Effects of Trace Metal Enrichment in Cobble Streams and Rivers on the Aquatic Midge (Diptera: Chironomidae)

by

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"One of the penalties of an ecological education is that one lives alone in a world of wounds. Much of the damage inflicted on land is quite invisible to laymen. An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise"

Leopold (1987)

ABSTRACT

Chironomidae (Diptera) are commonly used as indicators of environmental degradation. This study examined the effects of mine drainage on chironomid assemblages in northeastern New Brunswick rivers. At mine drainage receiving sites, significantly elevated concentrations of metals in water (Ba, Fe, K, Mn and Zn; $p<0.05$) and in periphyton (Cd, Co, Cu, Mn, Pb and Zn; $p<0.002$) were detected. Concentrations in periphyton were 10 - 100 times higher than in water, suggesting that metal uptake from diet may be a more important source of metals for primary consumers than surrounding water.

Chironomid composition was significantly different at mine drainage receiving sites than at reference sites. Fewer chironomid taxa were collected at mine sites ($p<0.025$). The relative abundance of Orthocladiinae taxa was higher while the abundance of Tanytarsini taxa was lower at mine drainage receiving sites ($p<0.05$). The incidence of mentum deformities was significantly elevated at mine drainage receiving sites, with the mean percentage approaching a doubling of that observed at reference sites ($p<0.05$).

To assess the effects of metal enriched periphyton on aquatic invertebrates downstream of metal mining facilities, periphyton was collected at mine drainage receiving (MIN) and reference (REF) sites in northeastern New Brunswick rivers and fed to *Chironomus riparius* in a 10-d bioassay. In the laboratory, larvae fed reconstituted, freeze-dried MIN periphyton exhibited significantly lower survival (mean ±1 SE, 16% ± 6, n=4) and mean biomass (mean ± 1 SE, 0.07 ± 0.01 µg AFDW, n=4) than REF-fed larvae (survival: mean ± 1 SE, 42% ± 8, n=4) (biomass: mean ± 1 SE, 0.42 ± 0.11 µg AFDW, n=4). Overall incidence of mentum deformities was low and not significantly different between treatments.

These results suggest that primary consumers feeding on periphyton are at risk of significant mortality and reduced growth, compromising the viability of most metal intolerant populations. Consequently, metal enrichment of periphyton could explain observed differences in chironomid communities of mine drainage receiving rivers.

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G. Lindsay and R. Parker (Environment Canada, Atlantic Region) assisted in the selection of metal mining facilities included in this study. Participation in a tour of metal mines in northeastern New Brunswick conducted by these individuals provided much information on the history of metal mining in this area, as well as pointing out specific monitoring sites. Access to mine drainage receiving sites on mine premises was generously granted by Noranda Inc. and Anaconda Inc. Specifically, M. Patterson and D. Scott (Heath Steele Mine, Noranda Inc.), R. Butler (Brunswick Mining and Smelting, Noranda Inc.), R. Prairie (Noranda Inc.), and R. Carreau (Caribou Mine, Breakwater Resources Ltd.) assisted in the identification of stream sites suspected of receiving acid mine drainage.

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CHAPTER 1: INTRODUCTION

GENERAL INTRODUCTION

In Atlantic Canada, mining is an important stress on stream systems. Water runoff from metal mining practices commonly contributes to elevated concentrations of trace metals in aquatic environments. Metal contamination can have various deleterious effects on aquatic organisms that can ultimately elicit significant changes in population and community dynamics, altering the structure and function of aquatic ecosystems (Cain et al. 1992; Canfield et al. 1994; Clements 1994; Beltman et al. 1999; Clements et al. 2000).

The purpose of this research was to evaluate the effects of metal contamination in lotic environments on the larvae of midges (Diptera: Chironomidae). Assemblages of chironomids were examined in streams receiving drainage from metal mining facilities and non-metal enriched reference streams to determine effects on community composition. In the laboratory, chironomids fed periphyton collected downstream of metal mining facilities or at reference sites, were examined to elucidate processes involved in structuring natural communities.

The present chapter is divided into two main sections. The first section discusses the use of aquatic organisms, specifically chironomids, as monitors of aquatic ecosystem integrity. A brief review of the effect of metal contamination on chironomid assemblages is presented, highlighting changes in bioaccumulation, abundance, diversity, presence/absence of indicator genera, and incidence of deformities. Laboratory studies examining the effects of metal exposure on the physiology of individual larvae are detailed. The bioavailability of metals through aqueous and dietary routes is discussed, focusing on metal accumulation at the base of the food chain.

The second section of Chapter 1 describes the study area and general experimental design of this project. A brief history of metal mining in New Brunswick is included, detailing the geology of this area and the mining activity at each of the metal mining facilities included in this study. Information on metal inputs into receiving waters and associated effects on benthos and fish communities are presented where available. Study objectives and experimental design of this project, highlighting the use of separate rivers as replicates of metal exposure, are described.

Natural variability in aquatic environments contributes to variable responses of aquatic communities to metal stress. Consequently, general conclusions on the response of aquatic organisms to metal contamination require evaluation of multiple locations (Hurlbert 1984). Exposure to metal contaminants may be demonstrated by bioaccumulation, reductions in richness and abundance and differences in community composition (Reice and Wohlenberg 1993). The effects of metal enrichment on natural chironomid assemblages downstream of New Brunswick metal mining facilities are presented in Chapter 2. Changes in chironomid generic richness, presence/absence of indicator genera, and the incidence of deformities are compared at mine drainage receiving and reference locations. Concentrations of trace metals in water and periphyton are also discussed.

Alterations in richness, community composition and incidence of deformities are associated with metal contamination (Beltman et al. 1999; Clements et al. 2000). Presumably, metal uptake from water or diet contributes to observed effects in field populations (Hare 1992). In many aquatic environments, metal contaminants in food resources contribute significantly to metal accumulation by subsequent trophic levels and the observation of deleterious effects (Woodward et al. 1994; Munger and Hare 1997; Roy and Hare 1999). Chapter 3 examines the effects of a periphyton diet,

collected at mine drainage receiving and reference sites, on a larval culture of *Chironomus riparius* in the laboratory. Survival, growth (as determined by final size), and the incidence of deformities are compared between larvae fed periphyton from mine drainage receiving sites and periphyton from reference sites.

The final chapter integrates the results of the above studies, discusses ecological implications of these results, and suggests possible directions for future research.

TRACE METALS AND CHIRONOMIDAE

Biomonitoring

Metals are frequent contaminants of aquatic environments receiving effluent or site runoff from active and abandoned mines, smelters, and other industries (Hare 1992; Clements 1994; Clements et al. 1988; Pacyna et al. 1995). Contamination can be quite severe in areas of intense industrial development. For example, prolific metal mining in the U.S. Northwest is responsible for one-third of Colorado's U.S. Environmental Protection Agency (EPA) Superfund Sites, making metal contamination one of the most significant environmental problems in Rocky Mountain streams (Clements et al. 2000). Distant industrial sources may also contribute to metal contamination of aquatic systems through atmospheric transport of metals or through the generation of acidic precipitation, which may release trace metals from soil (Franzin et al. 1979; Nriagu and Pacyna 1988).

Except in the most extreme circumstances, adverse effects of metal contaminants in nature are difficult to demonstrate. This is not because metal effects are minimal, but rather is due to our limited ability to detect and quantify these effects (Luoma and Carter 1991). Assessing environmental degradation has frequently

employed chemical analysis of water concentrations. However, measuring the concentration of trace metals in water from these environments at best gives only an instantaneous measure of ambient conditions (Steines and Wharfe 1987). It provides no indication of the extent of contamination experienced a month, a week, or even a day prior. Chemical analysis of water also does little to describe uptake and accumulation of contaminants by biota, and thus provides little information on the effect on these contaminants on aquatic organisms.

Alternatively, one of the most widely used approaches for assessing aquatic ecosystem integrity is biomonitoring. Biomonitoring uses the response of living organisms to identify and characterize changes in the environment (Clements 1991; Rosenberg and Resh 1993). Biotic data incorporate the effects of episodic as well as chronic contaminant exposure over time, respond to novel contaminants, and integrate the effects of contaminant mixtures (Steines and Wharfe 1987). This approach assumes that the composition of biological communities reflects the relative health of the system, and that structural change can be attributed to an anthropogenic influence (Clements 1991).

Benthic macroinvertebrates play an integral role in the structure and function of aquatic environments, making them excellent indicators of ecosystem "health" (Ciborowski et al. 1995). Benthic invertebrates are effective indicators of ecosystem integrity for a number of reasons (see Rosenberg and Resh 1993). Firstly, they are relatively sessile and thus their distribution reflects the extent of local contamination. Their relatively short life cycle allows benthic invertebrates to incorporate a recent history of contaminant exposure. Benthic invertebrates employ a range of trophic strategies and thus experience contaminant exposure through contact with their physical environment (i.e. water, sediment) and through biotic interactions (i.e. dietary

sources) (Hare 1992). Finally, these organisms readily accumulate contaminants, frequently exhibiting tissue concentrations orders of magnitude (i.e. 10x, 100x, 1000x) greater than their surrounding environment (Kiffney and Clements 1993; Rosenberg and Resh 1993).

Chironomidae as Bioindicators of Metal Contamination

General Biology and Ecology

Midge larvae (Diptera: Chironomidae) are holometabolous insects with four stages of development; egg, larval, pupal and adult (Fig. 1.1). Successful completion of the life cycle involves development primarily in aquatic environments, followed by a brief terrestrial (winged adult) stage of development. The chironomid life cycle begins with the oviposition of eggs, contained within a gelatinous matrix, on or below the surface of the water. Aquatic larvae hatch from the egg mass, developing through four instars. In lentic aquatic environments, first instar larvae are planktonic, with subsequent instars assuming a benthic lifestyle (Oliver and Roussel 1983). Following the final instar of development, pupation occurs. The adult emerges from the pupa, beginning the terrestrial portion of the life cycle. Adult chironomids live for only a few days, with minimal feeding occurring during this stage of the life cycle. Some adult chironomids ingest sugars, extending their life cycle by 1 – 2 d (Oliver 1971). Adult male chironomids form swarms, attracting the females, and copulation ensues. Aquatic environments are then colonized by egg-bearing female chironomids, who complete the life cycle by depositing the egg mass back into the aquatic ecosystem (Pinder 1986).

