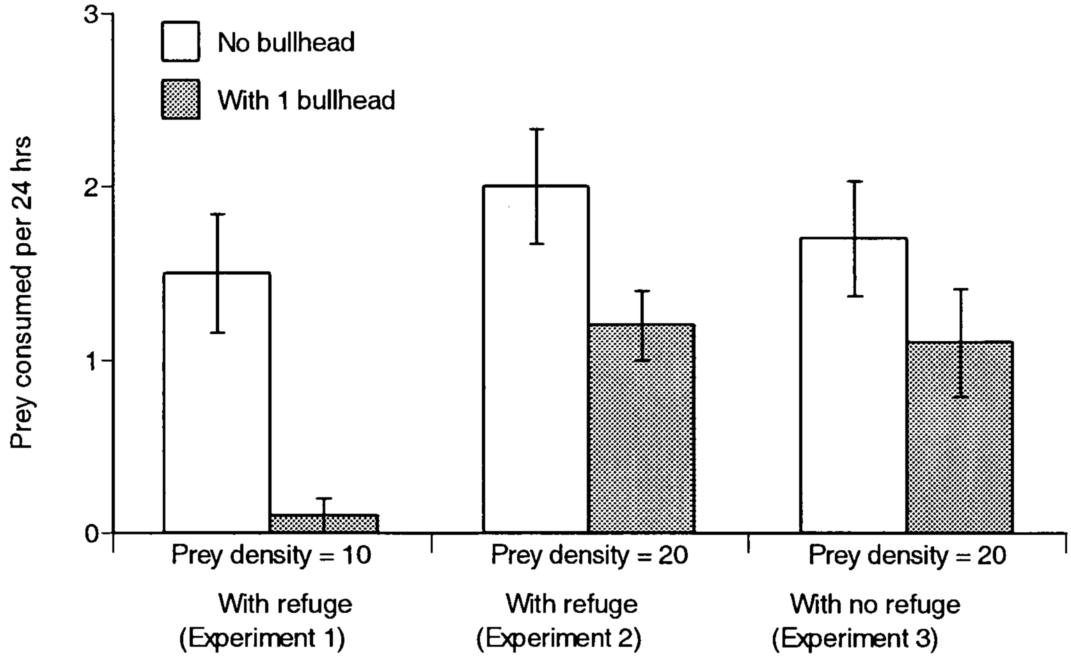

Figure 7.1: Mean prey capture rates ± 1 SE (n = 10) of stonefly nymphs with and without bullheads in three experiments.

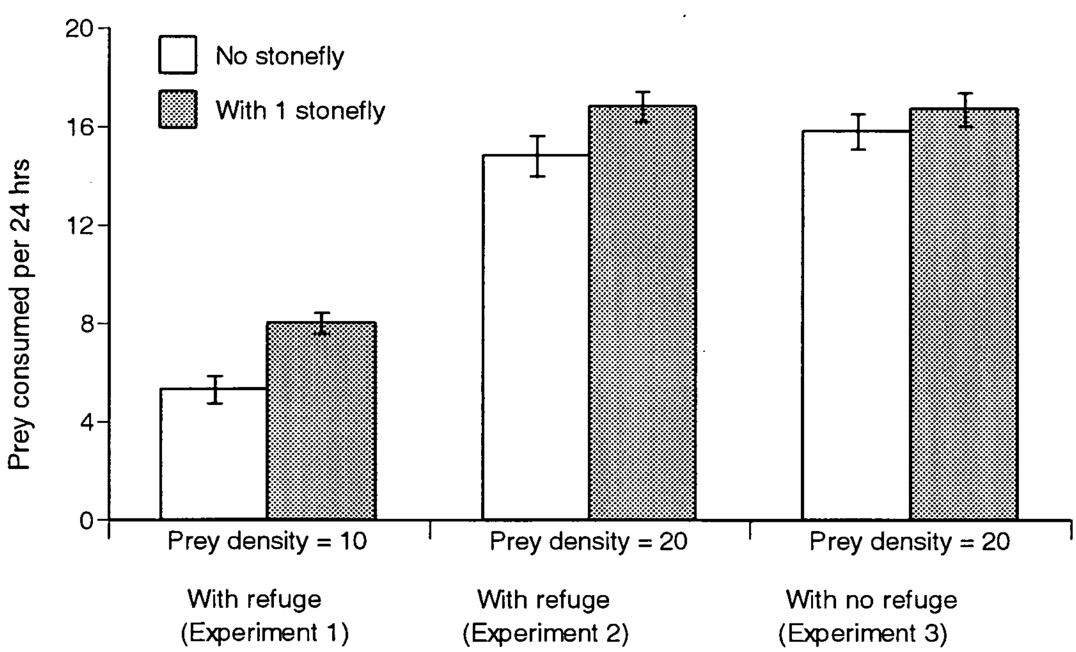

Figure 7.2: Mean prey capture rates ± 1 SE (n = 10) of bullheads with and without stonefly nymphs in three experiments.

Table 7.1: Two-way ANOVA for the prey capture rate of stonefly nymphs in Experiment 1 & Experiment 2.

Source	DF	MS	F	P
Fish presence	1	3.30	21.31	< 0.001
Prey density	1	2.61	16.82	< 0.001
Interaction	1	0.63	4.09	0.05
Error	36	0.15		
Total	39			

Table 7.2: Two-way ANOVA for the prey capture rate of stoneflies in Experiment 2 & Experiment 3.

Source	DF	MS	F	P
Fish presence	1	0.98	5.02	0.03
Refuge presence	1	0.19	0.97	0.33
Interaction	1	0.00	0	0.94
Error	36	0.19		
Total	39			

Table 7.3: Two way ANOVA for the prey capture rate of bullheads in Experiment 1 & Experiment 2.

