

# **Ecology of streams affected by iron precipitates and iron flocculants**

---

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

Master of Science in Zoology

in the

University of Canterbury

by

**Annabel R. Barnden**

---

University of Canterbury

June 2005

# Table of Contents

---

<b>Abstract</b>		i
<b>Chapter One</b>	<b>General Introduction</b>	1
	Thesis objectives	14
<b>Chapter Two</b>	<b>Study area</b>	
	Background	15
	Iron flocculent and iron precipitate study sites	22
	Infection study sites	32
<b>Chapter Three</b>	<b>Effects of iron flocculants and iron precipitate on water chemistry, algal biomass and benthic invertebrates</b>	
	Introduction	37
	Study design	39
	Methods	40
	Results	45
	Discussion	75
<b>Chapter Four</b>	<b>Leaf breakdown in streams effected by iron precipitates and iron flocculants</b>	
	Introduction	83
	Methods	84
	Results	90
	Discussion	101
<b>Chapter Five</b>	<b>Infections in benthic macroinvertebrates</b>	
	Introduction	106
	Methods	108
	Results	111
	Discussion	121
<b>Chapter six</b>	<b>Concluding discussion</b>	124
<b>Acknowledgements</b>		131
<b>References</b>		133

## Abstract

---

The effects of mining generated iron flocculants and iron precipitates on stream communities were investigated at 16 sites, near Reefton, South Island. Flocculent and precipitate streams were examined for water chemistry, algal biomass, leaf breakdown and benthic invertebrates between June 2004 and January 2005. Results showed that iron flocculent streams had higher pH (5-7) and lower conductivity ( $45-115 \mu\text{S cm}^{-1}$  at  $25^\circ\text{C}$ ) compared to precipitate streams (pH 3.3- 5.2, conductivity  $111-800 \mu\text{S cm}^{-1}$  at  $25^\circ\text{C}$ ). Algal biomass was similar between sites but reference sites showed greater variability. Leaf breakdown was fastest in reference streams ( $-0.003$  to  $-0.005 \text{ day}^{-1}$ ) and slowest in iron precipitate streams ( $0.0004$  to  $-0.002 \text{ day}^{-1}$ ). Benthic invertebrate taxa richness was lowest in precipitate streams (2-8) and higher in iron flocculent streams (8-22). Benthic invertebrate densities showed similar trends with lower densities in iron precipitate streams. Invertebrate community composition in iron flocculent streams did not change much longitudinally and communities were dominated by mayflies (e.g., *Deleatidium* spp.) and dipterans (e.g., Orthoclaadiinae). In contrast, benthic communities in iron precipitate streams had a number of low pH tolerate taxa (e.g., *Spaniocercoides* sp. and *Psilochorema* sp.).

Additionally, biological infections in benthic invertebrates were investigated in Carton Creek, near Reefton. Community infection rates were highest at sites with elevated concentration of dissolved heavy metals (e.g., Fe  $0.6 \text{ g m}^{-3}$  and Al  $0.42 \text{ g m}^{-3}$ ). The higher rates of infections may indicate that these concentrations of dissolved heavy metals are not directly toxic but may increase 'stress'.

This study demonstrates how iron precipitates may have a greater negative effect on stream communities than iron flocculants. Importantly, stream communities are also dictated by their tolerance to adverse water conditions and in this study water chemistry was vastly different between iron flocculent and precipitate streams. Furthermore, this study demonstrates that the effects of mine pollution may manifest through other avenues such as infections and disease.

## Chapter One

### General introduction

---

For at least the last 30 years it has been increasingly recognised that streams are intrinsically linked with their catchments (i.e., since Hynes 1975). Consequently, human activities within a catchment will directly and indirectly impact the stream environment and influence both water and habitat quality (Johnson et al. 1997, Quinn et al. 1997, Sponseller et al. 2001, Quinn and Stroud 2002). For example, land-use activities can result in changes in water temperature (Scott et al. 2002), increase sediment concentrations (Quinn et al. 1992, Allan et al. 1997) and interfere with linkages between the terrestrial and aquatic environments (Gregory et al. 1991, Allan and Flecker 1993).

The distribution and abundance of benthic macroinvertebrates within a stream are strongly influenced by a range of abiotic factors. These factors have often been described using a hierarchical framework (Poff 1997, Scott et al. 2002, Mykra et al. 2004) and probably operate at multiple spatial and temporal scales to influence stream environments. Patterns in freshwater fish and benthic invertebrate communities have been found to correspond with large regional scale or ecoregion characteristics (Biggs et al. 1990, Harding and Winterbourn 1997), because of differences in regional climate, vegetation, geology and altitude. However, stream communities do not always correspond directly with ecoregion boundaries, but are sometimes influenced more strongly by catchment or local scale features (Hawkins et al. 2000, Heino et al. 2002). Furthermore, catchment-scale influences on stream environments can sometimes be difficult to separate from the effects of human land-use as natural variation within catchments can disguise the full effect of land-use (Richards et al. 1996, Quinn et al. 1997, Richards et al. 1997). Conversely, studies in New Zealand have indicated that regardless of regional factors, changes brought about by human land-use can have a marked effect on benthic invertebrate communities (Quinn and Hickey 1990, Quinn et al. 1997).

Overseas, widespread natural acidification of freshwaters has been linked to a combination of two large scale catchment factors, vegetation and geology. Natural acidification of streams has been particularly important in Sweden where water emerging from bogs and wetlands have low buffering capacity, and weathering of catchment bedrock is low (Otto and Svensson 1983, Dangles et al. 2004). In New Zealand, freshwater systems with naturally low pH are common and widespread, particularly on the West Coast of the South Island (Winterbourn and Collier 1987, Winterbourn and McDuffett 1996). Their low pH is the result of leaching of high concentrations of organic acids (e.g., humic acids) from decomposing vegetation in their catchments (Winterbourn and McDuffett 1996). These systems have been referred to as humic brown waters and often have pH around 4 (Collier et al. 1990). Additionally, acid waters can be a consequence of geology and arise naturally through acid rock drainage (ARD). ARD occurs when natural weathering processes, or the fracturing of surface rock strata, expose sulphide minerals (e.g., pyrite) to air and water thereby allowing oxidation to occur (Grande et al. 2005). The oxidation of sulphide minerals is often an abiotic reaction, however, it can also be catalysed by sulphide-oxidising micro-organisms (e.g., *Acidithiobacillus thiooxidans* and *Metallosphaera sedula*) (Banks et al. 1997, Johnson and Hallberg 2003). Sulphuric acid, one of the primary by-products of pyrite oxidation is primarily responsible for the lowering of pH in receiving waters (Campbell et al. 2001).

Increasingly, acidification of freshwaters is occurring from anthropogenic sources, and much of this is due to acid precipitation or acid mine drainage (AMD). Acid precipitation is relatively common in the Northern Hemisphere where industrial combustion of fossil fuels releases large quantities of sulphur and nitrogen oxides into the atmosphere (Holden and Clarkson 1986). Acid precipitation has resulted in the acidification of lakes and streams in Scandinavia since the late 1800s (Parsons 1977), but it has only been since the 1970s that acid deposition has emerged as an important environmental stress affecting ecosystems in North America, Europe and Asia (Driscoll et al. 2001). Subsequently, acidification of freshwaters has spurred a more general interest on the effects of low pH in freshwater ecosystems (Townsend et al. 1983, Simpson et al. 1985, Hall and Ide 1987, Smith et al. 1990).

In New Zealand, acidification of freshwaters through acid precipitation has not been documented and rainfall generally has a pH of around 5.6 (its normal unpolluted condition) (Holden and Clarkson 1986). Instead, acidification of freshwaters, other than brown waters occurs primarily as a result of mining, and is commonly manifested as acid mine drainage (AMD) (Winterbourn and McDiffett 1996, Winterbourn 1998). AMD is brought about by the geochemical processes responsible for acid rock drainage, except that it is associated with and exacerbated by mining, which exposes reactive rock strata (Black and Craw 2001). Pyrite, which is responsible for the production of acid mine drainage, is commonly an abundant mineral in coal deposits and frequently occurs with other metals such as arsenic, cadmium, cobalt, lead and zinc (Black and Craw 2001). On the West Coast of the South Island, AMD is associated with coal seams within the Brunner Coal measures, which have high levels of sulphur containing minerals (Harding and Boothroyd 2004).

In New Zealand, the mining of coal and gold have been important industries especially in Otago, Southland, the West Coast, Waikato and Northland (Taylor and Smith 1997). The nature and extent of environmental impacts on freshwater systems associated with these mining activities is variable, with changes in water quality and benthic invertebrate habitat being influenced by the underlying geology (Leon and Anstiss 2002), climate (Kim and Kim 2004) and the type of extraction method (Kelly 1988). Coal is extracted in New Zealand using open cast (surface) and underground mining techniques, the former being on a larger scale than underground mining (Kelly 1988), and consequently having the potential to cause disturbance to freshwater systems across a greater proportion of a catchment. Nevertheless, the majority of acid mine drainage, in the Northern Hemisphere, comes from abandoned underground mines that continue to discharge polluted waters (Letterman and Mitsch 1978).

Within the last 20 - 30 years mining activities have received a higher profile due to an increasing awareness of their potential environmental impact on freshwater systems (Gray 1997). AMD has been recognised as a frequent and persistent by-product of mining and a significant source of water degradation, worldwide (Gray 1997, Cherry et al. 2000). AMD waters, although variable in their physico-chemical characteristics, often have low pH, high conductivity and high concentrations of dissolved iron and other heavy metals (Monterroso and Macias 1998, Garcia-Criado et al. 2002). At very

low pH (<3), metals such as iron, zinc, arsenic and copper, become increasingly mobile and soluble, resulting in high concentrations in receiving waters (McKnight and Bencala 1990, Boulton et al. 1994, Harding and Boothroyd 2004).

Most research into the effects of AMD on stream ecosystems has focused on changes to benthic invertebrate communities due to lowered pH and increased concentrations of heavy metals. However, stream communities affected by AMD often do not suffer solely from changes in water chemistry. In fact, aquatic organisms often have to contend with changes to their physical environment caused by metal precipitates (e.g., iron and aluminium). In particular, iron hydroxide (FeOH) precipitates are often associated with streams impacted by coal mining (Soucek et al. 2003, Harding and Boothroyd 2004). Iron precipitates effectively act in a similar manner to sedimentation, and affect in-stream habitat by smothering substrate and clogging interstitial spaces that provide habitat for some species (Gray 1996).

Iron precipitation is predominantly a pH dependent oxidation of primarily soluble ferrous iron (Fe<sup>2+</sup>) to particulate ferric iron (Fe<sup>3+</sup>) (Broshears et al. 1996). In streams with pH <3 iron will generally remain in its soluble form (Harding and Boothroyd 2004), however dilution of AMD waters from heavy rainfall and downstream tributaries can lead to an increase in the pH and subsequent precipitation of iron from the water column on to the surrounding substrate (Kim and Kim 2004). Streams affected by iron deposition generally have lower concentrations of dissolved iron than particulate iron due to the precipitation of iron out of the water column onto the streambed (Broshears et al. 1996, Kim and Kim 2004).

Whereas iron deposits commonly occur as hard encrusting iron precipitates, they can also manifest themselves as blooms or 'flocs' of iron-depositing bacteria. These bacteria (e.g., *Leptothrix* and *Sphaerotilus*) are probably the most obvious and easily recognised micro-organisms in the freshwater environment due to the accumulation of orange ferric iron (Johnson and Hallberg 2003). They are widespread in nature and can occur in freshwaters with near neutral pH (Ghiorse 1984) as well as those impacted severely by acid mine drainage (Ferris et al. 1989). Although the direct involvement of sulphur and iron-oxidising bacteria in the formation of AMD is well known (McGinness and Johnson 1993, Bond et al. 2000, Johnson and Hallberg 2003),

the role of bacteria in the formation of iron flocs is often complex (Clarke et al. 1997, Crundwell 2003, Kappler and Newman 2004). However, in oxygenated waters, iron hydroxides can form passively through the binding of dissolved ferric species to negatively charged polymers, or when soluble ferrous iron reacts spontaneously with dissolved oxygen to precipitate as ferric hydroxide on available nucleation sites (e.g., bacteria) (Konhauser 1998). Additionally, iron bacteria may promote ferric iron deposition actively by oxidising ferrous iron as an energy source (Clarke et al. 1997). The literature on the mineral composition of iron precipitates and iron bacteria flocculants indicates that these are complex issues. However, there is some agreement that pH has a strong influence on the mineral composition of deposited iron, and that iron deposited in near-neutral waters may be composed of complexes that differ from those in lower pH streams (Murad 2003, Kim and Kim 2004). Moreover, various kinds of iron complexes may differ in their potential toxicity to stream organisms.

AMD causes significant modifications to the chemical and physical characteristics of affected freshwater systems, and can lead to a range of biological and ecological changes. The effects of AMD on impacted streams are often at the very least two-fold, with the two most widely documented changes in water quality being lowered pH, and increases in the concentrations of soluble heavy metals. However, AMD contamination associated with active mines, also frequently involves an increase in suspended sediments, and precipitation of ferric iron hydroxide on to the substrate (Kelly 1988, Gray 1996). Not surprisingly, the magnitude of AMD contamination frequently varies spatially and temporally within freshwaters (McGinness and Johnson 1993, Boulton et al. 1994, Gray 1996, Olias et al. 2004) and as a consequence, interactions between stream communities and AMD can be difficult to interpret.

Heavy metals and low pH both affect benthic stream invertebrate communities adversely (Hall et al. 1980, Winner et al. 1980, Økland and Økland 1986, Rasmussen and Lindegaard 1988, Clements 1994) by lowering species diversity, taxonomic richness, benthic densities, and by shifting communities from pollution-sensitive to pollution-tolerant species. The mechanisms for these changes have been linked to a number of factors, including disturbances in ion regulation and calcium metabolism, increases in toxicity of metals, and changes in the quantity and quality of food resources (Townsend et al. 1983, Sutcliffe and Hildrew 1989).

Studies on the density and composition of benthic invertebrate communities in natural acidic and anthropogenically acidic waters, suggest there are differences in responses to acidity between the two systems. Dangles et al. (2004) investigated benthic invertebrate communities in six streams in Northern Sweden, three with naturally low pH (4.0 – 4.6) and three with pH closer to circum-neutral (pH 6.0 – 6.6). They found that benthic invertebrate taxonomic richness among these streams were not significantly different although, the naturally acidic streams supported a distinctive fauna, 27% of the taxa in them being exclusive to those streams. Earlier, Collier and Winterbourn (1987) investigated benthic invertebrate communities in two brown water streams with naturally low pH (pH 4.3 - 5.7) and three alkaline clear water streams (pH 6.6 – 8.0), on the West Coast of the South Island in New Zealand. They found that the number of taxa in the main insect orders was similar among sites although species richness was higher at alkaline sites than acid sites (64 and 47 taxa, respectively). Moreover, several species were common to all sites (e.g., *Deleatidium*), although their densities at alkaline sites were greater. Collier and Winterbourn (1987) suggested that the differences in species abundance may have been related to the availability and quality of food resources, which were in turn directly and indirectly related to stream water pH. This idea supports the notion put forward by Townsend et al. (1983) that acidity may act indirectly to affect invertebrate communities by altering food resources.

Human-induced acidity of previously uncontaminated streams results in obvious shifts in benthic invertebrate communities from acid sensitive species to acid tolerant species. Hall and Ide (1987) illustrated the effects of human induced acidification in a study of benthic invertebrate communities at three sites on two streams in Ontario, Canada. Benthic invertebrates at these sites had been sampled previously from 1937–1942 when stream water pH ranged from 6.1–6.8. Hall and Ide re-sampled the invertebrates in these streams in 1984-1985 when the pH was more variable and generally lower as a result of inputs of sulphuric and nitric acids from acid rain and snow. They found that one stream showed little change in acidity (pH 6.2-6.5) and had the same number of mayfly and stoneflies species in 1984-1985 as when sampled in 1937-1942. However, at the two sites on the second stream, a more acidic headwater site (pH 4.9-6.3) and a site (pH 5.6-6.2) 230 metres downstream there was

a notable reduction in the number of mayfly and stoneflies species (77% and 29%, respectively). Additionally, at the more acidic headwater site, seven mayflies known to be acid-tolerant species were collected in 1984-1985 that had not been collected in 1937-1942. It is common for streams in the Northern Hemisphere to display a relationship between pH and a reduction in benthic invertebrate taxa or abundance (Sutcliffe and Carrick 1973, Hildrew et al. 1984, Simpson et al. 1985, Smith et al. 1990, Rosemond et al. 1992, Dangles and Guerold 2000), with a distinct reduction in taxonomic richness below pH <5.7 (Sutcliffe and Hildrew 1989).

The results of studies on invertebrate communities in Northern Hemisphere acidic waters are often in contrast to findings from streams in regions where low pH occurs predominately as a result of natural processes. For example, on the West Coast of the South Island Winterbourn (1998) found some invertebrate species belonging to Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa in AMD-impacted streams with pH ranging from 2.9-4.2. Collier et al. (1990) suggested that widespread tolerance to low pH in West Coast stream invertebrates may have evolved because of the common occurrence of naturally acidic brown waters. Furthermore, heavy metals that are soluble and toxic at low pH in anthropogenically acidified waters may be less toxic in acidic brown water streams. For example, dissolved aluminium which is toxic in AMD affected systems (Soucek et al. 2001), binds to dissolved organic matter in brown water streams rendering it essentially non-toxic (Collier et al. 1990, Gensemer and Playle 1999)

Acid mine drainages frequently contain high levels of heavy metals, and the responses of benthic invertebrates to them vary considerably (Gower 1994). Findings from studies into the effects of low pH are often confounded by heavy metals (Hall et al. 1980) or visa versa; an issue that is especially relevant to AMD streams (Courtney and Clements 2002). However, it is apparent that metal toxicity generally results in reductions in invertebrate densities, and species richness, and a change in macroinvertebrate dominance (Johnson et al. 1993). For example, Winner et al. (1980) investigated the effect of heavy metal pollution on benthic invertebrate communities in two alkaline limestone streams in Ohio. One stream, Shayler Run, had six sites along it, and had a relatively constant discharge of copper during the study period. Copper concentrations declined downstream from the point of discharge from

120 µg/L to 23 µg/L. The second stream, Elam's Run had five sites along it and received higher and more variable concentrations of three metals: copper, cyanide and zinc in effluent from a metal-plating industry. The maximum concentrations of copper, cyanide and zinc over the duration of the study were 9590 µg/L, 8197 µg/L, and 2431µg/L, respectively. Insect densities in both streams were reduced directly below the point of metal discharge, however, only in Shayler Run, where there was an 81% decline in insect densities, was there a significant difference. Winner et al. (1980) also found a relationship between heavy metal concentration, and the dominance of chironomids in both streams. On Shaylar Run, at the site upstream of the copper discharge, chironomids averaged only 5% of the insects per sample, increased to 75% directly downstream of the copper discharge and then declined to 3%, 2.6 kilometres downstream. In Elam's Run, where metal concentrations were more variable, there was a smaller reduction in chironomid dominance from 86% at the site directly downstream of the metal discharge to 48% at the lowest site. These results illustrate how invertebrate communities can change in response to varying levels of metal contamination.

Periphyton biomass can be highly variable among AMD sites, however in general algal biomass is high in AMD affected streams (Hall et al. 1980, Mulholland et al. 1986). Possible explanations for observed increases in periphyton biomass in acidic waters include a reduction in grazing pressure by macroinvertebrates, a community switch to acid tolerant species, and a decline in microbial decomposition (Hall et al. 1980, Planas et al. 1989, Verb and Vis 2000). The latter may also be important in influencing decomposition rates of organic matter in acidified streams (Townsend et al. 1983). Declines in microbial activity and subsequent reductions in microbial respiration and production have been observed in low pH waters (Palumbo et al. 1987). Additionally, declines in microbial activity and colonisation have been linked to slower organic matter decomposition rates (Allard and Moreau 1986). Reductions in microbial activity may also lead to a reduction in food quantity and quality for detritivorous invertebrate species (Townsend et al. 1983). Organic matter breakdown may be particularly slow in streams suffering metal deposition. Thus, Gray and Ward (1983) observed that ferric hydroxide deposition directly inhibited the colonisation of both fungi and invertebrate shredders.

Finally, stream communities impacted by AMD can be affected by iron deposition, which can lead to ecosystem changes different from those seen in streams affected by low pH or heavy metals alone. The most obvious visual change is that the stream substrate is blanketed in characteristic orange-brown deposits, sometimes termed 'yellow boy' (Niyogi et al. 1999). McKnight and Feder (1984) investigated the effect of hydrous metal oxide precipitates on periphyton and stream benthic invertebrate communities in the Snake River in the Colorado Rocky Mountains. In contrast to previous investigations that had found an increase in algal biomass in waters with low pH, McKnight and Feder (1984) found that substrate covered with precipitates had lower algal biomass but similar algal species to sites that had low pH (<3.8) and/or high concentrations of heavy metals (Al, Fe and Zn). Furthermore, the diversity and abundance of benthic invertebrates was lower at sites with metal precipitates compared to sites suffering from increased acidity and/or metal enrichment. McKnight and Feder concluded from this study that metal precipitates may have a greater adverse effect on stream communities than high concentrations of soluble metals.

Although, iron deposits are most obvious on the stream bed, they can also affect stream invertebrates directly by coating their bodies. Iron precipitates on the gills and gut membranes of mayflies may reduce oxygen consumption, movement and feeding (Gerhardt 1992). However, Gerhardt and Westermann (1995) investigated the effect of iron hydroxide coatings on mayfly nymphs in a field experiment, and found that neither iron precipitates nor increases in iron concentrations affected the survival of the nymphs over three months. They speculated that the indirect effect of iron hydroxide may be more important in structuring stream communities than the direct smothering of iron hydroxide on an animal's body.

Mining-associated changes in water quality also may have chronic effects leading potentially to increases in infections and disease; however, this notion has received little attention.

## **Infections in benthic macroinvertebrates**

Current literature suggests that freshwater benthic invertebrate communities are primarily structured by physico-chemical conditions and habitat suitability (Minshall and Minshall 1978, Harding and Winterbourn 1995, Malmqvist and Hoffsten 2000). The importance of disease and infection in structuring communities has been poorly studied (although see Cummins and Wilzbach 1988). The influence of disease may be most important where aquatic environments have been contaminated by pollutants, leading to benthic invertebrates with increased stress, lowered immunity and therefore increased susceptibility to disease. Stress, although, as a result of mechanical (or physical) disturbance, has been associated with, lower immune defences, and increases in disease outbreaks in marine shellfish (Lacoste et al. 2001, Lacoste et al. 2002).

Numerous studies have investigated the degradation of freshwater associated with heavy metal inputs (Winner et al. 1980, Hoiland et al. 1994, Clements et al. 2000), lowered pH (Parsons 1977, Hall and Ide 1987), sedimentation (Ryan 1991, Quinn et al. 1992), industrial and domestic sewage (Donald 1980) and nutrient enrichment (Campbell 1978). Much of this research has focused on changes in community composition and species diversity (e.g., Dills and Rogers 1974, Clements et al. 1988, Courtney and Clements 1998, Mori et al. 1999, Clements et al. 2000). However, freshwater pollution and associated changes in habitat quality can also manifest themselves in the form of morphological deformities (e.g. Hamilton and Saether 1971, Donald 1980), growth impairment (e.g., Timmermans et al. 1992), behavioural changes (e.g., Vuori 1994) and increases in bacterial growths (e.g., Lemly 1998) in benthic macroinvertebrates.

Morphological deformities in midge (Diptera: Chironomidae) larvae are well documented (e.g., Warwick 1985, Warwick and Tisdale 1988, Jansens de Bisthoven et al. 1992, Bird et al. 1995, Jeyasingham and Ling 1997, Jansens de Bisthoven et al. 1998, Jeyasingham and Ling 2000, Servia et al. 2000) and Servia et al. (2000), put forward four hypotheses to explain them. These hypotheses were (1) teratogenic

(causing malformation in developing eggs) effects from contaminants in the water, (2) teratogenic effects from accumulation of contaminants accumulated by imagos (3) genetic inheritance of contaminant-induced mutations and (4) developmental deformities occurring independently of genetic or teratogenic effects. Although Servia et al. found no support for the first two hypotheses, deformities similar to those observed in larvae collected from contaminated sites were found in *Chironomus riparius* larvae hatched from eggs incubated in uncontaminated water. Secondly their results suggested that the type of deformities found in larvae from one egg mass may differ from the type of deformities found in larvae from another egg mass collected from the same locality. These findings support the third hypothesis, since deformities with consistent similarities were observed in larvae from the same egg masses. Finally, the authors reported deformities in larvae laid by non-deformed adults as support for the fourth hypothesis that deformities can occur independently of genetic or teratogenic effects. They concluded that the hypotheses are not mutually exclusive and that genetic effects and contaminants may both result in an increased frequency of deformities.

Field studies have indicated that metal contamination and deformities in Chironomidae larvae may be linked (Diggins and Stewart 1998). Furthermore, some kinds of heavy metal contamination may show a greater association with deformities than others. Jansens de Bisthoven et al. (1992) investigated accumulation of copper, zinc, cadmium and lead in the bodies of deformed *Chironomus thummi*-group larvae, and concluded that there was a relationship between accumulated lead and copper, but not cadmium or zinc, and higher incidence of deformities in larvae. Bird et al. (1995) investigated induced deformities in the menta of larval *Chironomus* species following the addition of the non-essential trace metal cadmium to an experimental lake in Ontario. Head capsules of chironomids from the lake were compared with individuals from reference lakes. The frequency of observed deformities did not increase over time, or following the additions of cadmium and moreover, four of the five reference lakes had higher proportions of deformed larvae than the experimental lake. From these results Bird et al. (1995) concluded that cadmium was not responsible for the deformities found in *Chironomus* and that the deformities observed were 'natural' abnormalities. Additionally, Warwick (1985) suggested that natural variation in water

quality parameters such as salinity, temperature, oxygen concentrations and sediment deposition may induce morphological deformities in Chironomidae.

Although some studies have indicated a link between levels of pollution 'stress' and abnormalities, with particular reference to chironomids, there is still no consistent evidence of an actual deformity-stress or deformity-fitness relationship (Hämäläinen 1999). To further complicate the issue, populations of aquatic invertebrates from 'unpolluted' and 'unstressed' environments may show signs of morphological deformities. Warwick (1985) proposed that deformities probably occur at uncontaminated sites because a proportion of the organisms are naturally weaker than others. However, in unstressed environments deformities probably occur less often than under stressful conditions. Additionally, the response to stress in the form of deformities is likely to differ among taxa with some individuals being more susceptible than others (e.g., Warwick and Tisdale 1988, Dermott 1991). Furthermore, without the establishment of clear boundaries between what is normal and what is abnormal, assessment of rates and the severity of deformities can be highly subjective (Warwick and Tisdale 1988, Hämäläinen 1999).

As well as having morphological deformities, freshwater invertebrates can be infected by disease and parasites. For example, in New Zealand, pre-parasitic (or encysted) hairworm (Nematomorpha: Gordioidea) larvae have been found in six invertebrate taxa (e.g., *Deleatidium* and *Olinga*) (Poinar 1991). Interestingly, as well as normally encysted hairworm larvae, melanized dead horse hair worm larvae were also observed in some of the invertebrates collected and has been found on encysting horsehair worms in mosquito larvae (*Culex pipiens*) in the laboratory (Poinar and Doelman 1974). The melanin release was considered to be a defence mechanism to eradicate the horse hair worm larvae.

Almost all that is known about disease (e.g. bacterial, viral, and fungal infections) in insects has been the result of continuing investigations of control methods for pest species (Burgess and Hussey 1971). For example, it is known that fungal diseases are common and widespread in insects and that virtually all insect orders are susceptible including dipterans (Scholte et al. 2004). Most information linking fungi and aquatic invertebrates stems from increasing interest in finding biological control methods for

mosquitoes. Scholte et al. (2004) reviewed the advantages and disadvantages of using entomopathogenic fungi as biological control agents for mosquitoes and cited species of *Lagenidium*, *Coelomyces*, *Entomophthora*, *Culicinomyces*, *Beauveria* and *Metarhizium* as being the most important entomopathogenic fungi in terms of mosquito control.

Entomopathogenic micro organisms enter a host's body, either by penetrating the mid-gut (bacteria, viruses and microsporidia), or through the external cuticle (Samson et al. 1988). The latter form of entry is unique and characteristic of fungi and three phases of infection have been identified; (1) spore adheres and germinates on the host cuticle, (2) a germ tube penetrates the insects integument and (3) the fungus develops inside the host's body and usually results in death (Samson et al. 1988). An insect with a damaged cuticle will be more susceptible to infection because of the ease in which a fungal germ tube will be able to enter the body (Samson et al. 1988). Moreover, a 'stressed' insect may be even more susceptible to infection.

The real question here is whether 'stress', as a result of heavy metal pollution, that can bring about morphological changes in benthic invertebrates can also act as a factor in increasing infection rates in a stream community.

## **Thesis objectives**

---

My research is in two parts. In part one I report on investigations into how stream communities respond to iron deposits in the form of chemical precipitate and iron bacterial blooms, ‘flocculants’, as a result of past and present mining. I focused on several key questions in this research.

- 1) Does water chemistry differ between streams characterized by iron flocculent, iron precipitate or no mine pollution?
- 2) Does taxonomic composition and abundance of benthic invertebrate communities differ between streams characterized by iron flocculent, iron precipitate or no mine pollution?
- 3) Does the rate of leaf breakdown and associated microbial activity, differ between streams characterized by iron flocculent, iron precipitate or no mine pollution?
- 4) Does water chemistry and therefore benthic invertebrate communities improve down the study streams?

In part two of the thesis I discuss ‘infection’ rates of benthic macroinvertebrates in a West Coast stream impacted by past gold mining. My research focused on answering the following question:

- 1) Do non-toxic concentrations of heavy metals, such as dissolved iron and aluminium, increase infection rates in benthic invertebrate communities?

## Chapter two

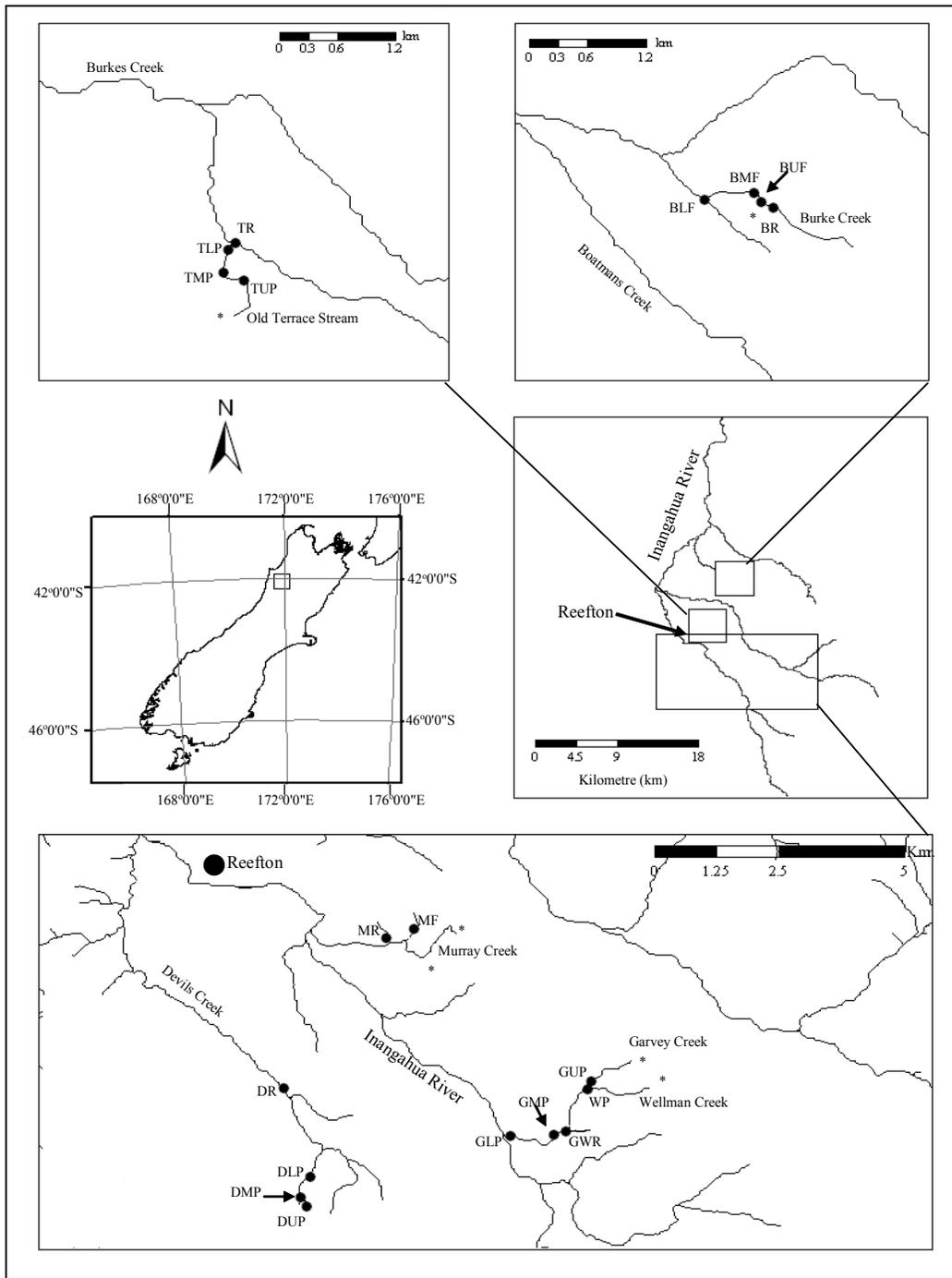
### Study area

---

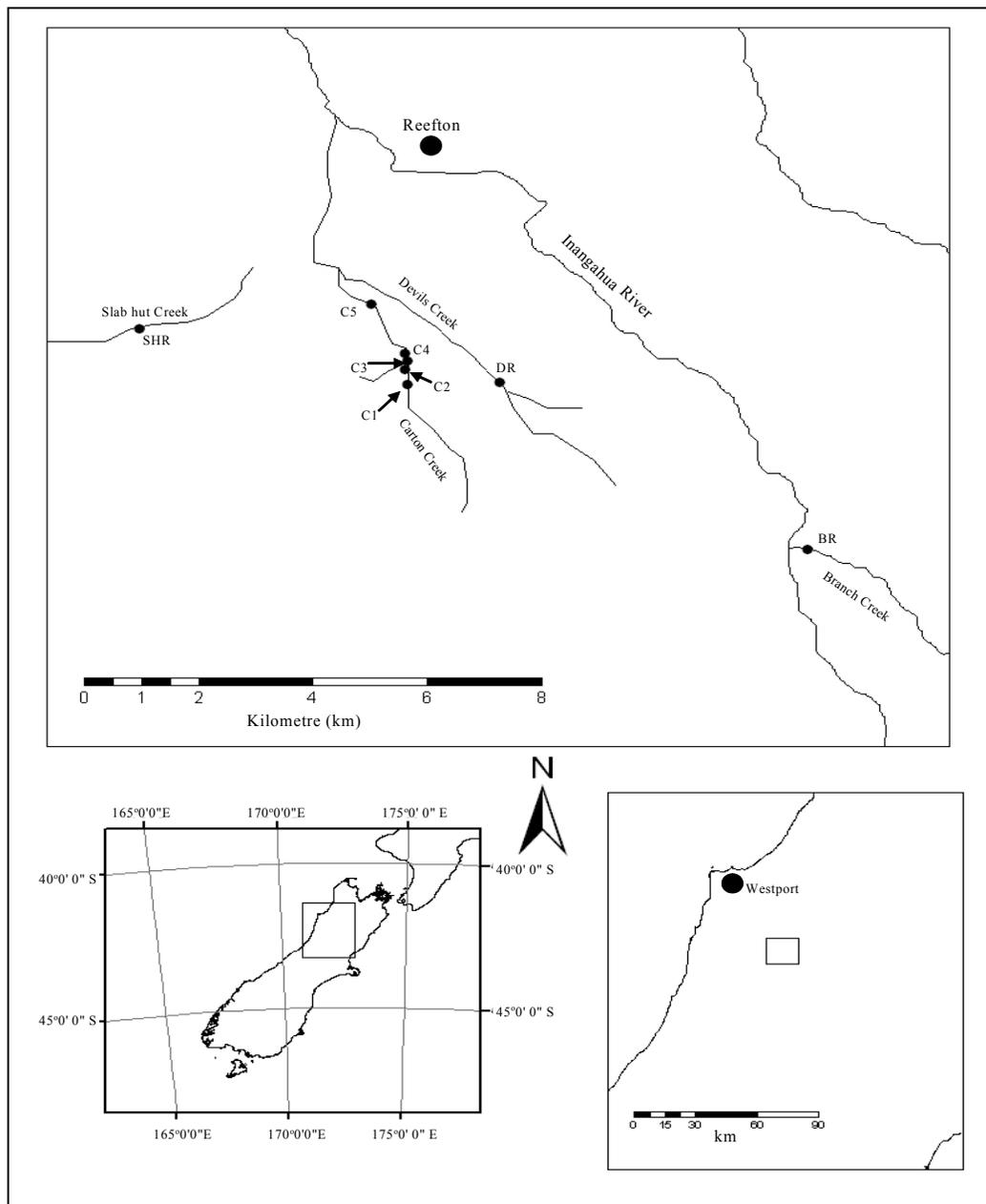
#### **Background**

This study was conducted near Reefton, inland of the Paparoa Ranges, on the West Coast of the South Island. For the first component of this study, 16 sites were used to examine water chemistry, algal biomass, benthic invertebrate communities, and leaf breakdown, four control or reference sites and 12 treatment sites. The 12 treatment sites consisted of sets of three sites located along four mine-polluted streams (Figure 2.1). The four mine-polluted streams comprised of two dominated by iron flocculants, and two with iron precipitates. Control sites were located either upstream or downstream of the impacted stream section or on an adjoining tributary with similar geology, hydrology, vegetation and climate. Additional iron flocculent, iron precipitate and control sites were added later, during the summer sampling period to increase replication for comparisons between broad-scale, flocculent, precipitate and or reference effects.