The length of the chironomid life cycle is variable, depending on species and environmental conditions. Many chironomid species, including *Chironomus* sp., complete more than one generation per year (multivoltine). Other species are slower

Figure 1.1: Life cycle of the midge (Diptera: Chironomidae) (adapted from McAlpine et al. 1981)

growing, completing their life cycle in one to seven years (Oliver 1971). Regardless of the number of generations, chironomids generally overwinter as larvae in Canadian environments (Oliver and Roussel 1983).

Chironomid larvae occupy various feeding niches, ranging from detritivores to predators (Oliver and Roussel 1983; Ferrington and Coffman 1996). The chironomid groups Diamesinae, Chironomini, and Orthocladiinae feed predominantly on algae and detritus. However, they employ slightly different methods for collection. Diamesinae and Orthocladiinae larvae are scraper-grazers, scraping algae and detritus from the surface of hard substrates. Chironomini larvae are collector-gathers, gathering detritus from soft sediments and hard substrates. Tanytarsini larvae feed predominantly on fine particulate organic matter (FPOM). Larvae are collector-gatherers, filtering suspended matter from the water column. Larvae of the tribe Tanypodinae are predators, engulfing small invertebrates including chironomids (Cummins and Klug 1979; Oliver and Roussel 1983).

Suitability as Biomonitors

Chironomids represent one of the most ecologically diverse groups of aquatic insects (Pinder 1986). They are found in cold and warm waters, fresh and saline waters, flowing and still water environments, and living in soft sediments and on hard substrates (Oliver 1971). In addition to their ubiquitous distribution, chironomids comprise a significant portion of the benthic invertebrate biomass in aquatic systems (Oliver 1971), making large numbers of chironomids easily collectable. Chironomids feed on algae and detritus, playing an important role in nutrient cycling. They also represent an important diet item for predatory insects and benthivorous fish (Pinder 1986; Woodward et al. 1994). Metal accumulation by chironomid larvae from food or

aqueous sources, thus represents an important link in the movement of contaminants from lower to higher trophic levels (Smock 1983a; Hare 1992).

Large differences in the sensitivity of chironomid genera to metal contaminants exist (Wiederholm 1984a; Gower et al. 1994; Kiffney and Clements 1994a). Certain chironomid tribes, genera, and species are recognized as being either metal tolerant or metal sensitive. As a result, many chironomid taxa, unlike some benthic invertebrates, can tolerate and thus continue to be studied in even the most severely contaminated aquatic environments (Hare and Carter 1976; Warwick et al. 1987). In addition, chironomid larvae possess morphological structures such as antennae, mentum, mandibles and epipharyngeal pecten that respond to contaminant exposure (Hudson and Ciborowski 1996a). Therefore, the importance of chironomids in aquatic ecosystems, their persistence in contaminated environments and the ability of morphological structures to act as biomarkers of contaminant exposure makes them excellent indicators of ecosystem integrity.

However, the use of chironomids as biomonitors of aquatic ecosystem integrity has been limited. Chironomid identification can be difficult and labour intensive, requiring slide mounting of specimens (Waterhouse and Farrell 1985; Rosenberg and Resh 1993). As a result, the level of taxonomic resolution in studies using chironomids varies widely, from family or tribe (Armitage 1980; Winner et al. 1980; La Point et al. 1984; Selby et al. 1985; Chadwick et al. 1986; Clements et al. 1988; Clements et al. 1989) to species level identification (Waterhouse and Farrell 1985; Kawai et al. 1989; Gower et al. 1994; Lindegaard and Brodersen 1995; Orendt 1999; Ruse et al. 2000). Considering the different contaminant sensitivities exhibited by chironomid taxa, the level of taxonomic resolution can limit the ability of chironomids to act as effective biomonitors (Resh and Unzicker 1975). In addition, metal contamination studies

exhibiting various levels of taxonomic resolution are difficult to compare, and limit the establishment of conclusions on the effectiveness of chironomids as bioindicators. Waterhouse and Farrell (1985) and more recently, Ruse et al. (2000) found that identification of chironomid genera appears to adequately reflect variability in sensitivity to contaminant exposure, without the loss of too much species-specific information.

Trace Metals and Chironomid Assemblages

In aquatic environments, metal exposure can be demonstrated through changes in bioaccumulation, abundance, richness, community composition, and incidence of deformities in chironomids. Changes in these variables can be used to make inferences about consequent changes in ecosystem-level processes (Reice and Wohlenberg 1993).

Bioaccumulation

Chironomids living in intimate contact with their environment experience contaminant exposure through contact with their physical environment (habitat) or through biotic interactions (diet) (Hare 1992). Upon exposure, chironomids can readily accumulate contaminants at concentrations orders of magnitude greater than occur in surrounding environments (Kiffney and Clements 1993; Rosenberg and Resh 1993; Mason 1996). Metal concentrations in biota, in concert with bioaccumulation factors (BAF's), can then be used to estimate metal contamination in surrounding environments (Hare 1992). Biological responses to metals are subsequent to metal accumulation, and thus bioaccumulation can be associated with changes in survival, growth and reproductive success. Metal bioaccumulation in chironomids may also describe the

movement of contaminants to higher trophic levels in both terrestrial and aquatic ecosystems (Reinfelder et al. 1998).

In metal contaminated environments, metals can be absorbed or adsorbed by chironomids. Essential metals, such as zinc and copper, are actively taken up. These metals are then incorporated into proteins or act as catalysts for enzyme activity (Hare 1992). Excess quantities of Zn and Cu are detoxified by binding to stress proteins and either stored in granules or excreted (Rainbow and Dallinger 1993) (Fig. 1.2). Cadmium and lead (i.e. non-essential metals) are not required by chironomid larvae. These elements when absorbed are detoxified, and predominantly stored (Rainbow and Dallinger 1993). Elimination of Cd and Pb is minimal (Krantzberg and Stokes 1989; Timmermans and Walker 1989; Hare 1992; Rainbow and Dallinger 1993). Bioaccumulation is a measure of the net quantity of metals stored or sequestered in an organism's body (Hare 1992; Rainbow and Dallinger 1993). Concentrations of metals detected in chironomids range from 0.20-46.04 mg/kg (Cd), 6.60-85.6 mg/kg (Pb), 13.47-200 mg/kg (Cu), and 149.92-8151 mg/kg (Zn) in metal contaminated rivers in North America, Europe and Asia (Saiki et al. 1995; Gupta 1996; Janssens de Bisthoven et al. 1998a).

Different physical and chemical properties of metals (i.e., solubility, valence, and reactivity) contribute to differences in bioaccumulation. Metal concentrations in waters receiving drainage from base metals mines, tend to exhibit relatively high concentrations of Zn and low concentrations of Cd. Consequently, chironomids tend to accumulate metals in the following order: Cd<Pb<Cu<Zn, with Zn concentrations often an order of magnitude (i.e., 10x) greater than other metals (Hare 1992; Goodyear and McNeill 1999). Higher concentrations of essential metals are accumulated than non-essential metals, reflecting the presence of active routes of uptake (ion pumps) for Cu

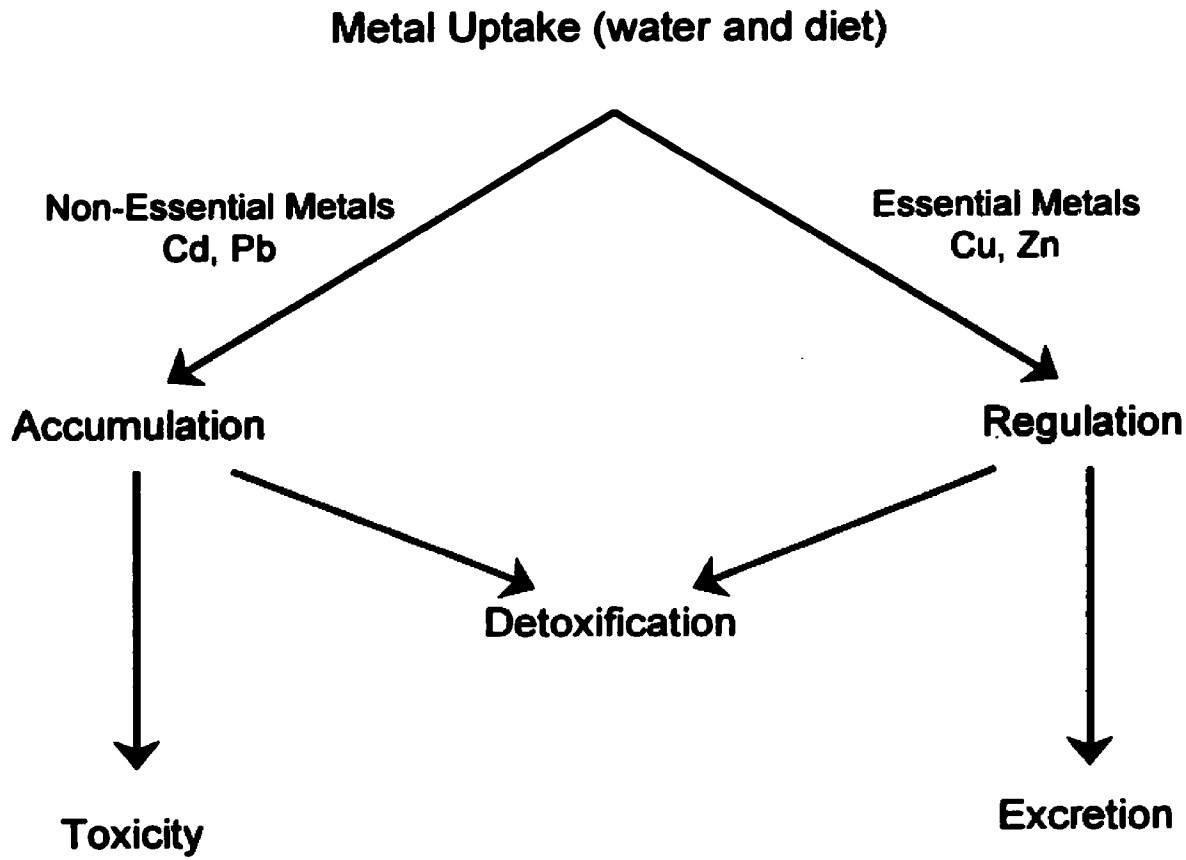

Figure 1.2: Internal process in chironomid larvae governing the transport, accumulation and excretion of metals taken up across gill membranes and through consumption of metals bound to organic and inorganic matter. Pathways for essential and non-essential metals differ.

and Zn (Goodyear and McNeill 1999).