Source	DF	MS	F	P
Stonefly presence	1	1.61	16.82	< 0.001
Prey density	1	19.96	207.5	< 0.001
Interaction	1	0.20	2.13	0.154
Error	36	0.09		
Total	39			

Table 7.4: Two-way ANOVA and for the prey capture rate of bullheads in Experiment 2 & Experiment 3.

Source	DF	MS	F	P
Stonefly presence	1	0.33	4.1	0.05
Refuge presence	1	0.03	0.41	0.52
Interaction	1	0.05	0.63	0.43
Error	36	0.08		
Total	39			

Table 7. 5: One-sample *t*-tests comparing mean observed combined consumption (O) in the 3 experiments to expected consumption (C_{fs}), and p-values. Bonferroni corrected p = 0.016

Test	C_{fs} ± SE	O ± SE	t	p
10 prey, refuge	6.0 ± 2.0	8.1 ± 0.3	5.52	0.0004
20 prey, refuge	15.3 ± 2.7	18.0 ± 0.4	5.69	0.0003
20 prey, no refuge	16.1 ± 3.2	17.8 ± 0.4	3.51	0.0066

7.4 Discussion

Bullheads did not facilitate the capture rates of Baetidae by *P. microcephala.* On the contrary, prey capture by the stonefly was always inhibited by the presence of fish. If bullheads do not generate a facilitation effect, then it is unlikely that trout would, as they tend to have weaker effects on prey behaviour and abundance than benthic feeding fish (Dahl, 1988a). Hence, there was no evidence that the increased condition of *P. microcephala* and other predators, in the stream of highest fish abundance (Chapter 5) was due to a positive effect of fish mediated by behaviour on prey behaviour.

Bullheads always interfered with stonefly feeding, because they reduced daytime foraging by the stonefly, and also decreased the abundance of prey in the experimental arenas. These results are consistent with other experiments which indicate a negative effect of Cottidae on the capture rates of predatory stoneflies (Soluk and Collins, 1988a, 1988b; Soluk, 1993). In this type of study, the trophic and behavioural processes are hard to separate. However, Soluk and Collins (1988a) observed a similar reduction in the feeding rates of stoneflies by bullheads with mouths sewn together, preventing prey consumption. This would indicate that reduced prey capture rates in the presence of fish are mainly due to reduced foraging activity of the stonefly, rather than prey depletion.

The stonefly always facilitated the feeding rates of bullheads on Baetidae, and this increased the impact of the two predators on prey abundance. Facilitation between invertebrate predators and fish has been observed in some situations, for example leeches facilitated the capture of Baetidae by bullheads (Dahl, 1988a) and stoneflies facilitated their capture by trout (Soluk and Richardson, 1997). In terms of their impact on prey populations, the interaction between bullheads and *P. microcephala* nymphs was non-additive. The increased prey capture rates of fish overwhelmed the reduced capture rates of stoneflies, and more prey were captured when predators were combined. Similarly (Soluk, 1993), the effect of reduced foraging by *A. capitata* on *Ephemerella* nymphs (Ephemeroptera) was over-compensated for by the increased capture rates of sculpin. However, other studies of predator-predator interactions in streams also report mutual interference (e.g. Dahl and Greenberg, 1997) or mutual facilitation (e.g. Resetarits, 1991), hence facilitation/interference effects appear to vary according to the species of fish, invertebrate predators and invertebrate prey. The effects of stream fish on the lower trophic levels may depend on the diversity and abundance of the invertebrate predator assemblage in streams, as they do in other systems (e.g. Carpenter *et al*, 1987; Power, 1990).

The facilitation effect of stoneflies on bullhead feeding rates contrasts with the interference observed by Soluk (1993), in an experiment using similar size arenas, assemblage of species (Cottidae, predatory stonefly, Baetidae), and prey densities. There may be several reasons for this difference. 1) Soluk used Perlidae nymphs, which are nocturnal foragers. The bullheads and stonefly had different activity patterns, reducing the likelihood of facilitation. 2) Soluk had several stonefly nymphs to an arena and the effect of intraspecific interference was unclear. 3) Soluk provided natural cobble substrate and therefore there were many alternative benthic refugia. In this experiment, prey had no true refugia in combined predator treatments. The difference in results between my experiment and Soluk's (1993) is consistent with the theory that the strength of trophic interactions varies with the heterogeneity of the habitat (Fuller and Rand, 1990). Generalisations about predator interactions in the field cannot be inferred without an understanding of the geomorphology of the stream bed.

The presence of a refuge had no effect on predator feeding rates, contrary to expectations. The removal of the refugium did not affect mayfly survival in combined treatments. This was expected, as, when the refugium was present, it did not provide shelter due to the presence of the stonefly nymph. The prey capture rates of *P. microcephala*, in isolation, were not affected by the absence of refugia, perhaps indicating that they captured *Baetis* when foraging at night, rather than ambushing them during the daytime in the refugia. When fish were present, baetids could have rated the predation risk from the fish lower than that from the stonefly, and chosen the arena over the refuge. Other studies also indicate that invertebrate predators cause a greater avoidance response in their prey than fish (Wooster and Sih, 1995). This may be because Baetidae have a greater chance of escaping fish, for example by entering the drift, than escaping *P. microcephala* that have very high success rates when attacking prey (Elliott, 2000, 2003a).

The presence of a refugium also had no effect on bullhead feeding rates, supporting the idea that facilitation occurred because of the avoidance response of the prey to stoneflies, rather than movement of prey in and out of the actual refugium. Bullheads probably detected mayflies from visual cues (Smyly, 1957), and these increased when *Baetis* responded to the stonefly by swimming away (Peckarsky, 1980), whether they encountered them in the refuge or in the open arena. Facilitation of fish prey capture rates by invertebrate predators may therefore occur across a wide range of substrate type and size. Bullheads select, like invertebrate predators may do (Chapter 4), for large stable stones (Roussel and Bardonnet, 1996). This association may reflect the avoidance of larger fish, rather than the exploitation of a resource patch (the stone) where foraging by invertebrate predators causes high prey fluxes at the sides of the stone, as suggested by Roussel and Bardonnet (1996).