For the second or 'infection' component of this study, five sites, longitudinally, on the historically mined, Carton Creek and three controls or reference streams were investigated (Figure 2.2).



**Figure 2.1.** Map of four study streams (Old Terrace Stream, Burke Creek, Devils Creek, and Garvey Creek), and 18 study sites, near Reefton, New Zealand. \* denotes approximate location of known coal mining activities.



**Figure 2.2.** Map of eight study sites, five longitudinal, on Carton Creek (C1 – C5), and reference sites, Slab Hut Creek (SHR), Branch Creek (BR), and Devils Creek (DR), sampled between March 2004 and January 2005. \* denotes approximate location of known gold mining activities.

## **Mining history**

The Reefton area, situated within the Victoria Forest Park is unusual from an economic view point in that it has rich deposits of both gold and coal, and the presence of these two minerals played an important role in the early mining history of New Zealand (Henderson 1917, Wright 1990). Financially profitable gold was first found on the West Coast in 1859 on the north bank of the Buller River near the boundary of what is often referred to as the Reefton subdivision (Henderson 1917). It was only a few years later in 1864-65 that the West Coast became recognised as an important source of gold with the opening of the Waimea gold fields. In 1865, prospectors centred in Hokitika and an area now part of Greymouth embarked on searching every gully in the surrounding districts for gold. It was in 1870 when gold-bearing quartz lodes were first discovered in Reefton by the Kelly's near the top of Murray Creek (Henderson 1917). For at least five decades after gold was discovered in Reefton it was the most significant industry in the region (Suggate 1957). However, during the 1930's further prospecting for gold failed to make any new discoveries and the importance of the industry began to decline in the area. During this period the coal mining industry was beginning to expand, with the increase in private demand leading to the opening of many new coal mines (Suggate 1957). Initially, extensive mining for coal in the Reefton area was most likely spurred by the need to obtain fuel to power plants associated with lode mining. The first coal mined in the area was probably from Murray Creek deposits which were used to fuel the Ajax power plant (Henderson 1917). At one time each gold mine had its own coal claim and this meant that the opening of coal mines was not done systematically (Henderson 1917). Additionally, gold miners required timber to build tramways and fluming for water races (Wright 1990), and thus timber production increased considerably with the onset of the gold industry (Henderson 1917).

## **Geology**

The geological history of Reefton and the surrounding area is complex and was first described in detail by Henderson (1917) and later by Suggate (1957). The Reefton Goldfield (or the Reefton Mineral Belt), is an area 30 – 40 km long by 15 km wide and is dominated by basement rocks argillates and greywackles belonging to the Greenland Group (Weber 1995). Gold consists in quartz veins, gravel and conglomerates containing detrital gold with the greatest numbers of quartz lodes of economic importance being contained in the Palaeozoic greywackes and argillites (Henderson 1917). The Reefton coalfield covers a smaller known area of about 16 square km extending from approximately 3.2 km south-west to about 9.5 km north-east of the Reefton township (Williams 1965). Coal seams lie within the Quartzose Coal Measures of Eocene age at the base of the Tertiary sequence and consist of conglomerates, sandstones, mudstones and coal (Williams 1965). The majority of coals from the Reefton coalfield fall within the high volatile C bituminous group and the sub-bituminous A group. Importantly the sulphur content in this coal is high and this has influenced water chemistry within the Reefton coalfield (varying from 1 – 7 %) (Williams 1965).

## **Climate**

The West Coast of the South Island has numerous micro-climates, but in broad-terms the weather is similar throughout the region, with relatively small ranges in mean monthly temperatures and high rainfall (Hessell 1982). The Southern Alps strongly influence the weather on the West Coast by acting as a barrier to prevailing westerlies. The westerly air stream is either deflected or forced to ascend resulting in the West Coast's characteristic rainfall which maybe often heavy and prolonged (Hessell 1982).

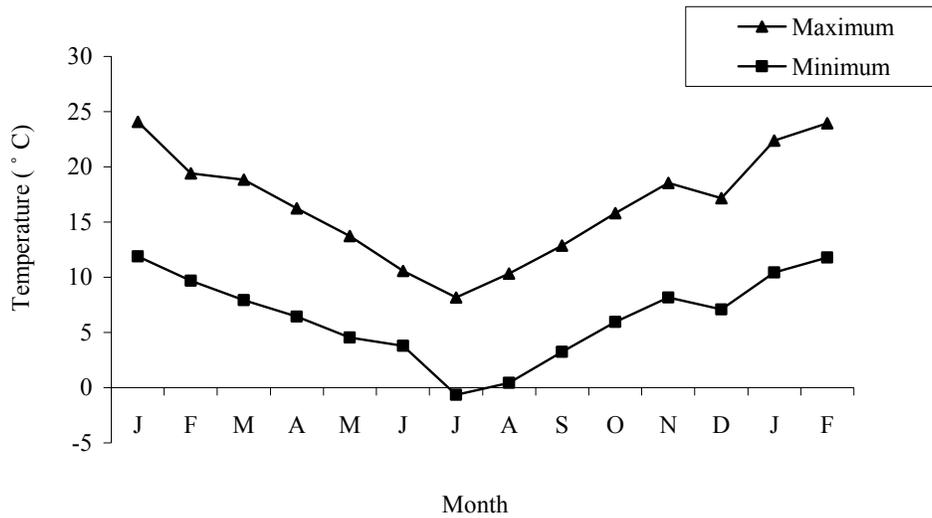
Reefton has its own micro-climate being sheltered by the Paporoa Ranges from the full impact of the West (or 'Wet') Coast weather (Wright 1990). Warm, nor'-westerlies frequently bring the heaviest rain during spring and early summer and in autumn temperatures drop, the wind turns south-west and rain eases to showers or snow. During the winter months southerlies are common and bring cool but clear weather (Wright 1990).

During the period of this study the maximum air temperature recorded at the Reefton weather station (lat 42°117'S, long 171° 86'E) was 24.1 °C in January 2004 and the minimum air temperature was just below zero at -0.6 °C in July 2004 (Figure 2.3a) (G. Furniss, pers. comm.). The mean monthly rainfall recorded at the Reefton weather station from 1<sup>st</sup> January 2004 to 28<sup>th</sup> February 2005 was 169.24 mm. The maximum recorded rainfall was 322mm in June 2004, and the minimum was 61mm in April 2004 (Figure 2.3b) (G. Furniss, pers. comm.).

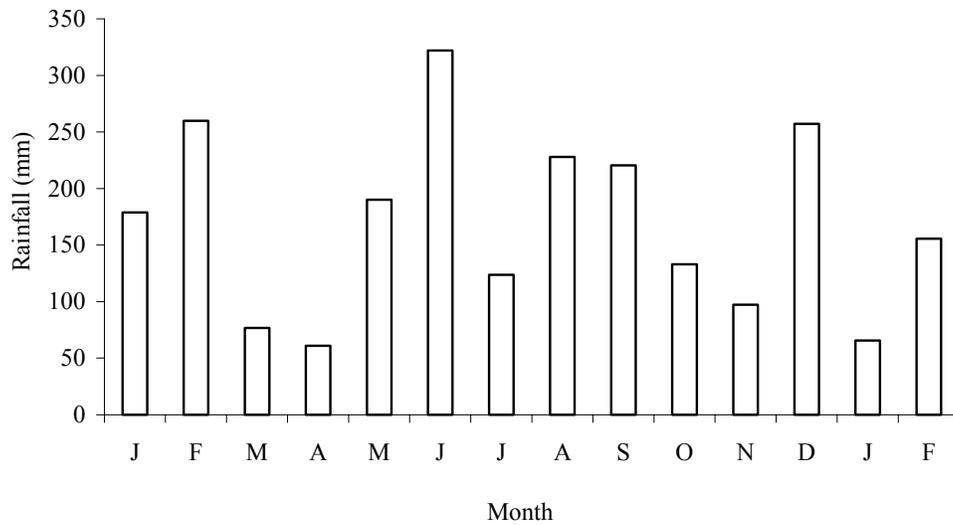
## Vegetation

Forest canopy in the east and north of Victoria Forest Park primarily consists of mixed beech (*Nothofagus* spp.), however toward the south podocarp species become more common and in some areas they can dominant the canopy. Four species of beech occur within this area, red (*Nothofagus fusca*), silver (*N. menziesii*), mountain (*N. solandri* var. *cliffortioides*), and hard beech (*N. truncata*) (Wright 1990). Rimu (*Dacrydium cupressinum*), miro (*Prumnopitys ferruginea*), kahikatea (*Dacrycarpus dacrydoides*) and kaikawaka (*Libocedrus bidwilii*) are the commonest podocarps (Wright 1990). Native species that are common in the understorey of these forests are kamahi (*Weinmannia racemosa*), rata (*Metrosideros robusta*) and tree fuchsias / kotukutuku (*Fuchsia excorticate*). Many areas of forest that had been cleared for mining operations in the past have regenerated, however sites that are poorly drained or infertile have reverted to pakihi (stunted scrub, which usually includes, native species maunka (*Leptosperum scoparium*) or bracken (*Pteridium aquilinum* var. *esculentum*) and introduced species such as gorse (*Ulex europaeus*)) (Wright 1990).

a)



b)



**Figure 2.3.** Climate data for the duration of the study, 1 January 2004 to 28 February 2005, showing a) maximum and minimum air temperatures and b) monthly rainfall (mm) recorded at the Reefton weather station (42° 117' S, 171° 86' E).

## **Iron flocculent and iron precipitate study sites**

### ***Burke Creek (Iron Flocculent)***

Burke Creek is a first order stream, approximately two kilometres in length, running through a primarily mixed *Nothofagus* - podocarp forest. It is a headwater tributary of in the Boatmans Creek catchment, approximately seven kilometres north of Reefton. It has been effected by historic coal workings, however these operations were small due to the coal seams being at most only a few feet thick (Suggate 1957).

### ***Burke upper flocculent (BUF), (Figure 2.4a)***

BUF, was the upper most impacted site on Burke Creek. Directly upstream of this site, thick orange-flocculants coated the true left side of the steep stream bank where mine waters entered the stream. Stream channel width was about 4 – 5 metres, with substrate consisting of small to medium sized cobbles (64 – 256 mm) and the occasional boulder sized (> 256 mm) (Wentworth classification, (Cummins 1964). The canopy cover consisting primarily of mixed *Nothofagus* species, was dense and light level was low.

### ***Burke middle flocculent (BMF), (Figure 2.4b)***

BMF, was the middle impacted site on Burke Creek approximately 100 metres downstream of BUF. Directly upstream of this site on the true right bank, a seepage drains from the hill side, and orange flocculants entered the stream. This section of Burke Creek was more open, with no forest canopy and consisted of two branches with the right hand side branch being primarily affected by iron flocculants. It was evident from this site in particular that Burke Creek was prone to severe flooding with tree branches strewn across the stream channel and significant areas of bank erosion. Channel width was about 5 – 6 metres, with substrate primarily consisting of small to medium sized cobbles. Riparian site vegetation consisted mainly of gorse, manuka and *Nothofagus* species.

### ***Burke lower flocculent (BLF)***

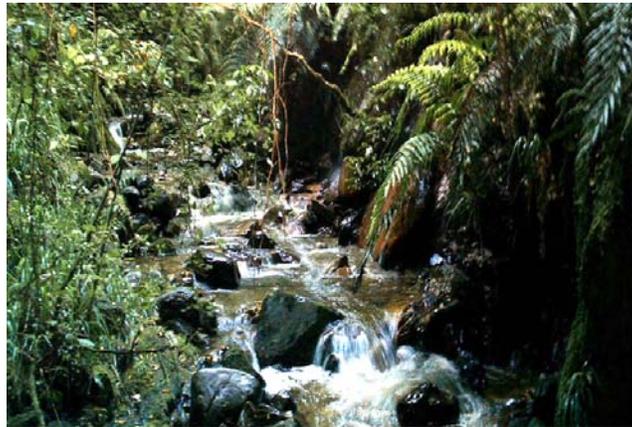
BLF, was the lowest impacted site on Burke Creek, approximately 500 metres downstream, from BMF, and about 5 – 10 m upstream of its confluence with Coal Creek. The stream channel narrowed at this site and was about 1.5 – 2 metres. The

substrate consisted of moderate to large pebbles (4-64 mm) with occasional medium-sized cobbles. The riparian vegetation consisted primarily of gorse and unmanaged grasses. Iron flocculants increased during periods of low flow, and were least obvious after periods of high rainfall.

***Burke reference (BR)***, (Figure 2.4c)

BR, was free of iron flocculants and was located about 60 – 100 metres upstream of the upper most impacted site, BUF, on Burke Creek and the mine discharge. Stream width, vegetation cover and light entry were similar to that described for BUF and bed substrate was made up of small to medium sized cobbles and large boulders.

a)



b)



c)



**Figure 2.4.** Burke Creek a) upper site (BUF), mine drainage enters on the right hand side of the picture, b) middle site (BMF), with orange coloured flocculants entering on the left hand side of the picture and c) reference site (BR), upstream of mine drainage and BUF. Photos taken January 2005.

***Old Terrace Stream (Iron Flocculent)***

Old Terrace Stream is a short (approximately 1.5 kilometres), first order stream that has been impacted by the mining activities at the adjacent Old Terrace Coal Mine. This stream is un-named, and for identification purposes here has been called, Old Terrace Stream.

***Terrace upper flocculent (TUF)***

TUF, was the uppermost impacted site situated on Old Terrace Stream. The width of the stream at this site was approximately 1 – 1.5 metres wide, with substrate consisting of moderate to large pebbles (4 – 64 mm) and small to medium sized cobbles. Riparian vegetation was dominated by *Nothofagus* species on the right, and gorse and grasses on the true left. Orange coloured iron flocculants covered the substrate within the stream channel which looked to be heaviest during periods of low flows.

***Terrace middle flocculent, (TMF) (Figure 2.5)***

TMF was the middle impacted site on Old Terrace Stream, approximately 200 metres downstream of TUF. Stream width, depth and riparian vegetation were comparable to those described for TUF.

***Terrace lower flocculent (TLF)***

TLF, was the lowest impacted site situated on Old Terrace Stream approximately 400 metres downstream of TMF. Channel width was approximately one metre wide with substrate consisting of moderate to large pebbles. Riparian vegetation surrounding this site consisted primarily of gorse and grasses.

***Terrace reference (TR)***

TR was situated on another stream called, Burkes Creek and was within 250 metres of TLF and represented the control or ‘reference’ condition for comparison with Old Terrace Stream. This stream was a second order stream, about 3 – 4 metres wide and had substrate dominated by medium to large pebbles with a few larger cobbles. Riparian vegetation consisted of gorse and manuka. No iron deposits were evident at this site.



**Figure 2.5.** Old Terrace Stream (TMF), showing the orange coloured flocculants characteristic of this stream, particularly during low flow as when this picture was taken. Photo taken; January 2005.

***Garvey Creek and Wellman Creek, (Iron precipitate)***

Garvey and Wellman creeks are approximately 15 kilometres SE of Reefton and drain a catchment that has been the focus of coal mining operations since 1947 (Suggate 1957). The main source of coal mining pollution to Wellman Creek is from the opencast mine, Island Block, while the main stem of Garvey Creek continues to receive inputs from the disused burning mine. The impact of coal mining is visually obvious on Wellman Creek and almost the entire length of Garvey Creek with the stream bed being heavily coated and stained by iron precipitates.

***Garvey upper precipitate (GUP) (Figure 2.6a)***

GUP, is the uppermost site on Garvey Creek, situated about 10 metres upstream of its confluence with Wellman Creek. This is a first order stream, approximately 4 metres wide with the streambed consisting of substrate sizes ranging from fine gravel and sand (< 2mm) to larger cobbles with a few boulders present. Iron deposits are evident on the stream bed particularly during periods of low flow. GUP was an additional site added in summer (2005) and was not sampled during winter (2004).

***Garvey middle precipitate (GMP)***

GMP is the middle site on to Garvey Creek, approximately 1 kilometre downstream of GUP. Riparian vegetation at this site consisted largely of gorse, black berry, grasses and low canopy trees. At this site, Garvey Creek is a second order stream, and

is approximately 3 – 4 metres wide, with substrate ranging from fine gravel and sand to pebbles to smaller sized cobbles.

***Garvey lower precipitate (GLP)***

GLP was the lowest impacted site on Garvey Creek, approximately 1 kilometre downstream from GMP, and was about 10 metres upstream from State Highway 7. The channel width was approximately 2 – 3 metres wide and substrate consisted of fine gravel, sand, pebbles and small sized cobbles. Riparian vegetation consisted mainly of gorse and pastoral grasses, and dairy farming occurred in the paddocks above this site. Iron precipitation declined at this site, but was still evident by the iron stained substrate within the stream channel.

***Wellman precipitate (WP)*** (Figure 2.6b)

Wellman Creek is a first order stream and tributary of Garvey Creek and WP was about 20 metres upstream of its confluence with Garvey Creek. Channel width was approximately 5 metres wide and consisted of unsorted but heterogeneous substrate including pebbles, cobbles and many large boulders. Heavy deposits of orange to dark yellow iron precipitates smothered and stained the stream bed.

***Garvey Wellman reference (GWR)*** (Figure 2.6c)

GWR was used as a reference site for the impacted Wellman and Garvey Creek sites and was situated on an un-named tributary of Garvey Creek. In the past, this creek has been used as a water supply to wash coal vehicles, and for identification purposes it will be referred to as Wash Creek. Wash Creek is a small (approximately 1 metre wide), first order stream that drains primarily through native podocarp forest. The bed substrate consisted primarily of gravel, small pebbles and cobbles.

a)



b)



c)



**Figure 2.6.** Garvey Creek Coalfield sites a) upper site (GUP), with brown iron precipitates, b) Wellman Creek (WP), the orange coatings on the stream bed is iron precipitate, and c) reference stream, Wash Creek (GWR), seen here emerging from the forest. Photos taken January 2005, except GWR which was taken June 2004.

***Devils Creek (Iron precipitate)***

Alborn Coal Mine lies within the Reefton Coalfield at the southern end of the Devils Creek Catchment, at the headwaters of Progress Creek. This mine was historically worked for a number of years to supply coal to the Globe-Progress gold mine, and later to supply local requirements. Mine workings cover several square kilometres (Weber 1995).

***Devils upper precipitate (DUP)***

DUP was situated on Progress Creek, a small, first order tributary of Devils Creek and was approximately 200 metres upstream from the car park on the Alborns Coal Mine walkway. Channel width was approximately 1 – 1.5 metres wide, and stream bed substrate consisted of coal, silt, gravel, pebbles and small cobbles. Riparian vegetation surrounding this site was open and consisted primarily of manuka, grasses and small silver beech. Dark brown iron precipitates were found on the stream bed at this site.

***Devils mid precipitate (DMP) (Figure 2.7a)***

DMP was also on Progress Creek, and was about 200 metres downstream of DUP, at the car-park at the start of the Alborns Coal Mine walkway. A large stockpile of waste coal lies adjacent to the stream at this site. The stream at this site was approximately 2 – 3 metres wide, with stream substrate consisting of silt, pebbles and small cobbles. The stream substrate was covered and stained by brown precipitates. Riparian vegetation at this site was similar to that of DUP however, a greater amount of shade was provided.

***Devils lower precipitate (DLP)***

DLP was located on Devils Creek and was the lowest, impacted site sampled, within the Devils Creek catchment. The stream at this site was second order, with a width at this site was approximately 2.5 – 3.5 metres wide, and stream bed substrate consisted of pebbles and various sized cobbles. Riparian vegetation consisted of a mixture of native low canopy species and mixed *Nothofagus*.

**Devils reference (DR) (Figure 2.7b)**

DR was used as a reference site and was located on Devils Creek, 30 metres downstream of Progress Junction Bridge, and about 2 kilometres downstream of DLP. Stream width at this site was approximately 5 – 6 metres wide and stream substrate consisted primarily of cobbles and boulders. This part of the catchment had been used for forestry and the surrounding vegetation was a mixture of exotic pines and eucalypts. This site was far enough downstream that no acid mine drainage effects could be detected in the water chemistry.

a)



b)



**Figure 2.7.** Devils Creek a) middle site (DMP), and b) the reference site (DR), looking downstream from Progress Junction Bridge. Photos taken; January 2005.

***Murray Creek tributaries***

***Murray flocculent (MF)*** (Figure 2.8)

MF was on a small, first order tributary of Murray's Creek and was accessed via the Murray Creek walkway. The stream was about 1.5 metres wide, with bed substrate consisting of pebbles, cobbles and small boulders. Vegetation surrounding this site consisted primarily of large and regenerating mixed *Nothofagus*. MF was affected by iron flocculants, and was an additional site added in summer (2005) and was not sampled in winter (2004).

***Murray reference (MR)***

MR was a small (about 1 m wide), steep, first order tributary of Murray's Creek and like MF was accessed via the Murray Creek walkway. The bed substrate consisted of gravels, pebbles and large cobbles. The vegetation surrounding this site consisted of *Nothofagus* forest and was similar to that of MF. This stream has several large woody debris jams. This site was also sampled in summer (2005), but not in winter (2004).



**Figure 2.8.** Site MF, tributary of Murray's Creek. This photo shows the characteristic orange coatings of iron flocculants. Photo taken; January 2005.

## **Infection study sites**

### **Carton Creek (C1-C5)**

Carton Creek is a third order tributary of Devils Creek, and rises in the foot hills of the Victoria Ranges, within Victoria Forest Park. For this part of the study, four sites longitudinally on Carton Creek and one tributary of Carton Creek were investigated. Three control or 'reference' streams with minimal, or no known mining histories were also sampled. Carton Creek was accessed via Quigley's Track, beginning at the end of Quigley's Road, approximately 3 kilometres south-west of Reefton.

#### **C1** (Figure 2.9a)

C1 was the upper most site situated on Carton Creek and was approximately 2 -3 metres wide, with bed substrate consisting primarily of boulders (>256mm). Forest canopy surrounding this site consisted of mixed *Nothofagus* species.

#### **C2** (Figure 2.9b)

C2 was approximately 300 metres downstream from C1, and was about 3 – 5 metres wide, with bed substrate consisting of small to moderate pebbles (4 – 64 mm), cobbles (64 – 256 mm) and boulders. Forest canopy at this site was similar to that found at C1 and consisted of mixed *Nothofagus* species.

#### **C3** (Figure 2.9c)

C3 was on a small, first order un-named tributary of Carton Creek, located about 170 metres downstream of C2. This stream was approximately 1 – 1.5 metres wide, and canopy vegetation consisting predominately of mixed *Nothofagus* species. Bed substrate consisted of a mixture of moderate to large pebbles (4 – 65 mm), cobbles and occasional boulders. This stream is thought to be in the vicinity of an old, small gold mine, Morning Star. I expected that this might be the source of elevated dissolved metals in Carton Creek.

#### **C4** (Figure 2.10a)

C4 was situated on the forest edge and had bed substrate ranging from large pebbles, cobbles and occasional boulders. At C4, the channel was quite wide but the wetted

channel area ranged from approximately 3 – 6 metres over the course of the study. The surrounding vegetation consisted primarily of beech (*Nothofagus*), and at some stage during the year a large beech tree fell across the channel.

**C5** (Figure 2.10b)

C5 was the bottom site on Carton Creek and was situated below the reach of Carton Creek that was diverted in 2003 for a mining operation. This site was accessed across a paddock and was approximately 4 - 5 metres wide, with riparian vegetation consisting primarily of introduced gorse, broom and exotic grasses. Bed substrate ranged from pebble to cobble sized.

***Reference streams***

***Branch Stream (BR)*** (Figure 2.11a)

BR, was a fast flowing, fourth order stream and was the largest stream sampled for this part of the study, being approximately 11 metres wide. This site was about 60 metres upstream of the State Highway 7 bridge, and had bed substrate consisting of large cobbles and boulders. Riparian vegetation at this site consisted of shrubby gorse and broom and provided little overhead shade.

***Devils Creek (DR)***

Refer to DR, of previous section for description of this site.

***Slab Hut Creek (SHR)*** (Figure 2.12b)

SHR, a third order stream, was accessed upstream of the State Highway 7 bridge between Reefton and Maimai, and was approximately 3 – 4 metres wide. Bed substrate was pebble to cobble sized and riparian vegetation was primarily introduced gorse, broom and blackberry.

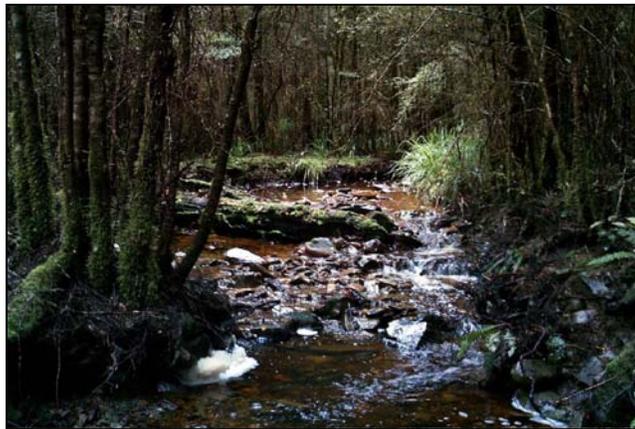
a)



b)



c)



**Figure 2.9.** Carton Creek sites a) C1, the uppermost site, b) C2, and c) C3, located on a tributary of Carton Creek. Photos taken; January 2005.

a)

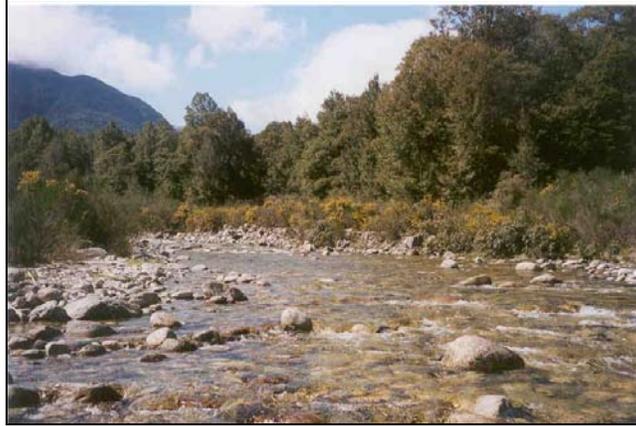


b)



**Figure 2.10.** Carton Creek a) C4, showing a large beech tree that fell across the channel during the course of the study, and b) C5, the furthest downstream site. Photos taken; January 2005.

a)



b)



**Figure 2.11.** Reference sites a) Branch Creek (BR), and b) Slab Hut Creek (SHR). Photos taken; January 2005.

## Chapter three

# Effects of iron flocculants and iron precipitate on water chemistry, algal biomass and benthic invertebrates

---

### Introduction

The extent and nature of environmental impacts on freshwater systems as a result of mining often vary both spatially and temporally (Letterman and Mitsch 1978, McKnight and Bencała 1990, McGinness and Johnson 1993, Munk et al. 2002). However, in many coal mine systems affected by acid mine drainage (AMD), pH is often significantly lower than other non-impacted or minimally impacted streams, and this ultimately affects concentrations and precipitation of dissolved metals (Gray 1998, Nordstrom et al. 2000, Black and Craw 2001).

Further, changes in water chemistry usually influence the spatial distribution, and diversity and abundance of both, algal communities, and benthic macroinvertebrates (Minshall and Minshall 1978, Anthony 1999, Courtney and Clements 2002, Bradley 2003). My study focuses on the various effects of iron deposition on benthic flora and fauna in a range of water chemistries.

In this chapter I compare water chemistry between streams characterised by iron precipitates and iron flocculants and investigate whether benthic algal biomass and benthic invertebrate communities responded differently to these two forms of iron deposition. I focus on two aspects, first a broad-scale comparison of precipitate and flocculent effects and secondly an assessment of longitudinal changes in responses. Specially, I asked the questions;

1. Are water quality parameters the same in the iron flocculent and iron precipitate streams?
2. Does algal biomass, benthic invertebrates (particularly total abundances, EPT densities and taxonomic richness), differ between flocculent, precipitate and reference streams?



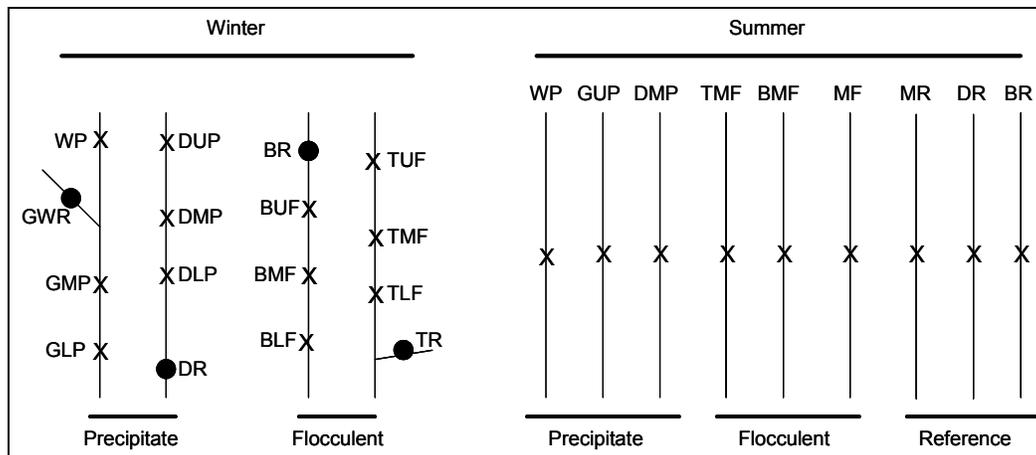
3. Does water quality show longitudinal change in flocculent and precipitate streams and if so do impacted sites shows longitudinally change in algal biomass and benthic invertebrate communities in response to this change in water chemistry?

## Study design

This study was designed to examine, water chemistry, algal biomass and benthic invertebrates across (1) broad scale ‘stream-treatments’ and (2) three longitudinal sites along study streams.

Sites were sampled in winter and summer. Initially in winter, the three sites on each stream were ‘pooled’ and used as a replicate in analysis, thus there were two iron precipitate, and two iron flocculent streams but four control sites (Figure 3.1). In summer, nine sites were used to examine stream-treatment, three flocculent, three precipitate and three references, this enabled increased replication of the stream-treatment effect (Figure 3.1).

Longitudinal change was investigated in winter (June 2004), and in summer (January 2005), using 16 sites, with three sites longitudinally, on two precipitate and two flocculent streams, and four reference sites (see Chapter two; Figure 2.2 for location and site details).



**Figure 3.1.** Study design used to examine stream-treatment effect, between flocculent, precipitate and reference streams in winter (left) and summer (right). Black closed circles in winter diagram represent reference sites. Letter codes (e.g. WP) represent actual sites sampled and are described in Chapter two.

## **Methods**

### **Water chemistry**

A suite of basic water chemistry parameters were measured on several occasions during the winter (2004) and summer (2005). Temperature, specific conductivity and pH were measured using an Oakton CON 10 Series meter on five different occasions at all sites during winter, and on either two or three occasions during summer. Turbidity (HACH 2100P Turbidimeter) was also measured three or four times at all sites in winter, and one to three times during the summer.

Freshwaters effected by acid mine drainage often have elevated concentrations of heavy metals (Leon and Anstiss 2002), and thus grab water samples were collected once in winter and once in summer and analysed for dissolved heavy metals. Water was collected in acid washed 250ml polyethylene bottles from two flocculent sites (BMF and TMF), two precipitate sites (DMP and GMP) and from two reference sites (BR and DR). In summer, two additional sites (MF, MR) were sampled. These samples were sent to a commercial laboratory (RJ Hill Laboratories, Hamilton) and analysed for dissolved aluminium, iron, nickel, arsenic and zinc. Results from GMP acted as a 'pooled' value for WP and GUP in statistical analysis.

### **Biological sampling**

Chlorophyll-*a* was used to estimate algal biomass at each site. Sites were sampled once in winter (June 2004), and once in summer (January 2005). Five randomly collected, similar sized cobbles, were taken from riffle sections at each site and returned to the laboratory within 24 hours where chlorophyll-*a* was extracted. Cobbles were placed with the upper surface faced down (the exposed surface in the stream) in to separate plastic containers with 100 ml of 90% ethanol and were left for 24 hr at 4°C in the dark. The extracted pigments were measured at absorbencies 665 nm and 750 nm using a spectrophotometer. Cobbles were labelled to correspond to extracted absorbencies, and dried.

Following chlorophyll-*a* extraction, cobble surface area was estimated. It was assumed that only the top surface of the cobble had epilithon, and thus the cobble surface area was estimated by tracing around the cobble on paper, cutting it out and

weighing the piece paper on a balance to the nearest 0.001g. A known area (1 cm<sup>2</sup>) was also cut out and weighed. The following equation (after (Steinman and Lamberti 1996) was used to estimate the unknown cobble surface (A<sub>c</sub>) :

$$A_c = \left( \frac{A_k}{W_k} \right) * W_{cf}$$

Where A<sub>k</sub> represents the known area, W<sub>k</sub>, known weight and W<sub>cf</sub>, weight of cut out piece of paper.