Metal are distributed on both exteriour and interior surfaces of chironomid larvae (Hare 1992). Metals bound to chitin on the surface of chironomid larvae, are lost during metamorphosis and not transferred to higher trophic levels (Timmermans and Walker 1989; Groenendijk et al. 1999). Internal distribution of metals in chironomid larvae involves binding to metallothionein proteins and storage granules (Hare 1992). Cadmium and Zn are preferentially stored in the midgut and anal papillae of chironomid larvae (Krantzberg and Stokes 1990; Craig et al. 1998).

Differences in metal bioaccumulation are also related to properties of the organisms themselves. Chironomids employing different trophic strategies or exhibiting different habitat preferences may experience different metal exposure and hence, contaminant accumulation (Smock 1983b; Goodyear and McNeill 1999). For example, chironomids associated with sediment will tend to accumulate more metals than those associated with detritus or periphyton (Smock 1983b), due to the high relative metal concentrations in sediment. Similarly, chironomids occupying collector-gather (Chironomini) or scraper-grazer (Orthocladiinae) guilds, tend to accumulate more Cd, Cu, and Zn than predatory chironomids. Goodyear and McNeill (1999) found that predatory benthic invertebrates exhibit higher Pb concentrations than other chironomid feeding guilds, implying that Pb is biomagnified in aquatic food webs. Zinc has been demonstrated to biomagnify in chironomids (Timmermans et al. 1989), while the issue of whether or not Cd is accumulated through trophic transfer remains disputed (Memmert 1987; Prosi 1989).

In addition to differences among taxa, metal concentrations can vary within a population according to the size, age, sex, and developmental stage (Hare 1992). Larvae accumulate higher concentrations of metal than pupae, which accumulate

higher concentrations of metals than adults (Timmermans and Walker 1989; Groenendijk et al. 1999). Because higher bioconcentrations occur in aquatic environments, trophic transfer of metal contaminants would be more significant for salmonids feeding on larval chironomids, than birds feeding on adult chironomids.

Abundance

Many factors contribute to the observed abundance of aquatic organisms. Availability of food and space, frequency and success of reproduction, and predation pressure can determine the abundance of aquatic organisms (Tilman 1999). Contaminants can also affect an organism's abundance through direct toxicity or indirectly through effects on predator-prey interactions.

Reduced chironomid abundance is frequently associated with highly contaminated aquatic environments (Wentsel et al. 1977a; Waterhouse and Farrell 1985; Leland et al. 1989; Kiffney and Clements 1994b; Janssens de Bisthoven et al. 1995; Diggins and Stewart 1998; Hickey and Clements 1998; Clements et al. 2000). Chironomid abundance is commonly 2 to 3 times lower at metal contaminated than at reference sites (Kiffney and Clements 1994b; Hickey and Clements 1998; Clements et al. 2000). However, chironomid abundance in a metal contaminated lake was over 30 times lower than at a nearby reference site (Wentsel et al. 1977a). Direct metal toxicity to chironomid larvae probably contributes most strongly to reduced abundance. However, food resources may also be limited due to reduced rates of photosynthesis (herbivores) and direct toxicity to prey items (predators) in metal contaminated environments (Arnegard et al. 1998; Genter and Lehman 2000; Ivorra et al. 2000; Paulsson et al. 2000).

High chironomid abundance, relative to that observed in reference sites, has also been observed in metal contaminated environments (Winner et al. 1980; Selby et al. 1985; Canfield et al. 1994; Clements 1994; Kiffney and Clements 1994a; Kiffney and Clements 1994b). Presumably, increases in the abundance of Chironomidae as a family can be attributed to the increased abundance of metal tolerant taxa, namely Orthocladiinae taxa (Clements 1994; Kiffney and Clements 1994a). Such taxa dominate the benthic communities in metal contaminated environments, taking advantage of reduced competition for resources and reduced rates of predation (Canfield et al. 1994).

Alternatively, the abundance of chironomid taxa at some metal contaminated environments has not differed from assemblages at reference sites (Beltman et al. 1999; Clements et al. 2000). However, no difference in chironomid abundance at metal contaminated and reference sites was observed in streams contaminated with low to moderate metal (Cd, Cu, Pb and Zn) concentrations (Clements et al. 2000). At higher metal concentrations, chironomid abundance was lower than at reference sites (Clements et al. 2000).

Taxa Richness

Metal contamination generally reduces richness by eliminating more sensitive taxa. The richness of chironomid taxa is infrequently measured in biomonitoring studies, due to difficulty in achieving greater taxonomic resolution. Few workers identify chironomids below the subfamily level. Most studies have found reduced chironomid richness in metal contaminated environments, ranging from 2 - 22 genera (Winner et al. 1980; Armitage and Blackburn 1985; Waterhouse and Farrell 1985; Yasuno et al. 1985; Wilson 1988; Diggins and Stewart 1998; Clements et al. 2000). Clements et al. (2000)

found that highly contaminated environments had 2.5 - 3 times fewer taxa than at reference sites.

Other workers have found metal contaminated sites that supported chironomid communities with greater richness than at reference sites (Canfield et al. 1994; Cranston et al. 1997). Cranston et al. (1997) examined tropical chironomid communities, and concluded that increased richness could be due to a larger pool of tolerant taxa inhabiting tropical environments. Increases in richness could also be attributed to reduced competition for food resources from other benthic invertebrates and reduced rates of predation (Canfield et al. 1994).

Tolerant and Sensitive Taxa

Aquatic organisms have particular requirements with respect to the physical and chemical properties of the environment. A species' absence in a particular aquatic environment may indicate that the habitat is not suitable, possibly due to environmental degradation. Likewise, the presence of a particular species indicates that the environment satisfies the physical and chemical requirements of that organism (Johnson et al. 1993). Metal contamination can make an environment uninhabitable for some taxa, while it does not prevent the colonization of others.

Metal contaminated environments commonly support substantial assemblages of Orthocladiinae larvae, suggesting that this tribe is fairly metal tolerant (Armitage 1980; La Point et al. 1984; Chadwick et al. 1986; Clements et al. 1988; Clements et al. 1989; Clements 1994; Clements and Kiffney 1994). Larvae of the subfamily Tanypodinae (e.g., *Larsia* and *Procladius*) also dominate metal contaminated waters (Winberg 1978; Winner et al. 1980; Canfield et al. 1994; Diggins and Stewart 1998). Larvae of the tribe Tanytarsini (Chironominae) are frequently classified as metal-

sensitive due to their reduced abundance in metal contaminated environments (Clements et al. 1988; Clements et al. 1989).

Within subfamilies, genera may exhibit different metal sensitivity. Orthocladiinae taxa, *Orthocladius* and *Tvetenia* were abundant in moderately contaminated environments (Armitage and Blackburn 1985; Clements et al. 2000; Ruse et al. 2000). However, *Eukiefferiella* and *Limnophyes*, also Orthocladiinae genera, were less abundant at metal contaminated sites than at reference sites (Wilson 1988; Ruse et al. 2000). Abundances of the Tanytarsini genera *Micropsectra* and *Tanytarsus* were reduced (Yasuno et al. 1985; Chadwick et al. 1986; Ruse et al. 2000), while *Stempellinella* was more abundant (Clements et al. 2000) in metal contaminated environments. Metal sensitivity of other chironomid genera is summarized in Table 1.1.

Species within the same genus may also display markedly different metal sensitivity. In the Arkansas River, *Cricotopus bicinctus* was more abundant at highly contaminated sites than at upstream sites. *Cricotopus infuscatus* was apparently more metal sensitive than *C. bicinctus*, with abundance increasing with distance from the initial metal input (Ruse et al. 2000). Likewise, *Eukiefferiella claripennis* is metal tolerant (Armitage and Blackburn 1985; Gower et al. 1994; Ruse et al. 2000), whereas some undescribed *Eukiefferiella* species are classified as intolerant (Ruse et al. 2000). Chironomid populations within the same species from different environments may also exhibit different metal sensitivities, suggesting a genetic determination of tolerance (Postma et al. 1995a).