Prey density reduced the magnitude of both interference and facilitation. Prey capture rates of both predators increased with prey density, as would be expected from the increase in encounter rates (Elliott, 2000). At the lower prey density, the stonefly prey capture rates were virtually nil with fish. Assuming that *P. microcephala* foraged out of the substrate refuge by night only (personal observation; Elliott 2000), depletion of prey numbers by the fish, prior to the start of stonefly foraging, may have been responsible for the strong interference effect. Reductions of prey availability in streams with fish, may explain why *P. microcephala* and the other predators relied heavily on algae as food (Chapter 6; Lancaster *et al*, 2005). The facilitation of bullhead prey capture rates at low prey density may have important consequences for their feeding in streams, particularly when invertebrate abundance is low, for example during the winter (Chapter 3), or after spates (McCabe and Gotelli, 2000). At the high prey density, more may have survived the period of fish activity, and thus were available to the night-time foraging nymphs. This increase in stonefly consumption may in turn, reduce the number of available prey to the fish, compensating partly for the daytime facilitation effect. The trophic effect of invertebrate predators on prey density and the behavioural effect on prey may thus have opposite consequences for bullhead prey capture rates.

It is important that stonefly nymphs avoided predation by bullheads in all trials, even though their feeding rates where compromised. Use of refugia and nocturnal foraging, presumably, were responsible for this, hence it was surprising that absence of a refugium was not associated with increased mortality of *P. microcephala* in combined treatments. This was partly explained by the inactivity of the nymphs when exposed to the fish, even in open arenas. The Baetidae were a far more conspicuous prey because they are very active and avoided the proximity of *P. microcephala* individuals by swimming away. Hence the stonefly nymphs derived an advantage from the *Baetis* response, because bullheads fed exclusively on the mayfly i.e. higher capture rates of other prey types by fish may reduce actual predation on stonefly nymphs. *Baetis* and *P. microcephala* were both potential prey to fish, but the invertebrate predator induced a response in shared prey that increased their consumption by fish. This type of indirect effect between two species that share a predator is referred to apparent competition, or competition for enemy-free space (Holt, 1977; Jeffries and Lawton, 1984). These effects can occur in host-parasite systems (Holt and Lawton, 1993; Bonsall and Hassell, 1997) and terrestrial invertebrate communities (Sih *et al*, 1985; Berdegue *et al*, 1996), and they may occur in streams too, but so far, they have rarely been considered when the 'competitors' are also a predator and its prey.

8 Conclusions

Are fishless sites better?

There was no indication that the habit of invertebrate predators is better suited to fishless streams, where their main predator is absent, than in streams with fish. On the contrary, the abundance, condition and diet of predatory invertebrates were little affected by the presence/absence of fish. There was some evidence the size class distributions of the smaller species were biased towards smaller individuals in streams with fish, leading to differences in biomass with and without fish. This pattern principally occurred in only one stream, and thus was hard to attribute purely to the effects of fish. The presence or absence of a top predator can have strong effects on invertebrate communities (Carpenter *et al*, 1987; Woodward and Hildrew, 2001), and often the intermediate predators are the most affected (Power, 1990). This study indicated that the effects of a top predator's presence/absence may be weak in some stream systems.

The heterogeneity of the stony substrate may have contributed to the weak effects of fish, because the high habitat complexity may reduce the impacts of predation by fish. However, the ecological traits of the invertebrate predators, several stoneflies and a caddisfly, were simply well suited to the presence of fish, perhaps reflecting the long term sympatry between salmonids and these invertebrate predators. Fishless streams are rare, at least in some geographical areas (e.g. the Tweed catchment area, Campbell, 1998) and there may be little advantage to traits that allow invertebrate predators to exploit these habitats. The introduction and removal of salmonids, for example because of angling, may have little impact on the invertebrate predator assemblage in these types of streams.

Fixed vs flexible foraging traits

Invertebrate predators could balance fish avoidance and foraging through fixed and flexible foraging traits, and these were reflected by their life cycle and growth requirements. Predators with long multivoltine life cycles and slow growth, e.g. Perlidae, can afford a fixed low foraging activity, for example a strict nocturnal habit. They are likely to incur little exposure to salmonids, and many aspects of their ecology were constant across all streams, irrespective of the presence of fish. Predators with short univoltine life cycles (e.g. Perlodidae) have higher growth requirements and a higher level of foraging activity, for example they can forage during the day and night. For these predators, flexible foraging activity and diet may be advantageous as this allows them to maximise the rate of resource acquisition according to the local predation risk. Despite exposure to fish during the daytime, their diet flexibility allowed them to avoid fish, and make use of prey that were more abundant with fish, such as midges, and possibly algae.

Principal effects of fish on invertebrate predators appeared to be indirect, mediated through their effects on the prey they share with invertebrate predators. However, these processes may be affected by how invertebrate predators interact with one another, and how fish modify these interactions. Does the abundance of fish affect the competitive and predatory interactions between invertebrate predators, and what effect does this have on the stream invertebrate community?

Effects of fish

This study allowed me to answer some of the questions concerning the effects of fish abundance on 5 species of stream invertebrate predators (summarised in Table 8.1), but some general patterns also emerged. The abundance of invertebrate predators across streams was difficult to compare because it varied seasonally. This may explain the equivocal patterns in the abundance of invertebrates across natural streams with and without fish (Allan, 1875, 1982a; Bowlby and Roff, 1986; Harvey, 1993).

There was some evidence that fish reduced the abundance of large individuals of some species, and this caused a difference in the overall biomass of invertebrate predators in streams with fish vs fishless streams. The flow of organic matter through the food webs of streams with and without fish may differ, and impacts on terrestrial food webs may differ also due to differences in the emergent biomass of invertebrates.

Table 8 - 1: Summary of the effects of fish on 5 species of stream invertebrate predators.