Chlorophyll- *a* was calculated using the following formula below.

$$\text{Chlorophyll-}a = \left( \frac{12 * \text{Abs (665 - 750)} * \text{ml ethanol}}{\text{area}} \right) / 2.6$$

Organic and deposited inorganic sediment on cobble surfaces was examined once in winter (June 2004) at 16 sites, and once in summer (January 2005) at all 19 sites. On each sampling occasion five similar sized cobbles were randomly collected from riffles at each site and returned to the laboratory. Organic and inorganic material was scrubbed from each cobble using a wire bristled brush into approximately 100 ml of water, and then vacuum filtered on to pre-weighed Whatman glass microfilters (GF/C). Samples were dried at 40°C for 24 hrs, weighed, ashed at 500°C for 1 hr and then reweighed. Filters were weighed to the nearest ± 0.001g. Organic and inorganic material per cm<sup>2</sup> of cobble surface was estimated first by estimating cobble surface area using the method and equation as described above for estimating cobble surface area for algal biomass per cm<sup>2</sup>. The initial dry weight (DW g) of the filtered material prior to ashing was determined using the equation after Steinman and Lamberti (1996) below:

$$DW = W_a - W_f,$$

where  $W_a$  is the dried material on the filter (g) and  $W_f$  is the filter weight (g). Following ashing the weight of material minus the filter weight was also calculated using this equation.

Ash free dry mass or alternatively organic deposits ( $\text{g}/\text{cm}^2$ ) was determined using the following equation after Steinman and Lamberti (1996):

$$\text{AFDM} = \frac{(\text{DW} - W_{\text{ash}})}{A_{\text{vr}}}$$

Where  $W_{\text{ash}}$  is the weight of the material on the filter after ashing and  $A_{\text{vr}}$  is the calculated surface area of cobble ( $\text{cm}^2$ ). Inorganic material (g) per surface area ( $\text{cm}^2$ ) of cobble was similarly calculated using the final weight remaining after ashing.

Quantitative benthic invertebrate data was collected by taking three Surber samples ( $0.30 \text{ cm}^2$ ,  $500 \mu\text{m}$  mesh) at 16 sites in winter (June 2004) and at 19 sites in summer (January 2005). Surber samples were randomly collected from riffles, and substrate was thoroughly disturbed to an approximate depth of 5 cm and surfaces of larger stones were wiped to dislodge attached fauna. All samples were preserved separately in the field with 70 % ethanol and returned to the laboratory for analysis.

Benthic invertebrate samples were washed through a  $500 \mu\text{m}$  mesh sieve and placed in to a white sorting tray where organisms were removed and identified using a microscope up to 40 x magnification. Invertebrates were identified to the lowest possible taxonomic level. Usually genus or species, except for Chironomidae which were identified only to tribe and Oligochaeta, Ostracoda, Acarina and Collembola which were not identified past order or class. Identifications were made using Winterbourn et al. (2000a).

## **Statistical analyses**

### **Treatment comparisons**

#### **Water Chemistry and inorganic sediments**

To determine if water chemistry, concentrations of dissolved heavy metals, and deposited inorganic sediment differed between the three stream-treatments and between winter and summer, a generalised linear model, analysis of variance (GLM ANOVA) was used, with stream-treatment and 'season' as factors (in all analyses) and water chemistry parameters (except pH which was not analysed), or inorganic sediments as responses. Stream-treatments using summer data, were compared using mean values from the three streams corresponding to each stream-treatment effect and were used as replicates in analysis. Stream-treatment effect, using winter data was examined by using a 'pooled' stream value, and was obtained by taking the mean value from each site longitudinally and then averaging across the three sites, to obtain one value, that was then used as a replicate in stream-treatment analysis. Data recorded from each reference site were averaged to gain an overall value for each site.

#### **Biotic Responses**

GLM ANOVA's were used to investigate whether there were any differences between the three stream-treatments, seasons or an interaction effect between treatment and season. Mean algal biomass and deposited organic deposits were examined between stream-treatments by averaging algal biomass across the five sampled cobbles at each site and then using these values as replicates in stream-treatment comparisons. Winter samples, of algal biomass and organic deposits were pooled in the same way as water chemistry.

Taxonomic richness, benthic invertebrate and EPT abundances were investigated using a GLM ANOVA, with stream-treatment, and season as factors and biotic variables as responses. The stream-treatment effect, using summer data, was examined by averaging the number of taxa, benthic invertebrate and EPT abundances at each of the nine sites (using the three Surber samples as replicates). These averages were used as replicates in stream-treatment analyses. Stream-treatment effect using longitudinal data, collected in winter, was examined by averaging, total benthic

invertebrate and EPT abundances at each longitudinal site (using Surber sample as replicates), on each stream, and then averaging across the three sites, to obtain an overall value which was used as a replicate in stream-treatment comparison. Benthic invertebrate and EPT abundances at reference sites were obtained by averaging the total numbers from between the three Surber samples, these values were used as replicates in stream-treatment comparisons. Total taxonomic richness at each stream was obtained by counting the total number of taxa recorded at each site and then averaging these values to obtain a mean value for each stream which was used as a replicate in stream-treatment comparisons.

### **Community composition patterns**

Patterns in community composition were examined using seasonal means of species abundances from the 16 sites sampled in both winter and summer, using a non-metric multidimensional scaling (NMDS) ordination with the PC-ORD statistical package (McCune and Mefford 1995).

Non-metric multidimensional scaling is an iterative search for the ranking and placement of samples in  $k$  dimensions (ordination axes) that minimises stress of the  $k$ -dimensional ordination configuration (Kahmen et al. 2005). The fit between dissimilarities and inter-object distances can be improved (stress reduced) by increasing the number of dimensions in the scaling, however more dimensions increases the difficulty of interpretation of the final ordination configuration (Quinn and Keough 2003). Thus, patterns in community composition have been examined using a two dimensional solution. The NMDS was run using 100 random number of seeds and as a distance measure to determine similarity between sites the Bray-Curtis coefficient was used. Bray-Curtis as a measure of similarity is well suited for species abundance data because it ignores multiple zeros (Quinn and Keough 2003).

## **Longitudinal patterns along impacted streams and comparison with reference sites**

Patterns in water chemistry, abiotic and biotic responses were examined at three sites in four streams longitudinally and compared to their corresponding reference site.

### **Water chemistry and inorganic sediment**

A GLM ANOVA was used to investigate differences in water chemistry parameters and deposited inorganic sediment between sites, season, and any interaction effect between site and season. Spot water chemistry measurements taken separately on each visit in winter and in summer were used as replicates for analysis. Deposited inorganic sediment on the five cobbles at each site was used as replicates in analysis.

### **Biotic responses**

A GLM ANOVA was used to investigate differences in biotic variable between sites, seasons, and any interaction effect between sites and season, using cobbles as replicates.

Differences detected by GLM ANOVAs, were tested using Tukey's honestly significant differenced (HSD) post-hoc mean tests to determine where the significant difference lay. Plots of residuals versus fitted values and normality plots were used to examine for normality and homoscedasticity of data. In instances where assumptions of normality and homoscedasticity were not met response variables were log transformed ( $\chi + 1$  where necessary) (Zar 1999). All statistical analyses were carried out using SYSTAT (Version 10).

## **Results**

### **Water Chemistry and inorganic sediment**

Comparisons of water chemistry between the three stream-treatments (flocculent, precipitate and reference streams) indicated that in general reference and flocculent streams had similar water chemistry, but precipitate streams were markedly different. For example, pH across precipitate streams ranged from 3.3 – 5.2 while flocculent and reference streams had pH ranging from 5 – 6.9 and 5.1 – 7.7, respectively (Table 3.1). Measured specific conductivity differed across stream-treatments and was

significantly higher in precipitate streams (Table 3.1). There was no difference in specific conductivity between seasons and there was no interaction effect between treatment and season (Table 3.1). This suggests that there is little seasonal variation in conductivity, with higher overall values in precipitate streams, than in flocculent and reference streams. There was no difference in turbidity across treatments, seasons or any interaction effect between stream-treatments and season (Table 3.1). Temperature did not differ significantly across stream-treatments but unsurprisingly there was a significant season effect with higher temperatures being recorded in all streams in summer (Table 3.1). There was no interaction effect between treatment and season.

Dissolved heavy metal concentrations were significantly different across treatments with one exception, arsenic which had similar values across all streams (Table 3.1). Dissolved iron, aluminium, nickel and zinc concentrations were highest in precipitate streams, and lowest in reference streams (Table 3.1). There were no seasonal differences, or an interaction effect, with dissolved metal concentrations remaining highest in the precipitate streams and lowest in the reference streams in either season.

Deposited inorganic sediment on cobbles was highest in the precipitate streams and lowest in the reference streams (Figure 3.2a). Significantly more inorganic sediment was deposited on cobbles in the precipitate streams, and more was deposited in winter (Table 3.2). However, there was no interaction effect between stream-treatment and season on deposited inorganic sediment, which was highest in precipitate streams and lowest in reference streams in both seasons (Table 3.2, Figure 3.2 a). Further, a Tukey (HSD) post-hoc test indicated that the amount of inorganic sediment in reference streams in summer, was significantly lower than inorganic sediments in precipitate streams in either season ( $P < 0.05$ ) (Figure 3.2a).

**Table 3.1.** Mean ( $\pm 1$ SE), except pH water chemistry across the three stream-treatments (flocculent, precipitate and reference) during winter (2004) and summer (2005). GLM ANOVA statistics are shown for stream-treatment (treatment), season, and interaction effects. Significant results are in bold.

	Winter			Summer			Treatment		Season		Treatment*Season	
	Flocculent	Precipitate	Reference	Flocculent	Precipitate	Reference	$F_{2,11}$	$P$	$F_{1,11}$	$P$	$F_{2,11}$	$P$
pH	6.2 (5.1 - 6.9)	4.2 (3.3 - 5.2)	6.8 (5.1 - 7.4)	6.8 (5.5 - 6.8)	4.2 (3.8 - 4.6)	7.1 (6.7 - 7.5)						
Conductivity ( $\mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ )	68 $\pm$ 3	300 $\pm$ 157	56 $\pm$ 8	71 $\pm$ 4	460 $\pm$ 197	91.3 $\pm$ 23	16.502	<b>&lt;0.001</b>	1.426	0.258	0.286	0.757
Turbidity (NTU)	10.6 $\pm$ 7.0	30.5 $\pm$ 11.2	7.4 $\pm$ 3.1	14.3 $\pm$ 6.17	5.2 $\pm$ 2.2	7.0 $\pm$ 3.50	2.926	0.096	2.897	0.117	3.297	0.076
Temperature ( $^\circ\text{C}$ )	8.9 $\pm$ 0.0	5.46 $\pm$ 3.2	7.7 $\pm$ 0.3	13.8 $\pm$ 0.87	13.4 $\pm$ 0.8	15.7 $\pm$ 0.54	1.832	0.206	137.28	<b>&lt;0.001</b>	2.839	0.115
							$F_{2,8}$	$P$	$F_{1,8}$	$P$	$F_{2,8}$	$P$
<i>Dissolved heavy metals</i>												
Iron ( $\text{g m}^{-3}$ )	0.32 $\pm$ 0.05	0.74 $\pm$ 0.13	0.16 $\pm$ 0.115	0.32 $\pm$ 0.067	1.16 $\pm$ 0.47	0.17 $\pm$ 0.106	7.527	<b>0.015</b>	0.099	0.761	0.074	0.929
Aluminium ( $\text{g m}^{-3}$ )	0.22 $\pm$ 0.17	2.62 $\pm$ 1.47	0.107 $\pm$ 0.066	0.145 $\pm$ 0.032	1.83 $\pm$ 0.96	0.131 $\pm$ 0.087	10.64	<b>0.006</b>	0.083	0.781	0.022	0.978
Arsenic ( $\text{g m}^{-3}$ )	0.001 $\pm$ 0	0.001 $\pm$ 0	0.025 $\pm$ 0.024	0.002 $\pm$ 0.001	0.001 $\pm$ 0	0.013 $\pm$ 0.011	22.73	0.165	<b>&lt;0.001</b>	0.99	0.094	0.912
Nickel( $\text{g m}^{-3}$ )	0.003 $\pm$ 0.001	0.054 $\pm$ 0.047	0.003 $\pm$ 0.002	0.003 $\pm$ 0.001	0.044 $\pm$ 0.039	0.001 $\pm$ 0.001	6.742	<b>0.019</b>	0.55	0.479	0.363	0.707
Zinc ( $\text{g m}^{-3}$ )	0.012 $\pm$ 0.004	0.214 $\pm$ 0.178	0.002 $\pm$ 0.001	0.010 $\pm$ 0.001	0.162 $\pm$ 0.139	0.002 $\pm$ 0.001	23.297	<b>&lt;0.001</b>	0.018	0.895	0.776	0.492

**Biotic responses**

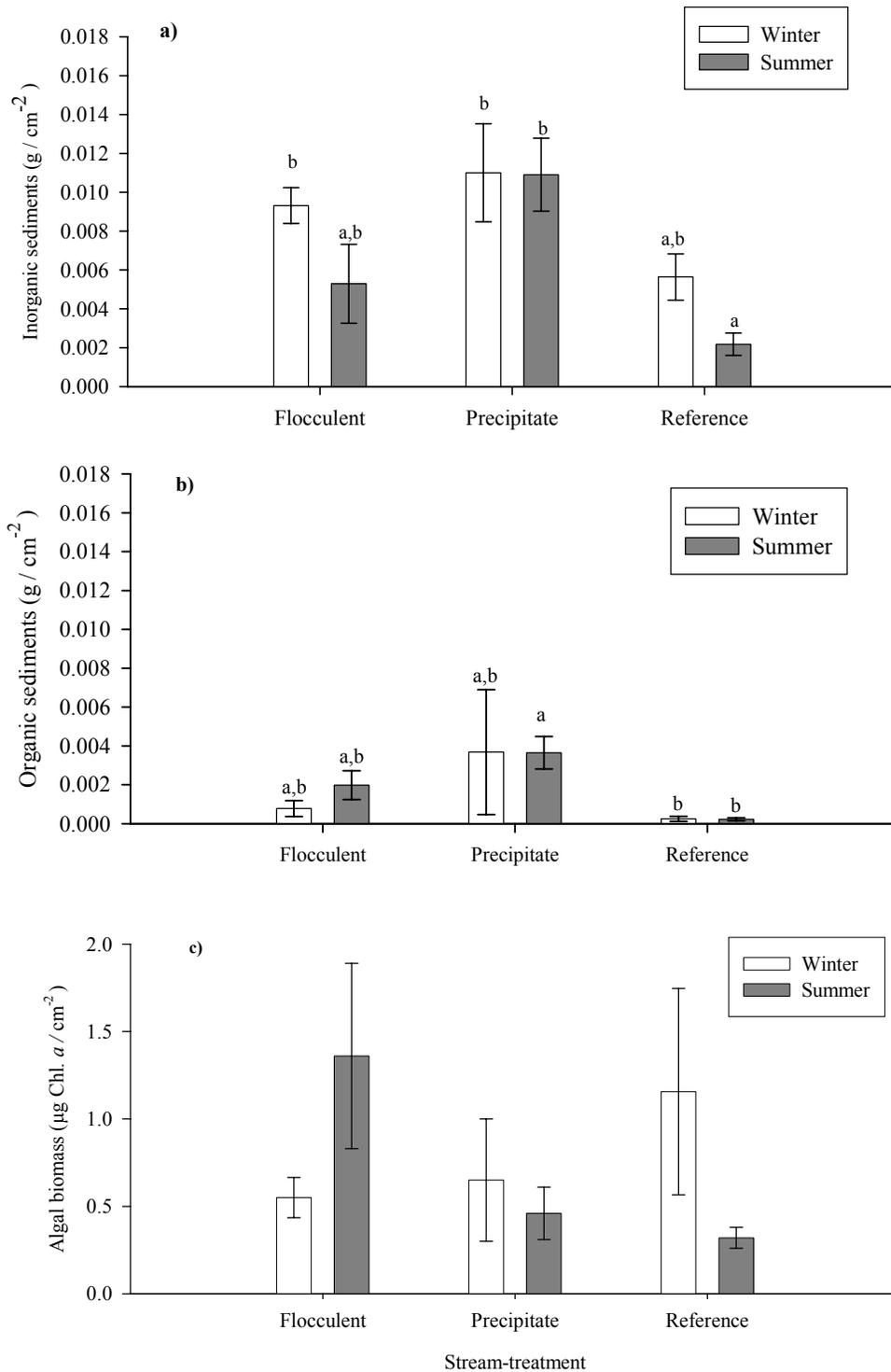
Organic deposits were also significantly different across stream-treatments, with precipitate streams having the greatest amount of organic sediments and reference streams having the least (Table 3.2). There was no effect of season on deposited organic sediments, and no interaction effect between stream-treatment and season (Table 3.2). A Tukey (HSD) post-hoc test indicated that there were significantly greater amount of organic sediment in precipitate streams, although only in summer, than in the reference streams ( $P < 0.05$ ) (Figure 3.2b).

In winter, algal biomass was highest in the reference streams, and precipitate and flocculent streams were similar. In summer, the reverse occurred and the greatest amount of algal biomass was found in flocculent streams and the lowest occurred in the reference streams (Figure 3.2c). However, there was no difference in algal biomass across the three stream-treatments or between the two seasons and there was no interaction effect between stream-treatment and season (Table 3.2).

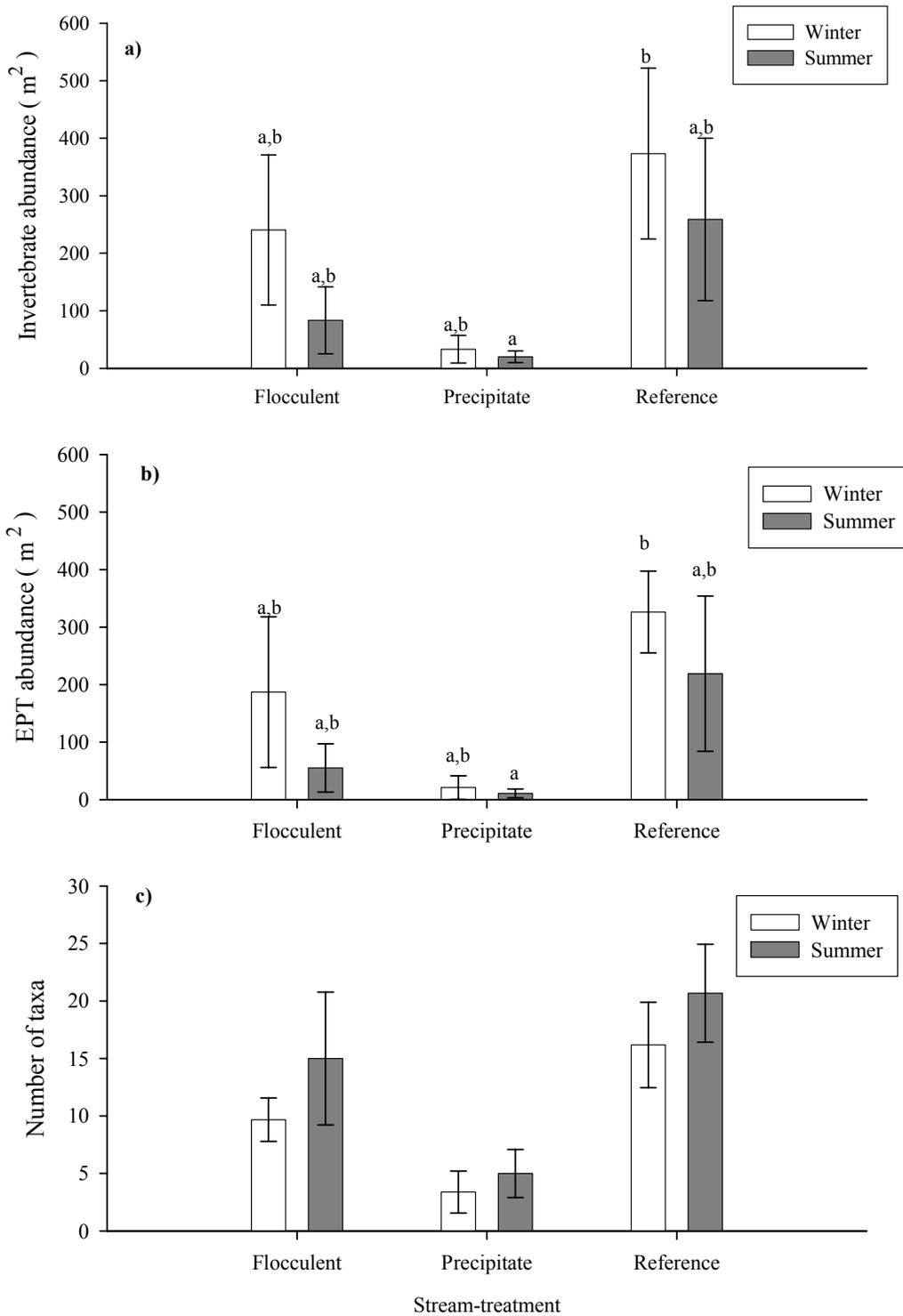
**Table 3.2.** Results from GLM ANOVAs examining differences in responses across stream-treatment (flocculent, precipitate and reference), seasons (winter and summer) and interaction effects (stream-treatment and season) on biotic and abiotic variables measured. Significant results are in bold.

	<b>Treatment</b>		<b>Season</b>		<b>Treatment * Season</b>	
	<i>F</i> <sub>2,11</sub>	<i>P</i>	<i>F</i> <sub>1,11</sub>	<i>P</i>	<i>F</i> <sub>2,11</sub>	<i>P</i>
<i>Biotic</i>						
Organic sediments (g cm <sup>-2</sup> )	8.893	<b>0.005</b>	0.002	0.967	0.848	0.454
Algal biomass (µg Chl. <i>a</i> cm <sup>-2</sup> )	0.594	0.569	0.339	0.572	0.852	0.453
Invertebrate abundance	6.991	<b>0.011</b>	2.118	0.174	0.391	0.686
EPT abundance	8.871	<b>0.005</b>	1.8	0.207	0.293	0.752
Taxonomic richness	8.046	<b>0.007</b>	0.583	0.461	<0.001	1
<i>Abiotic</i>						
Inorganic sediments (g cm <sup>-2</sup> )	8.33	<b>0.006</b>	8.672	<b>0.013</b>	2.196	0.158

Benthic invertebrate and EPT abundances varied markedly between stream replicates in flocculent and reference stream-treatments (Figure 3.3a and Figure 3.3b). However, benthic invertebrate and EPT abundances were significantly different between the three stream-treatments, with higher abundances in reference streams, and low abundances in precipitate streams (Table 3.2, Figure 3.3a and Figure 3.3b). Benthic and EPT invertebrate abundances did not differ between seasons nor was there an interaction effect between stream-treatments and season (Table 3.2), indicating that invertebrate and EPT abundances in precipitate streams are uniformly lower than reference streams, regardless of season. The number of invertebrate taxa was significantly different between stream-treatments (Table 3.2), with the lowest numbers being found in precipitate streams and highest numbers in the reference streams (Figure 3.3c) There was no seasonal difference, or an interaction effect between stream-treatment and season (Table 3.2).



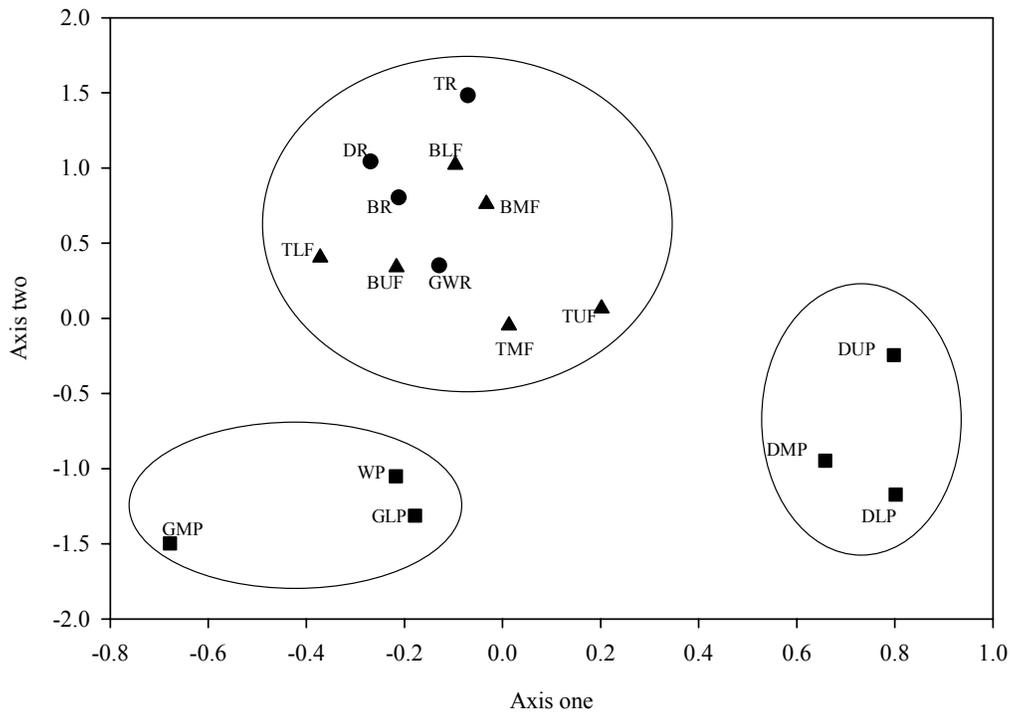
**Figure 3.2.** Comparisons of a) deposited inorganic sediments, b) organic sediments and c) algal biomass between the three stream-treatments (flocculent precipitate and reference) in winter (June 2004) and summer (January 2005). Means  $\pm$  1SE. In winter flocculent and precipitate (n=2), and reference (n=4). Summer means n=3. Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ).



**Figure 3.3** Comparisons of biotic indices a) benthic invertebrate abundances, b) EPT abundances and c) taxonomic richness between stream-treatments (flocculent, precipitate and reference) in winter (June 2004) and summer (January 2005). Means  $\pm$  1SE. In winter, flocculent and precipitate (n=2), and reference (n=4). Summer means n=3. Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ).

### Large scale community composition patterns

An NMDS ordination using seasonal means of invertebrate species abundances to examine community composition at 16 sites yielded a 2-dimensional solution with a relatively low stress value of 8.70. Overall the NMDS indicated strong patterns of similarity between treatments (Figure 3.4). The three Devils Creek precipitate sites (DUP, DMP and DLP) were grouped together, and had high axis one and low axis two scores. Similarly, the three precipitate sites in the Garvey Creek catchment (WP, GMP and GLP) were grouped together and had low axis one and axis two scores. Flocculent and reference sites were generally grouped together and had low axis one and high axis two scores (Figure 3.4).



**Figure 3.4.** Non-metric multidimensional scaling 2-dimensional ordination plot of seasonal means of benthic invertebrate species abundances from samples collected at 16 sites in June (2004) and January (2005). Triangles indicate flocculent sites, squares indicate precipitate sites and circles indicate reference sites.

There was a positive and significant correlation between pH and axis two from the NMDS analysis on seasonal mean benthic invertebrate abundances from the 16 sites (Spearman rank correlation  $P < 0.05$ ) (Table 3.3). Conductivity and turbidity were significantly negatively correlated with axis two scores from the NMDS analysis ( $P < 0.05$ ).

**Table 3.3.** Results of Spearman rank correlations of mean seasonal water chemistry, biotic and abiotic variables measured at 16 sites in winter (June 2004) and in summer (January 2005), with axis one and axis two of the non-metric multidimensional scaling analysis examining mean invertebrate communities at the 16 sites collected in winter and in summer. Significant correlation coefficients are in bold ( $P < 0.05$ ).

Variables	Axis one	Axis two
pH	0.003	0.882
Conductivity ( $\mu\text{s}_{25^{\circ}\text{C}}\text{cm}^{-1}$ )	0.015	-0.771
Turbidity (NTU)	-0.018	-0.776
Algal biomass ( $\mu\text{g Chl. } a\text{ cm}^{-2}$ )	0.271	0.176
Inorganic sediment ( $\mu\text{g} / \text{cm}^{-2}$ )	0.335	-0.162
Organic sediment ( $\mu\text{g} / \text{cm}^{-2}$ )	0.197	-0.479

When macroinvertebrate taxonomic composition was examined across stream-treatments both flocculent and reference streams showed more diverse communities than the precipitate streams (Table 3.4). Proportions of the six main taxonomic groups were almost equally represented in flocculent and reference streams, with one exception, mayflies which dominated the reference and flocculent streams but made up approximately 65% and 32%, respectively. Dipterans dominated benthic invertebrate communities in precipitate streams making up approximately 61% of the community, compared with 12% and 18% in reference and flocculent streams respectively.

Comparisons of functional feeding groups across treatments in summer indicated that flocculent and reference sites were dominated by collector browsers (79.3% and 86.2%, respectively) (Table 3.4). In precipitate streams, collector browsers and filter feeders were proportionally similar (43.1% and 35.3%, respectively). Precipitate streams had the highest proportion of algal piercers which were represented by one caddisfly species, *Oxyethira albiceps*, which were also the most numerically dominant taxon making up invertebrate communities in precipitate streams (Table 3.5).

Whereas, the mayfly, *Deleatidium* spp. was the dominant taxon found in both flocculent and reference streams, but was also found in precipitate streams. The five numerically dominant taxa found in the reference streams differed from the other treatments except for *Deleatidium* and orthoclads, and although, flocculent and precipitate streams had representatives from four different taxa, Orthocladiinae was the second most dominant taxon in these systems compared to being number five in reference streams (Table 3.5).

**Table 3.4.** Mean ( $\pm 1SE$ ) proportions of five taxonomic groups and ‘others’, which includes Collembolans, Acarina, Annelida and Megalopterans, and six functional feeding groups in the three stream-treatments (flocculent, precipitate and reference) in January 2005.

	Flocculent	Precipitate	Reference
<i>Taxa</i>			
Ephemeroptera	31.6 $\pm$ 9.4	12.1 $\pm$ 10.7	65.0 $\pm$ 9.3
Plecoptera	23.1 $\pm$ 14.1	2.0 $\pm$ 2.0	4.1 $\pm$ 1.2
Trichoptera	7.2 $\pm$ 6.0	19.6 $\pm$ 19.6	8.5 $\pm$ 1.5
Diptera	17.9 $\pm$ 9.4	60.7 $\pm$ 21.4	12.3 $\pm$ 7.5
Coleptera	15.2 $\pm$ 9.07	1.9 $\pm$ 1.9	4.2 $\pm$ 2.5
Other	5.0 $\pm$ 3.7	3.8 $\pm$ 1.9	5.9 $\pm$ 4.4
<i>Functional groups</i>			
Collector browser	79.3 $\pm$ 6	43.1 $\pm$ 30	86.2 $\pm$ 2
Filter feeder	2.3 $\pm$ 1	35.3 $\pm$ 32	4.8 $\pm$ 0.47
Predator	5.9 $\pm$ 3	2.9 $\pm$ 3	5.8 $\pm$ 0.62
Shredders	12.3 $\pm$ 11	0	3.3 $\pm$ 1
Scrapers	0	0	0.32 $\pm$ 0.32
Algal piercer	0.18 $\pm$ 0.18	18.6 $\pm$ 18.6	0
<b>Benthic invertebrate densities</b>	<b>83 <math>\pm</math> 58</b>	<b>20 <math>\pm</math> 10</b>	<b>260 <math>\pm</math> 141</b>

**Table 3.5.** The five most numerically dominant taxa collected in the three stream-treatment (flocculent, precipitate and reference) in January 2005.

Flocculent	Precipitate	Reference
<i>Deleatidium spp.</i> (Ephemeroptera)	<i>Oxyethira albiceps</i> (Trichoptera)	<i>Deleatidium spp.</i> (Ephemeroptera)
Orthoclaadiinae (Diptera)	Orthoclaadiinae (Diptera)	<i>Hydrobiosella sp.</i> (Trichoptera)
<i>Pycnocentrella eruensis</i> (Trichoptera)	<i>Deleatidium spp.</i> (Ephemeroptera)	<i>Austroperla cyrene</i> (Plecoptera)
Chironominae (Diptera)	<i>Austrosimulium sp.</i> (Diptera)	Elmidae (Coleoptera)
Elmidae (Coleoptera)	<i>Spaniocera sp.</i> (Plecoptera)	Orthoclaadiinae (Diptera)

## **Longitudinal patterns along mine polluted streams**

### **Water chemistry and inorganic sediment**

#### **Iron Flocculent**

Burke Creek, which was dominated by iron flocculent, showed little variation in water chemistry longitudinally, with all impacted sites having similar pH, conductivities, turbidity and temperature compared to its reference site in either season (Table 3.6). The only significance seasonal difference in water chemistry was temperature, and was higher in summer at all sites (Table 3.6; Tukey HSD post-hoc test  $P < 0.05$ ). Inorganic sediment was similar between sites, but higher deposition occurred in winter (Table 3.7; Figure 3.5a).

Old Terrace, the other iron flocculent stream, had a relatively consistent pH longitudinally, ranging from 5.5 – 6.5, and did not differ much from the pH recorded at its reference site, where pH ranged from 6.2 – 7.2 (Table 3.6). Conductivity on Old Terrace, did not differ significantly between sites, or when compared to its reference site, but was on average higher at TMF and lowest at TLF (Table 3.6). Consistently, higher recorded conductivity at TMF was unexpected and may be in part due to a small observed, but unmeasured tributary that enters the stream between TUF and TMF. Turbidity was significantly different between site and season, with TR having the lowest turbidity, and turbidity being highest in winter (Table 3.6). There was a significant difference in temperature, between site, and season, with temperature at TR being warmer, than the flocculent sites, and higher temperatures were recorded in summer (Table 3.6). There was an interaction effect between site and season, suggesting that to seasonal changes in temperature are not consistent between sites.

Deposited inorganic sediments were significantly different seasonally, and between sites (Table 3.7). Sediment levels were highest in winter, and the reference site (TR) had the lowest sediment levels, with TUF the highest (Figure 3.7a).

## **Iron Precipitate**

Devils Creek showed little change in water chemistry longitudinally (Table 3.6). Interestingly, the middle site (DMP) had an overall consistently lower pH, and higher turbidity and conductivity, than either DUP, or the lower site DLP (Table 3.6). This trend is similar to that observed in Old Terrace Stream, where there was no longitudinal improvement in water chemistry. The reference site (DR), showed marked differences in pH, ranging from 6.2 – 7.1, compared to pH 4.0 – 5.4, at the three impacted sites. Conductivity was significantly different between sites, and was lowest at the reference site (DR), and highest at the middle site (DMP) (Table 3.6). Turbidity was overall higher at the DMP site and lowest at the reference site (DR), however this difference was not significant (Table 3.6). Temperature generally declined downstream, along Devils Creek, but was slightly higher at DR, although this difference was not significant. There was a seasonal difference with higher temperature being recorded in summer (Table 3.6).

Inorganic sediments differed significantly between sites and seasons, with DR having the lowest deposited sediment and the middle site (DMP), having the highest (Figure 3.9a). There were significantly higher amounts of deposited sediment in winter, but no interaction effect between site and season (Table 3.7).