Differences in metal sensitivity may in part be attributable to differential exposure through habitat and dietary preferences. For example, contaminants in the water column or bound to fine particulate matter may be more available to the filter-feeding Tanytarsini larvae than other types of chironomids, resulting in the appearance

Table 1.1: Metal tolerant and metal sensitive chironomid genera as determined from prominent metal mining literature in the last 20 years

CHIRONOMID GENERA	METAL SENSITIVITY[1]	REFERENCE
Tanypodinae		
Lersia	Tolerant	Winner et al. 1980
Procladius	Tolerant	Canfield et al. 1994; Diggins and Stewart 1998[2]
Diamesinae		
Pagastia	Tolerant	Chadwick et al. 1986
Diamesa	Tolerant	Chadwick et al. 1986
Chironominae		
Chironomini		
Chironomus	Tolerant	Canfield et al. 1994; Diggins and Stewart 1998[2]
Cryptochironomus	Tolerant	Canfield et al. 1994
Tanytarsini		
Micropsectra	Sensitive	Chadwick et al. 1986
	Moderately Tolerant	Clements et al. 2000
Stempellinella	Moderately Tolerant	Clements et al. 2000
Tanytarsus	Sensitive	Yasuno et al. 1985; Ruse et al. 2000
	Tolerant	Canfield et al. 1994
Orthocladiinae		
Chaetocladius	Tolerant	Gower et al. 1994
Corynoneura	Tolerant	Gower et al. 1994
Cricotopus	Moderately Tolerant	Ruse et al. 2000
	Tolerant	Surber 1959; Winner et al. 1980; Yasuno et al. 1985; Chadwick et al. 1986; Ruse et al. 2000
Diplocladius	Tolerant	Winner et al. 1980
Eukiefferiella	Sensitive	Wilson 1988; Ruse et al. 2000;
	Tolerant	Armitage and Blackburn 1985[2]; Yasuno et al. 1985; Wilson 1988; Holland and Rabe 1992; Gower et al. 1994;
Krenosmittia	Tolerant	Wilson 1988; Ruse et al. 2000
Limnophyes	Sensitive	Ruse et al. 2000
Orthocladius	Moderately Tolerant	Armitage and Blackburn 1985[2]; Clements et al. 2000; Ruse et al. 2000
	Tolerant	Winner et al. 1980; Yasuno et al. 1985; Holland and Rabe 1992
Parametriocnemus	Tolerant	Chadwick et al. 1986
Rheocricotopus	Tolerant	Holland and Rabe 1992
Tvetenia	Moderately Tolerant	Clements et al. 2000

[1]Metal sensitivity classified by author, cited in corresponding reference column
[2]Environment contaminated with trace metals and organic pollutants

of enhanced sensitivity. Similarly, the dominance of Orthocladiinae larvae at metal contaminated sites may be partially attributable to release from competition for food and reduced predation by sensitive chironomids (Surber 1959; Paulsson et al. 2000). Within genera, differences in metal sensitivity may simply be a result of poor taxonomic resolution as evidenced by the difference in sensitivity of *Cricotopus* and *Eukiefferiella* species.

Chironomid populations living in metal contaminated environments have different capacities to deal with accumulated metals, either through enhanced detoxification or storage capacities (Wentsel et al. 1978; Krantzberg and Stokes 1989; Clements 1999; Postma and Groenendijk 1999). Populations of *Chironomus riparius* originating from metal contaminated environments exhibited increased capacity for Cd storage and enhanced excretion efficiency compared to larvae from non-metal contaminated environments (Postma et al. 1996). The induction of metal binding proteins (i.e., heat shock proteins, metallothioneins) may also contribute to enhanced ability to tolerate metal exposure (Yamamura et al. 1983; Seidman et al. 1986; Janssens de Bisthoven et al. 1998b).

Mentum Deformities

Warwick (1988) defined a deformity as any morphological feature that departs from normal configuration. In invertebrates, deformities most likely result from a physiological disturbance in development during the molting process (Janssens de Bisthoven et al. 1992; Vermeulen et al. 1998). The specific mechanisms involved may include mutations, interference with transcription and/or translation, disruption of cell division, and disruption of control proteins during development (Bird et al. 1995).

Metal contaminants are capable of disrupting development in invertebrates, causing deformities. Brinkhurst et al. (1968), studying Great Lakes populations of chironomids, first reported deformities at sites that were polluted with metals and organic contaminants. In 1971, Hamilton and Saether proposed examining chironomid deformity levels as an indicator of contaminant exposure. Since then deformities in the mentum, ligula, mandibles, maxillary palps, and antennae have been observed in many genera inhabiting a variety of environments (Warwick 1988). However, the use of deformities as an indicator of impaired ecosystem quality requires a precise estimation of the incidence of naturally occurring deformities (Burt 1998). Attempts to estimate background levels of deformities are complicated by the ubiquitous distribution of contaminants in our environment. Frequencies reported at "reference" sites range from 0.0 – 48.0 % (Wiederholm 1984b; Dickman et al. 1992; Janssens de Bisthoven et al. 1992; Lenat 1993; Bird et al. 1995; Burt 1998; Groenendijk et al. 1998; Janssens de Bisthoven et al. 1998b; Vermeulen et al. 1998). Warwick (1980a) estimated background deformity frequencies in the Great Lakes as <1.0 % prior to the 1950's and <2.0 % between the 1950's and 1970's. This has led some researchers to use 1 % as a deformity "benchmark", meaning >1 % deformity frequency signals a degraded environment (Wiederholm 1984b; Bird 1994).

The reported frequency of chironomid mouthpart deformities in metal contaminated environments ranges from 0.0 – 82.5 % (Warwick et al. 1987; Canfield et al. 1994; Burt 1998; Diggins and Stewart 1998; Groenendijk et al. 1998; Janssens de Bisthoven et al. 1998 a,b; Vermeulen et al. 1998). However, in many studies the incidence of mentum deformities at metal contaminated sites is below 10 %, and of these studies most incidences are below 5 % (Warwick 1980 a,b; Tennessen and Gottfried 1983; Cushman 1984; Burt 1998). High incidences of deformities are typically

associated with very small sample sizes (Warwick et al. 1987, based on a sample size of 6 individuals) and subjective criteria (Burt 1998; Hamalainen 1999).

Whereas an increased incidence of deformities in chironomid larvae indicates contaminant exposure, the observation of deformities may also act as an indicator of the relative fitness of individual larvae (Cervi 1996; Janssens de Bisthoven et al. 1998b). Elevated concentrations of Pb and Cu have been observed in deformed larvae compared to normal larvae (Janssens de Bisthoven et al. 1992; Janssens de Bisthoven et al. 1998a). In chironomid populations from the River Ijse, Gerhardt and Janssens de Bisthoven (1995) demonstrated reduced emergence success, less locomotion, and increased ventilation in deformed larvae. However in the same study, populations from the River Dommel, a metal contaminated river, showed no differences between deformed and normal larvae. No differences in length, weight and developmental stage of deformed of *in situ* deformed vs. normal larvae were demonstrated by Janssens de Bisthoven et al. (1992) or Janssens de Bisthoven et al. (1998b). However, in the laboratory, deformed larvae developed more slowly and were larger than undeformed larvae (Cervi 1996; Janssens de Bisthoven et al. 1998b). Differences between field-collected deformed and non-deformed larvae appear to be site specific, perhaps related to the history of contamination and the ability of populations to express adaptation (Janssens de Bisthoven et al. 1998b; Postma and Groenendijk 1999).

Trace Metals and Chironomid Larvae

Metal contamination affects chironomid assemblages, resulting in the observation of reduced overall abundance, richness and altered community composition. However, to understand the processes involved in generating these differences one must identify chironomid larvae to higher levels of taxonomic resolution

than the family. Ultimately, metal exposure reduces the survival and growth and development of individual chironomid larvae, which consequently affects the ability of populations to persist (Hare 1992). However, these endpoints are difficult to measure within field populations, and thus chironomids are frequently examined in laboratory experiments instead. The effect of metal exposure will depend on the metal, length of exposure, route of exposure, and various life-history attributes (Wiederholm 1984a; Johnson et al. 1993).

Survival

Metal exposure can elicit acutely toxic effects, reducing survival of chironomid larvae. Different metals exhibit different effects on survival. Anderson et al. (1980) studied a 10-d water-borne exposure of various metals to *Tanytarsus dissimilis* larvae. Cadmium was the most toxic metal (LC_{50}=0.0038 mg/L), followed by Cu (LC_{50}=0.0163 mg/L), Zn (LC_{50}=0.0368 mg/L), and Pb (LC_{50}=0.258 mg/L) (Anderson et al. 1980). Alternatively, Khangarot and Ray (1989) found that *Chironomus tentans* in a 24-h assay of water-borne metal effects was most sensitive to Cu (LC_{50}=0.701 mg/L), followed by Zn (LC_{50}=10.83 mg/L), Cd (LC_{50}=23.25 mg/L), and Pb (LC_{50}=52.87 mg/L). Similarly, different chironomid species may exhibit markedly different responses to metals. In the case of a 24-h water-borne Cu study, *C. plumosus* (LC_{50}=0.698 mg/L, Fargasova 1998) and *C. tentans* (LC_{50}=0.701 mg/L, Khangarot and Ray 1989) were most sensitive and *Polypedilum nubifer* was more tolerant (LC_{50}=7.91 mg/L; Hatakeyama 1988).

Exposure time will affect mortality upon metal introduction. A 10-d water-borne bioassay resulted in a 10-fold decrease in the LC_{50} of *C. tentans* for Cu (0.054 mg/L) and Zn (1.125 mg/L) compared to LC_{50} values for a 24-h exposure (Khangarot and Ray 1989; Phipps et al. 1995). A life cycle exposure of *P. nubifer* to water-borne Cu saw a

1000-fold decrease in the concentration resulting in significant mortality compared to a 24-h exposure (Hatakeyama 1988). Different stages of development will also differ in their sensitivity to metal exposure. First instar larvae of *P. nubifer* were 30 times more sensitive (LC_{50}=2.05 mg/L) than fourth instar larvae (LC_{50}=62.40 mg/L) in a 24-h water-borne Cu exposure (Hatakeyama 1988).

Mixtures of chemicals can also affect the survival of chironomid larvae. Bird et al. (1995) found that sediment contaminated with 35,000 mg/kg Pb caused 100 % mortality in *C. tentans*. No significant differences in larval survival were apparent in sediment contaminated with 0 – 5,000 mg/kg Pb (Bird et al. 1995). However, Harrahy and Clements (1997) reported that significantly lower concentrations of Pb (70 mg/kg) in combination with Cd (5 mg/kg), Cu (10 mg/kg) and Zn (300 mg/kg) resulted in a similar level of mortality. This would suggest the additive or synergistic effects of metal mixtures.