	D. cephalotes	*P. microcephala*	*I. grammatica*	*S. torrentium*	*R. dorsalis*
Relative size	Large	Large	Medium	Small	Medium
Life cycle	2 – 3 years	1 year	1 year	1 year	1 - 1½ years
Foraging activity	Nocturnal	Diurnal	Diurnal	Diurnal	Diurnal or nocturnal
Cross stream abundance	No effect	Weak positive	No effect	No effect	No effect
Size class distribution	No effect	-	No effect	Fewer large individuals	Fewer large individuals
Condition	No effect	Positive	Positive	-	Positive
Pre-emergent weights	No effect	Positive	Positive	Positive	Positive
Herbivory	More algae in diet	More algae in diet	More algae in diet	More algae in diet	More algae in diet
Prey in diet	No effect	More Chironomidae	Less Baetidae	No effect	No effect
Facilitation/interference	-	Interference	-	-	-

The condition of invertebrate predators was constant across steams for some species or increased with the abundance of fish, and this was because some species appeared to benefit from the effects of fish on the wider community. Omnivory, i.e. the presence of algae in the diet, always increased with fish and this could have allowed invertebrate predators to maintain their feeding, even if fish reduced the abundance or activity of the prey. The consumption of algae by invertebrate predators is little understood, and a recent study suggests some invertebrate predators assimilate algae and some do not (Lancaster *et al*, 2005). The species in my study which showed no change in condition with more algae in the diet, *D. cephalotes*, does not assimilate algae. Why then does it consume algae? The species which showed an increase in condition with an increase of algae in the diet, are species that assimilate algae. Do these species mix food types because of a nutritional requirement, or because a generalist feeding strategy provides flexibility across streams with varying amounts of fish, prey and algae? These questions await further investigation.

The behavioural and abundance effect of fish on prey had the potential to lower the capture rate of at least one invertebrate predator, *Perlodes microcephala*. However, there was evidence that an indirect effect could occur between invertebrate predators and prey, because the avoidance of the invertebrate predators by the prey increased their capture by fish. Hence, the foraging behaviour of the invertebrate predators caused a response in the prey which reduced their own vulnerability to fish, because fish fed principally on the invertebrate prey and not the invertebrate predators. Both types of invertebrates thus 'competed' to avoid a shared predator, the fish, a common effect between two hosts that share a parasite (Holt and Lawton, 1993) and that can occur in terrestrial invertebrate communities (Berdegue *et al*, 1996). These types of predator-mediated interactions have been reported in freshwater systems (Naeem, 1988), though rarely in cases where the two prey types are themselves a predator and prey (Wissinger and McGrady, 1993).

Invertebrate predators are intermediate predators when fish are present i.e. they are a consumer and a prey (Predation, Figure 8.1). They share prey with the top predator, and because of this indirect effects occur mediated by the abundance and behaviour of the prey (A in Fig. 8.1). Intermediate predators and their prey share a top predator, and this can also generate indirect effects (B in Fig. 8.1). All three processes can occur simultaneously but prey-mediated interactions (A, Fig. 8.1) appeared to be the most important in this study. The relative strength of these processes may affect invertebrate community structure, across ecosystems, and streams with and without fish are good model systems to study these effects. The use of laboratory experiments may allow to separate these interaction pathways from one another and help predict consequences of changes to predator/prey populations. In particular, the relative strengths of these processes cannot be predicted until the time lags necessary for effects to become detectable in predator and prey abundance, behaviour and condition are known.

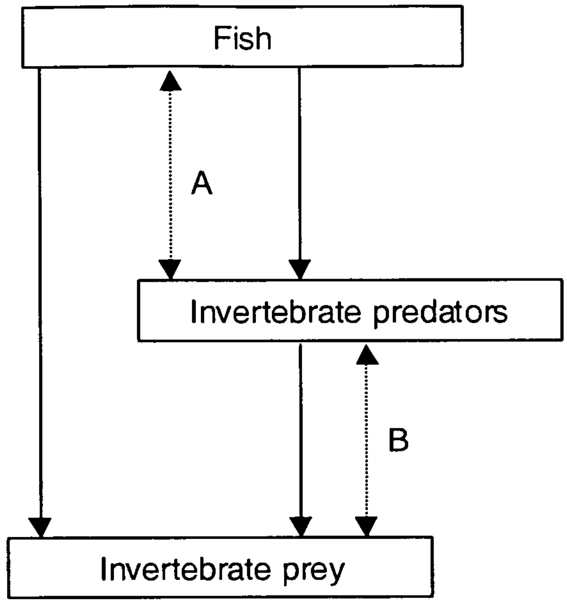

Figure 8 - 1: Pathways of interaction in a 3 trophic level system. Solid lines represent predation. Dotted lines represent indirect effects. A: indirect effect between intermediate and top predator mediated by a shared prey. B: indirect effect between intermediate predator and prey mediated by a shared top predator.

9 Appendix A: Length mass regressions of five species of predators.

In Chapter 4, abundance was converted to biomass from the head width measurements of invertebrates using the equations in Table A.1. These length mass relationships were derived from all available specimens used in this thesis and thus are based on data from more than one stream. The methods for specimen processing, and for calculating the head width to mass parameters are given in Chapter 5.

Table A - 1: Parameters of head width (mm) to dry mass (mg) regressions for 5 species of invertebrate predators. b = slope, a = intercept. n = number of specimens used, % R^2 = fit. All equations fit the model: dry mass = b × head width + a. All regressions are significant at $\alpha = 0.05$, $p < 0.001$

Species	n	% R^2	b	a
Dinocras cephalotes	189	91.7	2.78	0.34
Perla bipunctata	56	78.7	2.98	0.52
Isoperla grammatica	133	80.6	2.66	0.28
Siphonoperla torrentium	148	38.8	1.91	0.60
Rhyacophila dorsalis	134	71.8	1.55	1.55

10 Appendix B: Effects of preservation and drying on the weight estimates of freshwater invertebrates and consequences for the calculation of length-mass relationships.