Garvey Creek was the most severely impacted stream in this study, and showed little improvement in water chemistry downstream. pH was the lowest in the upper site, Wellman Creek (WP), where pH ranged from 3.2 – 4.1, but showed a small improvement downstream at GMP and GLP, although pH was still low (pH 3.7 – 4.5) (Table 3.6). pH at the reference site (GWR) was markedly higher than at the impacted sites and ranged from 5.1 – 6.5 (Table 3.6). Conductivity was significantly higher at WP, compared to all other sites, and declined downstream (Table 3.6). All longitudinal sites were markedly different to the reference site (GWR), where conductivity was never above  $55 \mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ , compared to sites along Garvey Creek where conductivity was never below  $300 \mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ . Turbidity was similar at all sites along Garvey Creek, which were similar to GWR, but turbidity was higher in winter (Table 3.6). Finally, temperature showed a seasonal difference, and was highest in summer (Table 3.6).

Benthic inorganic sediments decreased downstream, with the highest amounts being found at WP, however there was no difference between sites (Table 3.7; Figure 3.11a). Benthic inorganic sediments were significantly higher in winter (Table 3.7).

**Table 3.6.** Mean ( $\pm 1$ SE), except pH, water chemistry for 16 sites, measured in winter (2004) and in summer (2005). GLM ANOVA statistics are shown, for sites, seasons and interaction effects (sites and season). Significant results are in bold ( $P < 0.05$ ).

Flocculent streams	Winter				Summer				Site		Season		Site*Season	
	BUF	BMF	BLF	BR	BUF	BMF	BLF	BR	$F_{3,22}$	$P$	$F_{3,22}$	$P$	$F_{3,22}$	$P$
<i>Burke Creek</i>														
pH median	6.7	6.7	6.6	7	7	7	7.2	7.4						
pH range	(6.4-6.8)	(6.5-7)	(6.3-6.9)	(6.8-7.3)	(6.8 - 7.2)	(6.7 - 7.1)	(7.1 - 7.2)	(7.0 - 7.5)						
Conductivity ( $\mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ )	63 $\pm$ 5	68 $\pm$ 6	65 $\pm$ 6	54 $\pm$ 4	56 $\pm$ 9	66 $\pm$ 10	58 $\pm$ 7	54 $\pm$ 6	1.434	0.26	0.629	0.436	0.143	0.933
Temperature ( $^\circ\text{C}$ )	8.9 $\pm$ 0.6	8.9 $\pm$ 0.6	8.9 $\pm$ 0.7	8.6 $\pm$ 0.7	12.3 $\pm$ 0.2	13.3 $\pm$ 0.64	13.5 $\pm$ 0.7	13.2 $\pm$ 0.8	0.233	0.873	64.062	<b>&lt;0.001</b>	0.31	0.818
									$F_{3,17}$	$P$	$F_{1,17}$	$P$	$F_{3,17}$	$P$
Turbidity (NTU)	4.7 $\pm$ 1.6	2.9 $\pm$ 0.8	3.2 $\pm$ 0.9	2.3 $\pm$ 0.8	0.7	4.9 $\pm$ 2.9	1.7 $\pm$ 0.6	0.8 $\pm$ 0.1	1.788	0.188	4.376	0.052	1.5	0.25
	<b>TUF</b>	<b>TMF</b>	<b>TLF</b>	<b>TR</b>	<b>TUF</b>	<b>TMF</b>	<b>TLF</b>	<b>TR</b>	$F_{3,21}$	$P$	$F_{3,21}$	$P$	$F_{3,21}$	$P$
<i>Old Terrace Stream</i>														
pH median	5.59	6.13	5.74	6.87	6.3	5.8	5.9	6.9						
pH range	(5.5-6.5)	(5.7-6.3)	(5.6-6.2)	(6.2-7.2)	(6.0-6.5)	(5.5-6.5)	(5.7-6.1)	(6.7-7.1)						
Conductivity ( $\mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ )	76 $\pm$ 4	78 $\pm$ 4	59 $\pm$ 3	57 $\pm$ 5	62 $\pm$ 0.3	66 $\pm$ 2	65 $\pm$ 20	56.1 $\pm$ 6.5	2.758	0.068	1.559	0.226	1.181	0.341
Temperature ( $^\circ\text{C}$ )	9.6 $\pm$ 0.7	8.88 $\pm$ 0.9	8.32 $\pm$ 0.9	8 $\pm$ 0.7	16.3 $\pm$ 0.9	15.5 $\pm$ 0.5	17.1 $\pm$ 3.1	15.2 $\pm$ 3.4	7.597	<b>0.001</b>	32.525	<b>&lt;0.001</b>	6.23	<b>0.003</b>
									$F_{3,16}$	$P$	$F_{3,16}$	$P$	$F_{3,16}$	$P$
Turbidity (NTU)	15.9 $\pm$ 5.6	14.8 $\pm$ 4.4	21.8 $\pm$ 6.8	5.4 $\pm$ 1.4	4.3 $\pm$ 2.2	11.8 $\pm$ 6.2	3.5 $\pm$ 0.2	1.3 $\pm$ 0.2	4.139	<b>0.024</b>	12.388	<b>0.003</b>	0.664	0.586

**Table 3.6 cont'd.**

Precipitate Streams	Winter				Summer				Site		Season		Site*Season	
	DUP	DMP	DLP	DR	DUP	DMP	DLP	DR	$F_{3,22}$	$P$	$F_{3,22}$	$P$	$F_{3,22}$	$P$
<i>Devils Creek</i>														
pH median	4.9	4.3	4.6	6.6	4.9	4.3	4.9	7						
pH range	(4.3-5.1)	(4.2-4.5)	(4.4-5.2)	(6.2-7.0)	(4.5-5.3)	(4.0-4.4)	(4.5-5.4)	(6.2-7.1)						
Conductivity ( $\mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ )	132 ± 20	133 ± 17	107 ± 13	76 ± 11	146 ± 62	173 ± 40	111 ± 36	88 ± 16	3.9	<b>0.022</b>	1.11	0.303	0.229	0.875
Temperature (°C)	7.8 ± 0.7	7.1 ± 1.0	7 ± 1.0	7.7 ± 0.8	14.4 ± 1.9	14.7 ± 0.8	14.3 ± 0.8	15 ± 1.1	0.138	0.936	85.591	<b>&lt;0.001</b>	0.076	0.972
									$F_{3,16}$	$P$	$F_{3,16}$	$P$	$F_{3,16}$	$P$
Turbidity (NTU)	9.6 ± 3.3	34.4 ± 23.2	11 ± 3.5	5.4 ± 1.8	11.1 ± 7.2	14 ± 5.2	7 ± 0.8	7.3 ± 1.6	0.697	0.568	0.809	0.382	0.083	0.968
	<b>WP</b>	<b>GMP</b>	<b>GLP</b>	<b>GWR</b>	<b>WP</b>	<b>GMP</b>	<b>GLP</b>	<b>GWR</b>	$F_{3,21}$	$P$	$F_{3,21}$	$P$	$F_{3,21}$	$P$
<i>Garvey Creek</i>														
pH median	3.6	4.2	4.2	6.2	3.3	4.2	4.1	6.3						
pH range	(3.3-4.1)	(3.7-4.4)	(3.7-4.5)	(5.1-6.5)	(3.2-3.8)	(4.0-4.4)	(3.9 - 4.4)	(6.1-6.4)						
Conductivity ( $\mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ )	636 ± 96	374 ± 46	361 ± 50	38 ± 2	835 ± 169	424 ± 112	418 ± 112	45 ± 10	22.22	<b>&lt;0.001</b>	1.566	0.225	0.51	0.68
Temperature (°C)	8.6 ± 0.7	8.6 ± 0.8	8.7 ± 0.8	7.4 ± 0.9	16.5 ± 0.8	16.3 ± 1.6	18.9 ± 3.4	15.6 ± 2	1.052	0.39	89.711	<b>&lt;0.001</b>	0.408	0.749
									$F_{3,17}$	$P$	$F_{1,17}$	$P$	$F_{3,17}$	$P$
Turbidity (NTU)	48.4 ± 14.7	27.7 ± 11.9	52.1 ± 42.4	16.4 ± 5.2	2.5 ± 0.5	3.5 ± 1.3	3.5 ± 0.5	2.3 ± 0.1	0.483	0.699	23.051	<b>&lt;0.001</b>	0.601	0.623

## **Biotic responses**

### **Iron flocculent**

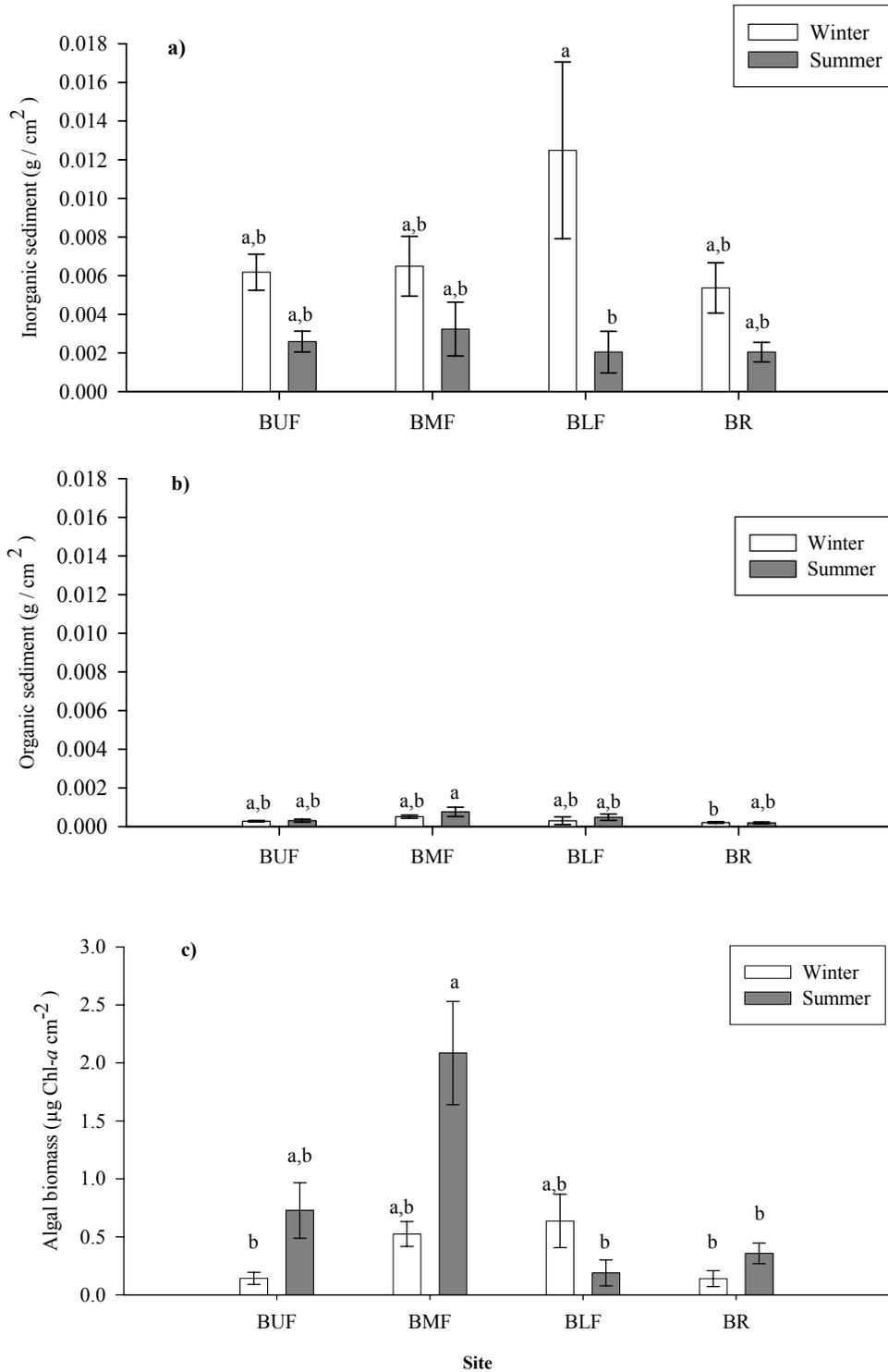
#### **Burke Creek**

Organic sediments were relatively low across all sites (Figure 3.5b), but were significantly lower at the reference site (BR) and higher at the middle site (BMF) (Figure 3.5b; Table 3.7). Algal biomass was significantly lower at the reference site (BR) than at the other sites (Figure 3.5c; Table 3.7). Algal biomass was significantly higher in summer than in winter, and there was an interaction effect between site and season, with higher algal biomass observed in summer at all sites except the lowest impacted site (BLF), where in winter, higher biomass was observed (Figure 3.5c; Table 3.7).

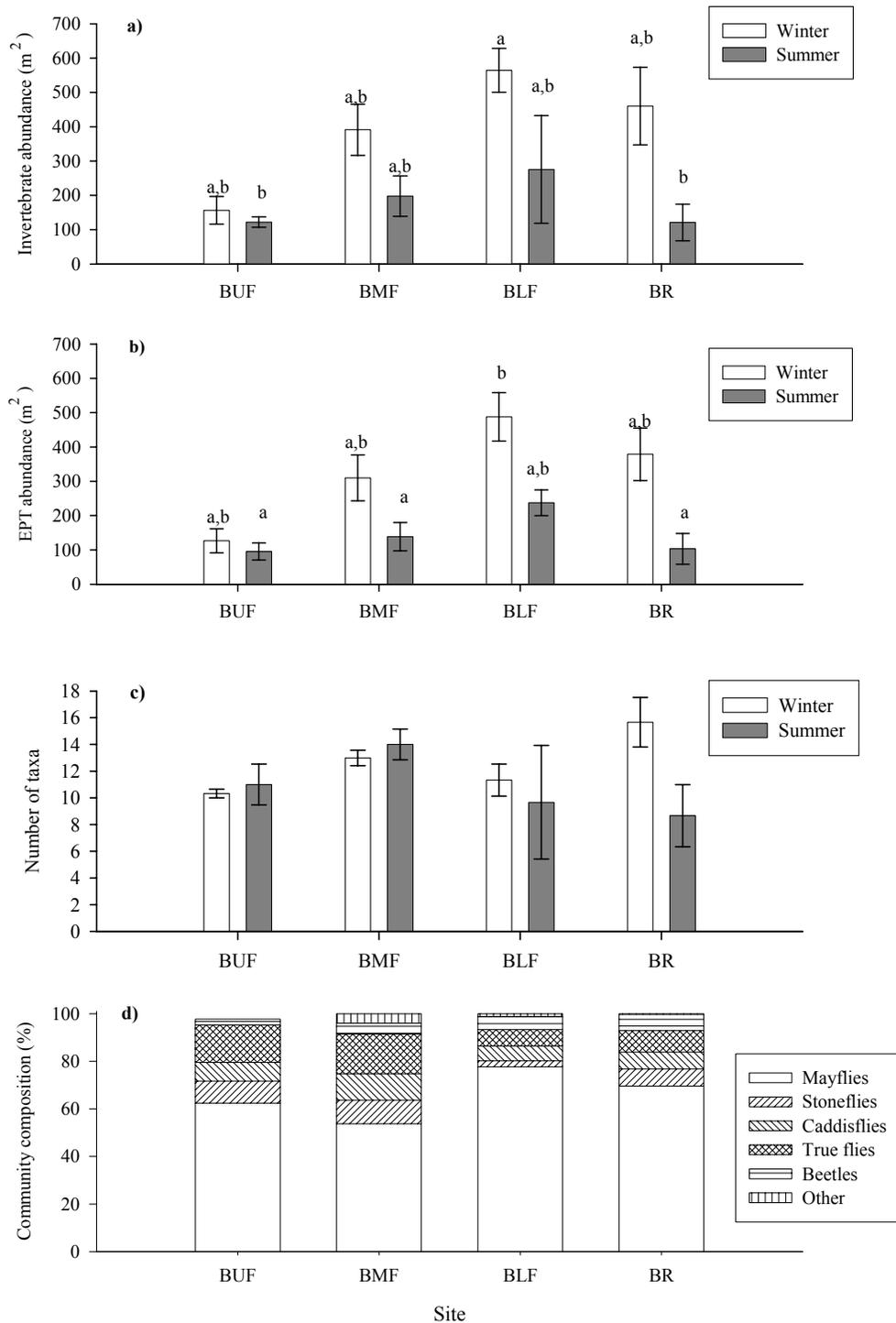
Mean benthic invertebrate abundances differed significantly between sites with abundances increasing downstream, and there was a significant effect of season with higher values in winter (Figure 3.6a; Table 3.8). Mean EPT abundances differed significantly between sites with higher abundances being recorded at the lowest site (BLF) (Figure 3.6b; Table 3.8). There was a significant season effect, with on average higher EPT abundances being found in winter (Table 3.8). Taxonomic richness did not differ significantly between site or season, and there was no interaction effect between site and season (Figure 3.6c; Table 3.8). All sites along Burke Creek, had similar benthic invertebrate communities to the reference site (BR) (Figure 3.6d), and were dominated by >50% mayflies, which were mostly the common mayfly, *Deleatidium*.

**Table 3.7.** Results from GLM ANOVAs comparing algal biomass, inorganic and organic sediments along two flocculent streams, Burke Creek and Old Terrace Stream and two precipitate streams, Devils Creek and Garvey Creek, in winter (June 2004) and in summer (January 2005), with reference sites. Results shown are for sites, seasons, and interaction effects between sites and season. Significant results are in bold ( $P < 0.05$ ).

	Site		Season		Site * Season	
	$F_{3,32}$	$P$	$F_{1,32}$	$P$	$F_{3,32}$	$P$
<b><i>Iron flocculent</i></b>						
<i>Burke Creek</i>						
Algal biomass ( $\mu\text{g Chl. } a \text{ cm}^{-2}$ )	6.616	<b>&lt;0.001</b>	5.649	<b>0.024</b>	<b>4.83</b>	<b>0.007</b>
Organic sediments ( $\text{g cm}^{-2}$ )	4.872	<b>0.007</b>	0.752	0.92	0.83	0.487
Inorganic sediments ( $\text{g cm}^{-2}$ )	0.341	0.796	13.686	<b>0.001</b>	1.452	0.246
<i>Old Terrace Stream</i>						
Algal biomass ( $\mu\text{g Chl. } a \text{ cm}^{-2}$ )	3.291	<b>0.03</b>	6.153	<b>0.019</b>	3.536	<b>0.026</b>
Organic sediments ( $\text{g cm}^{-2}$ )	17.777	<b>&lt;0.001</b>	13.94	<b>0.001</b>	1.674	0.192
Inorganic sediments ( $\text{g cm}^{-2}$ )	10.516	<b>&lt;0.001</b>	19.488	<b>&lt;0.001</b>	2.041	0.128
<b><i>Iron Precipitate</i></b>						
<i>DevilsCreek</i>						
Algal biomass ( $\mu\text{g Chl. } a \text{ cm}^{-2}$ )	2.919	<b>0.049</b>	12.671	<b>0.001</b>	0.127	0.943
Organic sediments ( $\text{g cm}^{-2}$ )	51.741	<b>&lt;0.001</b>	7.424	<b>0.01</b>	2.688	0.063
Inorganic sediments ( $\text{g cm}^{-2}$ )	4.726	<b>0.008</b>	5.547	<b>0.025</b>	1.482	0.238
<i>Garvey Creek</i>						
Algal biomass ( $\mu\text{g Chl. } a \text{ cm}^{-2}$ )	0.897	0.453	2.378	0.133	3.284	<b>0.033</b>
Organic sediments ( $\text{g cm}^{-2}$ )	23.851	<b>&lt;0.001</b>	51.726	<b>&lt;0.001</b>	23.459	<b>&lt;0.001</b>
Inorganic sediments ( $\text{g cm}^{-2}$ )	11.177	0.085	2.41	<b>0.002</b>	0.18	0.909



**Figure 3.5.** Comparisons of a) inorganic sediments, b) organic sediments and c) algal biomass at three longitudinal flocculent sites and one reference site on Burke Creek in winter (June 2004) and in summer (January 2005). Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ). Means  $\pm$  1SE; n=5.



**Figure 3.6.** Comparisons of a) benthic invertebrate abundance, b) EPT abundances and c) taxonomic richness, at three longitudinal flocculent sites and one reference site (BR), on Burke Creek in winter (June 2004) and in summer (January 2005) and d) seasonal mean community composition. Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ). Means  $\pm$  1 SE; n=3.

**Table 3.8.** Results from GLM ANOVAs comparing biotic responses along two flocculent streams, Burke Creek and Old Terrace and two precipitate streams, Devils Creek and Garvey Creek, in winter (June 2004) and in summer (January 2005), with reference sites. Results shown are for sites, seasons, and interaction effects between sites and season. Significant results are in bold ( $P < 0.05$ ).

	Site		Season		Site * Season	
	$F_{3,16}$	$P$	$F_{1,16}$	$P$	$F_{3,16}$	$P$
<b><i>Iron flocculent</i></b>						
<i>Burke Creek</i>						
Invertebrate density	3.829	<b>0.031</b>	13.297	<b>0.002</b>	1.305	0.307
EPT abundances	4.621	<b>0.016</b>	14.429	<b>0.002</b>	1.316	0.304
Taxonomic richness	0.818	0.503	1.856	0.192	1.585	0.232
<i>Old Terrace Stream</i>						
Invertebrate density	41.099	<b>&lt;0.001</b>	8.606	<b>0.01</b>	1.912	0.168
EPT abundances	55.095	<b>&lt;0.001</b>	1.587	0.226	0.898	0.464
Taxonomic richness	31.62	<b>&lt;0.001</b>	4.446	0.051	0.128	2.2
<b><i>Iron precipitate</i></b>						
<i>Devils Creek</i>						
Invertebrate density	22.051	<b>&lt;0.001</b>	0.264	0.614	0.69	0.624
EPT abundance	25.773	<b>&lt;0.001</b>	0.331	0.573	0.676	0.573
Taxonomic richness	9.678	<b>0.001</b>	0.1	0.756	0.473	0.706
<i>Garvey Creek</i>						
Invertebrate density	14.815	<b>&lt;0.001</b>	0.026	0.875	2.123	0.138
EPT abundance	56.616	<b>&lt;0.001</b>	0.062	0.807	4.519	<b>0.018</b>
Taxonomic richness	45.619	<b>&lt;0.001</b>	2.381	0.142	0.54	0.662

### Old Terrace Stream

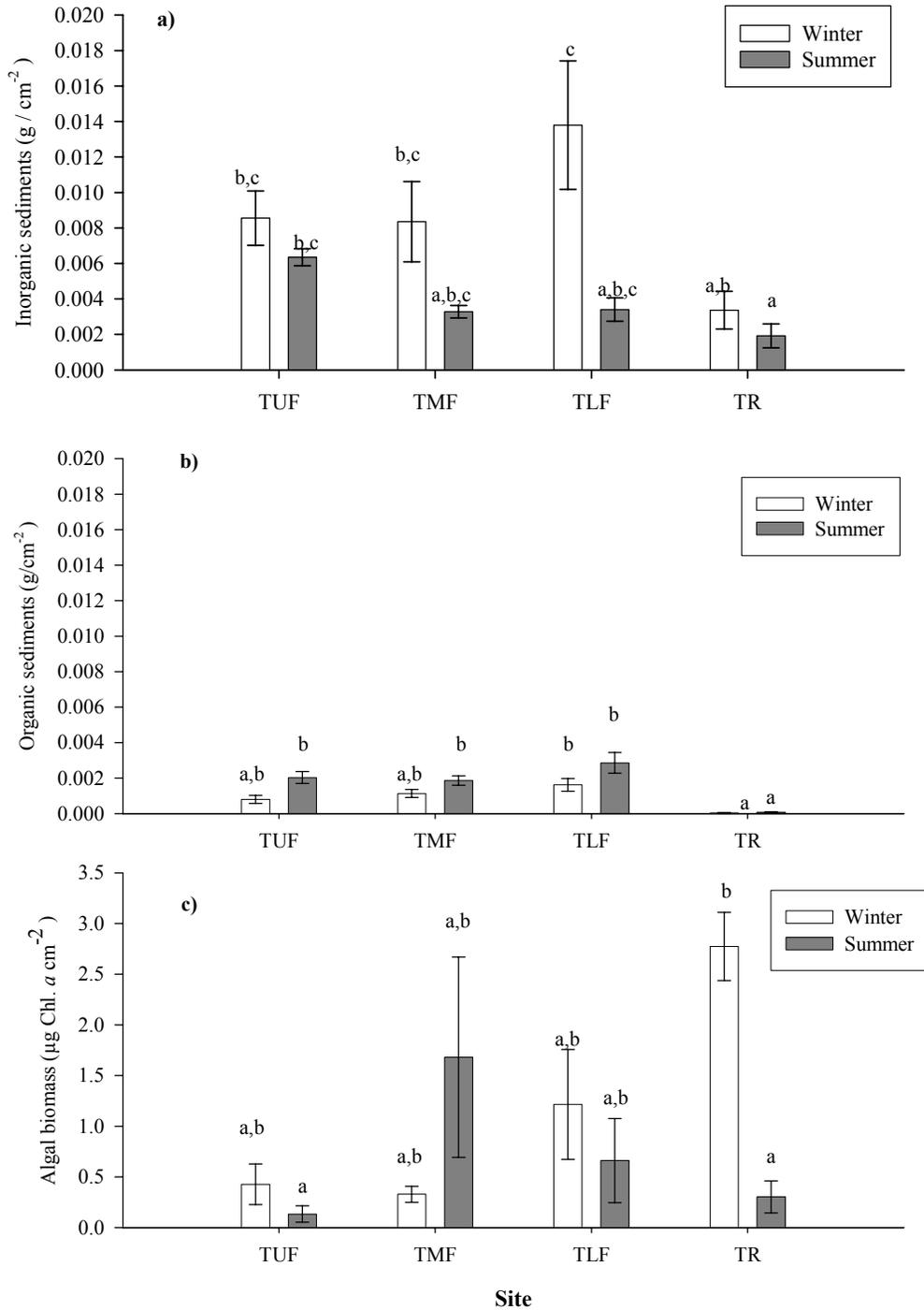
The amount of organic deposits increased marginally downstream on Old Terrace (Figure 3.7a). There was a significant difference in the amount of organic sediment between sites, with the greatest amount being found at the lowest impacted site (TLF), and the smallest amount being found at the reference site (TR) (Figure 3.7a; Table 3.7). There were significantly higher amounts of organic sediments in summer (Table 3.8), which is interesting, in that this is the opposite of inorganic sediments, which were lower in summer. Algal biomass was variable between and within sites, however, generally showed an increase downstream in both seasons, except in summer, where the middle site (TMF) breaks the trend, and had the highest algal biomass (Figure 3.7c). The upper impacted site (TUF) had significantly less algal

biomass than all other sites, including the reference site (TR), and there was significantly more algal biomass recorded in winter (Figure 3.7c; Table 3.7). Further, there was a significant site and season effect with all sites having higher algal biomass in winter, except for the middle site (TMF), where the highest algal biomass was recorded in summer.

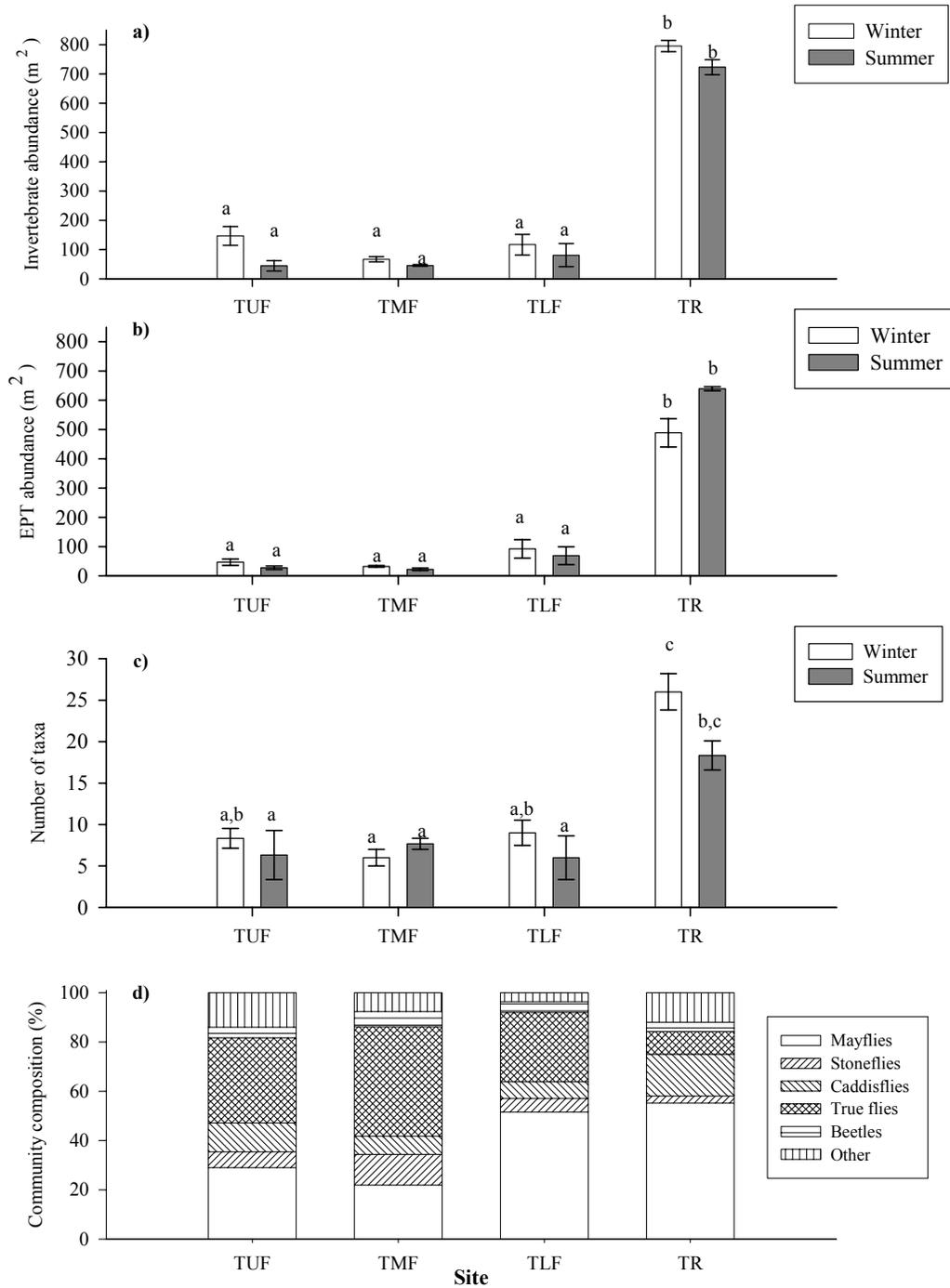
Benthic invertebrate abundances were significantly higher at the reference site (TR), and were on average 5 to 11 times higher in winter, and 9 to 16 times higher in summer than benthic invertebrate abundances at the three impacted sites (TUF, TMF and TLF) along Old Terrace (Figure 3.8a; Table 3.8). A significant seasonal difference was also found, with winter higher benthic invertebrates occurring in winter (Table 3.8). Importantly, a Tukey (HSD) post hoc test indicated that the three sites along Old Terrace were significantly different from the reference site (TR), in both seasons (Figure 3.8a).

EPT abundances followed a similar trend with significantly higher abundances being observed at the reference site (TR), but there were no seasonal differences (Figure 3.8b; Table 3.8). Further, the reference site (TR) had significantly more invertebrate taxa, than the three impacted sites along Old Terrace Stream (Figure 3.8c; Table 3.8).

Mean seasonal community composition, showed that the percentage of mayflies along Old Terrace increased downstream, with the highest percentage (51%), being found at the lowest site (TLF), which was similar to the percentage of mayflies (55%) found at the reference site (TR) (Figure 3.8d). There were higher percentages of dipterans at the sites along Old Terrace (28 – 44%), compared to TR, where there was less than 10%. The commonest mayfly found along Old Terrace was *Deleatidium*, which was also relatively abundant at the reference site, along with *Coloburiscus humeralis* and *Neozephlebia scita*. Orthocladiinae was the most abundant dipteran at all sites.



**Figure 3.7.** Comparisons of a) inorganic sediments, b) organic sediments and c) algal biomass at three longitudinal flocculent sites on Old Terrace Stream and one reference site on Burkes Creek (BR) in winter (June 2004) and in summer (January 2005). Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ). Means  $\pm$  1SE;  $n=5$ .



**Figure 3.8.** Comparisons of a) benthic invertebrate abundance, b) EPT abundances, c) taxonomic richness, at three longitudinal flocculent sites on Old Terrace Stream and one reference site, on Burkes Creek (TR), in winter (June 2004) and in summer (January 2005), and d) seasonal mean community composition. Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ). Means  $\pm 1SE$ ;  $n=3$ .

## Iron precipitate

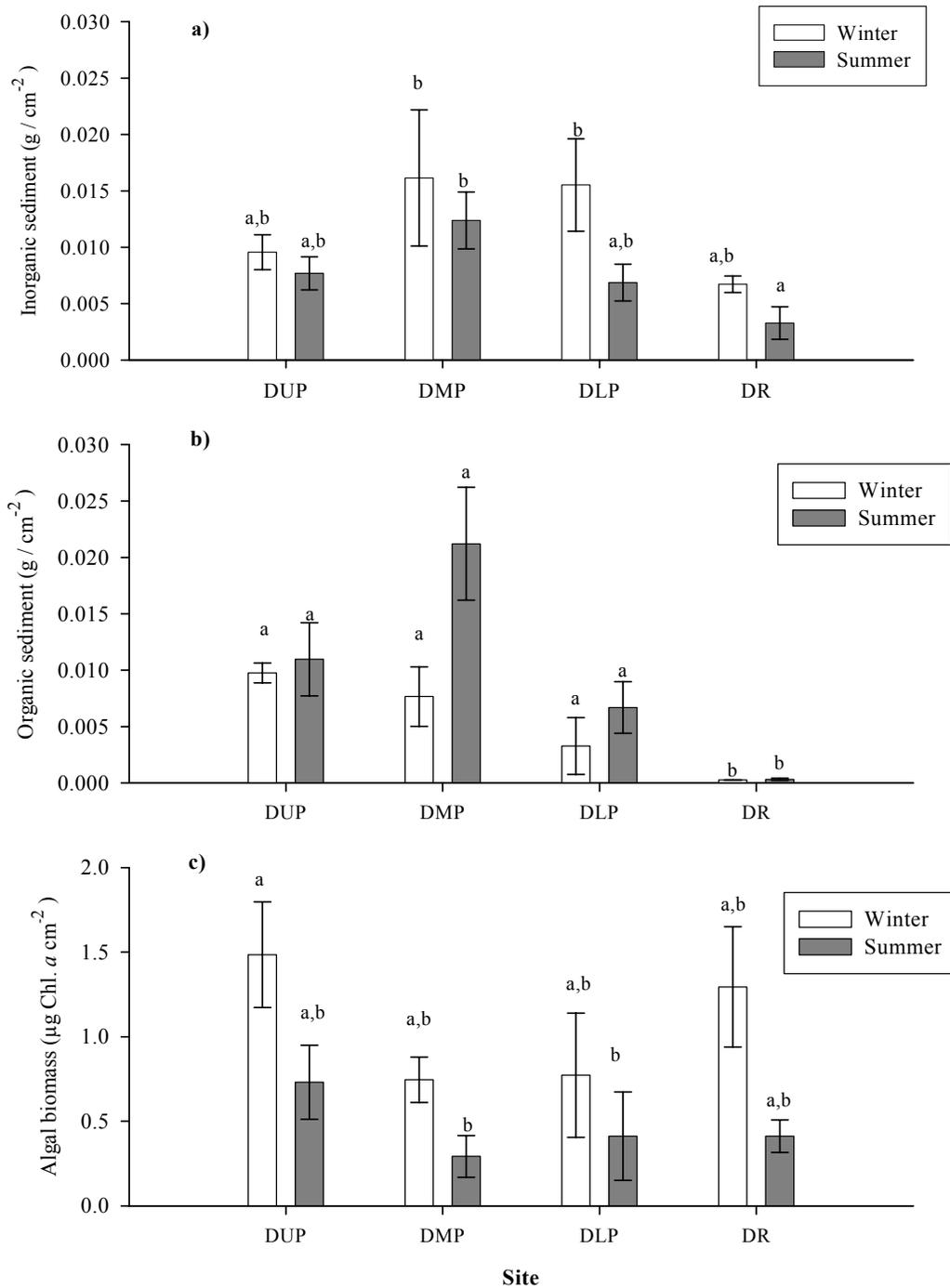
### Devils Creek

There was significantly less benthic organic deposits at the reference site (DR), than at sites along Devils Creek, and there was significantly more organic deposits found in summer (Figure 2.9a; Table 2.7).