Growth and Development

Laboratory bioassays typically indicate that chironomid larvae reared in the presence of metals exhibit slowed growth and delayed rates of development. Exposure of *C. riparius* to low levels of Cd (0.010-0.15 mg/L) and Zn (0.1-1.0 mg/L) resulted in slowed development, with the larvae spending more time in the first instar (Timmermans et al. 1992a; Pascoe et al. 1989). With Cd exposure, time spent in the fourth instar was much shorter than non-metal exposed larvae (Timmermans et al. 1992a).

As a result of slower growth, larvae exposed to metals had significantly reduced length and biomass compared to control larvae. Larvae inhabiting Cu contaminated sediment (1602 mg/kg) were 50% smaller than larvae reared in uncontaminated sediment (Kosalwat and Knight 1987). Larvae exposed to a mixture of Cd (1030

mg/kg), chromium (1640 mg/kg), and Zn (17300 mg/kg) in sediment were 55% smaller in terms of length and 93 % smaller in terms of biomass than larvae inhabiting uncontaminated sediment (Wentsel et al. 1977b). Biomass was significantly less when larvae were exposed to water-borne Cu (0.64-2.56 mg/L) and Cd (1.0-10 mg/L) (Hatakeyama and Yasuno 1981; Heinis et al. 1990).

Time to emergence is also affected by metal exposure. Zinc (0.1-1.0 mg/L) exposure retarded adult emergence of *C. riparius*, whereas Cd exposure (0.010-0.025 mg/L) caused larvae to emerge sooner than controls (Timmermans et al. 1992a). Exposure to low concentrations of Cd (0.002-0.006 mg/L) delayed emergence of *C. riparius* by 3 to 4 d whereas exposure to 0.016 mg/L delayed emergence by more than 10-d (Postma et al. 1994). Copper concentrations (1,800 mg/kg sediment) delayed emergence (Kosalwat and Knight 1987). Hatakeyama and Yasuno (1981) reported that 0.16-0.64 mg/L of Cu delayed the onset of emergence from one to seven d relative to controls (Hatakeyama and Yasuno 1981).

Reproductive Success

The ability to mate successfully and produce viable egg masses is imperative to survival. Studies of the reproductive success of metal-exposed chironomids have given variable results. Few studies have demonstrated an observable effect (Hatakeyama and Yasuno 1981). For example, rearing *C. riparius* larvae in water containing 0.002-0.016 mg/L of Cd did not affect the fecundity of emerging females (Postma et al. 1994). Similarly, Cu exposure had no effect on the oviposition success or hatchability of egg masses of *Chironomus decorus* (0.1-5 mg/L) and *Polypedilum nubifer* (0.010-0.040 mg/L) (Kosalwat and Knight 1987; Hatakeyama 1988). However, Cu (0.37 mg/L) did reduce the number of eggs produced per *Paratanytarsus parthenogeneticus* female

(Hatakeyama and Yasuno 1981).

Trace Metal Bioavailability: Routes of Uptake

Biological responses of Chironomidae to metal contamination are ultimately determined by metal bioavailability and uptake (Luoma and Carter 1991; Hare 1992). Bioavailability is the proportion of contaminant present in the environment in a form(s) that can be taken up by organisms and potentially cause adverse effects (Hare 1992; Plette et al. 1999). Reliably relating metal concentrations in chironomid larval tissues to concentrations in the environment is critical if chironomids are to be effective biomonitoring tools (Hare and Tessier 1996).

In aquatic environments, metals are distributed among the water column, biological matter, and sediment (Tessier and Campbell 1987; Luoma 1989; Amyot et al. 1994). Within each of these compartments, metals exist as free ions and in various complexes, resulting in complex metal speciation (Fig. 1.3). For example, Florence (1977) found that Cu was predominantly associated with organic matter, Pb with both organic and inorganic matter, Zn was present as both a free ion and bound to inorganic matter, and Cd was present as a free ion. Factors affecting the bioavailability of metals include such physical characteristics as pH, salinity, alkalinity and dissolved organic carbon concentrations of water (Luoma 1989) and chemical properties of the elements (Krantzberg 1989). Biological processes such as metal uptake by aquatic organisms will also affect metal distribution among environmental compartments (Luoma 1989; Hare 1992; Rainbow and Dallinger 1993; Bervoets and Blust 1999).

Metals can be taken up through direct absorption of dissolved metal species across gill membranes or body surfaces, or by ingestion of particulate matter to which metals are bound (Hare 1992). Both modes ultimately contribute to adverse effects

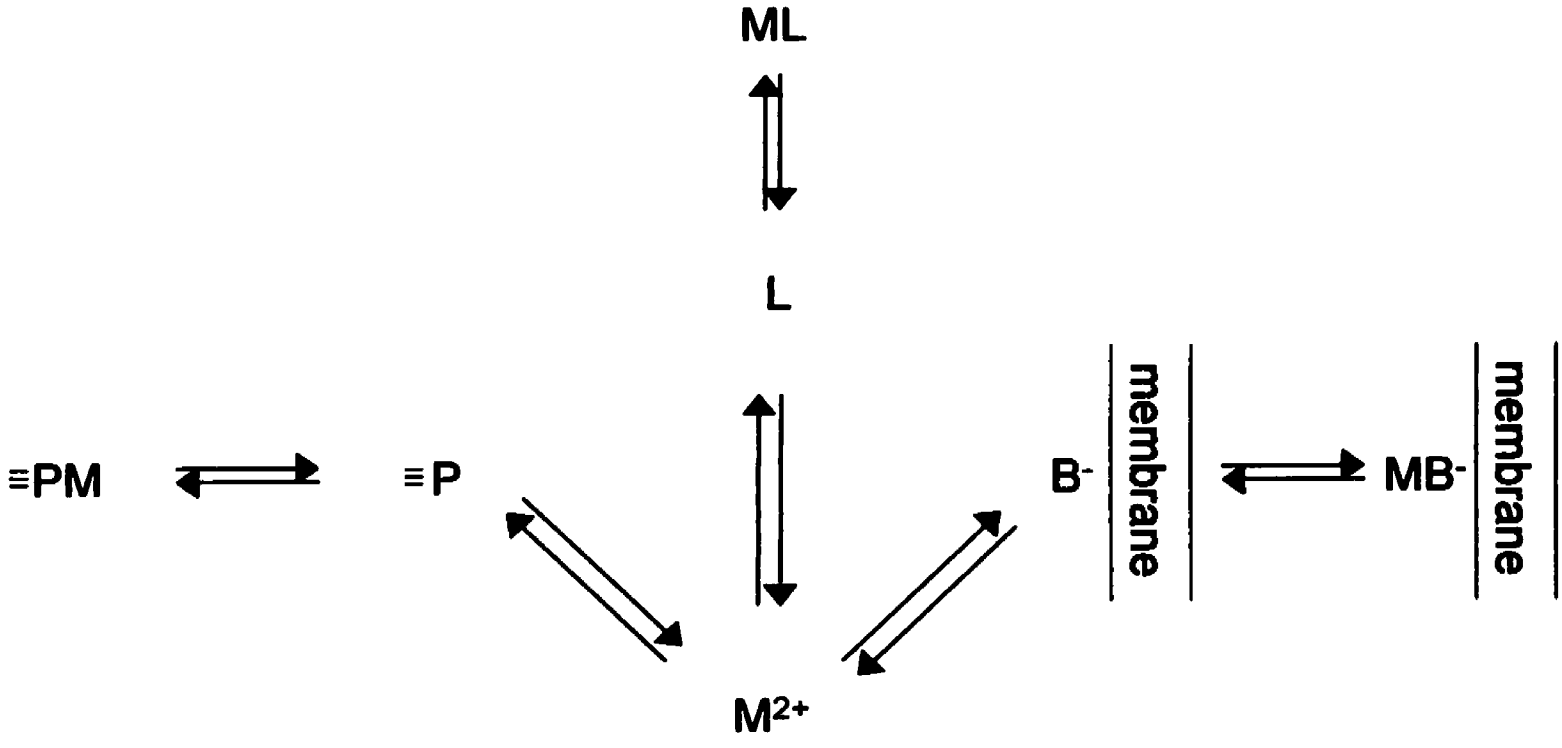

Figure 1.3: Metal bioavailability in aquatic environments, where M represents the metal as a free ion (M2+), or in association with ligands (L), particle matter (P), or biological membranes (B) (from Hare 1992).

(Luoma 1989; Hare 1992; Rainbow and Dallinger 1993; Bervoets and Blust 1999). The relative amounts of metal uptake from water and food are primarily determined by the metal concentration in each medium. However, uptake from water can also depend on the rate of water movement across the body, surface area for adsorption, and the ease with which absorbed metals transverse biological membranes (Hare 1992). Likewise, metal uptake is determined by the size of the food particles, strength of metal association, the rate of food moving across the gut, pH of the gut and presence of enzymes in the gut (Hare 1992). Knowledge of the relative importance of each of these routes contributes to an understanding of metal accumulation and our ability to predict fate and effects of metals in aquatic systems (Hare 1992; Luoma et al. 1992; Hare and Tessier 1996; Roy and Hare 1999).

Water Sources of Metals

Although both water-borne and dietary exposure contribute to metal uptake in aquatic organisms, most research has been devoted to the investigation of aqueous metal exposure, implying that this route is most significant (Anderson et al. 1980; Hatakeyama and Yasuno 1981; Kosalwat and Knight 1987; Hatakeyama 1988; Postma et al. 1994). Accordingly, environmental quality guidelines for the protection of aquatic life are derived from toxicity tests of water-borne contaminants. The role of other routes of exposure has been neglected. Thus, environmental guidelines potentially underestimate the integrated exposure of organisms in the field (Schlekat and Luoma 2000).

Toxic effects of metals have been most commonly attributed to free metal ions, whereas bound metal complexes were considered to be less toxic (Pagenkopf et al. 1974; Anderson et al. 1978; Allen et al. 1980). The free ion activity model (Morel 1983)

approximates water borne metal exposure to this toxicant form. This model proposed that for aquatic organisms obtaining metals from water, bioaccumulation or toxicity is best predicted by the concentration (activity) of the free metal ion (Morel 1983). This model has been successfully applied to Cd and Zn bioavailability (Sundra et al. 1978; Engel and Flower 1979; Allen et al. 1980; De Lisle and Roberts 1988; Blust et al. 1992; Hare and Tessier 1996). For example, Cd accumulation of the phantom midge, *Chaoborus*, is most significantly correlated to dissolved water concentrations in Canadian Lakes (Hare and Tessier 1996).