10.1 Introduction

Ecology does not yet have a standard method for producing mass-length regressions. In fact, from over 25 published studies on the regression equations of freshwater invertebrates cited in Chapter 5, no two use the same methods. Length of preservation, type of medium, drying procedure and the dimension used as a predictor of weight vary. It is hard to evaluate the bias in weight estimates inherent to each study, and often equations cannot be compared across studies. Estimates of biomass, derived from the literature, are routinely used to describe the ecology of populations and communities, when it is impractical to weigh specimens directly. Common species such as the freshwater shrimp *Gammarus pulex* are assigned a wide variety of length-mass parameters in the literature, and this is a problem for other ecologists wishing to calculate, for example, secondary production of invertebrates from size class frequencies (Hynes and Coleman, 1968), or the fluxes of organic matter in food webs (*sensu* Benke and Wallace, 1997). It is unclear whether the constants of the length-mass regressions truly vary with the environment of the animals, or simply vary with the method used. Sources of error, such as preservation and gut contents of specimens, must be identified and quantified. Though gut contents can be removed, this in itself may affect the weight estimate as the specimen is damaged and the gut itself is also usually removed. A protocol that minimises errors within data sets, and estimates the difference between true weight and predicted weight, is required for length-mass regressions to be a useful tool of comparisons across systems. In this appendix, I quantify the effects of preservation in alcohol on weight loss and test whether gut removal introduces a bias to the weight estimates of large predatory stonefly nymph.

The chemical preservation of freshwater invertebrates causes a leaching of organic matter from the specimens into the medium (Howmiller, 1972), leading to a loss in body mass, though Trichoptera larvae have sometimes shown an increase in weight (Stanford, 1972). Alcohol causes a greater mass change than any other preservative (e.g. formalin) in both freshwater (Leuven *et al*, 1985; Cressa, 1999b) and marine invertebrates (Mills *et al*, 1982), but alcohol is the most common preservative for benthic invertebrates. Although most studies that use or compile length-mass regressions acknowledge this source of error, quantitative estimates are surprisingly scarce and this decreases the confidence in these predictive equations. Howmiller (1972) observed sizeable losses in wet weight in 70% ethanol for tubificid worms (50% mass loss after 3 weeks) and chironomid larvae (40% loss). The body of these animals has little chitinous exoskeleton, and this may facilitate the exchange of organic matter with the preservative medium. For a heavily sclerotised invertebrate, *Pteronarcys californica*, in 70% alcohol, Stanford (1972) estimated a 10 to 15 % wet weight loss over a month, 25 % for small size classes (< 2.5 cm long). Howmiller (1972) and Stanford (1972) unnecessarily used centrifugation to remove surface moisture, which could have caused excessive losses of body fluids and body parts.

Leuven *et al* (1985) blotted the animals dry before weighing and observed a 10 to 25% loss in dry mass across a wide range of freshwater invertebrates. Gonzalez *et al* (2002) and Cressa (1999b) observed that length-mass regressions obtained from the dry mass of animals preserved in 70% alcohol consistently predicted lower weights than ones derived from non-preserved animals.

A stabilisation period in the rate of loss of organic matter to the preservative medium occurs a few weeks after initial preservation (e.g. Howmiller, 1972; Dermott and Paterson, 1974; Donald and Paterson, 1977; Leuven *et al*, 1985). This period is preceded by a period of rapid mass loss and followed by a period of very slow mass loss. This corresponds to a rapid initial exchange of water between the organism and the medium, followed by very slow leaching of body lipids to the medium (Howmiller, 1972; Stanford, 1972; Burgherr and Meyer, 1997). The stabilisation period, once determined, thus provides a 'window' in which specimens can be processed for weight measurements, while maintaining the bias due to preservation constant. If the bias is quantified *a priori*, a correction constant can be applied to the length-mass regression equations.

Invertebrates exhibit significant length changes following preservation in alcohol due to curling of the abdomen. Data about shrinkage are scarce, although Britt (1953) observed a 12% loss in body length for the mayfly *Ephemera simulans* preserved in 95% alcohol for 10 days, by which time shrinkage had stabilised. Head width is less affected by preservation, if at all, but the magnitude of changes between instars is less than for body length and hence accounts for less variation in weight (Meyer, 1989; Towers *et al*, 1994; Burgher and Meyer, 1997; Johnston and Cunjak, 1999) leading to poorer correlation of size and weight. If body length is used, it is necessary to estimate, and correct for, the 'curling' effect.

Gut contents of the specimens add to the overall mass, and this is a sizeable effect for engulfers such as predatory Plecoptera, which may have several whole prey items in their gut (Hynes, 1976). Furthermore, fullness of the gut varies greatly between individual predators (Allan, 1982b, Plecoptera; Wotton *et al*, 1993, Trichoptera) and the error in the weight estimates cannot be corrected by the use of a constant. Although many studies of freshwater invertebrate length-mass relationships mention this source of error (e.g. Smock, 1980; Burgherr and Meyer, 1997; Benke *et al*, 1999; Johnston and Cunjak, 1999) only Dudgeon (1989) removed the gut contents of specimens (Odonates) to be dried and weighed. Cutting open the exoskeleton, however, may facilitate the evaporation of lipids and other volatile organic matter from the thoracic and abdominal body cavities, and removal of the gut (either via an incision or via the mouth) may also remove other internal tissue. An alternative way of correcting for weight of gut contents is to starve the specimens before processing them, but this is often impractical as it requires knowledge of gut passage time and may lead to weight loss through the metabolisation of fat reserves. Removal of the gut is hence a practical way of correcting for what could be a very large error in weight estimates, but its inherent biases must be quantified.