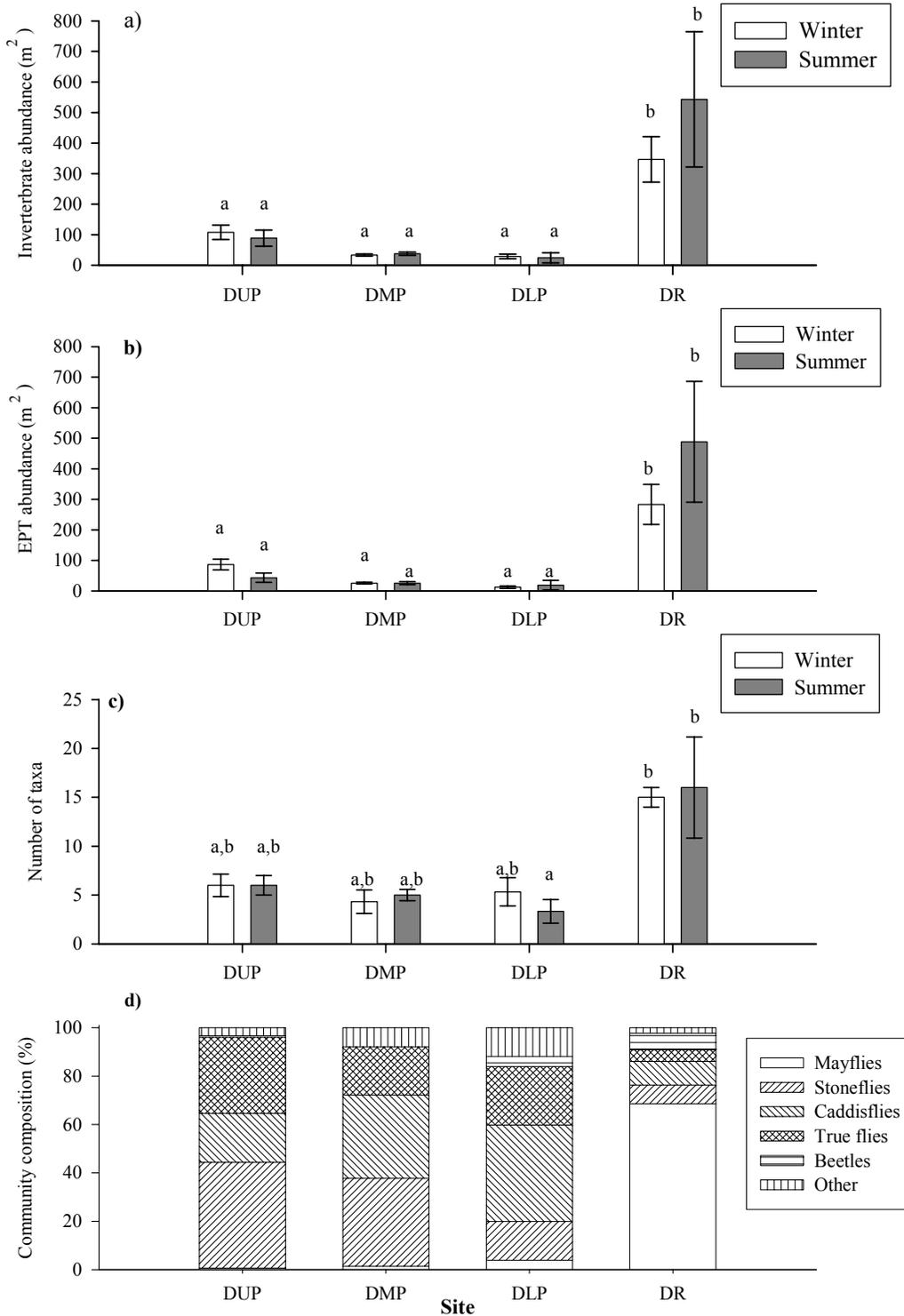
Algal biomass also differed between site and season, where the upper site (DUP) had the highest algal biomass and greater amounts were found at all sites in winter (Figure 3.19c; Table 2.7).

Benthic invertebrate abundances were significantly higher at the reference site (DR) and were on average 3 to 11 times higher in winter, and 6 to 21 times higher in summer than benthic invertebrate abundances at sites along Devils Creek (Figure 3.10a; Table 3.8). Benthic invertebrate abundance did not change seasonally, and there was no interaction effect, with average abundance declining downstream (Figure 3.10a; Table 3.8). A Tukey (HSD) post hoc test indicated that invertebrate abundances at the reference site (DR), were significantly higher than the three sites, DUP, DMP and DLP, in both seasons (Figure 3.10a). The reference site (DR) had significantly higher EPT abundances than the three impacted sites, DUP, DMP and DLP, but there were no seasonal difference, or an interaction effect between site and season (Table 3.8; Figure 3.10b). The average number of taxa was significantly higher at the reference site (DR), than at DUP, DMP and DLP, but there was no seasonal difference, or an interaction effect between site and season (Figure 3.10c; Table 3.8).

The percentage of mayflies increased downstream on Devils Creek (0.6% - 3.8%), but were markedly lower than the percentage of mayflies at the reference site (DR) (68%). But the percentage of caddisflies, stoneflies and true flies were higher at DUP, DMP and DLP, than the reference site (DR) (Figure 3.10d). *Deleatidium* was the most common mayfly found at the reference site, and the only genus of mayfly found along Devils Creek. *Spaniocercoides* and *Oxyethira albiceps* made up the largest proportion of stoneflies and caddisflies respectively, along Devils Creek. Whilst at the reference site, the stonefly, *Austroperla cyrene* and the caddisfly *Hydrobiosella* were the most important. Orthocladiinae were the most common dipteran at all sites.



**Figure 3.9.** Comparisons of a) inorganic sediments, b) organic sediments and c) algal biomass at three longitudinal precipitate sites and one reference site on Devils Creek (DR) in winter (June 2004) and in summer (January 2005). Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ). Means  $\pm$  1SE;  $n = 5$ .



**Figure 3.10.** Comparisons of a) benthic invertebrate abundance, b) EPT abundances and c) taxonomic richness, at three longitudinal precipitate sites and one reference site (DR) on Devils Creek in winter (June 2004) and in summer (January 2005) and d) seasonal mean community composition. Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ). Means  $\pm 1$  SE;  $n=3$ .

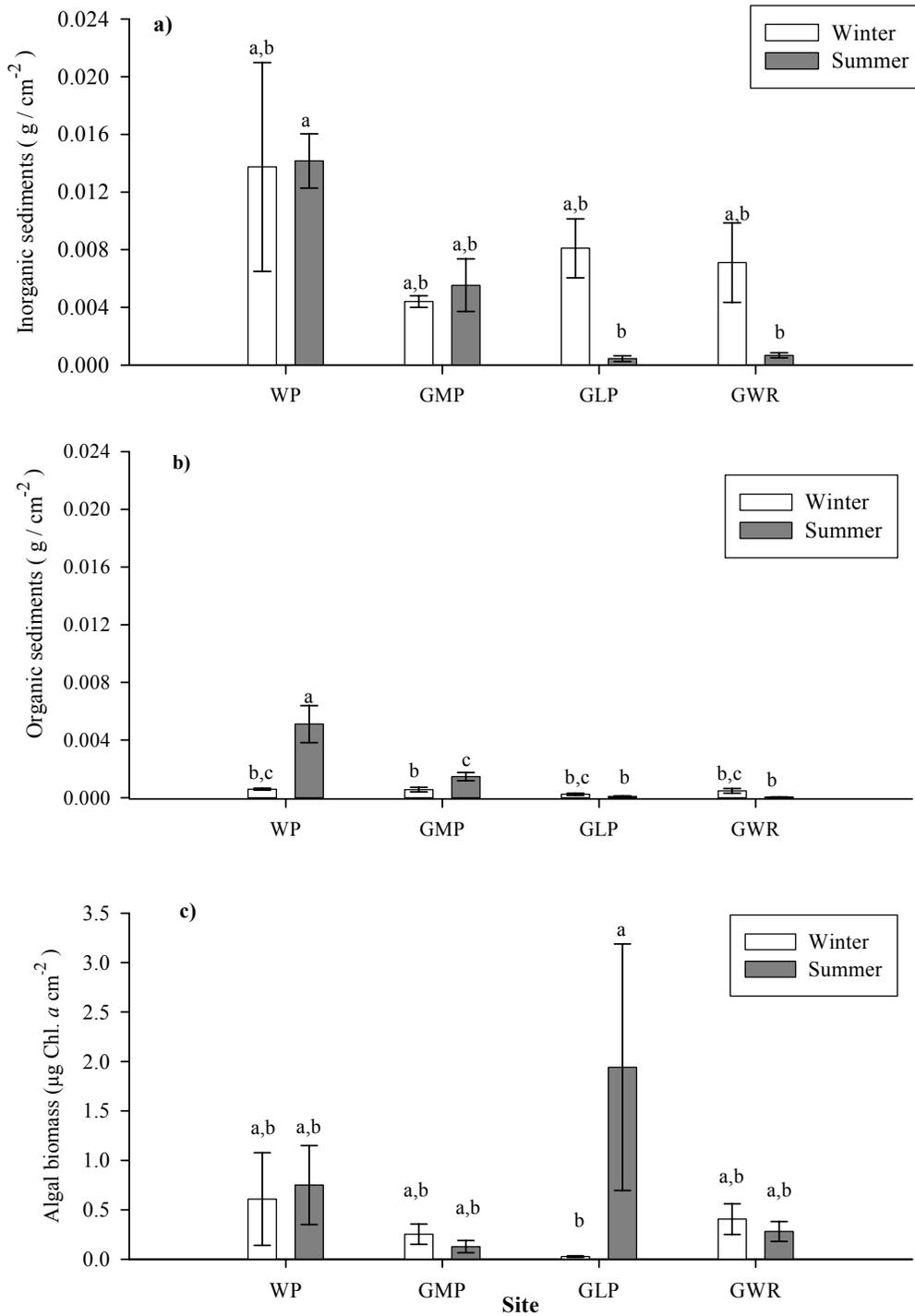
### **Garvey Creek**

The amount of benthic organic deposits varied between sites and season, with significantly higher amounts recorded at the upper site on Wellman Creek (WP), and in summer (Table 3.7). There was an interaction effect between site and season, with a downstream decline in organic deposits occurring in winter, and the opposite occurring in summer where the amount of deposits increased upstream (Figure 3.11b; Table 3.7).

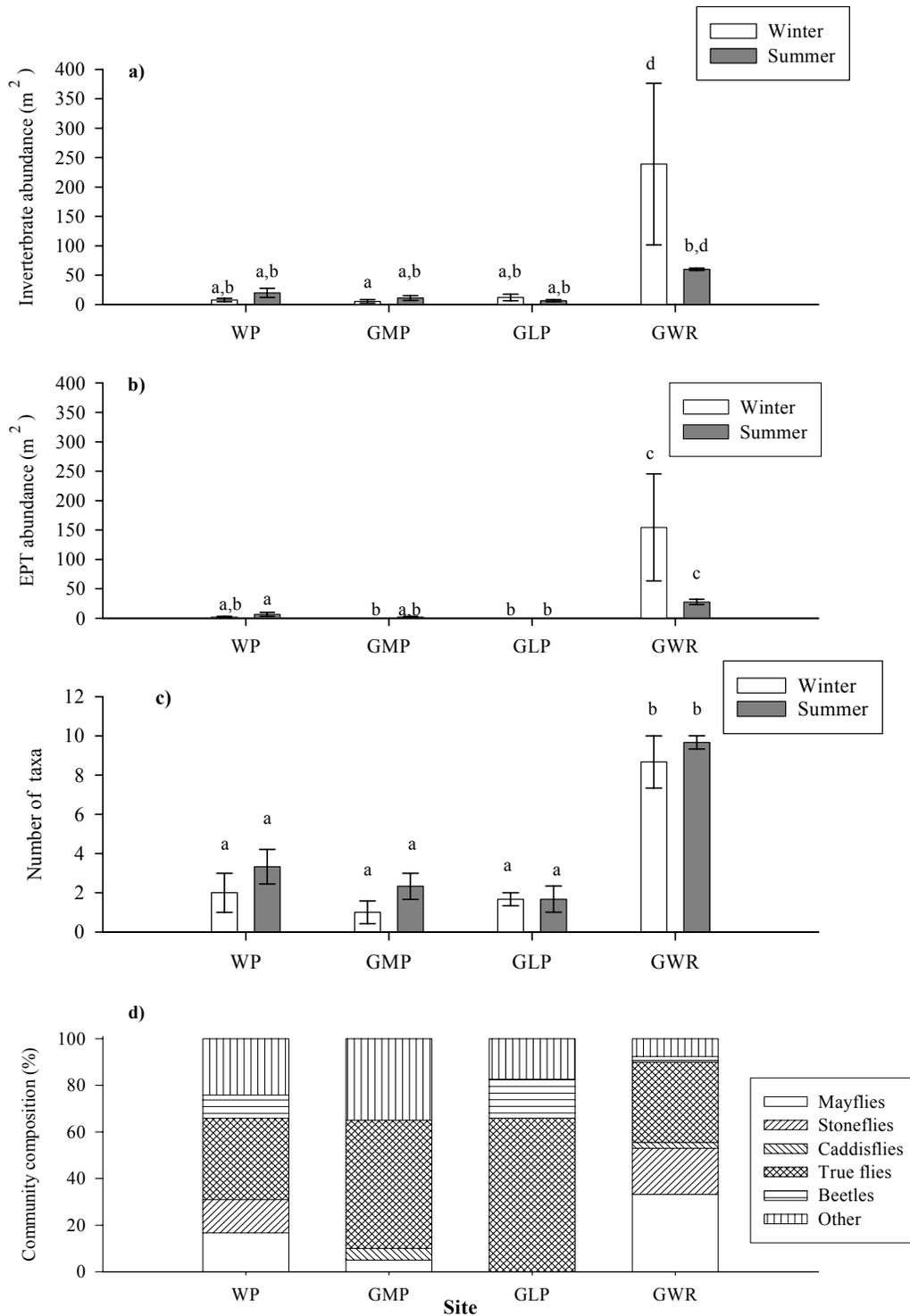
Algal biomass did not differ across sites or season (Table 3.7). However, there was an interaction effect between site and season, and algal biomass was higher in winter at the middle site (GMP) and reference site (GWR), but highest in summer at WP and the lower site (GLP) (Figure 2.11c; Table 3.7).

The reference site (GWR), had significantly higher benthic invertebrate abundances than the three impacted sites on Garvey Creek (Figure 3.12a; Table 3.8). There was no seasonal difference or an interaction effect between site and season with similar abundances being found in both seasons. EPT abundances also fared poorly along the three impacted sites on Garvey Creek, with significantly higher EPT abundances collected at the reference site (GWR) (Figure 3.12b; Table 3.8). Taxonomic richness was also significantly higher at the reference site (GWR), than at the other three sites, but did not differ between seasons (Figure 3.12c; Table 3.8).

Garvey Creek sites displayed low diversity in benthic invertebrate composition. Wellman Creek (WP) was dominated by dipterans (34.9%) and ‘other’ (Oligochaetes) (24%), but surprisingly also had a small percentage of mayflies (*Deleatidium*) (16.6%) and stoneflies (*Spaniocercoides*) (14.3%). The middle (GMP) and lower (GLP) sites were dominated by dipterans (55% and 66%, respectively). These percentages should be viewed with caution due to the low number of benthic invertebrates at these sites. GWR was dominated almost equally by mayflies (33%), and dipterans (34%) (Figure 3.12d). *Deleatidium* was the most abundant mayfly and Orthocladiinae were the most abundant dipteran at the reference site (GWR).



**Figure 3.11.** Comparisons of a) inorganic sediments, b) organic sediments and c) algal biomass at three longitudinal precipitate sites on Garvey Creek and one reference sites on Wash Creek in winter (June 2004) and in summer (January 2005). Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ). Means  $\pm$  1SE;  $n=5$ .



**Figure 3.12.** Comparisons of a) benthic invertebrate abundance, b) EPT abundances and c) taxonomic richness, at three longitudinal precipitate sites on Garvey Creek and one reference site (GWR), in winter (June 2004) and in summer (January 2005) and d) seasonal mean community composition. Letters above the bars show Tukey (HSD) post-hoc test ( $P < 0.05$ ). Means  $\pm 1$  SE;  $n=3$ .

## **Discussion**

My findings illustrate that mine-impacted streams can display a range of water chemistries, and although water chemistry between precipitate streams was variable, they conform more to what is generally considered to be 'mine polluted', than did the iron flocculent streams (e.g., Koryak et al. 1972, Dills and Rogers 1974, DeNicola and Stapleton 2002). For example, precipitate streams, were characterized by low pH (pH 3.3 - 5.15), and high mean conductivities ( $>300\mu\text{S}_{25^{\circ}\text{C}}\text{cm}^{-1}$ ), compared to iron flocculent streams, that had higher pH (5.05-6.9) and lower conductivities ( $<75\mu\text{S}_{25^{\circ}\text{C}}\text{cm}^{-1}$ ). Dissolved iron was higher in precipitate streams, than in flocculent streams ( $0.61\text{--}1.63\text{ g m}^{-3}$  and  $0.23\text{--}0.43\text{ g m}^{-3}$ , respectively), as was dissolved aluminium ( $0.866\text{--}4.08\text{ g m}^{-3}$  and  $0.052\text{--}0.384\text{ g m}^{-3}$ , respectively). Reference streams, collectively, showed water chemistry markedly different to precipitate streams but similar to those recorded for flocculent streams, in this study, although there were some differences. For example, pH ranged from 5.1 – 7.5, and mean conductivity was surprisingly higher ( $<115\mu\text{S}_{25^{\circ}\text{C}}\text{cm}^{-1}$ ). Dissolved iron and aluminium across reference streams was variable, but often fell within the range recorded for streams characterised by iron flocculants (e.g. Fe,  $0.04\text{--}0.38\text{ g m}^{-3}$  and Al,  $0.041\text{--}0.3\text{ g m}^{-3}$ ). Mine drainage can have circum-neutral pH when buffered by carbonates, and thus metals that are usually soluble at low pH, will become less soluble as pH rises (Niyogi et al. 2002a). The range in water chemistry recorded in my study may be related to factors such as, differences in underlying geology, or the history, age and type of the coal mining operations.

Overseas, metal deposition described as 'precipitates' have also been recorded in streams with diverse water chemistries. For example, Koryak et al. (1972), described iron hydroxide precipitates in Turtle Creek, Pennsylvania at two sites, receiving acid mine drainage, with characteristic low pH (pH 3.3 – 3.8). DeNicola and Stapleton (2002), recorded precipitates, in Slippery Rock Creek, Pennsylvania, as AMD precipitates, where pH was perhaps at the high end (pH 5.5 – 6.6), of what is generally characteristic of acid mine drainage streams, but similar to pH recorded in the flocculent streams in my study. Dissolved iron and aluminium concentrations were both elevated in Slippery Rock Creek ( $0.20\text{--}1.88\text{ mg l}^{-1}$  and  $0.55\text{ mg l}^{-1}$ , respectively), and fell within the range recorded across precipitate and flocculent

streams in my study. Iron hydroxide precipitates have also been recorded in a stream receiving coal mining pollution, in South Wales, where the pH (6.3 – 7.9), was similar to pH recorded in my flocculent and reference streams (Scullion and Edwards 1980). Iron deposition, described as ‘flocs’, and associated with iron-bacteria have been recorded in near-neutral pH waters (e.g., pH 6.2), but iron (both total and dissolved) is often elevated (Sheldon and Skelly 1990, Wellnitz et al. 1994, Sheldon and Wellnitz 1998), similar to my iron flocculent streams. It is possible that both abiotic and biotic factors may have been responsible for the iron deposition observed my streams, but I did not have time to investigate this further. Additionally, exploring the chemical composition and metal concentration in iron precipitates and iron flocculants, was out of the scope of this study, but could be important to consider in future projects because of potential toxicity of differing iron complexes on stream benthic communities.

In my broad-scale comparison of precipitate, flocculent and reference streams, algal biomass did not differ statistically. This was potentially due to the large variation in algal biomass across the streams in my study, which when considered longitudinally, also showed variability between, and within sites. There are several possible explanations why there may be high variation in algal biomass between these streams. These factors can be explained, in part, by the habitat matrix conceptual model (Biggs et al. 1998). Although, this model was designed for un-shaded streams, and my streams are mostly shaded, it suggests several factors that may be responsible for the variation in algal biomass found in my streams. The general idea behind the model is that algal biomass is controlled by resources, disturbance and invertebrate grazers, and is highest in streams with lots of available resources, infrequent disturbance events, and few invertebrate grazers. Firstly, the availability of light resources may vary between my sites, although most of my streams were in forested areas the amount of shade was variable, secondary my sites may be subject to different degrees of flood disturbance which will scour rocks clean of periphyton. Additionally, the abundance of invertebrates that feed on the organic layer of stones differed between treatments with the highest abundance being found at the reference streams and the lowest in the precipitate streams. The higher abundance of collector-browsers, greater flood disturbance and limiting light may partially explain why algal biomass was not higher in the reference streams. Algal biomass on substrates in precipitate and

flocculent streams may also be similar to reference streams because of a reduction in the amount of invertebrates or algae covered in precipitates and flocculants may have low palatability, and are thus avoided. Further, I did not investigate algae species in my streams, but filamentous algae, observed in Wellman Creek and the upper site on Devils Creek, for example, may be growing faster than iron precipitates are forming, and thus increasing algal biomass at these sites. Still, my results are surprising, as I expected to find results similar to overseas studies with a clear distinction between reference sites, and streams affected by either forms of iron deposition.

In several overseas studies it has been suggested that algal colonisation, growth and diversity are often reduced within regions of bacterial iron flocculants. Sheldon and Skelly (1990) investigated algal communities over a one kilometre stream reach in Vermont affected by ferromanganese-depositing bacteria (*Leptothrix ochracea*). They found that taxa richness was highest upstream and outside the bloom of *Leptothrix*, decreasing by up to 80% at their first site within the bloom of *Leptothrix*. Coincidentally, the stream in Sheldon and Skelly's study was not impacted by coal mining, and had elevated metals because of digging in the vicinity of the stream. Dissolved iron was 0.04 mg/L upstream of the bloom but increased to 0.9 mg/L at the start of the bloom, whereas dissolved manganese increased from 0.01 mg/L to 0.35 mg/L. Further, Sheldon and Wellnitz (1998) investigated alga colonisation on glass slides within blooms of *Leptothrix* in the same stream. They reported similar results to Sheldon and Skelly (1990) where the highest taxa richness was found in areas outside the ferromanganese-depositing bacteria.

Iron precipitates can also have similar affects on algal communities. Sode (1983), reported a reduction in the number of periphyton species and biomass in a Danish stream affected by ferric hydroxide. Further, dissolved iron in Sode's study ( $0.79 \pm 0.05$ ), fell within the range recorded for dissolved iron in the precipitate streams in my study, but the stream was not mine-impacted, and pH ( $7.14 \pm 0.16$ ) was similar to that recorded in my reference streams. In an experimental study conducted by DeNicola and Stapleton (2002) in an Ohio stream, substrates with Al and Fe precipitates from an AMD stream, were placed in a non-impacted reference stream, to investigate the response of periphyton in 'clean' water but on substrate with AMD precipitates. After four weeks, results revealed that periphyton biomass was about

40% higher on the substrates with metal precipitate coatings, in the 'clean' water. However as my study, variation between replicates was high and thus there was no significant difference was found. The authors suggest that the impacts of AMD metal precipitates may have been reduced in their study because the precipitate coating was thin and adhered to the rock surface, rather than being a loose iron hydroxide 'floc'.

Sheldon and Skelly (1990), suggested several explanations for the reduction in alga inside blooms of iron-depositing bacteria. Firstly, ferromanganese encrusted sheaths produced by the bacteria may limit space and nutrients, and secondly they may directly smother and inhibit the colonisation and growth of diatoms, filamentous alga and cyanobacteria (Sheldon and Skelly 1990). Sode (1983), further suggested that the coating of bed substrates by ferric hydroxides, may reduce periphyton growth by reducing the light available for photosynthesis.

Water chemistry and habitat quality in reference streams appears to be more favourable, with more taxa, and higher total and EPT invertebrate abundances, than in precipitate and to some extent flocculent streams. In precipitate streams multiple factors (low pH, elevated concentrations of heavy metals and iron precipitates) may be acting to influence invertebrate abundances and taxa richness. In comparison, flocculent streams had pH and heavy metal concentrations that were elevated, but comparable to those observed in reference streams. While benthic invertebrate densities in flocculent streams were highly variable, they often fell within the range recorded in the precipitate and reference streams. This suggests that even though water quality has improved, iron flocculants are preventing colonisation, or reducing the survival of macroinvertebrates in these areas.

In overseas studies, reductions in benthic invertebrate abundances have also been reported in streams with iron bacterial flocculants, and iron precipitates. Wellnitz et al. (1994), investigated the response of macroinvertebrates to blooms of iron depositing bacteria (*Leptothrix* spp.), in Vermont. They found that macroinvertebrate diversity was reduced inside the iron bacteria blooms. Moreover, species diversity was lowest when the iron deposits were thickest and concentrations of iron and manganese were highest but increased again after the iron bloom had thinned out and receded in size.

Similarly, McKnight and Feder (1984), reported a reduction in benthic invertebrate abundances, at two sites in a Colorado stream, with hydrous metal oxide precipitates and pH 5.2 – 6.4. Interestingly, another site in their study had an adhering metal precipitate, low pH (<4), and high dissolved heavy metals, but invertebrate abundances were similar to that of a non-impacted site (pH 7). DeNicola and Stapleton (2002) suggested that a loose, flocculent like, iron precipitate may have a greater adverse affect on benthic communities than an encrusting, adhering precipitate. However, the findings from my study suggest the opposite to this, with precipitate streams, being more adversely affected, but having encrusting deposits that adhere to rock surfaces, compared to flocculent streams, which have loose coatings of iron deposits.

Reasons for benthic invertebrate decline in streams affected by iron flocculants, and iron precipitates may be related to several factors, and include, reduction in food quality, clogging of the hyporheic and interstitial spaces, and direct toxic effects of iron deposits adhering to an animal's body. For example, Wellnitz et al. (1994) investigated the quality of iron bacteria (*Leptothrix*), as food for macroinvertebrates over 10 days, in three scrapers (two mayflies and one caddisfly). Their results showed that all three scrapers ingested iron bacteria, but only one mayfly species gained weight while on the diet. The second mayfly species has similar modes of feeding, but after 10 days of feeding on iron bacteria, its mouthparts became clogged. The final scraper, a caddisfly, had consumed the iron bacteria, but did not gain weight suggesting that *Leptothrix* was not an adequate food. Further, they investigated survival rates of nine species, from three orders, mayflies, stoneflies and caddisflies, within and outside blooms of iron bacteria. Of the nine species, the three mayflies displayed significantly higher mortality within the iron bacterial bloom, but the other species did not show any differences in rate of survival. They suggested that mortality of the mayfly species in their study may be related to their low tolerance of elevated heavy metals (e.g., their study, Fe 0.19-1.84). Additionally, they suggested that mayflies may be particularly susceptible to coatings of iron deposits.

Gerhardt (1992), experimentally investigated the effect of sub-acute doses of iron at two pH (pH 4.5 and 7) regimes, on feeding, and iron precipitate coatings, in the

mayfly (*Leptophlebia marginata*) over a one month period. Mayflies were given a diet of fine detritus, in two pH treatments, and three differing total iron concentrations (10, 20, or 50 mg Fe<sub>tot</sub> l<sup>-1</sup>). At pH 7, more than 90% of the mayflies continued to eat, regardless of iron concentrations, however mayflies in the iron treatments, at pH 4.5, stopped feeding as their mid-guts became filled with food, and they became constipated. This would essentially lead to starvation. Further, the uptake of iron onto the bodies of mayflies was greatest in the low pH and highest iron treatments. Soucek et al. (2000) investigated the toxicity of pure ferric hydroxide precipitate in an experiment using *Daphnia magna* and found that after 48 hours there was 100% mortality, but the dissolved concentration of iron in the water column were below detection limits (0.002mg/L), supporting previous studies, that iron precipitates, may be toxic to aquatic organisms.

The results from Wellnitz et al. (1994) are particularly interesting, because in my study the mayfly species *Deleatidium*, was numerically dominant not only in the reference streams, but also in the flocculent streams. *Deleatidium* larvae, feed primarily on the organic layer of stones (Collier and Winterbourn 1990), suggesting that either this genus can browse on substrates and ingest iron flocculants, or that iron flocculent areas can be avoided. In comparison, the dominant taxa in precipitate streams were a caddisfly *Oxytheria albiceps* and a dipteran Orthocladiinae. The mayfly *Deleatidium* ranked number three in the precipitate streams, and supports previous studies that suggest that this mayfly tolerates a wide range of water chemistries. Winterbourn (1998), recorded *Deleatidium* in a stream, on the West Coast of the South Island, receiving AMD waters with a measured pH of 3.5. Similar to my study, Winterbourn (1998) also found the caddisfly *Oxytheria albiceps* (pH >3.5) and dipterans belonging to Orthocladiinae (pH >2.9). The fact that I found a range of species in my flocculent and precipitate streams demonstrates that these stream invertebrates can not only tolerate adverse water quality, often with low pH, and elevated levels of dissolved metals, but that they can also tolerate to at least some extent habitat change as a result of iron deposition.

Numerous benthic invertebrates were observed to be coated in iron flocculants and precipitates in my study (pers. obs.). It is possible that mayflies (and possibly other invertebrates) have adapted to being coated in iron flocculants and precipitates, and

respond by increasing molting frequently. This has been reported by Gerhardt and Westermann (1995), who suggested that molting in the mayfly *Leptophlebia marginata* may be a mechanism to rid the body of iron precipitates, as newly molted mayflies showed no sign of precipitates. This idea could be further investigated using the mayfly *Deleatidium* under laboratory conditions.

Finally, longitudinal water chemistry and benthic invertebrate communities yielded varying results. In Burke Creek (a flocculent stream), water chemistry and benthic invertebrate communities and abundances were similar to the reference site. This suggests that under near-neutral pH, and low conductivities, a wide range of stream invertebrates may be able to tolerate iron flocculants. In Old Terrace, the other flocculent stream, water chemistry and benthic community composition was similar to the reference site, but invertebrate abundances were markedly lower. This begs the question why are benthic invertebrate abundances different in the reference site in Old Terrace when in Burke Creek abundances are similar? One reason might be that flocculants in Old Terrace are thicker and cover more of the stream channel (pers. obs.), these thicker flocculants may be having a greater smothering effect on stream communities, than the thinner flocculants in Burke Creek.

Water chemistry and invertebrate abundances at the impacted sites on Devils Creek (a precipitate stream), show little change downstream, and were markedly different to the reference site. Interestingly, community composition indicated that stoneflies decreased, but caddisflies increased downstream, and dipterans were similar at all sites. Importantly, community composition at all longitudinal sites was different to the reference site, where mayflies dominated. Further, the reference site on Devils Creek is approximately two kilometres downstream of the lowest site (DLP), and while recovery is not obviously occurring within the one kilometre stretch, that the three impacted sites are located along, it has occurred further down. The change in water chemistry and benthic communities is probably related to dilution of AMD waters from tributaries.

The water chemistry through Garvey Creek, the other precipitate stream was poor, and this is mirrored by low invertebrate densities. But although community composition was dominated by dipterans at all sites, the mayfly *Deleatidium* was

recorded at the Wellman Creek site, and the middle site on Devils Creek. Although, *Deleatidium* has been found in a wide range of water chemistries in other studies, on the West Coast (Winterbourn 1998, Anthony 1999), I was surprised when it was found here. However, the reference site, Wash Creek was dominated by mayflies (*Deleatidium*) and thus this could be a source of colonists.

It is perhaps not surprising that water chemistry and biological sampling indicated little change longitudinally in my precipitate and flocculent streams. This is arguably particularly true for the more mine-polluted streams, Devils Creek and Garvey Creek, than it is in the flocculent streams in this study. Never the less, the study sites along the streams were relatively close together, usually within a one to two kilometre stretch, and thus giving little opportunity for dilution from clean-water tributaries between sites.

My results illustrate the complexity, and range of factors that influence benthic invertebrate communities. Firstly, underlying water chemistry is probably acting to initially determine the range of species that can inhabit the stream. Secondly, stream invertebrates need to be able to tolerate possible changes in habitat and food quality imposed by iron deposition whether it is in a precipitate or flocculent form. Precipitate streams in this study were affected by low pH, and high dissolved heavy metals, making it hard to separate the affects of water chemistry from the affects of iron precipitates on benthic communities. But iron flocculent streams, often had similar pH and benthic communities to my reference streams, but metals, especially iron was elevated. This makes me ask the question is water chemistry, particularly low pH, acting as the initial filter for stream invertebrates and algal colonisation in these stream systems. Further, if precipitate streams are having a greater adverse affect on the structure of stream communities, will this be mirrored by a reduction in leaf breakdown?

## Chapter four

# Leaf breakdown in streams effected by iron precipitates and iron flocculants

---

### Introduction

Terrestrial vegetation that enters a stream is a significant source of energy for stream communities and can provide an important food and habitat resource for macroinvertebrates (Fisher and Likens 1973, Petersen and Cummins 1974, Anderson and Sedell 1979). Leaf breakdown, regarded as a key ecosystem process, undergoes at least three distinct phases after entering a stream (leaching, conditioning and fragmentation) (Cummins 1974, Petersen and Cummins 1974). Within the first 24 hours of submersion of leaves in a stream abiotic leaching which involves the rapid release of soluble compounds begins. In the second stage, leaves undergo conditioning which involves the colonisation of leaf surfaces by micro-organisms (i.e., bacteria and fungi) and subsequently increases the palatability of leaves for macroinvertebrate detritivores. The third stage, fragmentation involves the reduction of coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) and can occur through physical (e.g., mechanical abrasion) and biological (e.g., ingestion by macroinvertebrates) agents.

However, the role of macroinvertebrates in this process can be influenced by anthropogenic stress, which effects community structure and ecosystem functioning. For example, mine drainage can exert a range of stresses on a stream ecosystem, stresses which can act individually or collectively to affect and modify in-stream processes such as leaf decomposition. These stresses can include, lowered pH, high concentrations of dissolved heavy metals and deposition of metal oxides (e.g., iron hydroxides) (Kelly 1988).