However, until recently, other routes of exposure have received little attention (Reinfelder et al. 1998). This has resulted in confusion over how significant water exposure is and what role it plays in bioaccumulation. Even in instances where the free ion model has been successfully applied, doubt remains as to the interpretation of these results. Hare and Tessier (1996) suggested that the bioaccumulation of *Chaoborus* fit the free ion model not because it directly experienced exposure through water but perhaps their prey accumulate metals directly from the water.

Dietary Sources of Metals

Metals bind preferentially to organic and inorganic matter (Schlekat and Luoma 2000). As a result, metal concentrations in food resources tend to be orders of magnitude higher than concentrations in water (Schlekat and Luoma 2000). Organisms feeding on a contaminated diet are thus exposed to elevated concentrations of metal (Hare 1992). Food and particulate matter may be a much more important source of metals than water for some animals (Woodward et al. 1994; Munger and Hare 1997; Lee et al. 2000).

Dietary uptake of Cd, Cu, Pb and Zn is an important pathway for metal accumulation in fish and invertebrates. In marine environments, benthic-feeding flounder and plaice incur significant metal uptake from their food (Pentreath 1973a,b; Hoss 1964). For the planktonic-feeding phantom midge, *Chaoborus*, metal uptake from water was insignificant compared to that taken up through diet (Munger and Hare 1997). Crane fly larvae (*Tipula*), take up over 50 % of their body burden of metals through diet (Elwood et al. 1976). Dietary uptake is also significant in chironomid larvae (Pascoe et al. 1990). In *Chironomus thummi*, 63 – 81% of Cd was taken up through diet (Seidman et al. 1986). Significant metal uptake from dietary sources, highlights the possibility that metals could be transferred from one trophic level to another and potentially biomagnified (Luoma et al. 1992; Fisher and Reinfelder 1995; Wang et al. 1996; Munger and Hare 1997).

Feeding strategy plays a role in relative amounts of metal uptake among benthic invertebrates. Filter-feeding and deposit-feeding invertebrates accumulate more metals than predatory invertebrates (Goodyear and McNeill 1999), perhaps due to higher metal concentrations in their food sources (Smock 1983b; Lee et al. 2000).

Periphyton

In shallow, lotic environments, periphyton coats the upper surfaces of substrata, forming the base of the food web (Lock et al. 1984). Periphyton is an assemblage of algae, bacteria, fungi and detritus attached to hard substrates (Newman and McIntosh 1989; Ledger and Hildrew 1998). Light penetration (Hill et al. 1995), nutrient concentrations (Grimm and Fisher 1986), temperature (Darley 1982), flow (Peterson and Stevenson 1992), substratum composition (Bott 1983), disturbance (Biggs and

Thomsen 1995) and pH of the water (Maurice et al. 1987; Ledger and Hildrew 1998) determine the amount and type of periphyton growth.

The presence of metals can also affect periphyton growth. Reductions in periphyton abundance (Leland and Carter 1985; Genter and Lehman 2000) and the number of algal taxa present in the periphyton community have been observed in association with metal contamination of streams (Austin and Deniseger 1985; Leland and Carter 1985; Genter and Lehman 2000). Community composition in metal exposed streams shifts from diatoms, typical of reference sites, to green algae and cyanobacteria. Unicellular green algae dominate in highly contaminated environments (Patrick 1978; Austin and Deniseger 1985; Genter and Lehman 2000; Soldo and Behra 2000). Algae exhibit a range of sensitivity to metals (Newman and McIntosh 1989). Metal-sensitive species include *Diatoma vulgare* and *Melosira varians* (Medley and Clements 1998). Metal tolerant taxa include *Achnanthes minutissima*, *Chamaesiphon subglobosus*, *Fragilaria vaucheriae*, *Navicula cryptocephala*, *Nitzschia palea*, *Oocystis* spp., *Phormidium* spp. and *Stigeoclonium aestivale* (Austin and Deniseger 1985; Leland and Carter 1985; Takamura et al. 1990; Soldo and Behra 2000). Dominance of green algae, a less palatable food resource, in metal contaminated environments may result in a shortage of food resources of high nutritional quality (Newman and McIntosh 1989).

In metal-contaminated environments, periphyton readily accumulates contaminants through both active uptake and adsorption. Due to its sessile nature, periphyton can provide a time-integrated estimate of contaminant exposure, acting as a surrogate to sediment analysis (Newman and McIntosh 1989). Periphyton exhibit high bioconcentration factors for many trace metals (Trollope and Evans 1976; Patrick and Loutit 1977; Johnson et al. 1981), accumulating metals at concentrations up to 100x

higher than observed in surrounding water (Table 1.2).

Benthic invertebrates that rely on periphyton as a food source may ingest high trace metal concentrations (McIntosh 1991; Beltman et al. 1999). Invertebrates associated with periphyton can accumulate metals by feeding, adsorption, and absorption through gills (Selby et al. 1985). Kiffney and Clements (1993) argued that invertebrates in the Arkansas River accumulated Cd, Cu, and Zn primarily through diet, as indicated by the correlation between bioaccumulated metals and levels in periphyton. Aquatic organisms ingesting metal-contaminated periphyton can assimilate between 11 and 88% of Cd, and 16 and 88% of Zn (Wang and Fisher 1999). This indicates that trophic transfer of metals from periphyton to its consumers occurs and suggests that periphyton concentrations can indirectly provide an estimate on potential effects on herbivores, and ultimately the structure and functioning of the aquatic ecosystem (Johnson et al. 1978; Friant and Koerner 1981; Ramelow et al. 1987; Newman and Macintosh 1989).

Benthic Invertebrates

Little information exists on the transfer of metals from invertebrates to higher trophic levels. What does exist supports the idea that substantial trophic transfer occurs, making diet an important means of metal uptake in predatory invertebrates, benthivorous fish, and avian predators. Rainbow trout fed metal-contaminated benthic invertebrates experienced significant reductions in survival and growth, and histopathological abnormalities relative to controls (Woodward et al. 1994). Conversely, Miller et al. (1992) found no correlation between metal concentrations in field collected invertebrates and white suckers. However, no distinction was made in metal concentrations among the invertebrates that would normally be a part of the diet of

Table 1.2: Reported levels of metals in periphyton from mine drainage receiving and reference sites. Concentrations are expressed as a mean (± 1 SE, n) or a range of concentrations observed. Bioconcentration factor = ratio of metal concentration in periphyton : metal concentration in water at mine drainage receiving sites

LOCATION	METAL	REFERENCE PERIPHYTON CONCENTRATION (ug/g)	METAL EXPOSED PERIPHYTON CONCENTRATION (ug/g)	CONCENTRATION FACTOR	CITATION
Arkansas River, Colorado[1]	Cd	<10 (n = 1)	<10 – 30 (n = 5)	10	Kiffney and Clements 1993
	Cu	<50 (n = 1)	<50 – 125 (n = 5)	1	
	Zn	<2,000 (n = 1)	<1,000 – 5,000 (n = 5)	1	
Arkansas River, Colorado	Cd		24 – 80 (n = 3)	100	Clements and Kiffney 1994
	Cu		70 – 375 (n = 3)	100	
	Zn		5,000 – 17,000 (n = 3)	100	
Blackbird Mine, Idaho	Cu	0 (n = 3)	0 – 3370 (n = 7)	1	Beltman et al. 1999
Coromandel Peninsula, New Zealand	Cd	0.4 – 1 (n = 2)	0.03 – 4 (n = 5)	1	Hickey and Clements 1998
	Cu	10 – 11.5 (n = 2)	20 – 100 (n = 5)	100	
	Pb	1 – 1.1 (n = 2)	2 – 200 (n = 5)	10	
	Zn	200 (n = 2)	40 – 100,000 (n = 5)	100	
Heath Steele Mine, New Brunswick	Cd	1.65 (0.69, n = 6)	16.71 (6.56, n = 10)	100	BEAK 1998
	Cu	8.28 (2.59, n = 6)	682.3 (173.2, n = 10)	10	
	Pb	17.60 (7.58, n = 6)	415.3 (118.01, n = 10)	100	
	Zn	367.83 (189.00, n = 6)	4,791 (1,353, n = 10)	100	
North Fork Iron Creek, Idaho	Cd	<0.5 (n = 2)	<0.5 – 2 (n = 4)	1	Genter and Lehman 2000
	Cu	<40 (n = 2)	20 – 550 (n = 4)	10	
	Pb	0 – 4.5 (n = 2)	0 – 10 (n = 4)	1	
	Zn	7.5 – 35 (n = 2)	5 – 55 (n = 4)	1	

[1]Data presented from August 1991 sampling

Table 1.2: Cont'd

LOCATION	METAL	REFERENCE PERIPHYTON CONCENTRATION (ug/g)	METAL EXPOSED PERIPHYTON CONCENTRATION (ug/g)	CONCENTRATION FACTOR	CITATION
Shillong, Meghalaya State, India[2]	Cd	0.21 (0.07, n = 1)	1.16 (0.06, n = 1)	1	Gupta 1996
	Cu	22.7 (4.9, n = 1)	113.1 (51.9, n = 1)	10	
	Pb	14.3 (3.5, n = 1)	26.0 (5.2, n = 1)	1	
	Zn	38.3 (10.0, n = 1)	109.2 (40.9, n = 1)	10	
Various Rivers, Colorado	Zn	7.3 (0.6, n = 8)	542.6 (51.8, n = 9)[3]	1	Medley and Clements 1998

[2]Data presented from metal concentrations in algae only
[3]Data presented from zinc concentrations at most highly contaminated sites

white suckers and those that would not. In the megalopteran *Sialis velata*, Cd uptake occurred primarily through consumption of *Cryptochironomus* larvae (Roy and Hare 1999). *Sialis* subsequently assimilated and stored in gut tissues approximately 50% of the metal content of the chironomid larvae (Roy and Hare 1999). Caddisflies and predatory water mites also accumulated much of their Cd content through consumption of benthic invertebrate prey, while Zn was taken up predominantly from water (Timmermans et al. 1992b).