Using nymphs of the stonefly *D. cephalotes* as an example, I quantified the effects of preservation in 70% ethanol on the weight and body length of specimens and determined the length of time necessary for these effects to stabilise. Because the large stoneflies are morphologically very similar, these data could be applied to some of the species used in Chapter 5 (though this is not necessary for the cross-stream comparisons of Chapter 5) to correct the underestimate in weight estimates which occurs due to preservation. This makes the equations more useful to other ecologists for the purpose of cross-study comparisons, particularly as they may apply the correction factors to their own data when alcohol is used as a preservative. I also tested whether the weight loss on drying of gutted specimens is different than that of intact specimens with empty guts. If the two methods yield similar weight loss on drying, then removal of gut contents would be the simplest way to minimise variance in length-mass relationships.

10.2 Methods

Two experiments were carried out. In the first, the weight and length of specimens kept in alcohol was monitored over preservation time. In the second, the effect of gut removal on weight estimates was tested. Two treatments were compared; a control group which consisted of starved animals, which hence had empty guts, and a treatment group, which consisted of nymphs from which the foregut had been excised.

Individuals of *Dinocras cephalotes* were collected from the Faseny Burn (55°51'35''N, 2°35'54''W), an upland (310 m) second order stony stream tributary (mean width 2.5 m) of the Whiteadder Water, SE Scotland. Mean annual pH in this burn is 7.3 and mean annual conductivity 222 $\mu S.cm^{-1}$. The stonefly nymphs were captured in kick net samples taken within the same 100 m reach, on one sampling occasion (12th January 2001). Care was taken to select specimens of the same cohort (head width greater than 3.5 mm only), as two cohorts were present at the time of sampling. Specimens were kept in buckets containing stones taken from the stream until removed to an unheated outdoor facility.

Nymphs to be starved were placed in individual enclosures also used in Chapter 7, consisting of plastic cups placed in trays filled 5 cm deep with water. A pump supplying air to two diffusers at each end of the tray provided oxygenation and water circulation. Two square sections (~ 1 cm^2 and 5 mm from the bottom) had been removed from opposite sides of each cup, and were replaced by a piece of 300 μm mesh. This allowed water to circulate in and out of the cups, but the animals could not escape. A small pebble (~ 3 cm long) was placed in each cup to provide shelter and weigh down the cups. Pebbles had been cleaned with a brush, and placed in an oven at 80 °C for 72 hours, then brushed again to remove prey items, detritus and algae. A Perspex roof allowed a natural photoperiod for that time of year (14:10 D:L). Daytime water temperature was approximately 8 °C.

To estimate gut clearance time, an initial subsample of 5 nymphs, selected at random, was killed in 70% alcohol and examined for gut contents. A similar subsample of starved nymphs was examined daily until all 5 nymphs had empty gut passages (excluding prey setae which can remain in the gut until shedding of the lining with each moult). It took 5 days for guts to clear.

Effects of preservation

Mass loss and shrinkage in alcohol were determined by weighing and measuring preserved specimens after 5, 10, 15, 30 and 60 days. Ten individuals with cleared guts were killed in 70% alcohol, blotted dry for 30 s, then weighed to the nearest 0.01 mg (Sartorius MC5 microbalance). Head width at the widest point and body length (excluding appendages) were measured to the nearest 0.01 mm using digital callipers (Mitutoyo CD-6). The ten individuals were placed in individual glass vials containing 20 ml of 70% ethanol. The vials were kept at room temperature, in darkness, for the duration of the experiment. On each sample date, individuals were removed from the vials, blotted dry for 30 s between two sheets of tissue paper, then weighed and head width and body length measured as above. Individuals were then returned to their original vial, which was topped up with alcohol to 20 ml. Weight loss over time was compared directly with other similar data from the published literature. Correlation analysis (Pearson's) was used to examine the relationship between weight loss and size of the specimens.

Effect of gut removal

Nymphs with cleared guts (n = 30) were killed in 70% alcohol. Each carcass was blotted dry, weighed and head width measured as above. All nymphs were exposed for the same amount of time to air and preservative, and carcasses were returned to alcohol. Individuals were assigned at random to one of two treatments: the 'test' group, guts would be removed; the 'control' group were left intact. Guts were (test group) by slicing along the ventral surface, cutting both thorax and abdomen in half. Foreguts were removed from the buccal cavity to the malphigian complex and the carcasses were weighed again to 0.01 mg. All specimens were oven-dried for 48 hours at 80°C, in individual foil trays. The 15 removed guts (all empty) were pooled and similarly dried. Trays were removed from the oven and allowed to cool for ten minutes at room temperature. Contents of the trays were weighed to 0.01 mg in a random order. Differences in initial head width, wet weight and final dry weight were compared between the two treatments using a t-test ($\alpha = 0.05$, null hypothesis of no difference between control and test groups). I used Pearson's correlation to examine the relationship between body size and weight loss on drying in both treatment groups.

10.3 Results

Effects of preservation

The wet weight (WW) of the specimens decreased with preservation time (Figure B.1). Weight loss was rapid over the first 10 days of preservation, but stabilised to approximately 75 % of initial wet weight for the next 3 weeks. WW of individuals decreased more gradually thereafter and was ~ 60% of initial wet weight after two months. Head width (HW) of specimens ranged from 4.01 to 5.79 mm and body length (BL) from 14.23 to 21.46 mm. Weight loss after 60 days was negatively correlated with HW, BL and initial WW (Table B.1), hence smaller nymphs lost proportionally more weight. BL also decreased, to ~ 90% of initial BL within the first 2 weeks (Figure B.2), but stayed constant thereafter for the duration of the experiment. Decrease in BL after 60 days was not correlated to HW, WW or initial BL (Table B.1). The minimum time in preservative for both curling and mass loss to stabilise was hence two weeks.