In streams receiving mine drainage, leaf breakdown is often slower than rates of leaf breakdown in 'non-impacted' streams (Carpenter et al. 1983, Maltby and Booth

1991). Leaf breakdown may be altered in accordance to the sensitivity of benthic macroinvertebrates, fungi, and bacteria to mining related changes in water and habitat

quality. For example, some fungi, bacteria, and macroinvertebrates are known to be sensitive to lowered pH (Townsend et al. 1983, Allard and Moreau 1986, Palumbo et al. 1987, Smith et al. 1990), heavy metals (Clements et al. 1988, Rasmussen and Lindegaard 1988, Niyogi et al. 2002b) and metal deposition (McKnight and Feder 1984, Niyogi et al. 2001).

The aim of this chapter was to assess leaf breakdown, and colonisation of leaves by invertebrates in streams containing; (a) precipitates, (b) flocculants, and (c) reference conditions.

## **Methods**

### **Study design and sites**

In winter, 16 sites were used to examine leaf breakdown and water chemistry, four control sites and 12 treatment sites. The 12 treatment sites consisted of sets of three sites located along four mine-drainage impacted streams. To allow treatment comparisons during winter, data from the three longitudinal sites on each impacted stream were pooled and used as a replicates; thus there were two iron flocculent and two iron precipitate streams, but four control sites (see study design; Chapter three; Figure 3.1). In summer, leaf breakdown was examined at nine stream sites, three flocculent, three precipitate and three reference (see study design; Chapter three; Figure 3.1). This design enabled increased replication of the stream treatment effect. Sites are described in Chapter two.

### **Water chemistry**

Temperature, specific conductivity, and pH were measured on five occasions in winter, and on three occasions in summer, using an Oakton CON 10 Series meter. Turbidity (HACH 2100P Turbidimeter), was also measured three or four times at all sites in winter and one to three times during summer.

Grab water samples were taken once in each season and sent to a commercial laboratory (RJ Hills Laboratory, Hamilton) where they were analysed for five

dissolved heavy metals, iron, aluminium, arsenic, zinc and nickel (see Chapter three, methods, for sampled sites).

### **Leaf breakdown**

Abscised American Sycamore (*Platanus* spp: Platanaceae) leaves were collected in early May and returned to the laboratory where they were allowed to dry at room temperature and stored until subsequent use.

### **Winter leaf packs**

One hundred and twenty eight leaf packs were prepared using dried American Sycamore leaves, weighed to 20 grams dry weight ( $\pm 0.005\text{g}$ ) and placed into nylon onion bags with 5 mm mesh. On 19 May 2004, eight leaf packs were randomly placed at each of the 16 sites. Leaf packs were secured to boulders or riparian vegetation. Two leaf packs were subsequently recovered from each site at two, four, seven and nine weeks. Leaf packs were placed in separate zip-lock plastic bags and stored in a chilly bin with ice until being returned to the laboratory and frozen prior to analysis. In the laboratory, frozen leaves from each leaf pack were thawed, and washed through a 500  $\mu\text{m}$  mesh sieve to remove fine debris and invertebrates. Leaves from each leaf pack were placed into labelled containers and dried at 40°C for 96 hours, after which they were then reweighed to the nearest 0.005g. The average dry weight of leaves remaining at each site was obtained by averaging the remaining weights from the collected and dried leaf packs. Invertebrates colonising leaf packs were removed by hand picking and sieving, and identified using methods outlined in Chapter three. The average number of total invertebrates and invertebrate shredders across the two leaf packs recovered at each site was divided by the average weight of remaining leaf pack to obtain the number of invertebrates per gram leaf pack remaining.

### **Summer leaf packs**

During winter several leaf packs were physically abraded and lost in floods, and so for the summer experiment eighteen leaf pack canisters were constructed using PVC pipe cut into 30 cm lengths. One centimetre diameter holes were drilled along the length of each pipe, to allow water flow and invertebrate colonisation. Plastic caps with

additional holes were attached to each end to prevent leaf pack loss in the stream (Figure 4.1).

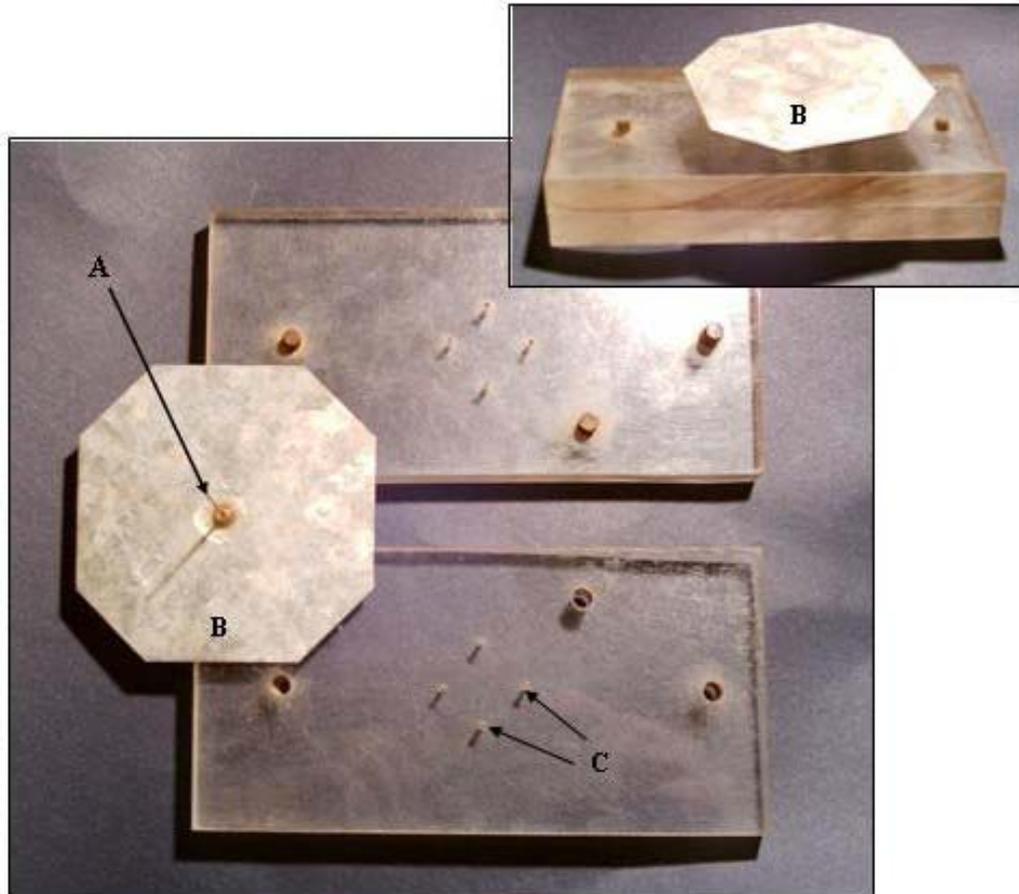


**Figure 4.1.** Leaf pack canister used during summer (2005) to prevent physical abrasion.

One hundred and eight, five gram leaf packs were constructed using dried American Sycamore leaves, placed into five mm mesh nylon onion bags. Two canisters, each with six leaf packs were submersed and secured at each of the nine sites, on the 23 December 2005. Three leaf packs were recovered from each stream at two, five, seven and nine weeks. Leaf packs were recovered and treated using the same procedure used in winter.

After being rinsed of invertebrates and attached sediments, leaves were tested for toughness using a penetrometer (Feeny 1970, Quinn et al. 2000) (Figure 4.2). Penetrance is a relative measure of leaf toughness, and is defined as the weight required to force a blunt rod through a leaf (Young et al. 1994). In this investigation I recorded the weight of lead shot (grams) needed to force a 1 (0.075) mm rod through a wet leaf. Fifteen measurements were made on leaves randomly drawn from the leaf pack. Veins were avoided because they might have different penetrance values.

Penetrance pressure (PEN, kPa) was calculated after Quinn et al. (2000) using the formula  $9.807 \text{ m} / 0.785$  (i.e., mass x gravitational force/area). The weight of the penetrometer stand was 22 grams and thus the kPa was 275. When the rod penetrated the leaf without applying any additional weight half this penetrance value was recorded. Young et al. (1994), commented that freezing may alter leaf toughness but because all leaf packs received the same treatment this would have affected all leaves, equally.



**Figure 4.2.** Penetrometer used to measure leaf toughness in summer leaf packs (2005), showing the two halves that leaves are placed between, and A, pin, B, stand for lead shot to sit on, and C, holes for pin. Penetrometer ready for use (inset).

After testing for leaf toughness a sub-sample of leaves was removed from each leaf pack, to quantify the percentage of ash free dry weight (AFDW) of leaf remaining. Fifteen, one centimetre diameter leaf disks were cut using a cork borer from randomly

selected leaves in the leaf pack. Leaf disks were placed individually into pre-weighed aluminium boats and dried at 40°C for 24 hours, weighed to the nearest 0.0001g, ashed at 500°C for 1 hour and reweighed. The weight of the inorganic and organic (AFDW) sediments from the fifteen leaf disks were added and then averaged between the three leaf packs. Because the initial weight of the 15 leaf disks removed was variable, percentage ash free dry weight of the 15 leaf disks was used to compare among treatment streams and was determined with the following equation;

$$\text{AFDW (\%)} = \left[ \frac{\text{Dry weight} - \text{Ash weight}}{\text{Dry weight}} \right] * 100$$

This process was undertaken because results in winter indicated that iron precipitates deposited on leaves may lead to an increase in leaf pack weight. I predicted that (1) iron deposition would result in a higher percentage of inorganic sediment on leaves in the flocculent and precipitate streams than the reference streams, and (2) that the percentage of inorganic sediment on leaves would increase the longer the leaf packs remained in the stream. Thus, percentage ash free dry weight (AFDW) of the sub-samples should have been lower in the flocculent and precipitate streams than the reference streams because the proportion of inorganic sediment should be greater in the former two.

The remaining leaf pack was dried as described above and the total dry weight of the 15 leaf disks removed was added to the dry leaf weight of the leaf pack, to calculate total weight loss.

## **Statistical analyses**

### **Water Chemistry**

To determine if water chemistry parameters and heavy metals differed between the three stream treatments a one-way analysis of variance (ANOVA) was used. In winter, the mean value for each stream was used as a replicate in the analysis as explained on page 84 (except pH which was not analysed). Measurements taken at

control sites were averaged to gain an overall value for each stream. In summer, mean values from the three streams within each treatment effect were used as replicates for analysis.

### **Leaf breakdown**

Dry leaf weight remaining, leaf toughness, and percentage AFDW, were tested for differences across treatments using a generalised linear model ANOVA, with stream treatment and time (weeks) as factors. In winter, dry leaf weight remaining was investigated by averaging the weight of the two leaf packs collected at each site. Values for the three treatment sites were then averaged and this 'pooled' value was used as a replicate. Dry leaf weight at reference sites was averaged across the two leaf packs and used as a replicate. In summer, dry leaf weight, leaf toughness, and percentage AFDW of the three leaf packs was averaged, and this value was used as a replicate.

In addition, the decomposition rate was modelled as a negative exponential function using the equation below after Peterson and Cummins (1974);

$$W_t = W_o e^{-kt}$$

Where,  $W_t$  = the dry weight remaining after time (t) in days,  $W_o$  = the initial weight,  $e$  = base of natural logarithm, and  $k$  = breakdown rate constant ( $d^{-1}$ ). A logarithmic transformation gave;

$$\ln W_t = \ln W_o - kt$$

To calculate the breakdown constant ( $k$ ), linear regressions of  $\ln W_t$  versus time were performed. Thus,  $k$ , is the slope of the line obtained from the linear regression. Breakdown rates were calculated for winter and summer, with leaf weight remaining being averaged across sites within each stream treatment and then regressed against time.

## **Biotic responses**

The number of taxa in leaf packs, and the number of invertebrates per gram of leaf pack, were examined using a generalised linear model ANOVA. In winter, the number of taxa and number of invertebrates were examined by averaging the numbers across the two leaf packs at each longitudinal site and then determining the average for the three sites. This 'pooled' value was used as a replicate. Taxa richness and number of invertebrates at reference sites were averaged across the two leaf packs and used as a replicate in the analyses. In summer, the three leaf packs from each site were averaged and this value was used as a replicate.

Differences detected by ANOVAs were tested using Tukey's post-hoc test (HSD) to determine which treatments were different. Plots of residuals versus fits and normality plots were used to test for normality and homoscedasticity of data. Where assumptions of normality and homoscedasticity were not met response variables were log transformed (in case of zeros,  $\chi + 1$ ) (Zar 1999).

## **Results**

### **Water chemistry**

Median pH was markedly lower in precipitate stream treatments and mean conductivity was significantly higher than in flocculent and reference streams (Table 4.1). Temperature was the only water chemistry variable that varied seasonally, and was higher in summer (Table 4.1).

Flocculent and precipitate sites had higher concentrations of dissolved iron, aluminium, nickel and zinc than reference streams (Table 4.1). However, in winter no significant difference in concentrations of heavy metals were found between the three stream treatments. In summer iron was significantly lower in the reference streams than in flocculent and precipitate treatment streams, and whereas aluminium was variable it tended to be much higher in precipitate streams (Table 4.1; Tukey (HSD) post-hoc  $P < 0.05$ ).

**Table 4.1.** Mean ( $\pm 1$ SE) water chemistry across the three stream treatments, reference, flocculent and precipitate in winter 2004 and summer 2005. Results displayed are from a one-way ANOVA. Significant results are shown in bold. Tukey (HSD) post-hoc results are shown, with sites in italics having no significant differences ( $P < 0.05$ ).

<i>Winter</i>	<b>Flocculent</b>	<b>Precipitate</b>	<b>Reference</b>	<i>F</i> <sub>2,5</sub>	<i>P</i>
pH median	6.23	4.17	6.75		
pH range	(5.1 - 6.9)	(3.3 - 5.2)	(5.1 - 7.4)		
Conductivity ( $\mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ )	<i>68.15</i> $\pm$ 2.85	300 $\pm$ 156.67	<i>56.25</i> $\pm$ 7.79	9.055	<b>0.022</b>
Turbidity (NTU)	10.6 $\pm$ 7.0	30.5 $\pm$ 11.2	7.38 $\pm$ 3.1	3.65	0.105
Temperature ( $^\circ\text{C}$ )	8.9 $\pm$ 0.0	5.46 $\pm$ 3.2	7.7 $\pm$ 0.3	1.978	0.233
<i>n</i>	2	2	4		
<i>Heavy metals (g m<sup>-3</sup>)</i>				<i>F</i> <sub>2,3</sub>	<i>P</i>
Iron	0.32 $\pm$ 0.05	0.74 $\pm$ 0.13	0.155 $\pm$ 0.115	8.366	0.059
Aluminium	0.218 $\pm$ 0.166	2.162 $\pm$ 1.465	0.107 $\pm$ 0.066	4.776	0.117
Arsenic	0.001 $\pm$ 0	0.001 $\pm$ 0	0.024 $\pm$ 0.023	1	0.465
Nickel	0.003 $\pm$ 0.001	0.053 $\pm$ 0.046	0.003 $\pm$ 0.002	0.184	0.84
Zinc	0.012 $\pm$ 0.004	0.214 $\pm$ 0.178	0.002 $\pm$ 0.001	7.289	0.071
<i>n</i>	2	2	2		
<i>Summer</i>	<b>Flocculent</b>	<b>Precipitate</b>	<b>Reference</b>	<i>F</i> <sub>2,6</sub>	<i>P</i>
pH median	6.83	4.2	7.1		
pH range	(5.5 - 6.8)	(3.8 - 4.6)	(6.7 - 7.5)		
Conductivity ( $\mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ )	<i>70.5</i> $\pm$ 4.3	460 $\pm$ 197	<i>91.3</i> $\pm$ 22.6	9.114	<b>0.015</b>
Turbidity (NTU)	14.33 $\pm$ 6.17	5.2 $\pm$ 2.2	7.03 $\pm$ 3.50	1.23	0.356
Temperature ( $^\circ\text{C}$ )	13.8 $\pm$ 0.87	13.4 $\pm$ 0.8	15.7 $\pm$ 0.54	2.7	0.143
<i>n</i>	3	3	3		
<i>Heavy metals (g m<sup>-3</sup>)</i>				<i>F</i> <sub>2,5</sub>	<i>P</i>
Iron	<i>0.32</i> $\pm$ 0.0133	<i>1.16</i> $\pm$ 0.44	0.17 $\pm$ 0.11	3.017	<b>0.047</b>
Aluminium	0.15 $\pm$ 0.03	1.83 $\pm$ 0.96	0.13 $\pm$ 0.086	5.2	0.051
Arsenic	0.002 $\pm$ 0.0007	0.001 $\pm$ 0	0.013 $\pm$ 0.011	0.79	0.503
Nickel	0.003 $\pm$ 0.006	0.044 $\pm$ 0.003	0.001 $\pm$ 0.006	2.15	0.211
Zinc	0.01 $\pm$ 0.0009	0.16 $\pm$ 0.14	0.002 $\pm$ 0.001	2.37	0.188
<i>n</i>	3	2	3		

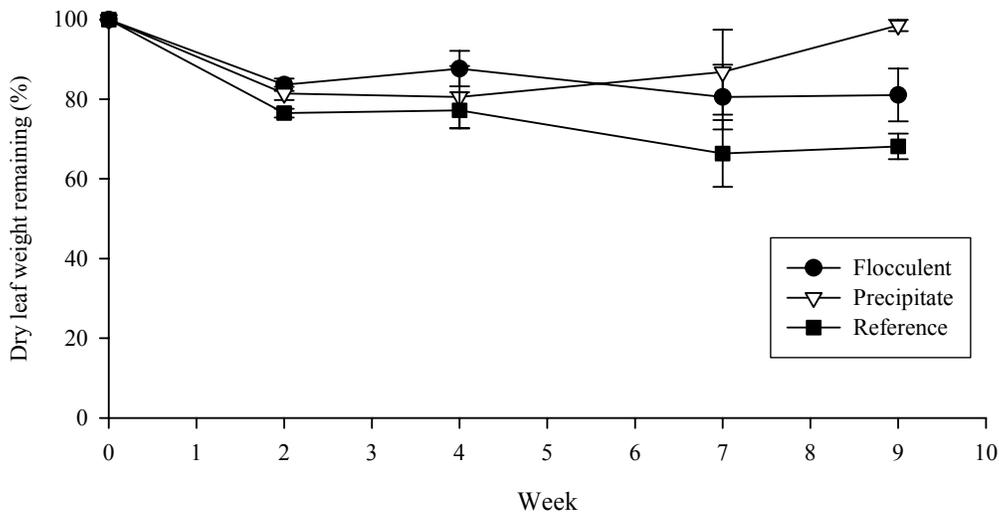
### Leaf pack processing

During winter leaf packs in precipitate streams initially lost weight during the first two weeks, and then began to gain weight, whereas leaf packs in flocculent and reference streams continue to breakdown (Figure 4.3). However, after nine weeks, only the reference streams had significantly less leaf material remaining than in the precipitate stream treatments (Table 4.2; Tukey (HSD) post-hoc test  $P < 0.05$ ). There

was no significant difference over time, or an interaction effect between stream treatment and length of time in stream on leaf weight (Table 4.2).

In summer, leaf breakdown was similar across all treatment streams but in precipitate streams leaf weight increased by 10 % between five and seven weeks (Figure 4.4a). The increase in leaf weight observed in precipitate streams was not significantly higher than that seen in flocculent or reference streams (Table 4.2). There was no interaction effect between stream treatment and length of time in stream on remaining leaf pack weight. Interestingly, although there was no difference in leaf weight between the three stream treatments, visual examination of portions of leaf packs differences in the degree of leaf breakdown. For example, leaves from a leaf pack in a precipitate stream showed build up of inorganic sediments and little decomposition, whereas leaves from a reference stream, were highly fragmented although this weight did not change (Figure 4.5).

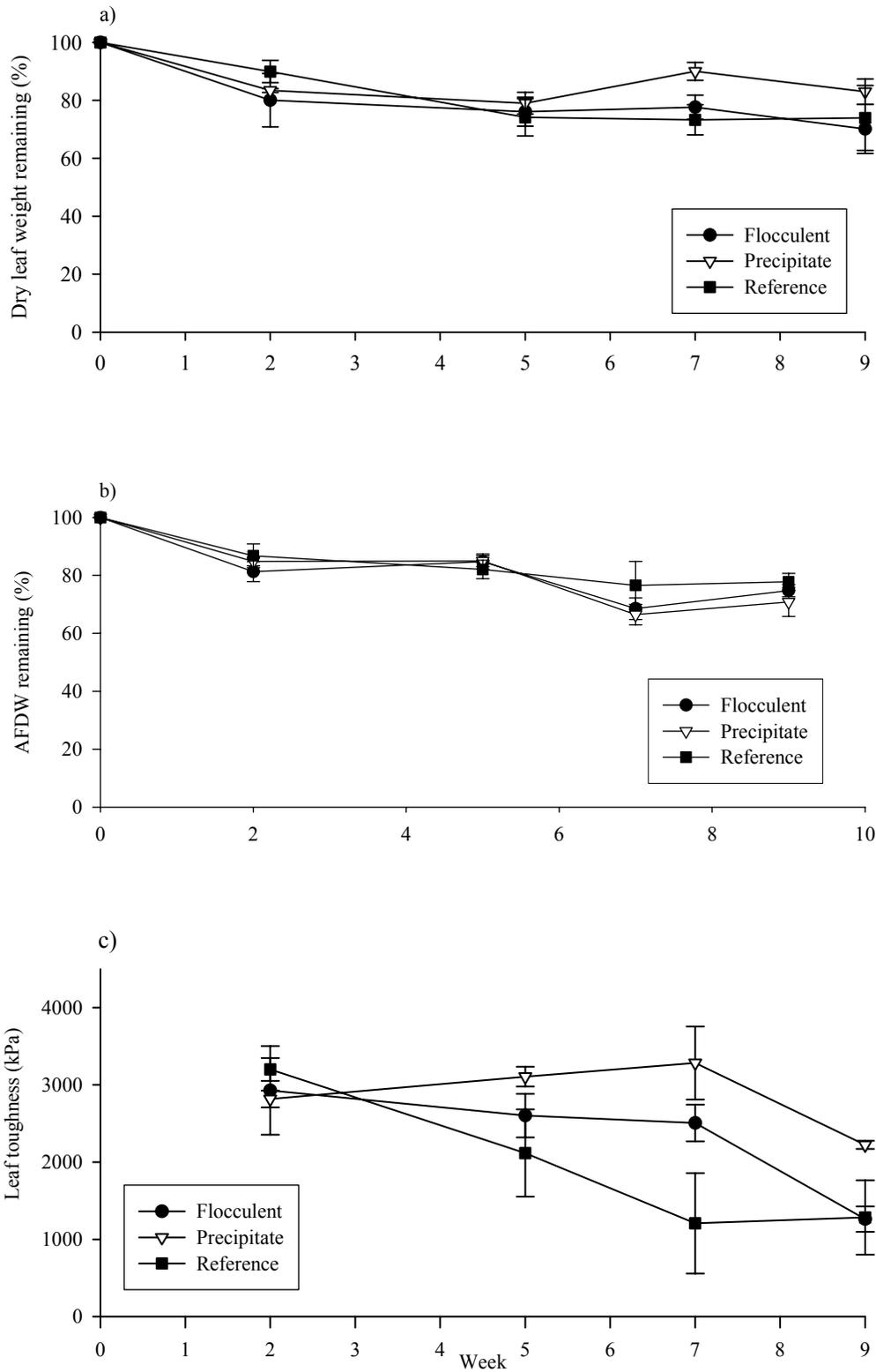
In winter, the exponential rate of leaf weight loss ( $k$ ) over the duration of the study ranged from  $0.0004 \text{ day}^{-1}$  in the precipitate streams, to  $-0.003 \text{ day}^{-1}$  in the flocculent and reference streams (Table 4.3). In summer, leaf weight loss ( $k$ ), was also slow in all streams, but again was slowest in the precipitate streams. However, precipitate stream,  $k$  values indicated that leaves were breaking down rather than gaining weight as observed in winter (Table 4.3). All  $k$  values indicated slow rates of leaf breakdown (Petersen and Cummins 1974).



**Figure 4.3.** Comparison of leaf pack weight loss between the three treatment streams, flocculent, precipitate and reference over a period of nine weeks in winter (2004). Means  $\pm$  1SE.

**Table 4.2.** GLM ANOVA comparison of leaf pack breakdown, and biotic responses across three stream treatments (flocculent, precipitate and reference), week (period of submersion) and interaction effects (stream treatments and weeks) for both winter (2004) and summer (2005), separately. Significant results are in bold.

	Treatment		Week		Treatment * Week	
	<i>F</i> <sub>2,20</sub>	<i>P</i>	<i>F</i> <sub>3,20</sub>	<i>P</i>	<i>F</i> <sub>6,20</sub>	<i>P</i>
<i>Winter</i>						
Remaining leafpack (g)	4.408	<b>0.026</b>	0.153	0.927	0.819	0.568
Invertebrates (No. per 1g leaf pack)	26.98	<b>&lt;0.001</b>	1.447	0.759	0.948	0.484
Shredders (No. per 1g leaf pack)	6.51	<b>0.007</b>	0.553	0.652	0.297	0.94
Number of taxa	16.66	<b>&lt;0.001</b>	0.639	0.599	0.2	0.966
	<i>F</i> <sub>2,24</sub>	<i>P</i>	<i>F</i> <sub>3,24</sub>	<i>P</i>	<i>F</i> <sub>6,24</sub>	<i>P</i>
<i>Summer</i>						
Remaining leafpack (g)	2.302	0.122	1.25	0.314	0.863	0.536
Leaf toughness (kPa)	5.716	<b>0.009</b>	7.17	<b>0.001</b>	1.966	0.111
% AFDW	1.211	0.315	9.31	<b>&lt;0.001</b>	0.679	0.668
Invertebrates (No. per 1g leaf pack)	3.76	<b>0.038</b>	0.471	0.705	0.262	0.949
Shredders (No. per 1g leaf pack)	5.767	<b>0.009</b>	2.808	0.061	1.909	0.121
Number of taxa	8.18	<b>0.001</b>	0.935	0.439	0.511	0.794



**Figure 4.4.** Comparisons of a) leaf weight remaining (DW), b) AFDW, expressed as a percentage from total leaf disc dry weight, and c) leaf toughness in the three stream treatments (flocculent, precipitate and reference) in summer (2005). Means  $\pm$  1SE.



**Figure. 4.5.** Leaf packs from a precipitate, flocculent and reference stream (from left to right) showing differences in extent of leaf breakdown after nine weeks exposure during summer 2005. Note: Inset photo of precipitate leaf showing signs of inorganic sediment deposition.

**Table 4.3.** The exponential breakdown rates ( $k$ ), for leaf packs in flocculent, precipitate and reference streams, in winter (2004) and summer (2005).

	$k$ (day <sup>-1</sup> )	
	Winter	Summer
Flocculent	-0.003	-0.004
Precipitate	0.0004	-0.002
Reference	-0.003	-0.005

AFDW was similar between stream treatments until week seven when leaves from precipitate and flocculent streams weighed less (Figure 4.4b). This suggests that there may have been a higher proportion of inorganic sediment building up on the leaves in these leaf packs. No significant difference in AFDW was found between treatment streams but there was a significant difference in time, with weeks seven and nine having a lower AFDW than weeks two and five (Table 4.2; Tukey (HSD) post-hoc test  $P < 0.05$ ).

Reference stream treatments showed a consistent decline in leaf toughness over time until week seven after which leaf toughness changed little. Leaves in both flocculent and precipitate streams declined slowly in leaf toughness until week seven, after which leaf toughness declined rapidly, and by week nine it was similar to that of reference stream leaves (Figure 4.4c). A GLM ANOVA indicated there was a significant difference in leaf toughness between stream treatments with precipitate stream leaves being slow to weaken than flocculent and reference stream leaves (Table 4.2; Tukey (HSD) post-hoc test  $P < 0.05$ ). Similarly, a significant difference was detected in leaf toughness between weeks with leaves being tougher in weeks two and five than on week nine (Table 4.2; Tukey (HSD) post-hoc test  $P < 0.05$ ). Obviously, over time leaves become more decomposed, however there was no interaction effect between stream treatment and time on leaf toughness.

## Invertebrates

In winter, the number of invertebrates in leaf packs differed between the three stream treatments, with lower taxa richness in precipitate streams than in the flocculent and reference streams (Table 4.2; Figure 4.6a; Tukey (HSD) post-hoc test  $P < 0.05$ ). Interestingly, taxa richness did not change over time, nor was there an interaction effect between the treatments and time (Table 4.2). A similar response was observed in summer when significantly more taxa were recorded in the reference streams (Table 4.2) (Tukey (HSD) post-hoc test  $P < 0.05$ ).

In winter, leaf packs from reference and flocculent streams had significantly higher number of invertebrates than precipitate streams (Table 4.2; Figure 4.7a) but the density of invertebrates did not change over time. In summer, significantly higher densities of invertebrates were found only in leaf packs from the reference streams (Table 4.2). There was no effect of week and no interaction effect between week and treatment on invertebrate densities.

Few shredders were collected in the three stream types. In winter, however, reference and flocculent streams had higher shredder abundances than precipitate streams (Table 4.2; 4.4). In contrast, shredder abundance was significantly higher in the reference streams than the flocculent and precipitate streams in summer (Table 4.2). No significant difference in shredder abundance was detected between weeks (time of submersion) and there was no interaction between week and treatment (Table 4.2).

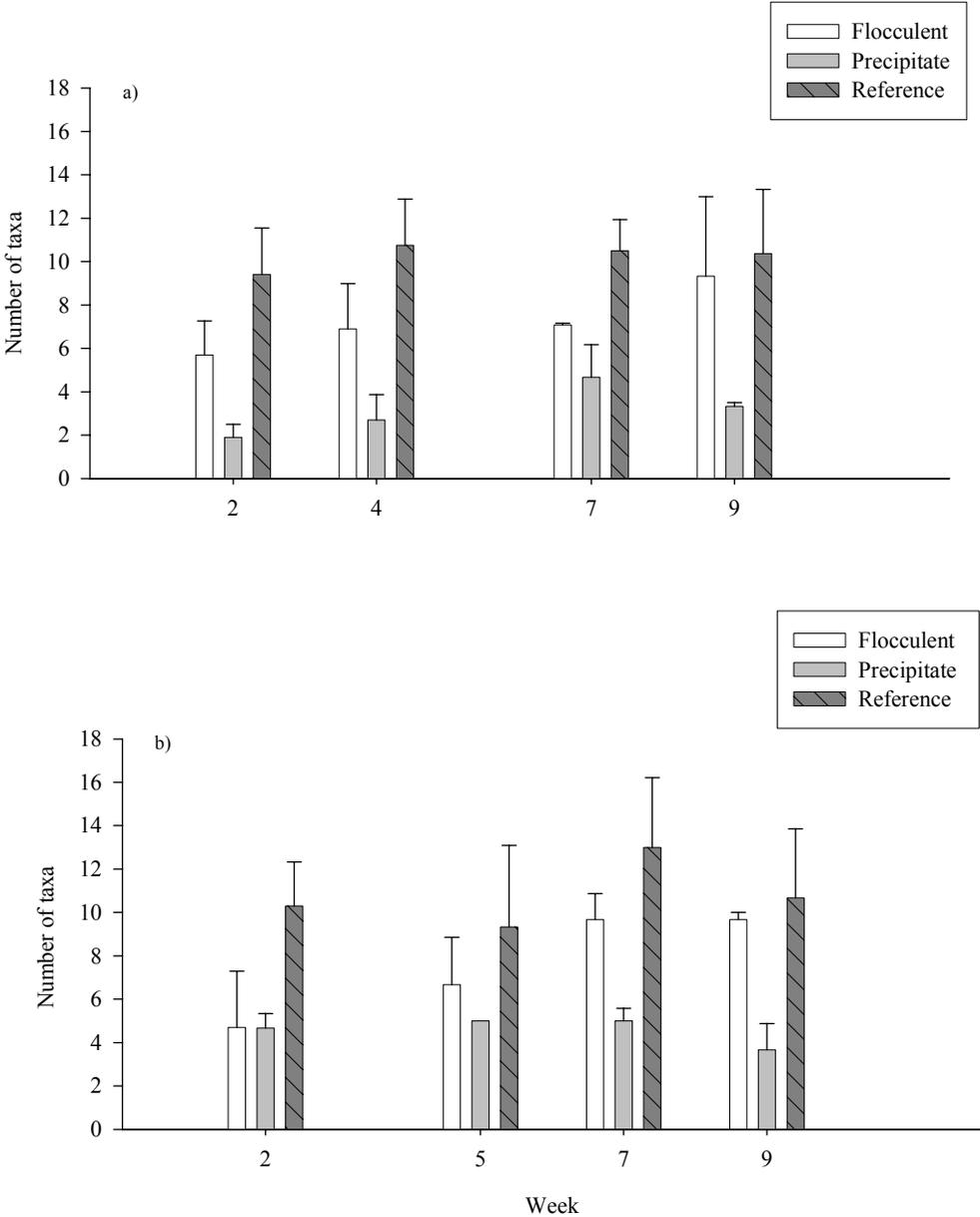
In winter, flocculent and precipitate streams had similar numerically dominant taxa in leaf packs, including the chironomid subfamilies, Orthocladiinae, Tanypodinae, and Chironominae, and a stonefly, *Spaniocerca* (Table 4.5). In reference streams, *Spaniocerca* was the dominant taxon, but of the dipterans only Orthocladiinae were in the five numerically dominant group of taxa (Table 4.5). In summer, leaf packs in flocculent streams were dominated by two caddisfly taxa, two dipterans and a stonefly, whereas leaf packs in precipitate and reference streams were dominated primarily by dipterans (Table 4.5).

**Table 4.4.** Number of shredding invertebrates in the three stream treatments, (flocculent, precipitate and reference) in winter (2004) and summer (2005). Means  $\pm$ 1SE.