STUDY SITES AND EXPERIMENTAL DESIGN

Metal Mining in New Brunswick

Background

A series of uplifting and folding events of continental and oceanic rocks, intrusion of igneous bodies, and erosion ending in the Palaeozoic era formed the area of partially eroded mountains of the Appalachian region (Environment Canada 1995). As a result of these processes, the geology of New Brunswick is comprised of a rich tapestry of rock types and landscapes. The bedrock of the Appalachian region is primarily sedimentary with some intrusive and extrusive igneous rocks (Stanley 1986). Ore deposits in northern New Brunswick are described as volcanogenic massive sulphides, resulting from submarine volcanic activity (Eckstrand 1984; Guilbert and Park 1986). "Massive sulphides" are rocks primarily composed of metallic minerals associated with sulphur. Ore minerals commonly found in northern New Brunswick include chalcopyrite (Cu ore), galena (Pb ore), pyrite (Fe ore), and sphalerite (Zn ore) (Eckstrand 1984; Guilbert and Park 1986). Similar deposits are found in Flin Flon, Manitoba and Timmins, Ontario (Environment Canada 1995).

While base metal mining occurred for many years across New Brunswick, the 1950's brought the discovery of a massive sulphide deposit in the northeastern region of the province. A flurry of mining activity resulted, making the region one of the world's largest Zn producers. Currently, the annual value of metal production in New Brunswick is estimated at more than $600 million and constitutes approximately 70 % of the total value of mineral production in the province (Natural Resources and Energy 1998). This represents a substantial source of revenue for the province and is a foundation of the local economy.

Metal Mining Processes

Ore can be extracted from surface or underground deposits. Surface, or open pit, mines extract ore that is close to the surface while underground mines extract deeper and more irregularly shaped ore bodies (Environment Canada 1995). Rock that is of lesser ore content is extracted to reach the ore body, and is referred to as waste rock. While both surface and underground mining methods produce waste rock, more is generated through surface mining (Environment Canada 1995). Frequently, waste rock was used for constructing roads, rail beds and other infrastructure on the mine site. Extracted ore is milled to remove valuable minerals from those of no economic value. This is accomplished through crushing, grinding and separation processes. Tailings are waste products generated during the milling process (Environment Canada 1995).

Environmental Effects on Aquatic Ecosystems

Much waste is generated during the metal mining process. Groundwater enters both open pits and underground shafts, and must be pumped out (i.e., mine de-watering). Water must also be provided for drilling, dust suppression, and other uses

and subsequently removed from the mine (Environment Canada 1995). Other waste products are generated from waste rock, process water in milling, and transport of tailings. Much effort has been expended to minimize the effects of metal mining on aquatic ecosystems that receive effluent. All mines employ some sort of water treatment facility to treat liquid effluents. Tailings are treated by passing water through a sequence of tailings ponds that accumulate precipitated metal hydroxides generated through lime addition. However, many mines still substantially affect aquatic ecosystems through the release of untreated tailings effluent and from acid mine drainage generated when surface water percolates through waste rock piles (Environment Canada 1995).

Acid mine drainage (AMD) or acid rock drainage, is generated by the oxidation of sulphides, producing sulphuric acid (Mills 1995). Both oxygen and water are required for AMD to occur. Metallic minerals (i.e. chalcopyrite, galena, pyrite, or sphalerite) are present as trace quantities in waste rock and may thus be a major constituent of acid mine drainage. Acid mine drainage can depress pH in receiving aquatic ecosystems and provide a source of trace metals. Many mining facilities have attempted to gather waste rock and collect run-off water for treatment. However, this has been difficult due to the diffuse dispersal of waste rock on mine sites (Environment Canada 1995).

Study Objectives

The objectives of this project were to evaluate the effect of metal contamination on field collected and laboratory cultured chironomids and project the potential effect on populations, communities and the ecosystem. Specifically, my goals were to:

1). determine the effect of metal enrichment on natural chironomid communities through the examination of community richness and composition and the incidence of mentum deformities; and

2). examine the effect of metal-enriched periphyton, collected in the vicinity of metal mining facilities, on survival, growth and development, and incidence of mentum deformities on the midge *Chironomus riparius* in the laboratory.

Examination of chironomid communities for changes in richness, community composition, and incidence of deformities at mine drainage receiving and reference sites documented the association of these biological responses with metal enrichment. The role of metal contaminated periphyton in bringing about changes in survival, growth and incidence of deformities in *Chironomus riparius* established its role in contributing to adverse effects associated with metal contamination. Therefore, the role of periphyton as a substantial route of metal uptake for primary consumers, contributing to the observance of individual, population and community effects was examined.

Metal Mining Facilities

All of the mines examined during this study are located in northeastern New Brunswick (Fig. 1.4) and belong to the "Bathurst Mining Camp". All the mines in this study were in existence prior to the establishment of Metal Mining Liquid Effluent Regulations (Fisheries Act 1977), and are not required to meet the standards set by this regulation. However, Metal Mining Liquid Effluent Guidelines (MMLEG) have been established for these mines, setting objectives for desired liquid effluent quality (Environment Canada 1995).

I examined biota in streams receiving AMD from 5 separate mines, located on different lotic systems. Mines were chosen according to ore deposits exploited (base

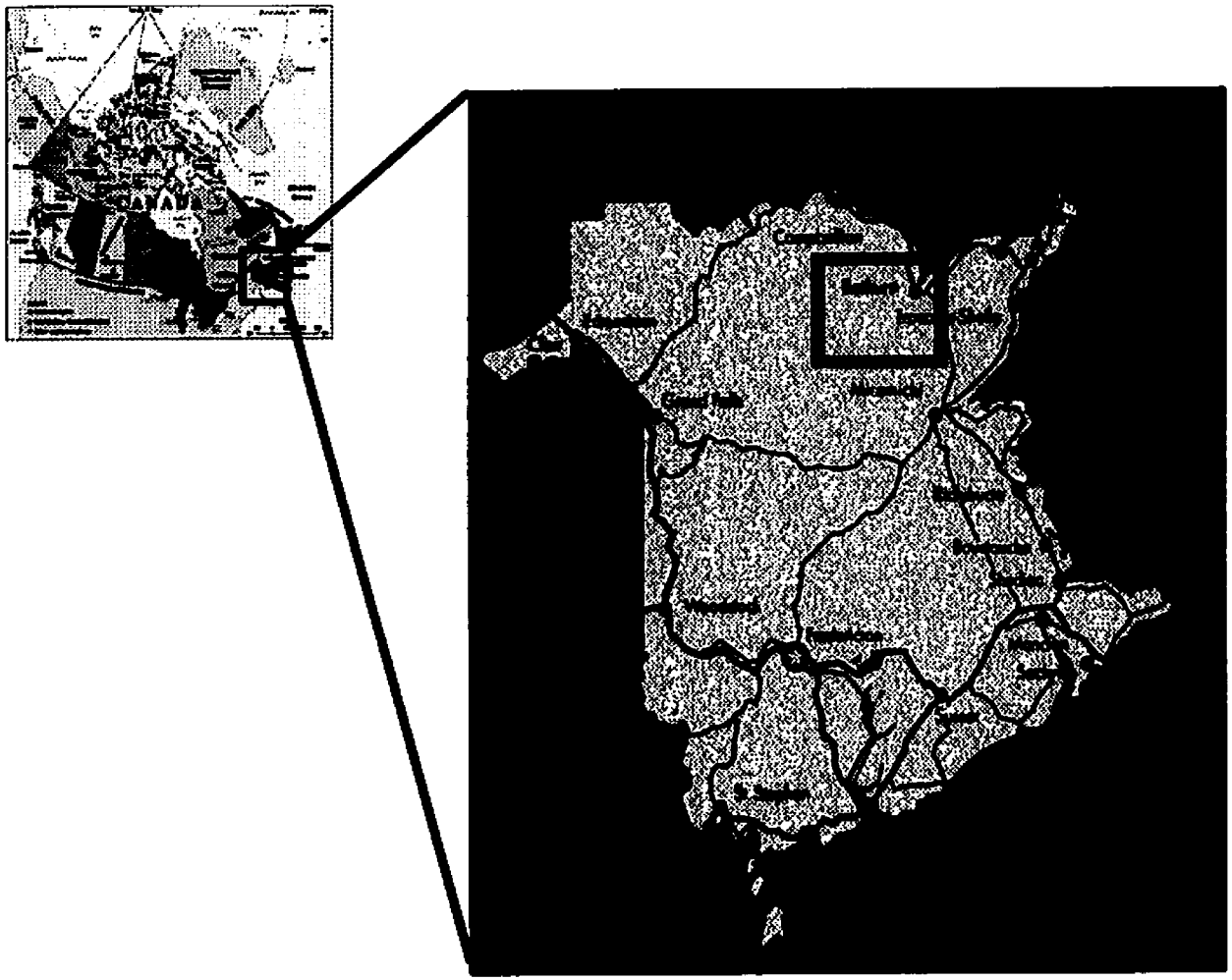

Figure 1.4: Map of New Brunswick, highlighting the general location of metal mining facilities included in this study

metal mines), receiving aquatic environments (mid-sized streams, cobble substrates), accessibility, and proximity to other similar mining facilities.