Effect of gut removal

Weight loss due to gut removal was minimal, the DW of gut tissue was 16.50 mg for 15 guts, equivalent to approximately 1.1 mg per gut. For the size range of specimens used, this value represented from 1.6 % to 4.1 % of the overall individual dry weight. Wet weight, dry weight and head width were not significantly different across treatments (Table B.2). Expressed as a percentage of initial wet weight, weight after drying was similar in both treatments (Figure B.3). A t-test supported the null hypothesis of no difference between percentage weight loss of control and test groups ($t = -1.22$, $p = 0.23$, $df = 28$, $\alpha = 0.05$). For both treatments, correlation between % weight loss and HW was not significant ($r_{13} = -0.43$, $p = 0.104$ and $r_{13} = 0.40$, $p = 0.138$ for control and test groups respectively) indicating that the error associated with the gutting procedure was size invariant at least for this size class of animals.

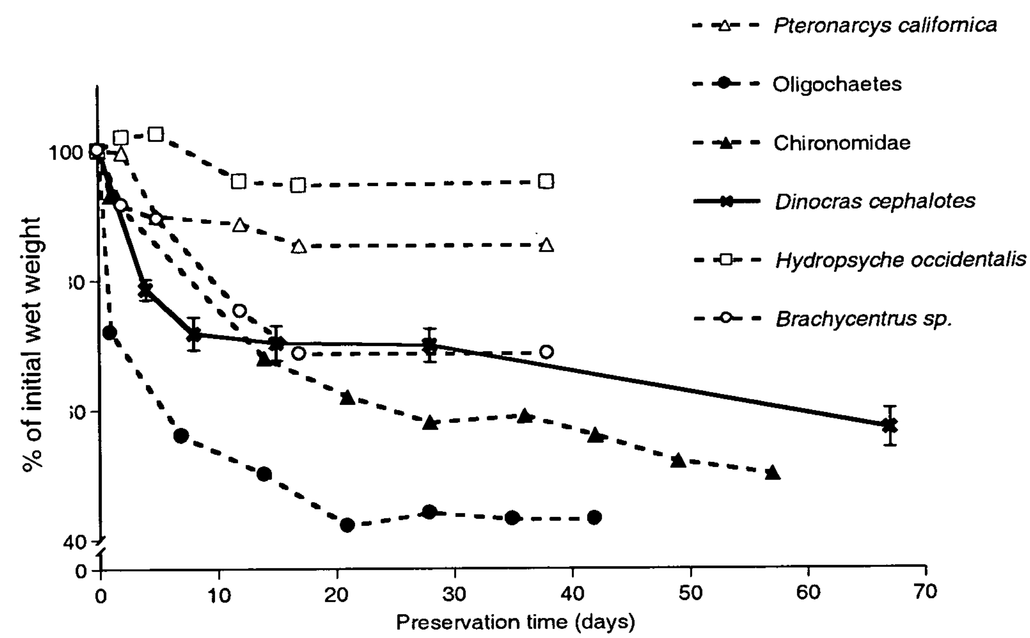

Figure B - 1: weight as mean % of initial weight (± 1SE) with preservation time for 10 *D. cephalotes* in 70% alcohol (crosses). Data for other species was taken from Stanford (1972) for *P. californica* and Howmiller (1972) for all others.

Table B - 1: Correlation coefficient and p value of wet weight lost (mg) and body length lost (mm) during 60 days of preservation, with initial head width (mm), body length and wet weight for 10 individuals of *D. cephalotes*.

	WW lost		BL lost	
	r	p	r	P
HW	-0.90	0.013	0.52	0.289
BL	-0.89	0.017	0.47	0.343
WW	-0.90	0.013	0.45	0.372

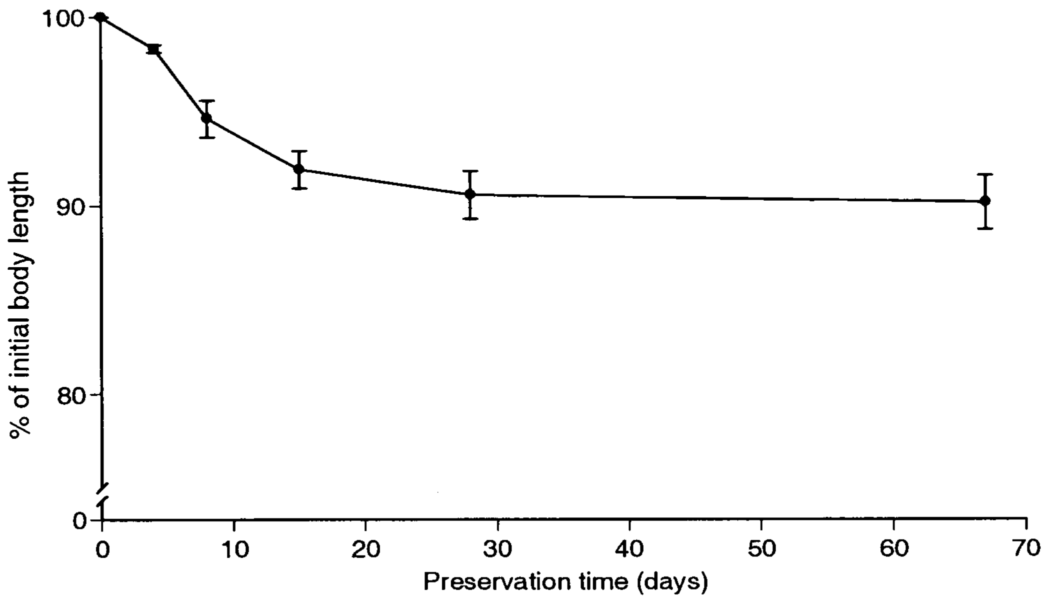

Figure B - 2: Body length of 10 *D. cephalotes* over preservation time as a % of initial body length (± 1SE) .

Table B - 2: Results of t-tests comparing head width (HW), wet weight (WW) and dry weight (DW) of control (starved) and test (gutted) groups of *D. cephalotes*. All data were normally distributed and homoscedastic.