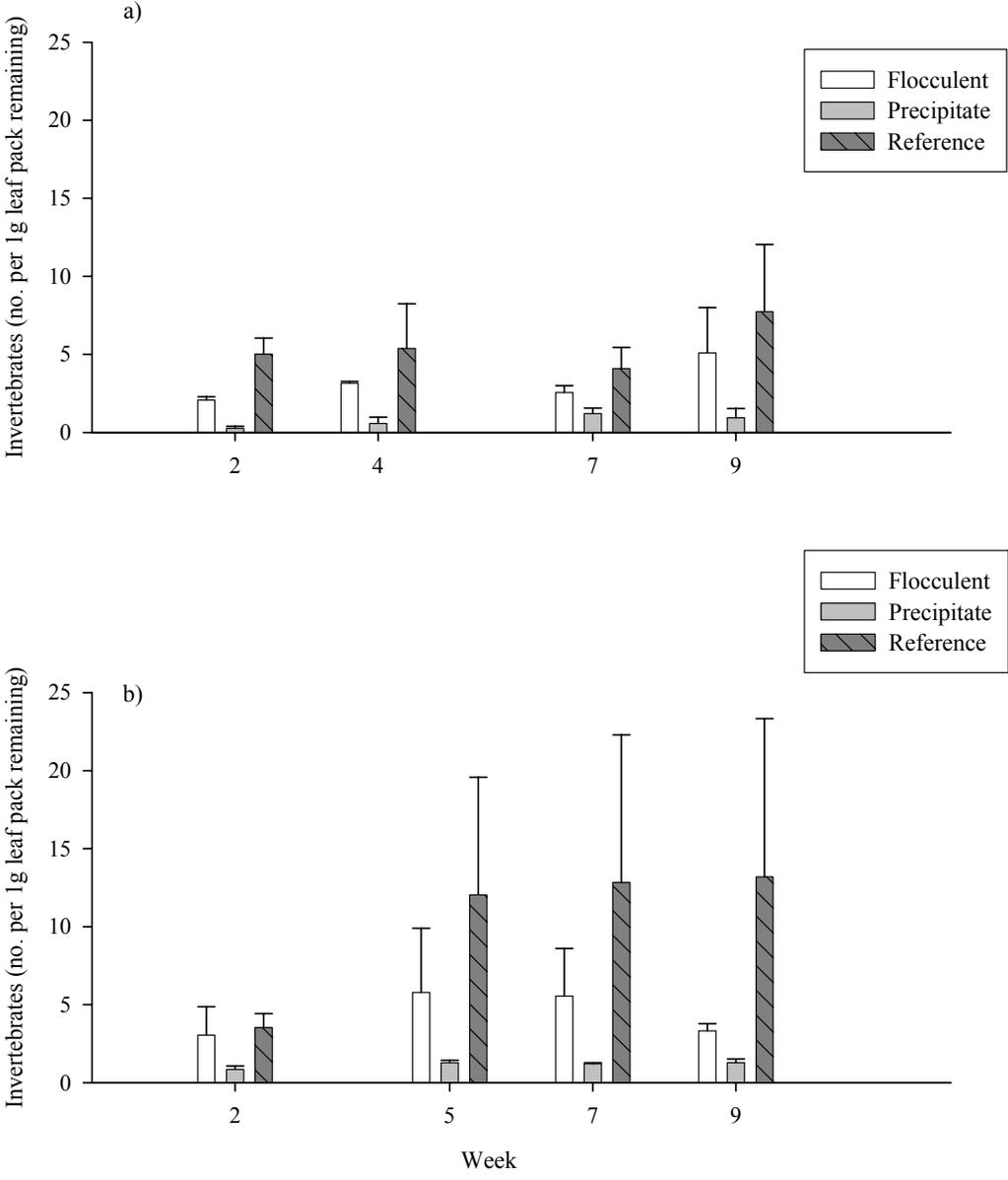
Weeks	Flocculent (no. 1gm leaf)	Precipitate (no. 1gm leaf)	Reference (no. 1gm leaf)
<i>Winter 2004</i>			
2	0.03 $\pm$ 0.02	0	0.14 $\pm$ 0.06
4	0.03 $\pm$ 0.001	0.04 $\pm$ 0.04	0.27 $\pm$ 0.24
7	0.04 $\pm$ 0.01	0.02 $\pm$ 0.02	0.27 $\pm$ 0.08
9	0.03 $\pm$ 0.01	0	0.42 $\pm$ 0.22
<i>n</i>	2	2	4
<i>Summer 2005</i>			
2	0	0	0.02 $\pm$ 0.02
5	0	0	0.09 $\pm$ 0.01
7	0.17 $\pm$ 0.03	0	0.61 $\pm$ 0.11
9	0.04 $\pm$ 0.04	0	0.04 $\pm$ 0.04
<i>n</i>	3	3	3

**Table 4.5.** The five numerically dominant taxa collected in leaf packs in flocculent, precipitate and reference streams in winter (2004) and summer (2005). Functional feeding group designations for each taxon are also shown.

Flocculent	Precipitate	Reference
<i>Winter</i>		
Orthocladiinae (Diptera) Collector-browser	<i>Spaniocercoides</i> sp. (Plecoptera) Collector-browser	<i>Spaniocerca</i> sp. (Plecoptera) Collector-browser
<i>Spaniocerca</i> sp. (Plecoptera) Collector-browser	<i>Spaniocerca</i> sp. (Plecoptera) Collector-browser	<i>Potamopyrgus antipodarum</i> (Mollusca) Grazer
Tanypodinae (Diptera) Predator	Orthocladiinae (Diptera) Collector-browser	<i>Deleatidium</i> spp. (Ephemeroptera) Collector-browser
Chironominae (Diptera) Collector-browser	Chironominae (Diptera) Collector-browser	<i>Neozephebia scita</i> (Ephemeroptera) Collector-browser
<i>Deleatidium</i> spp. (Ephemeroptera) Collector-browser	Tanypodinae (Diptera) Predator	Orthocladiinae (Diptera) Collector-browser
<i>Summer</i>		
<i>Pycnocentrella eruensis</i> (Trichoptera) Collector-browser	Tanypodinae (Diptera) Predator	Chironominae (Diptera) Collector-browser
Chironominae (Diptera) Collector-browser	Orthocladiinae (Diptera) Collector-browser	Empididae (Diptera) Predator
Empididae (Diptera) Predator	Scirtidae (Coleoptera) Collector-browser	<i>Spaniocerca</i> sp. (Plecoptera) Collector-browser
<i>Hydrobiosella</i> sp. (Trichoptera) Net filterer	Chironominae (Diptera) Collector-browser	Tanypodinae (Diptera) Predator
<i>Spaniocerca</i> sp. (Plecoptera) Collector-browser	Empididae (Diptera) Predator	Orthocladiinae (Diptera) Collector-browser



**Figure 4.6.** Taxonomic richness in the three stream treatments, flocculent, precipitate and reference, in a) winter (2004) and b) summer (2005). Means +1SE.



**Figure 4.7.** Numbers of invertebrates in leaf packs in flocculent, precipitate and reference streams in a) winter (2004) and b) summer (2005). Means +1SE.

## **Discussion**

Leaf breakdown was slow across all stream treatments in this study, and essentially stopped after about five weeks. This slow leaf breakdown, may reflect the relatively short period of exposure of the leaf packs compared to those used in other New Zealand studies (Young et al. 1994, Harbrow 2001), and elsewhere (Mackay and Kersey 1985, Baldy et al. 1995, Casas and Gessner 1999). However, shorter studies similar to this one have been undertaken, successfully, to investigate processes related to leaf breakdown (Gray and Ward 1983, Gessner 1991, Parkyn and Winterbourn 1997). Furthermore, the use of pre-dried leaves may have given a false indication of the rate at which leaves would breakdown in these streams and introduced tree species may have influenced shredder invertebrate colonisers. However, air drying leaves does not necessarily affect the overall rate of leaf weight loss (Gessner 1991) and leaves from introduced trees may actually be preferred by some New Zealand invertebrate shredders (Parkyn and Winterbourn 1997). Overall, whilst a stronger leaf breakdown response may have been achieved over a longer period of time I do not think that the period of time, or the leaves used to investigate leaf breakdown in this study were major contributing factors to the slow rates of breakdown observed. Instead, possible explanations for the slow leaf breakdown are, drying and rewetting of leaves, and deposition of iron onto leaves.

Although my results indicated that differences in weight loss between treatments only occurred between streams in winter, leaf breakdown in both seasons suggested that reference streams had faster leaf breakdown than the mine-polluted streams (precipitate and to some extent flocculent streams). Faster rates of leaf breakdown observed in reference streams in winter were probably due to physical abrasion which did not affect all streams equally, and it seems unlikely that leaf litter would be retained naturally in these streams for long periods because of flood frequency. Winterbourn et al. (1981) also made reference to the general lack of CPOM retention in many New Zealand streams which they considered were non-retentive. Surprisingly, even though the rate of leaf breakdown in my study appeared to be very slow, a comparison with studies using leaves from the same tree family (Platanaceae) suggests that my breakdown rates ( $k$ ) may actually be similar to those found

elsewhere (i.e., ranging from -0.005 to -0.001) (Benfield et al. 1979, Webster and Benfield 1986). Leaf breakdown in the streams in my study may be influenced by several factors, including variation in water flow, water quality, and inorganic sediment deposition on leaves.

The, slow leaf breakdown rates I observed could be attributed to the position of leaf packs in stream channels. While great care was taken to find 'suitable' areas of the channel for leaf pack submersion, leaf packs may have been inadvertently placed in areas of the channel, which between collection dates, had low flow, or formed pools. This would have not affected all streams, or all leaf packs equally, but may have contributed to the variable reduction in leaf breakdown observed, particularly for reference streams, where continued leaf breakdown would be expected. In support of this suggestion, Maamri et al. (1997) examined leaf breakdown in two tree species, *Salix pedicellata* (willow) and *Nerium oleander* (oleander), in a permanent pool, and in two intermittent areas of a Moroccan river. They found that over the one year duration of their study, leaf breakdown was continuous, and faster in the pool ( $k \text{ day}^{-1} = -0.009$  for willow and  $-0.007$  for oleander), than in the intermittent flowing reaches of the river, where weight loss was slow ( $k$  between  $-0.003$  and  $-0.005$ ). Further, they found that leaf breakdown in intermittent reaches was faster during periods of water flow than in the pool. Breakdown rates in the flocculent and reference streams in my study are similar to the breakdown rates reported for the intermittent reaches in the study of Maamri et al. (1997). Thus, variation in water levels and flow rates may partially explain slow leaf breakdown.

Secondly, and from my studies perspective, more importantly, rates of leaf breakdown can be influenced by water quality (Maltby and Booth 1991, Griffith and Perry 1993, Suberkropp and Chauvet 1995, Siefert and Mutz 2001). Comparisons of water chemistry in this study showed that flocculent streams, had elevated levels of iron, aluminium, zinc and nickel but had similar pH and leaf breakdown rates to reference stream treatments. In comparison, precipitate streams had the slowest leaf breakdown, and elevated heavy metals and pH markedly lower than flocculent and reference streams. Allard and Moreau (1986), showed that in an experimentally acidified stream channel in Canada, leaf decomposition can be markedly reduced in water at pH 4.0 compared to pH 6.2-7.0. Furthermore, they suggested the reduction in leaf breakdown

was in response to a reduction in microbial activity in the acidified waters, and not a reduction in macroinvertebrates because macroinvertebrate densities were similar in channels of either pH (pH treatments 4.0 and 7.0) throughout the study.

Additionally, the activity of important fungal (e.g., hyphomycetes) decomposers may be affected adversely by elevated metals (e.g., iron and nickel). In the UK, Bermingham et al. (1996) investigated rates of leaf decomposition in a stream receiving coal mining effluent with elevated heavy metals, and found that leaf decomposition and microbial, particularly fungal activity, was markedly reduced relative to non-impacted upstream control sites. Bermingham et al. (1996) used mesh size that excluded macroinvertebrates from the leaf packs, and thus concluded that the reduction in leaf breakdown was associated with the matched reduction in fungal activity due to elevated levels of heavy metals. In future studies of leaf decomposition I recommend that additional investigations of microbial activity be made (for example through respiration studies) to gain a greater understanding of how microbial activity may differ on leaves in different stream systems.

Thirdly, inorganic sediment may reduce the rate at which leaves can breakdown. In the summer leaf packs I examined, the proportion of AFDW was used as an indicator of inorganic build-up on leaves. These results suggested that over time leaves in precipitate streams may accumulate large inorganic deposits. Deposition of inorganic sediments may potentially lead to a reduction in leaf decomposition by smothering or preventing microbial activity. Additionally, the deposition of inorganic sediments was probably responsible for the large weight gain observed in leaf packs from precipitate streams during winter. Gray and Ward (1983), found that the rate of alder leaf breakdown in a stream receiving treated mine effluent was slower than at a non-impacted site but was similar to leaf decomposition rates in a stream receiving untreated mine effluent. They concluded that although mine effluent had improved water quality the deposition of ferric hydroxide on the surface of leaves was inhibiting the colonisation of microbes and invertebrates and led to the reduction in leaf breakdown.

Leaf toughness may have been the best indicator of leaf processing and decomposition rates in this study. As leaves are colonised by microbes, structural

compounds of leaves are broken down and the leaf tissue become softer (Young et al. 1994). Reference streams had a rapid decline in leaf toughness during the study, whilst flocculent and precipitate stream treatments had a much slower decline, with signs of leaf softening being observed only after seven weeks of submersion in the stream. Leaf penetrance suggests that flocculent and precipitate streams had much slower rates of leaf conditioning (or microbial activity) than reference stream leaves.

Other studies have linked differences in leaf conditioning (or leaf softening) to temperature or the nutrient concentration of leaves and/or water (Young et al. 1994, Molinero et al. 1996, Quinn et al. 2000, Niyogi et al. 2003, Woodcock and Huryn 2005), with generally higher rates of softening occurring at high temperatures and in nutrient rich leaves and/or stream water. In my study there was no difference in temperature between the three stream types, and thus it is unlikely that temperature can explain the differences in leaf breakdown between the stream treatments. I did not however, measure nutrient concentrations in my study however Anthony (1999), sampled nitrate-nitrogen and reactive phosphate in mine drainage and non-impacted stream water in the same study area and found no significant difference in concentration of these nutrients between mine drainage and reference streams. Furthermore, as my streams were forested mountain streams they could be expected to have very low baseline nutrient concentrations (Harding et al. 1999).

Invertebrate taxonomic richness and the number of invertebrates per pack were higher in the reference streams than precipitate streams, with flocculent streams being intermediate. Additionally, whilst the number of invertebrate shredders was low, precipitate streams were virtually depauperate of shredders compared to flocculent and reference streams. The low number of shredders in the leaf packs overall suggests that leaves were not an important food resource in these streams and that leaf breakdown was probably not related directly to invertebrate shredding activity. Additionally, it is likely that invertebrate colonisers may be using these leaf packs as refuges, particularly during floods. This is supported by the dominance of non-shredding taxa in the leaf packs throughout the duration of the study. The use of leaf pack canisters in summer may illustrate this further with a slightly higher number of taxa and higher invertebrate densities being observed when the leaf pack canisters were used. This

finding also indicates that leaf pack canisters did not prevent access of invertebrates to the leaf packs.

The rates of leaching, conditioning and fragmentation, and thus the rate of leaf breakdown are influenced by a number of factors such as water chemistry (Griffith and Perry 1993, Suberkropp and Chauvet 1995), microbial activity (Sridhar and Barlocher 2000) and macroinvertebrate feeding (Hieber and Gessner 2002). In my study the contribution of invertebrate shredding to leaf breakdown was probably small compared to microbial conditioning and subsequent fragmentation processes such as physical abrasion. Leaf breakdown in precipitate streams was slower than in reference and flocculent streams suggesting that adverse water quality and inorganic sediment build-up were limiting leaf decomposition in these systems. Furthermore, flocculent streams had similar pH to reference streams but were suffering from elevated heavy metals and iron deposition and subsequently had rates of leaf breakdown intermediate between reference and precipitate treatment streams. Overall, my study illustrates that iron precipitates, and associated water chemistry can markedly reduce leaf breakdown and invertebrate shredders.

## Chapter five

### Infections in benthic macroinvertebrates

---

#### Introduction

Community structure and population densities of freshwater invertebrates can be highly variable temporally and spatially, depending on a range of abiotic and biotic factors. For example, land use, physico-chemical conditions and predator-prey interactions can all influence invertebrate populations and community structure (Sutcliffe and Carrick 1973, Hildrew et al. 1980, Hildrew et al. 1984, Lenat 1987, Quinn et al. 1997). One poorly studied, but perhaps equally important biotic mechanism influencing freshwater invertebrates and consequently communities, is the role of disease and infection (Kohler and Wiley 1992, Jaenike 1995, Kohler and Wiley 1997).

Research on entomopathogens in the freshwater environment has largely been descriptive, documenting hosts, modes of infection and the variety of insect pathogens (Couch et al. 1974, Whisler and Zebold 1974, Sweeney 1975, Zebold et al. 1979, Blair 1983). The focus of much of this work has been seeking to identify, or test these entomopathogens as possible biological control agents, particularly for mosquitoes (Couch et al. 1974, Zebold et al. 1979, Wong and Pillai 1980). In this regard, freshwater dipterans have been widely studied for their susceptibility to disease and infection (see review Lacey and Undeen 1986). In comparison, little is known about disease and infections in other stream invertebrates, although research has found that they can be regularly infected (Poinar 1991, Vance and Peckarsky 1996, Stirnadel and Ebert 1997).

There are a range of entomopathogens known to infect freshwater invertebrates, including, nematodes (e.g., mermithid nematodes) (Benton and Pritchard 1990, Pritchard and Zloty 1994), protozoa (e.g., Microspora: Cougourdellidae) (Kohler and Wiley 1992), bacteria (e.g., *Bacillus thuringiensis israelensis*) (Dickman 2000), and fungi (e.g., *Coelomomyces* sp.) (Zebold et al. 1979).

In terrestrial and aquatic environments, entomopathogens may affect their host in several ways. Firstly, entomopathogens may be directly fatal, causing mortality in host populations and this can consequently act to regulate invertebrate populations (Anderson and May 1978, Myers and Rothman 1995). Although in the field, pathogen-induced host mortality is difficult to prove because dead hosts are rarely found, and if they are, it is often difficult to attribute host mortality to a pathogen (McCallum and Dobson 1995). Alternatively, pathogen infection may also influence a host, by altering the behaviour or increasing vulnerability of predation (Benton and Pritchard 1990, Vance and Peckarsky 1997).

At some time, most natural populations are subjected to disturbance or environmental fluctuations, these environmental disturbances may cause 'stress' to an organism (Jokela et al. 2005). During an episode of 'stress' an organism's immune system can be affected, but the magnitude of response depends on the species, nature of the stressor, the immune parameter studied and the physiological status of the individual (Jokela et al. 2005). A reduction in the function of an organism's immune system during periods of increased stress may be linked to an increase in the rate of pathogen infections (Lacoste et al. 2002).

In this chapter, I investigate whether 'stresses' which may arise through higher concentrations of heavy metals, increase disease and infection in benthic macroinvertebrates. Additionally, invertebrate densities and community composition are examined to establish whether the presence of a pathogen(s) is influencing the composition of benthic macroinvertebrate communities.

## **Methods**

### **Study Sites**

In this study, five sites (C1-C5) along Carton Creek, and three reference sites, Branch Creek (BR), Devils Creek (DR) and Slab Hut Stream (SHR), near Reefton were selected. Reference sites were selected because they had little or no known mining history. The location and description of each site are described in chapter two.

### **Water chemistry**

Basic water chemistry parameters, pH, specific conductivity and temperature were measured at each site on four separate occasions (once per season), using an Oakton CON 10 Series meter. Turbidity (HACH 2100P Turbidimeter), was measured at all Carton Creek sites on four occasions and two or three times at the reference sites.

Spot water samples were collected from each site in acid-washed unpreserved 250ml polyethylene bottles on four occasions (once per season) except at Carton Creek sites, C3 and C4, which were sampled three times. Water samples were sent to a commercial laboratory (RJ Hills Laboratories, Hamilton), where they were analysed for five dissolved heavy metals, iron, aluminium, arsenic, zinc and nickel. Dissolved zinc was tested for at Carton Creek sites 1-4, on three of the four sampling occasions.

### **Benthic sampling**

Quantitative benthic macroinvertebrates samples were collected by taking four Surber samples ( $0.30 \text{ cm}^2$ ,  $500 \mu\text{m}$ ) at each site seasonally, between March 2004 and January 2005. But in autumn (2004), Carton Creek sites C2 - C5 were sampled one month earlier than C1 on Carton Creek and the three reference streams. Thereafter, all sites were sampled on the same day. On the last sampling occasion, due to equipment damage only two Surber samples were collected from Branch Stream. Surber samples were randomly collected from riffles and substrate was thoroughly disturbed to an approximate depth of 5 cm, and surfaces of larger stones were wiped to dislodge attached fauna. All samples collected were preserved separately in the field with 70% ethanol and returned to the laboratory.

In the laboratory, benthic invertebrate samples were washed through a 500 µm mesh sieve and placed in to a white sorting tray where invertebrates were removed and identified using a microscope up to 40 x magnification. Invertebrates were identified to the lowest possible taxonomic level usually genus or species, except for Chironomidae which were identified only to tribe and Oligochaeta, Ostracoda, Acarina and Collembola which were not identified past order or class. Identifications were made using Winterbourn et al. (2000a).

During identification, benthic invertebrates were examined visually for signs of 'infection'. Infections, in this study were defined as black, mould-like masses, and in most cases were seen in areas where appendages were broken (Figure 5.1). Occurrences of infection were classes into one of four categories, antennae and/or cerci, legs, body or under multiple sites (where combinations of infected sites occurred). Benthic invertebrates, with no sign of infection were also counted and recorded. Scanning electron microscopy was conducted on several specimens and in some cases fungal matrices were observed. But as I was not able to confidently determine the nature or type of these infections identification of infections was done visually.

Blackened appendages from five individuals were examined by scanning electron microscopy, to see whether there was any sign of fungi or bacteria which may be associated with the blackened parts. Invertebrates were dehydrated in an ethanol series (half an hour of each of 30, 50, 70, 80, 90 and 100% ethanol), air dried, mounted on SEM stubs with conductive carbon paint and coated with 60 nm of gold-palladium. Stubs were viewed with a Leica S440 scanning electron microscope at accelerating voltages of 10-18 kV. Some time was spent on this and determining the nature of infections was difficult and was not investigated further.



**Figure 5.1.** *Deleatidium* collected from Carton Creek (2004), showing blackened area where cerci have broken off (inset).

### **Statistical analyses**

To determine if water chemistry (except pH) and concentrations of dissolved heavy metals differed between the eight sites, seasonal data was pooled from each site, and tested using a one-way analysis of variance (ANOVA).

A one-way ANOVA was also used to test for differences in benthic invertebrate densities and taxonomic richness between sites. Taxonomic richness and macroinvertebrate densities were obtained, for each season, by averaging the number of taxa and invertebrate densities from the four Surber samples taken from each site. Seasonal averages were used as replicates to test across sites. The density of the mayfly *Deleatidium* and the percentage of infected *Deleatidium* were tested across sites using a one-way ANOVA on seasonal averages as replicates. The mayfly *Deleatidium* was selected because it was the most abundant taxon found at all sites.

Simple linear regression analyses were used to examine the relationship between dissolved heavy metals (Fe, Al and Zn), pH, the percent of the community infected and the percent of *Deleatidium* infected, with the percentage of infection as the dependent variable. Nickel was not regressed because an ANOVA detected no significant difference between sites, and arsenic concentrations were the same at all sites except Devils Creek, where they were higher, thus data was skewed. Pooled and averaged seasonal data was used in analyses.

Differences detected by ANOVA's were tested using Tukey's post-hoc test (HSD) to determine which sites were significantly different. For all analyses, plots of residuals versus fits and normality plots were used to examine for normality and homoscedasticity of data (Zar 1999). Data was analysed using the statistical package STSTAT (Version 10).

## **Results**

### **Water chemistry**

Median pH and pH range was similar at Carton Creek sites C1 to C4 but the tributary (C3) had a lower median pH and a narrower pH range. All reference sites had a median pH >6 (Table 5.1). Mean specific conductivity was significantly higher at Devils Creek than at all other sites (Table 5.1; 5.2; Tukey (HSD) post-hoc test  $P<0.05$ ). Temperature did not differ between sites (Table 5.1), whereas Branch Creek had significantly lower turbidity than all sites except Carton Creek sites, C2 and C5 (Table 5.1; 5.2; Tukey (HSD) post-hoc test  $P<0.05$ ).

Concentrations of dissolved iron and aluminium were approximately two times higher in Carton Creek than they were in, Devils Creek and Slab Hut reference streams, while they were about 20 and four times higher, respectively, than concentrations in Branch Stream (Table 5.1; Table 5.2).

**Table 5.1.** Seasonal mean ( $\pm 1$ SE) water chemistry taken from, Carton Creek (C1-C5) and reference streams(BR, DR, SHR), from March 2004 – January 2005 (n=4).

	Sites							
	C1	C2	C3	C4	C5	BR	DR	SHR
Median pH	5.7	5.5	5.2	5.5	6	7.1	7	6.5
pH range	(5.3 - 6.4)	(5.3 - 6.0)	(5.1 - 5.5)	(5.2 - 6.0)	(5.7 - 6.4)	(6.5 - 7.4)	(6.2 - 7.4)	(6.1 - 6.8)
Conductivity ( $\mu\text{S}_{25^\circ\text{C}}\text{cm}^{-1}$ )	$29.9 \pm 0.3$	$28.9 \pm 2.8$	$37 \pm 1.3$	$31.7 \pm 1.2$	$34.9 \pm 2.0$	$31.3 \pm 2.3$	$78.38 \pm 10.6$	$31.0 \pm 1.0$
Temperature ( $^\circ\text{C}$ )	$9.2 \pm 1.4$	$9.4 \pm 1.3$	$9.2 \pm 1.3$	$9.5 \pm 1.2$	$11.8 \pm 1.7$	$9.4 \pm 1.8$	$9.9 \pm 1.2$	$11.2 \pm 1.4$
<i>n</i>	4	4	4	4	4	4	4	4
Turbidity (NTU)	$1.2 \pm 0.1$	$1.3 \pm 0.2$	$1.9 \pm 0.7$	$1.2 \pm 0.1$	$2.8 \pm 0.4$	$0.45 \pm 0.1$	$3.7 \pm 2.1$	$1.7 \pm 0.04$
<i>n</i>	4	4	4	4	4	3	2	2
<i>Heavy metals (g m<sup>-3</sup>)</i>								
Iron	$0.43 \pm 0.10$	$0.47 \pm 0.12$	$0.6 \pm 0.14$	$0.44 \pm 0.08$	$0.44 \pm 0.05$	$0.02 \pm 0$	$0.26 \pm 0.05$	$0.23 \pm 0.05$
Aluminium	$0.36 \pm 0.05$	$0.39 \pm 0.05$	$0.42 \pm 0.04$	$0.40 \pm 0.05$	$0.37 \pm 0.03$	$0.08 \pm 0.02$	$0.16 \pm 0.05$	$0.20 \pm 0.04$
Nickel	$0.003 \pm 0.002$	$0.003 \pm 0.002$	$0.001 \pm 0.000009$	$0.002 \pm 0.002$	$0.003 \pm 0.002$	$0.0005 \pm 0$	$0.001 \pm 0.0002$	$0.0005 \pm 0$
Arsenic	$0.001 \pm 0$	$0.001 \pm 0$	$0.001 \pm 0$	$0.001 \pm 0$	$0.001 \pm 0$	$0.001 \pm 0$	$0.05 \pm 0.009$	$0.001 \pm 0$
<i>n</i>	4	3	3	4	4	4	4	4
Zinc	$0.003 \pm 0.0004$	$0.003 \pm 0.0006$	$0.003 \pm 0.0003$	$0.003 \pm 0.004$	$0.002 \pm 0.0003$	$0.001 \pm 0$	$0.003 \pm 0.0006$	$0.001 \pm 0.0003$
<i>n</i>	4	3	3	3	3	4	4	4

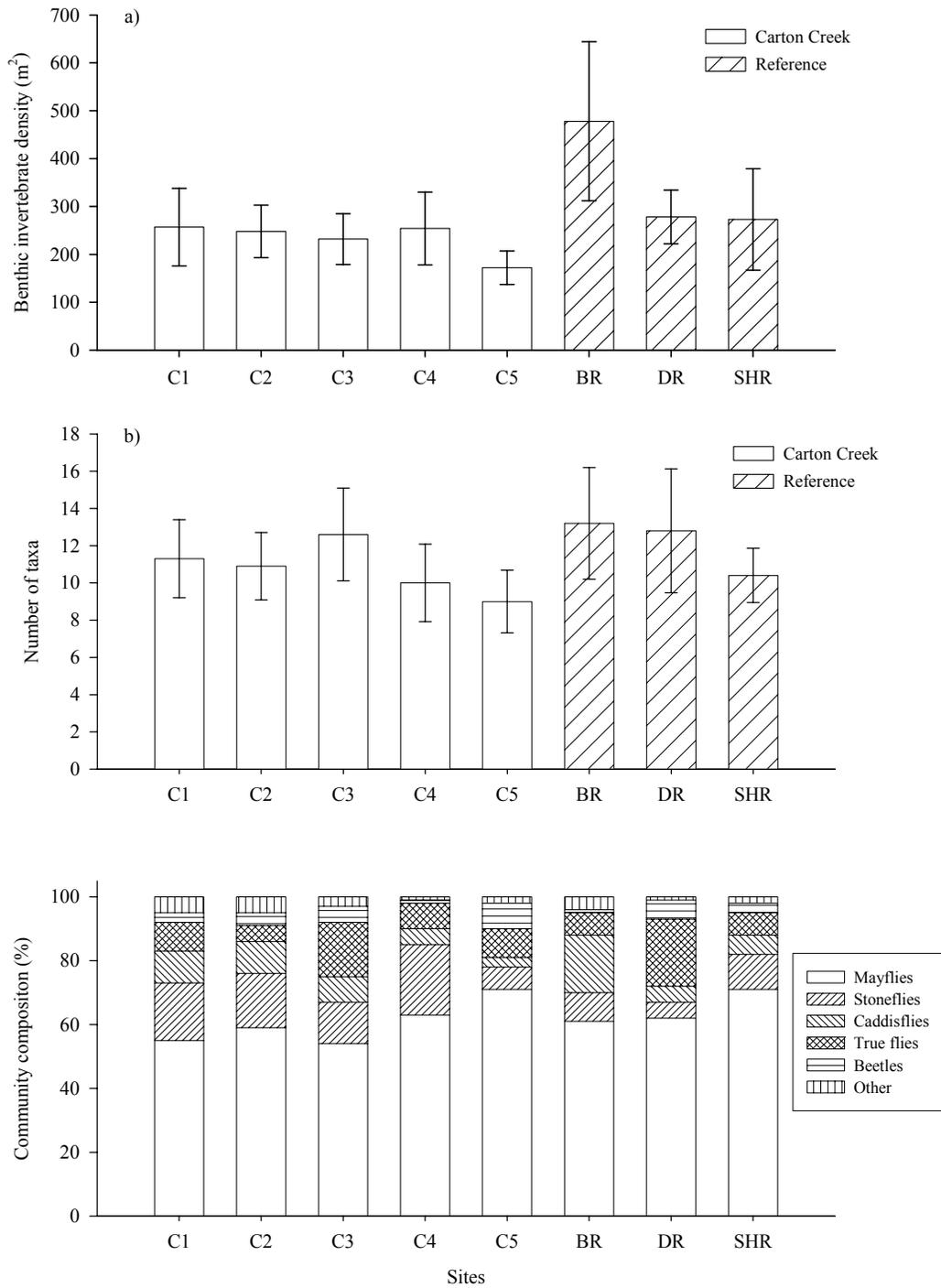
**Table 5.2.** Results from one-way ANOVA of water chemistry and biotic responses between five Carton Creek sites and three reference streams. Significant results are in bold.

	<i>F</i> <sub>7,24</sub>	<i>P</i>
<i>Water chemistry</i>		
Conductivity ( $\mu\text{S}_{25^{\circ}\text{C}}\text{cm}^{-1}$ )	20.272	<b>&lt;0.001</b>
Temperature ( $^{\circ}\text{C}$ )	0.474	0.844
	<i>F</i> <sub>7,18</sub>	<i>P</i>
Turbidity (NTU)	7.18	<b>&lt;0.001</b>
<i>Heavy metals (g m<sup>-3</sup>)</i>		
	<i>F</i> <sub>7,22</sub>	<i>P</i>
Iron	5.251	<b>0.001</b>
Aluminium	9.012	<b>&lt;0.001</b>
Nickel	1.832	0.131
Arsenic	32.54	<b>&lt;0.001</b>
	<i>F</i> <sub>7,20</sub>	<i>P</i>
Zinc	3.091	<b>0.022</b>
<i>Biotic responses</i>		
	<i>F</i> <sub>7,24</sub>	<i>P</i>
Invertebrate density ( $\text{m}^2$ )	1.027	0.438
Taxonomic richness	0.258	0.964
<i>Deleatidium</i> density ( $\text{m}^2$ )	1.586	0.188
<i>Deleatidium</i> infected (%)	5.752	<b>0.001</b>

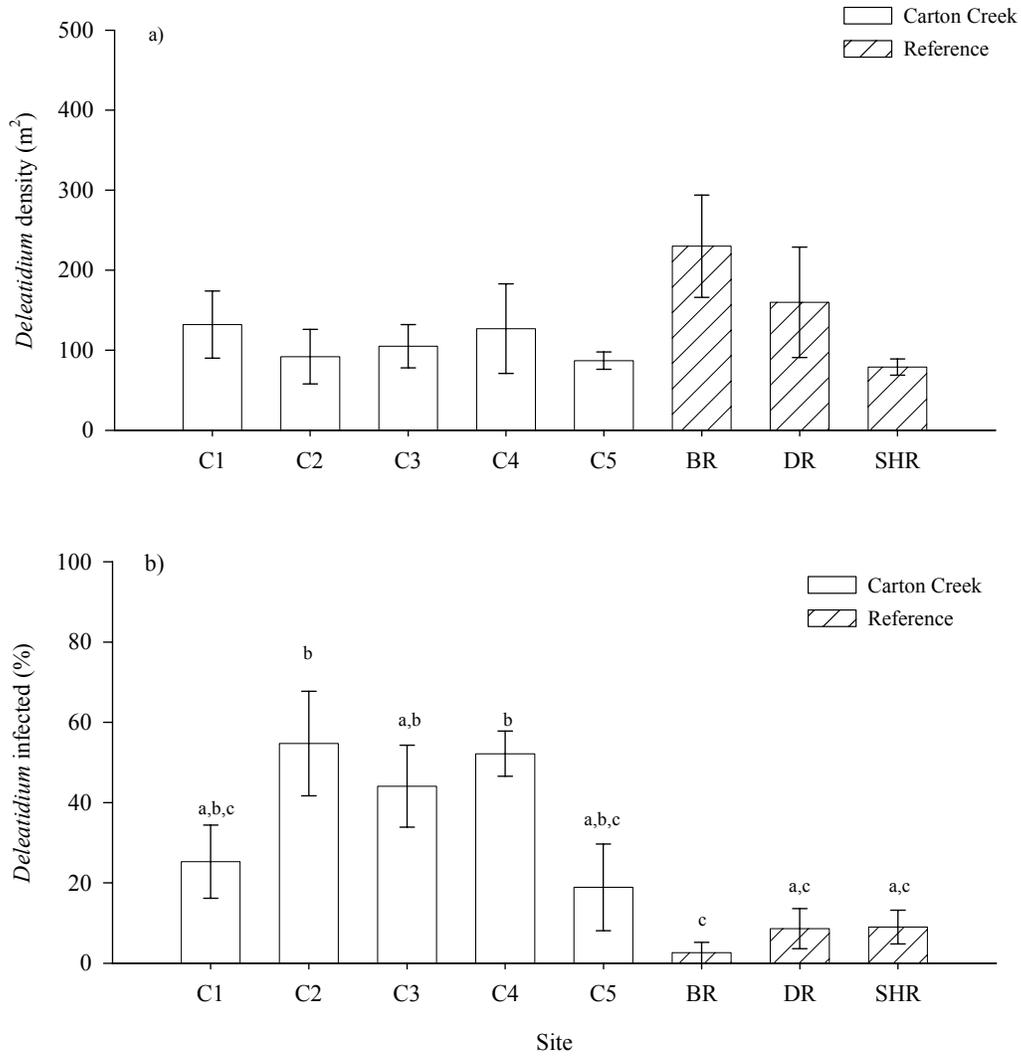
### Biotic responses

There was no significant difference in the density of benthic invertebrates or taxonomic richness between sites (Figure 5.2a; 5.2b; Table 5.2). All sites were dominated by mayflies, and had similar proportions of the other major taxonomic groups, i.e., stoneflies, caddisflies, dipterans and beetles (Figure 5.2c).

Furthermore, there was no significant difference in the density of *Deleatidium* between sites (Figure 5.3a; Table 5.2). However, Carton Creek sites C2, C3, and C4, showed markedly higher levels of infection in *Deleatidium* than the reference streams. Branch Creek, Devils Creek and Slab Hut Stream, had a significantly lower percentage of infected *Deleatidium* than C2 and C4. Site C3 on Carton Creek had significantly higher infected *Deleatidium* than Branch Creek (Figure 5.3b; Table 5.2; Tukey (HSD) post-hoc test  $P < 0.05$ ).



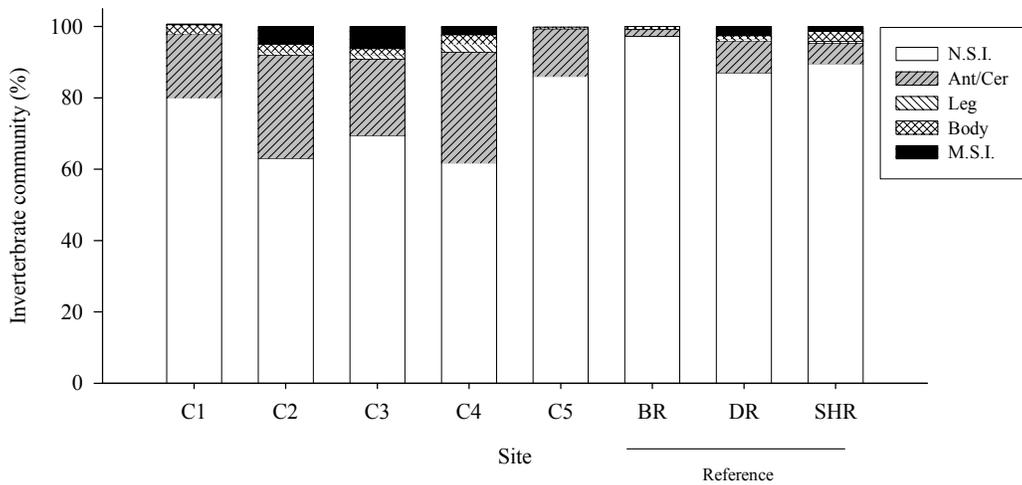
**Figure 5.2.** Comparison of a) benthic invertebrate densities and b) taxonomic richness and c) community composition across Carton Creek sites (upstream to downstream) and reference streams, Branch (BR), Devils (DR) and Slab Hut Creek (SHR). Seasonal means  $\pm 1SE$ .



**Figure 5.3.** Comparison of a) *Deleatidium* density and b) percentage of *Deleatidium* ‘infected’ between Carton Creek and reference streams, Branch (BR), Devils (DR) and Slab Hut Stream (SHR). Carton Creek sites are displayed, upstream to downstream. Letters above bars show Tukey (HSD) post-hoc test. Seasonal means  $\pm$  1SE.

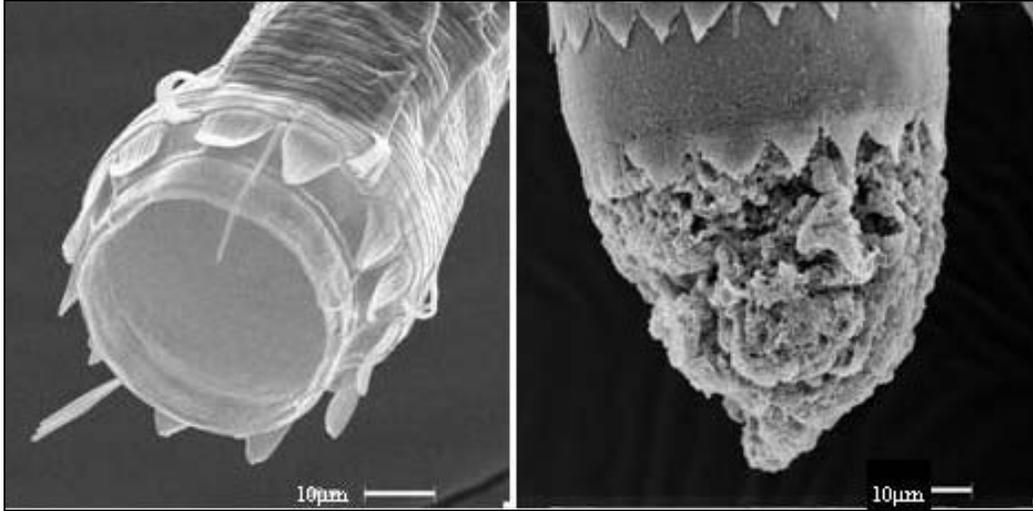
When community infection rates were compared, Carton Creek sites had higher percentages of infection rates than reference streams (Figure 5.4). Carton Creek sites, C2, C3, and C4, had the highest infection rates, and more macroinvertebrates with multiple infections on their bodies. There was no clear decline in infection rates downstream. Antennae and cerci were the most commonly infected appendages across all sites and benthic taxa with infected legs, bodies or multiple infections (>2) uncommon (Figure 5.4).

Overall, the rates of infections were lowish at C1, increased from C2 to C4, and then dropped again at C5 (Figure 5.4). The increased infection rates at these sites may have been partially related to increased stress, through sources such as lower median pH and the slightly elevated iron recorded at C2 and C3 (Table 5.1).



**Figure 5.4.** Comparison of percentage of invertebrate community showing no signs of infection (N.S.I.) and areas of body infected between Carton Creek (upstream to downstream) and reference streams, Branch (BR), Devils (DR) and Slab Hut Creek (SHR). M.S.I. = multiple sites of infection. Seasonal means.

Scanning electric microscopy was inconclusive. Areas of distinct fungal or bacterial activity could not be identified clearly. In some cases cerci and antennae were clearly broken, while in others amorphous matrices were observed. These matrices did not have obvious surface bacteria and fungi activity (Figure 5.5).



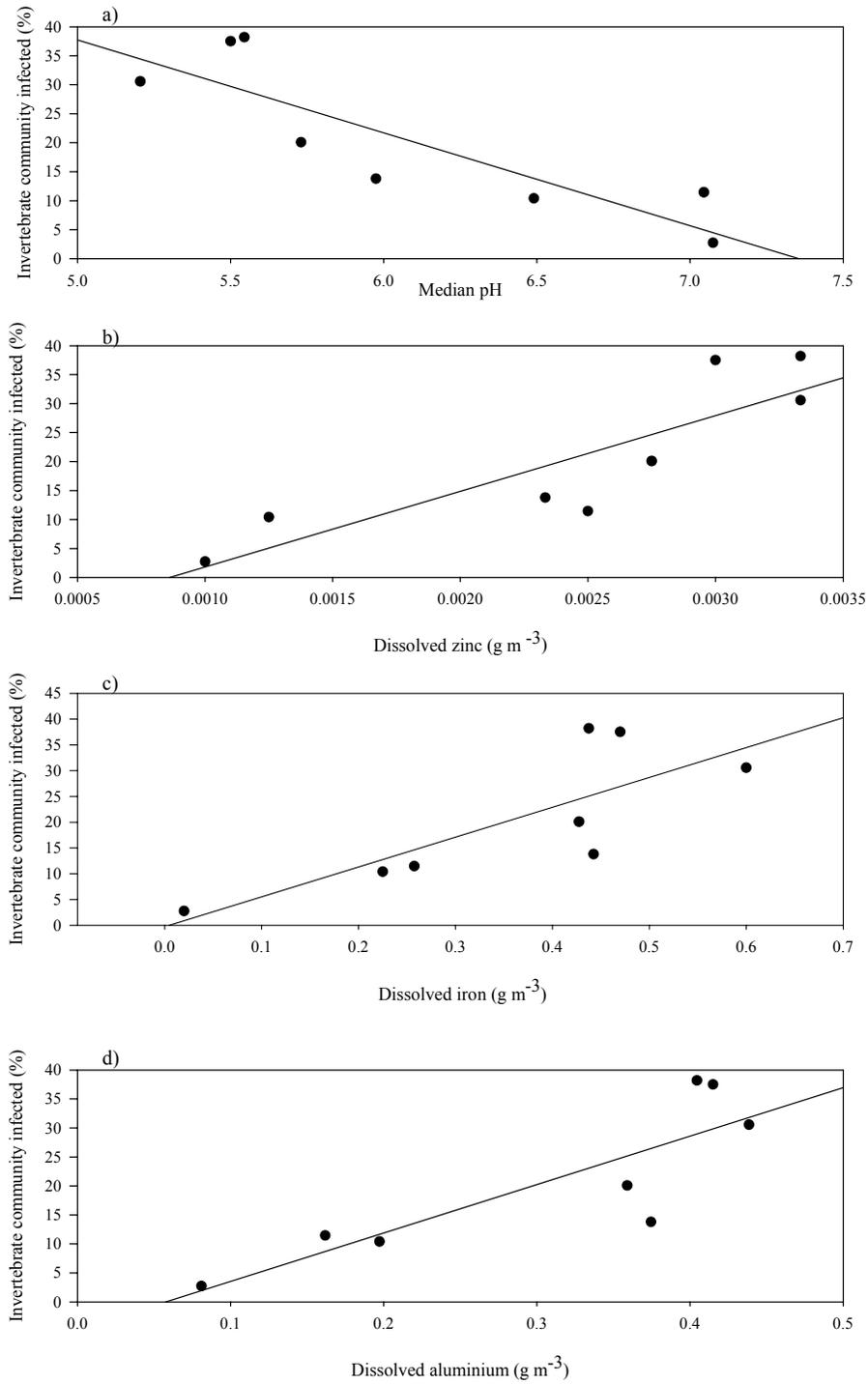
**Figure 5.5.** Scanning electron microscopy photographs of *Deleatidium* cerci showing clean break (left) and extruding area, where blackening was observed (right). There is no obvious fungal hyphae or bacteria present.

Interestingly, a microbial technician, Nic Cummins, in the School of Biology, did isolate a fungus (*Tolyposcladium inflatum*) from several of my stream invertebrates, displaying characteristic blackening. Some species belonging to the genus *Tolyposcladium* have been recognised as having species that are entomopathogenic to invertebrates (e.g., *T. cylindrosporum*) (Steenberg and Ogaard 2000, Scholte et al. 2004).

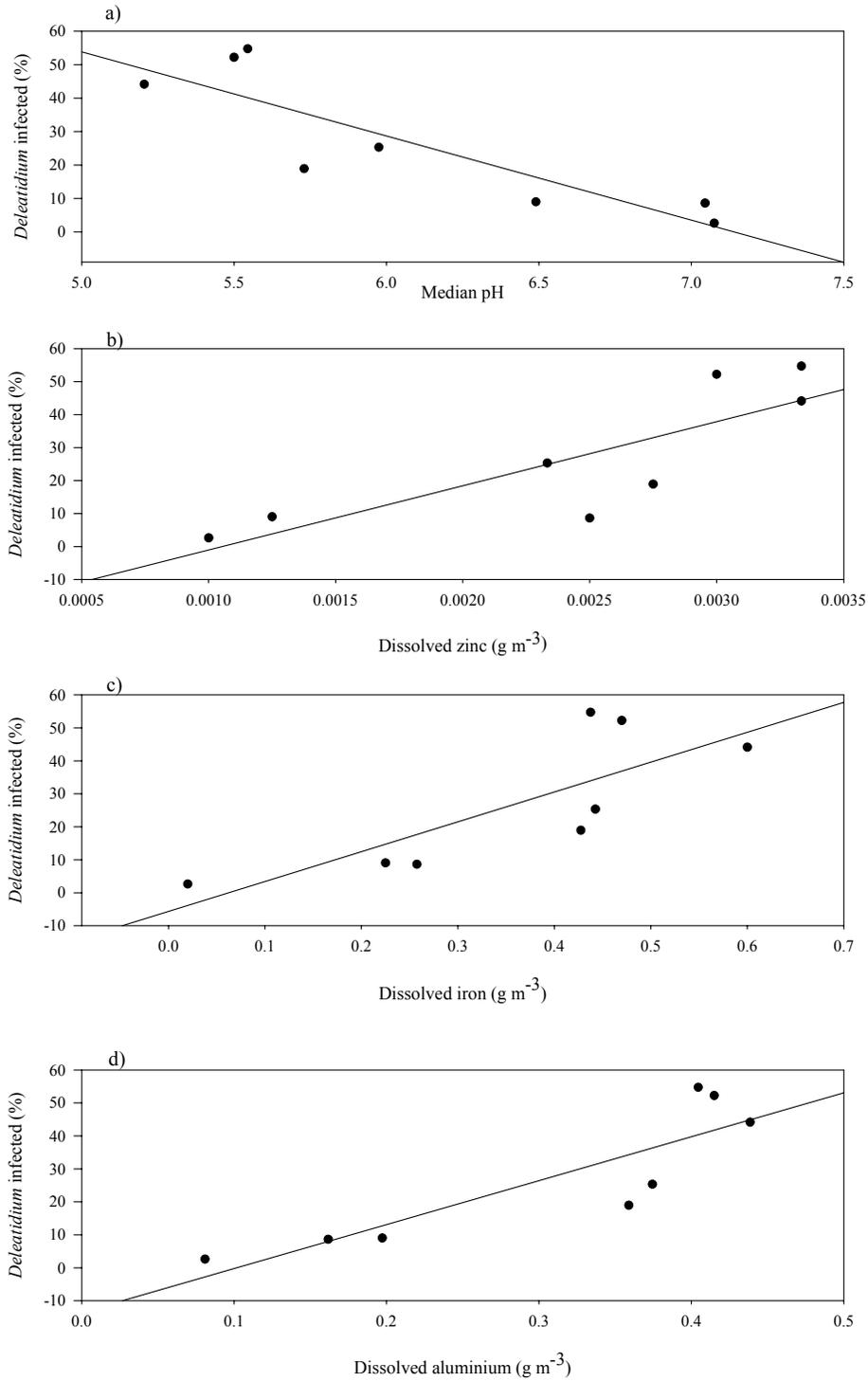
Linear regression analyses on pH and percent of community infected indicated a significant, negative relationship, with higher infection rates being observed as pH decreased (Figure 5.6a,  $F=17.2554$ ;  $P=0.006$ ;  $r^2=0.74$ ). In comparison, regressions of dissolved heavy metals, zinc, iron, and aluminium showed was a strong, positive relationship, with higher percentages of community infections being observed at

higher metal concentrations (Figure 5.6 b ( $F=21.6812$ ;  $P=0.0035$ ;  $r^2=0.78$ ), 5.6c ( $F=9.8740$ ;  $P=0.02$ ;  $r^2=0.62$ ), and 5.6d ( $F=15.9053$ ;  $P=0.0072$ ;  $r^2=0.73$ ).

Linear regression analyses of pH and percent of *Deleatidium* infected also indicated a strong negative relationship, with higher infection rates observed as pH decreased ( $F=18.584$ ;  $P=0.005$ ;  $r^2=0.756$ ) (Figure 5.7a). In comparison, dissolved heavy metals, zinc, iron and aluminium indicated that there was a significant, positive effect, with higher percentages of infections being observed at higher metal concentrations (Figure 5.7b. ( $F=13.4173$ ;  $P=0.01$ ;  $r^2=0.690$ ), 5.7c ( $F=10.116$ ;  $P=0.002$ ;  $r^2=0.63$ ) and 5.7d ( $F=19.2518$ ;  $P=0.005$ ;  $r^2=0.76$ ).



**Figure 5.6.** Linear regression of percentage of invertebrate community infected showing a negative, relationship with, a) pH and positive, relationships with b) dissolved zinc, c) dissolved iron, and d) dissolved aluminium.



**Figure 5.7.** Linear regression of percentage of *Deleatidium* infected showing a negative, relationship with a) pH, and a positive, relationship with b) dissolved zinc, c) dissolved iron and d) dissolved aluminium.

## **Discussion**

My findings indicate that, taxonomic richness, invertebrate abundance and community composition differed little between Carton Creek and the reference streams, suggesting that if these stream communities are affected by disease and infection, then the pathogen/s responsible for infections is having a chronic, rather than an acute effect on benthic invertebrate populations. Although invertebrate communities are not obviously structured by these 'infections' they may be effected in other ways, for example reductions in growth, moulting efficiently or fecundity.

One possible explanation for the lack of differences in invertebrate communities between highly infected and less infected communities, in this study, may be that, these stream invertebrates have evolved immune defence responses towards pathogens infecting them. This is not unlikely, as immune defences to infections have been observed in other invertebrates. For example, in response to a viral infection (*Autographa californica* nuclear polyhedrosis virus), in an lepidopteran host, infected midgut cells were rejected by the insect, and the insect then continued normal growth (Keddie et al. 1989). Additionally, defence mechanisms against infection have also been observed in the mosquito (*Culex quinquefasciatus*) (Da Silva et al. 2000). Da Silva et al., (2000) found that after injecting yeast cells (*Candida albicans*) into the thorax of adult mosquitoes, there was a marked reduction in the number of yeast cells in the hemolymph. They also found that there was a gradual increase in the number of hemocytes, up until six hours after yeast cells had been injected. After this period, the number of hemocytes declined, and at 24 hours after injection, hemocyte counts were similar to non-infected individuals. Yeast cells were also phagocytosed and melanized nodules formed around the yeast cells. Da Silva et al., (2000) suggested that the immune defence reaction initiated after injection with yeast cells indicated that mosquitoes may be capable of removing pathogen infections. In my study, it may also be possible that blackened limbs are lost after moulting and thus 'infection' could be removed or 'cleared'. I did not have time to pursue this idea however it could be investigated under laboratory conditions.

The results from this study indicate that there may be a relationship between, low pH, and the heavy metals, iron, aluminium and zinc and the percentage of benthic

invertebrate community infected. The results from the dissolved heavy metal analysis are interesting, not only in terms of infections, but also because the higher concentrations of heavy metals in Carton Creek, did not result in a significant reduction of invertebrate densities or large shifts in community composition, as has been reported overseas. For example, Rasmussen and Lindegaard (1988) found that iron concentrations of  $0.3 \text{ mg Fe}^{2+1-1}$  in a river in Denmark, reduced benthic invertebrate taxa from 67 to 53. My study indicates that elevated concentrations of dissolved metals (e.g. Fe,  $0.6 \text{ g m}^{-3}$  and Al,  $0.42 \text{ g m}^{-3}$ ) are not necessary directly toxic to invertebrates, but may instead act to 'stress' or weaken immune defences. Furthermore, significant linear regression does not necessary imply that the tested abiotic variables are directly responsible for the 'infections', although they may be acting as factors of 'stress' and increasing vulnerability of benthic invertebrates to disease. Furthermore, because blackening was usually observed in areas of missing or broken appendages it could be speculated that a damaged cuticle would make easy access particularly for pathogenic fungi that gain entry to an insect through the cuticle (Samson et al. 1988).

Alternatively, what may be occurring is that infected individuals may have slower growth rates. A pathogen-induced reduction in invertebrate growth rate is not a new idea and has been found in overseas studies. For example, Vance and Peckarsky (1996) investigated mermithid nematode (*Gasteromermis* sp.) infections on the growth of *Baetis bicaudatus* mayfly nymphs. Results indicated that during the later stages of development (stage III and IV), the size of head capsules in parasitized mayflies were significantly smaller than that of un-parasitized mayflies. Un-parasitized, stage IV, mayfly nymphs also showed signs of developing eggs and testes, while parasitized individuals at the same stage had no sign of development. Similar results were found when the effects of a terrestrial intracellular parasite (*Nosema* sp.) on the on the Indian tasar silkworm, *Antheraea mylitta* were examined. *Nosema* is a protozoa and develops within the host cells and infests almost all the tissues of the silkworm (Rath et al. 2003). Rath et al., (2003), found that *Nosema* infected fifth instar *A. mylitta* showed a significant reduction in weight over a period of about one month, compared to non-infected individuals that gained weight across the same period. Energy that would usually go towards growth may be used for other resources such as immune defences. Although, this study is a terrestrial invertebrate

example it indicates that parasite induced weight reductions in host organisms are probably common. I was not able to investigate possible effects on moulting and growth, but that maybe an avenue for further research.

The paucity of research on bacterial and fungal effects on aquatic insects makes interpretation of these results difficult and, this study has merely brushed the surface of what is really a large and complicated subject. Doubt remains on what was causing the observed blackening of appendages, and future studies would have to investigate the cause behind them. Stream communities regardless, appeared to be un-affected when compared to reference streams. There is potential for there to be future studies on the influence of pathogens on New Zealand stream invertebrates.

## Chapter six

### Concluding discussion

---

My thesis research was divided into two main components. Firstly, I investigated water chemistry, algal biomass, benthic invertebrate communities, and the rate of leaf breakdown in streams receiving two types of mine drainage impacts (i.e., iron flocculants, or iron precipitates). Secondly, I investigated the influence of heavy metal inputs from old mine workings in increasing the occurrence of infections and disease in stream invertebrate communities.

The streams considered in this study displayed quite variable water chemistries, but precipitate streams conformed more to what is generally considered to be ‘mine polluted’, than did iron flocculent streams (e.g. Koryak et al. 1972, Dills and Rogers 1974, DeNicola and Stapleton 2002). Further, streams (not always mine-impacted), affected by iron precipitates are often reported to have low pH < 4 (Koryak et al. 1972, McKnight and Feder 1984, Niyogi et al. 1999), although precipitates, as well as, iron-bacterial flocculants have also been reported in waters with pH >5 (Letterman and Mitsch 1978, Scullion and Edwards 1980, Wellnitz et al. 1994). The results of my study are consistent with overseas studies, and demonstrate that iron deposition (in either form) can occur in waters ranging in pH from 3.3-6.9.

The highly variable water chemistry recorded from streams affected by metal deposition in overseas studies and this study, indicate that factors controlling metal deposition are complex, and that impacted streams cannot be classified easily into categories based on types of iron deposits (e.g., precipitate and flocculent). Also, words like ‘flocculent’ and ‘precipitate’ are often used loosely when describing metal deposition and can lead to confusion. For example, McKnight and Feder (1984), described metal precipitates occurring in a Colorado mountain stream with pH between 5.5 and 6.5 as, “...‘flocculent’, brownish-white ‘precipitate’ of hydrous Al and Fe oxides....”. In my study, I used the term iron ‘precipitate’ specifically to describe chemically (or abiotically) derived encrusting iron deposits, and ‘flocculants’ to describe potentially bacterially-driven (or biotic) gelatinous iron deposits. Further,

iron precipitates and flocculants are not always associated with AMD, and may occur in waters that have elevated iron because of other factors such as natural weathering of iron enriched rock strata, or disturbance due to human land-use (other than mining activities) (e.g., Sheldon and Skelly 1990). Because they may occur together this may make the distinction behind iron flocculants and iron precipitate streams even harder.

In my study, I found few invertebrate taxa, in precipitate streams, whereas flocculent streams had greater taxonomic richness, but it was usually lower than in reference streams. In general terms, iron deposits of any description probably have a similar effect on stream benthos as both smother the stream substrate, remove habitat space, and reduce the quality and availability of food. Differences in benthic invertebrate communities found among iron precipitate and iron flocculent streams, may be partially explained by water chemistry. For example, in some Northern Hemisphere streams with low pH, resulting from either acid precipitation or AMD there is often a change in faunal composition at pH about 5.7, with some taxa (mayflies, some caddisflies, crustaceans and molluscs) absent or becoming scarce at lower pH (Tomkiewicz and Dunson 1977, Sutcliffe and Hildrew 1989). Some studies on the West Coast of the South Island, New Zealand have reported a small number of EPT taxa (e.g., *Deleatidium* spp., *Spaniocercoides* spp., and *Psilochorema* spp.) in streams affected by AMD with a pH of 4 or even lower (Winterbourn 1998, Anthony 1999). In my study, one genus in particular, the mayfly *Deleatidium* was common in flocculent and reference streams, and although it was rarely found in precipitate streams my results concur with these previous studies that have reported the same taxa common in 'reference' and also AMD streams. This is interesting, because in studies overseas there is often a reported absent of taxa found in 'clean' streams, in areas affected by iron deposition, of any kind, regardless of their water chemistry (Hoehn and Sizemore 1977, Letterman and Mitsch 1978, Scullion and Edwards 1980).

Leaf breakdown was slow in all my streams, but was markedly slower in the precipitate streams, indicating they were more adversely affected. Furthermore, my results indicated that shredder abundance was low in all streams, and that physical abrasion in summer (when leaf canisters were used) would have been minimal. This suggests that the observed leaf breakdown was most likely due primarily to microbial

activity, which is likely to have been lower in precipitate streams. Slow leaf breakdown reported in overseas studies, has been associated with a reduction in microbial and fungal activity, from stressors characteristic of AMD, including low pH, elevated heavy metals and deposition of metals onto leaves (Carpenter et al. 1983, Gray and Ward 1983, Griffith and Perry 1993, Griffith et al. 1995, Bermingham et al. 1996, Schlieff 2004). Therefore, it is probable that a combination of these factors was inhibiting leaf breakdown in my precipitate streams. Notably, my flocculent streams had pH similar to that of reference streams and although they had elevated concentrations of heavy metals and iron flocculants, leaf breakdown in them was similar to that in reference streams. Arguably, shredders are not an ideal functional feeding group to use in comparisons between treatments because shredders overall, are not generally well represented in New Zealand (Thompson and Townsend 2000).

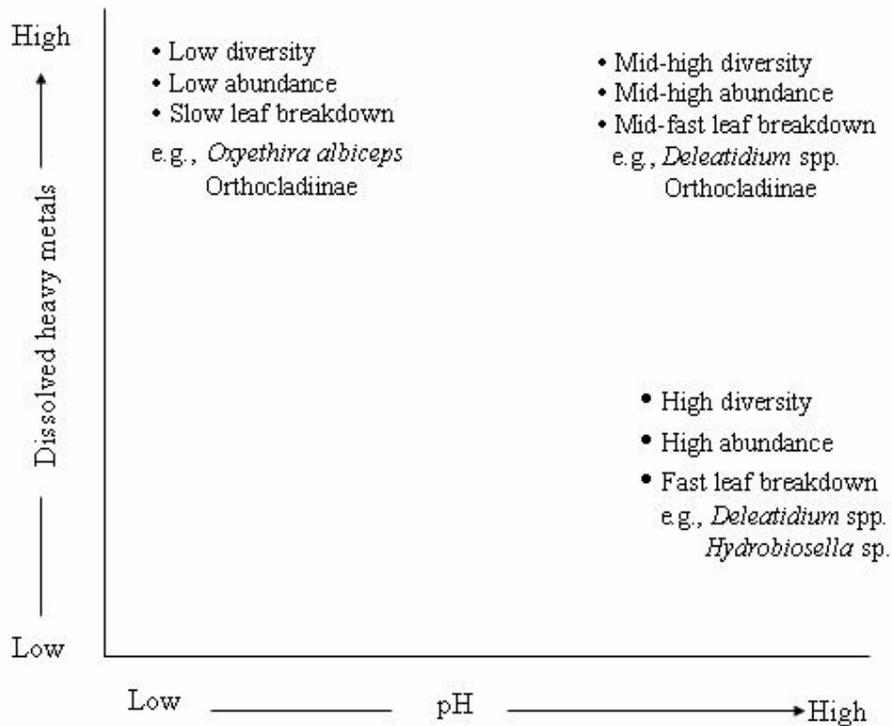
My results illustrate the complexity and range of factors that influence benthic invertebrate communities. Firstly, underlying water chemistry probably determines the range of species that can inhabit the stream. Secondly, stream invertebrates need to be able to tolerate possible changes in habitat and food quality imposed by iron deposition, whether it is in a precipitate or flocculent form. In streams impacted by low pH (but not iron deposition) a reduction in numbers of invertebrate species and lowered abundance are matched by a reduction in the number of functional feeding groups. For example, Townsend et al. (1983) investigated the importance of physicochemical factors in structuring invertebrate communities in Southern England and reported that collectors, shredders and predators were present at the most acidic sites and that as pH increased, grazers and filter feeders were added to the community. Townsend et al. (1983) suggested that low pH may have an indirect impact on the stream community by altering the availability of food resources. This notion was also supported by Sutcliffe and Carrick (1973) and obtained some support from a later study by Winterbourn et al. (1985).

In my study, algal biomass on stones was similar between treatments (reference, flocculent, and precipitate), indicating that the availability of algae as a food resource to benthic invertebrates should have been similar between stream types. Thus, benthic invertebrates that feed on the stone surface organic layers might have been expected to occur in all three treatments. I found this to be true for the flocculent and reference

streams, which were dominated by collector-browsers (over 70% of individuals) but in precipitate streams collector-browsers made up only 43% and filter feeders were much more abundant (35%). However, macroinvertebrate abundance in precipitate streams was low. Two possible explanations for the reduction in numbers and relative abundance of collector-browsers in my precipitate streams might be that the quality of alga as a food resource is reduced by the smothering of iron precipitates, or that algae tolerant of low pH (e.g., *Ulothrix*) are unpalatable to invertebrates.

An important question to ask is why benthic invertebrate communities and leaf breakdown differ in the iron precipitate and iron flocculent streams? The most obvious difference between the flocculent and precipitate streams was pH, although, heavy metal concentrations were also slightly higher in precipitate streams. In general however, precipitate streams had low pH, elevated heavy metals and were characterised by low invertebrate diversity and abundance and slow leaf breakdown (Figure 6.1). In contrast, flocculent streams had higher pH, mid-high concentrations of heavy metals, and mid-high invertebrate richness and abundance, as well as faster leaf breakdown (Figure 6.1). These results indicate that precipitate streams were more adversely impacted than flocculent streams, primarily I suggest because of their lower pH, which is responsible for the precipitation of iron.

The range and distribution of species in my streams, and in other studied streams on the West Coast reflect tolerance of West Coast stream invertebrates to naturally acidic, low pH waters (Collier et al. 1990). However, stream invertebrate species vary considerably in their tolerance to low pH and associated changes in water chemistry (Collier et al. 1990) and may explain why some species (e.g., *Spaniocercoides* spp. and *Oxyethira albiceps*) inhabit low pH sites whereas others do not. The ability of West Coast stream invertebrates to tolerate a wide range of water chemistries enables them to inhabit and survive in streams that are stressed by low pH. This characteristic of the West Coast stream fauna may be beneficial for recovery from AMD impacts, especially if low pH is the major factor inhibiting colonisation of benthic invertebrates.



**Figure 6.1.** Generalised diagram illustrating how pH and dissolved heavy metals may influence the structure and function of benthic stream communities.

My findings support the results of other studies carried out on the West Coast of the South Island, that have also reported a variety of stream invertebrates in a range of mine impacted streams (Winterbourn and McDiffett 1996, Winterbourn 1998, Anthony 1999, Winterbourn et al. 2000b, Harbrow 2001, Bradley 2003). In addition, my results indicate that benthic invertebrates can tolerate iron deposition in a range of water chemistry environments, although flocculent streams generally had water chemistry similar to my reference streams. Further research could investigate a variety of factors that may help to explain the distribution of benthic invertebrate communities in these stream systems. For example, the ability of benthic invertebrates to feed on iron precipitates and flocculants, growth rates when fed these diets, and the direct toxicity of the different iron precipitates and iron flocculants under different pH regimes, could be investigated under laboratory conditions.

Streams that have been impacted by past coal mining operations are often affected for a long time, as polluted mine waters usually continue to enter streams from adits for

decades. Even streams that have not been influenced by AMD, but have been mined historically for gold, such as Carton Creek, may suffer long term stress as their waters carry elevated concentrations of heavy metals. Effects of elevated heavy metals need not be acute but may manifest themselves as increased rates of biological infections (as investigated here) in stream communities. My findings suggest there may be a relationship between heavy metal concentrations (e.g., zinc and aluminium) and the percentage of a stream community infected. However, the higher rates of infection at sites with elevated heavy metals was not matched by a reduction in stream community abundance, as found by Kohler and Wiley (1992), Kohler and Wiley (1997) and Lemly (1998). Instead it is possible that infections reduce growth and fecundity of infected individuals, a subject that could be investigated further in the laboratory. Whether the rate of infections in a stream community is a measure of stress is yet to be decided, and really needs to be investigated further so that the mechanism(s) behind the infections can be fully appreciated.

Understanding the relative roles of substrate quality as well as water chemistry on benthic communities, is essential for successful remediation of mine-polluted stream systems. However, it can be difficult to distinguish the key factor or factors driving stream communities (e.g. low pH, elevated heavy metals, iron deposition), and this can often be a problem when studying them in AMD-receiving waters (Sode 1983, Gray 1997, Niyogi et al. 1999, Niyogi et al. 2002a). Water chemistry may have a greater influence than substrate or habitat quality (Soucek et al. 2000, DeNicola and Stapleton 2002, Bradley 2003) but not always (Scullion and Edwards 1980, McKnight and Feder 1984).

In cases where water quality has been improved, recovery of stream systems may still be slow, especially if bed substrates are coated in iron deposits (Gray 1997). Lack of stream recovery (community structure and function), may be due to several factors that may include toxicity of precipitates and flocculants to stream invertebrates, continuing habitat limitation, smothering of periphyton, food chain simplification, and lack of colonists (McKnight and Feder 1984, Wellnitz et al. 1994, Gray 1997). Furthermore, 'recovery' of stream communities following a mining disturbance, is hard to demonstrate if there is no prior biological, or physico-chemical information on a stream. In some cases, the best option may be to sample a non-impacted stream, in

the same catchment, and demonstrate at least partially the structure of a historic benthic community and the rate of stream functioning in un-stressed conditions.

## Acknowledgements

---

Well here I am nearing the end of the road, I think it's at this point that I am meant to shed a tear, light a candle, or throw a flower into the wind, and thank all of those that have encouraged and guided me throughout this study. Firstly, I can't thank my supervisor, Jon Harding enough for his support, ideas, proof reading and enthusiasm to help me in the field. It still amazes me that he always managed to find time for his students among everything else that he managed to fit into his day. Secondary, thanks to my co-supervisor Mike Winterbourn whose guidance and wisdom has been more than invaluable. I am honoured to have known them both. Thanks also to Angus McIntosh, Solid Energy, Dave Wilson, and especially Trish who lent me those fashionable high-visibility safety vests to wear.

I also wish to acknowledge the assistance of a line of technical staff, especially Linda Morris, Nick Etheridge, Neil Andrews, Gavin Robinson and Jan McKenzie whose help I am indebted for. I am also grateful to Nic Cummins who assisted me in the fungi arena and Graham Furniss for the climate data.

On a personal level, thanks to those that shared an office with me, Sarah, Duncan and Paul (plus of course those who lived next door, Michelle, Hamish, and Phil!!!!!!). You are all absolute legends, firstly for putting up with me (?) and secondly for answering my questions. Thanks especially to my coffee and lab buddy Sarah, for keeping my caffeine levels on high and my sanity questionable with that infamous question 'so how many Surbers have you done today??' Duncan, your uncouth comments tested my patience on more than one occasion but I can't deny that I would have been lost without your advice and field assistance.

To my other non-university 'acquaintances' and my best mate Amanda, that on the most part had absolutely no idea what I was doing and probably thought I had gone completely mad- thanks. You fortunately forgave me when I neglected you for weeks on end. Thanks also to Simon, who will never know it, but who gave me the incentive to start this whole process in the first place. To my Mum and Dad, thanks for your support and encouragement even if you didn't understand. I am incredibly grateful for

all that you have done for me. And to all of those that I have not mentioned in person here but helped me somewhere along the way- thanks!