	HW (mm)	WW (mg)	DW (mg)
Control mean ± 1SE	4.2 ± 0.14	147.5 ± 13.0	41.9 ± 4.4
Treatment mean ± 1SE	4.4 ± 0.12	178.8 ± 16.0	45.6 ± 3.2
t-value	-1.33	-1.52	-0.68
d.f.	28	28	28
p value	0.20	0.14	0.51

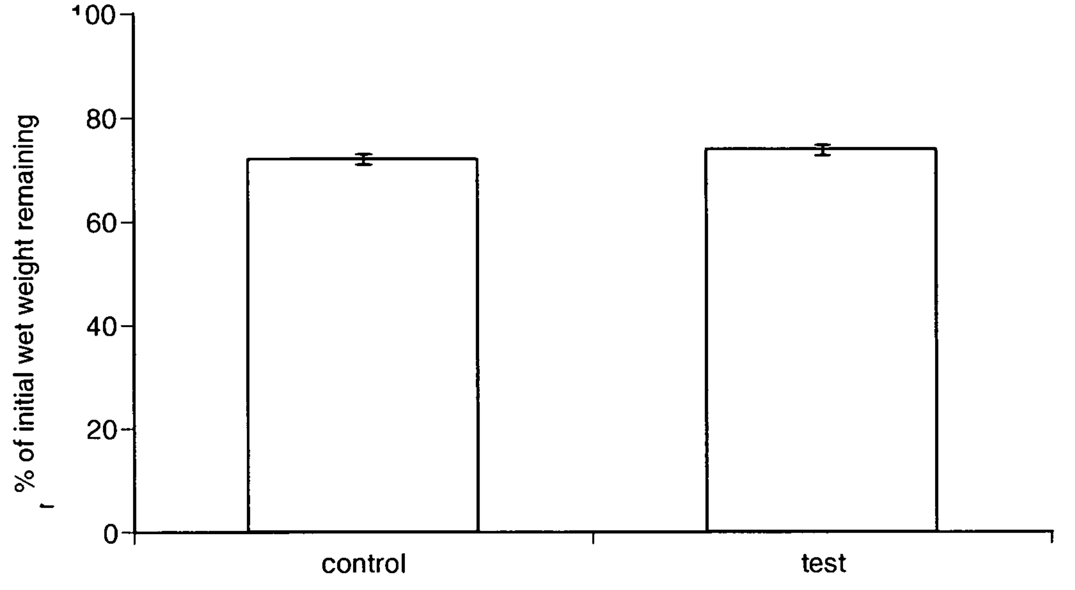

Figure B - 3: Weight remaining after drying as mean % of initial wet weight (± 1SE) for control (starved) and test (gutted) groups of *D. cephalotes*.

10.4 Discussion

Weight loss occurred rapidly in preserved *D. cephalotes* over the first two weeks of preservation, at which time it stabilised and decreased much more slowly. The final loss of 40 % of initial weight after 6 weeks was within the ranges of other studies (Howmiller, 1972, Stanford, 1972, Leuven *et al*, 1985, Cressa, 1999b). Weight loss of *Dinocras cephalotes* was 10 % greater than Stanford (1972) observed for *Pteronarcys californica*, despite the morphological similarity of the two species. Stanford's initial weight measurements were based on live weights (rather than simply fresh) and this study used preserved (and hence dead) weights. Tissues and cavities of the specimens killed in alcohol will be saturated with the preservative and hence the dead weight is likely to be greater than live weight. This is the likely explanation for the initial increase in weight with preservation observed for some species by Stanford. My study avoided this effect, but effectively overestimated initial weight. In addition, individuals were stored individually in 20 ml vials, whereas in most survey work, many organisms are preserved together, hence the ratio of organic matter to preservative varies greatly. Weight measurements of preserved specimens are underestimates of fresh weights and such biases must be accounted for in length-mass regressions developed from preserved specimens.

Percentage weight loss was negatively correlated with specimen size, consistent with differences observed by Stanford (1972). Rates of weight loss in preservation may therefore be larger for smaller species, such as *I. grammatica* and has important consequences for across-species comparisons. The volume of preservative in the vials was marginally higher for small nymphs in this experiment than in Stanford's (1972) which used a wider size range of animals. Rate of weight loss in small volumes of alcohol may decrease as the medium becomes saturated with leached organic matter, and this would occur first in the vials holding larger individuals. Furthermore, because small quantities of alcohol were lost with each blotting in this experiment, the vials had to be replenished to keep volume constant, and the addition of fresh preservative may have reduced the concentration of leached organic matter in the medium, thus affecting the rates of weight loss.

Loss of initial BL due to curling of 10% was similar to that observed by Britt (1953) for *E. simulans*, and head width did not decrease over preservation time. The curling effect was not affected by overall size and thus is consistent across small and large nymphs, though data cannot be extrapolated to the very small size classes. Ephemeroptera and Plecoptera have roughly similar body plans, and curling may be more pronounced in Trichoptera, which have proportionally longer and unsclerotised abdomens. The overlap in stabilisation time of curling and mass loss effects is advantageous, as during this period both are constant and both can be quantified.

The dry weight of specimens with guts removed was similar to starved nymphs and thus gut removal did not appear to introduce a bias to the estimate of dry weights. Removed guts accounted for a maximum of 4% of overall DW, a small error considering whole prey items would be likely to weigh much more than the gut itself.

The weight of the dissected gut can be corrected for in the final weight estimate, whereas variability in gut contents cannot be. Removal of gut contents, for these large Plecoptera, thus improved the accuracy of the weight estimate.

On the basis of these results, I recommend that if accurate length-mass regressions are to be calculated, specimens should be stored in a standard amount of preservative, and that they should not be processed until two to three weeks after initial preservation. I recommend that, if possible, specimens be gutted, particularly if they are predators which engulf other prey whole. I am confident that the equations presented in Chapter 5 are accurate, and hope they can be of use to others. If true weights are required, rather than just comparative values such as in Chapter 4, predicted weight may be corrected by approximately 20 % to allow for the effects of preservation.

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