Heath Steele Mine

Heath Steele Mine (Noranda Mining Exploration Inc.), located approximately 47 km NW of Miramichi, NB (NBGIC 1997), lies in the drainage basin of the Tomogonops R. The Tomogonops R. subsequently enters the Northwest Miramichi R. 22-km downstream of Heath Steele Mine (Fig. 1.5). This mine exploits complex massive sulphide ores found in the volcanic and sedimentary rock of the Tetagouche Group. Exploration began at this site in 1953, and mining began in 1955 using a combination of surface and underground mining techniques (Environment Canada 1995). Zinc, Cu and Pb were the primary minerals extracted, and at peak production 2,700 t/d of ore were processed (Environment Canada 1995). Shut down periods have occurred from 1958-1960, 1983-1989, and June 1993-October 1994 (Fig. 1.6). Recently, the mine was decommissioned, with the ore processing ending in 2000 (BEAK 1994, 1998; Environment Canada 1995).

Final effluent from the tailings pond, discharged to the South Branch Tomogonops R., has usually met MMLEG objectives (Environment Canada 1995). Acid mine drainage continues to seep from waste rock piles and site infrastructure and enters the Little South Branch and the South Branch Tomogonops R. (Environment Canada 1995). This has resulted in extensive aquatic effects downstream, including elevated water and sediment concentrations of Zn, Pb, and Cu, reduced fish abundance, and reduced density and diversity of benthic communities (BEAK 1994, 1998).

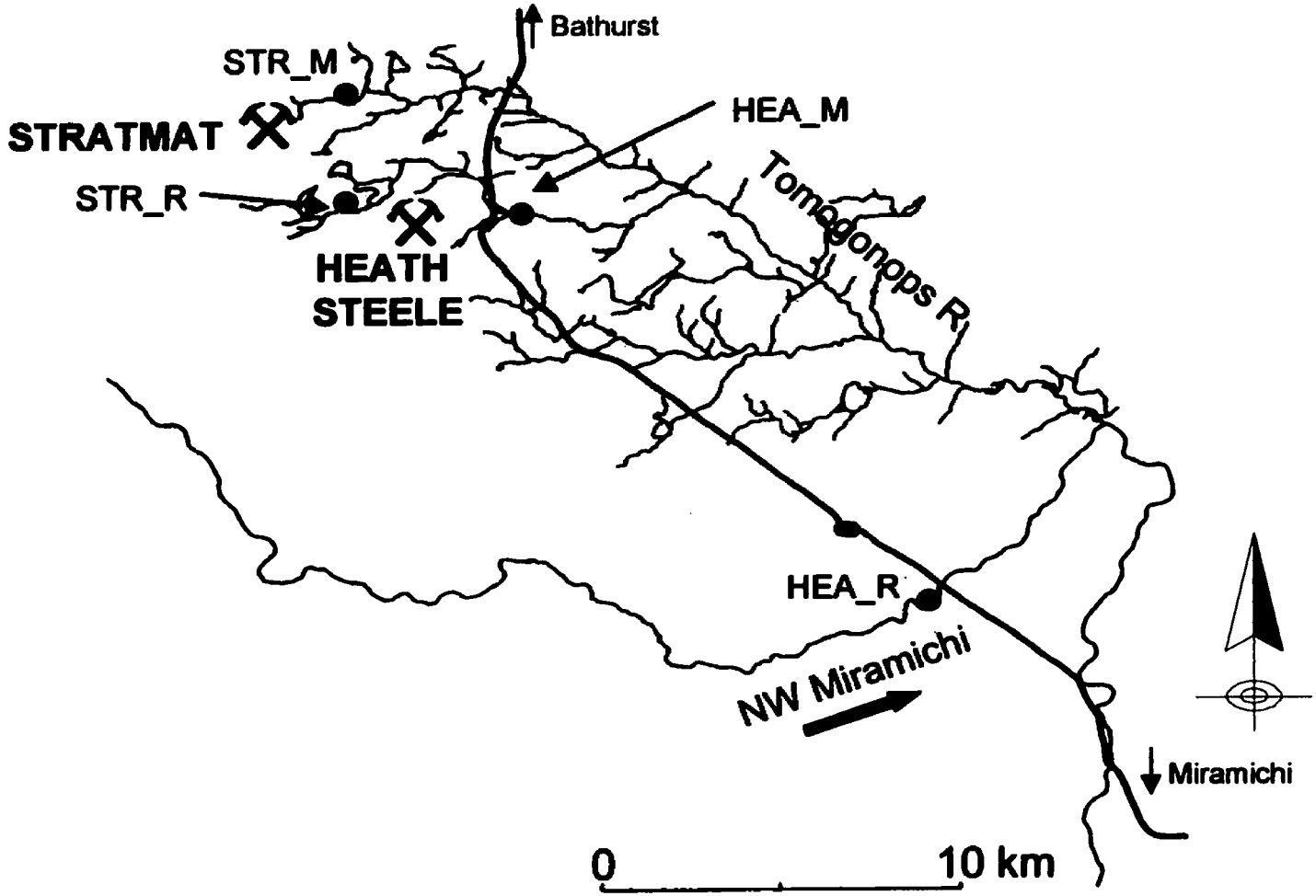

Figure 1.5: Location of Heath Steele and Stratmat Mines and corresponding sampling sites (HEA_R, HEA _M; STR_R, STR _M).

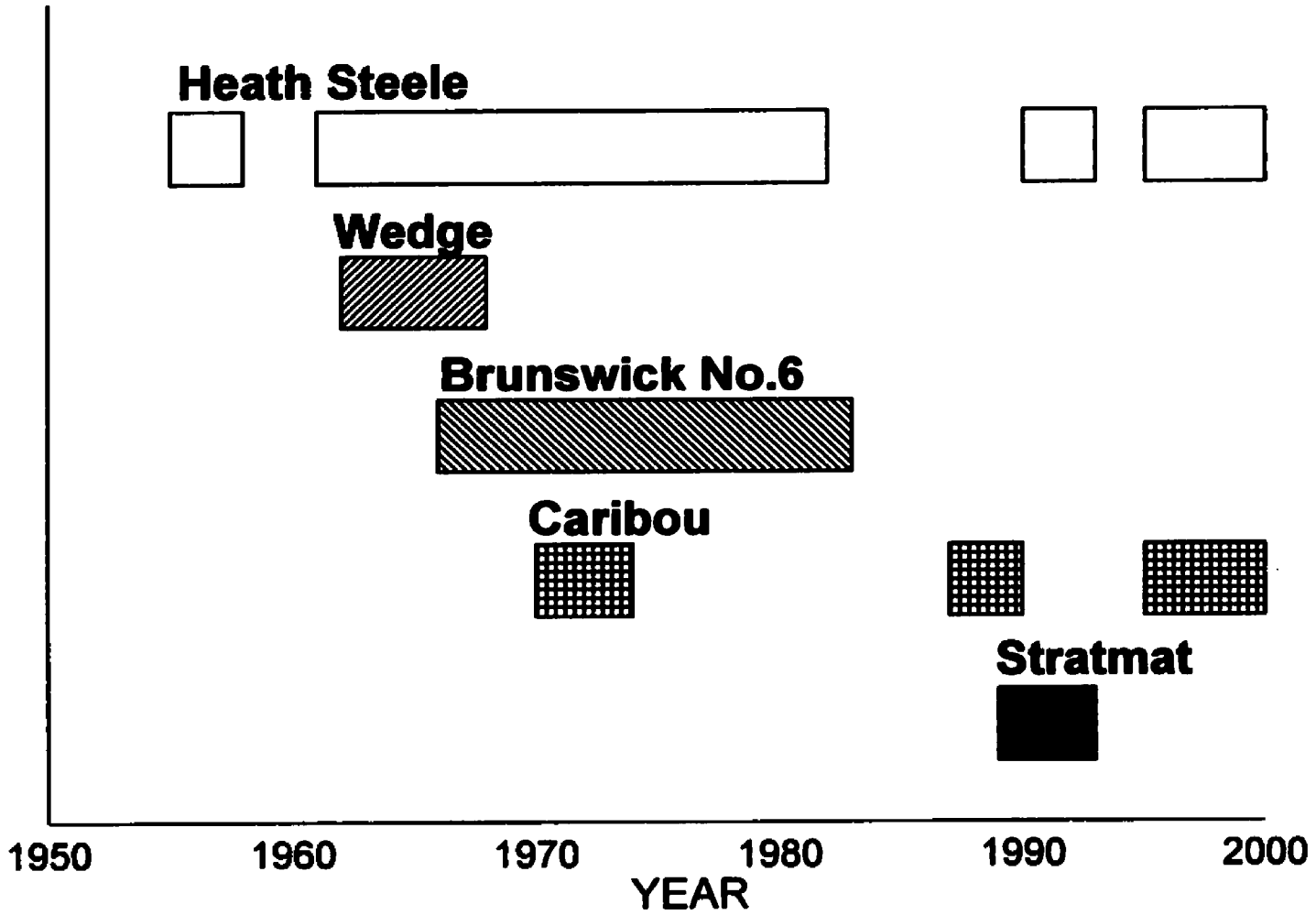

Figure 1.6: History of active metal mining at mines in northeastern New Brunswick

Stratmat Mine

Stratmat Mine (Noranda Mining Exploration Inc.) is located approximately 5 km NW of Heath Steele Mine (NBGIC 1997) and also lies in the drainage basin of the Tomogonops R. (Fig. 1.5). All ore extraction (primarily Zn, Cu and Pb ores) occurred through surface mining. Stratmat was developed in 1987-1988, and came into operation in 1989. This operation closed in 1993 (Fig. 1.6) (Environment Canada 1995).

Acid mine drainage has routinely been a problem at this site. Seepage enters the North Branch Tomogonops R. via Mosquito Pond and Mosquito Brook. While the effects of this seepage are considered minor and localized, elevated Zn concentrations in water, reductions in the abundance of fish populations, and alterations of the benthic community have been observed (BEAK 1994, 1998).

Brunswick Mining and Smelting (No. 6)

Brunswick Mine (No. 6) (Noranda Mining Exploration Inc.), located approximately 29 km SW of Bathurst, NB (NBGIC 1997), lies in the drainage basins of Knight and Austin brooks (Fig. 1.7). Both of these brooks subsequently enter the Nepisiguit R. This mine exploits complex massive sulphide ores. Open pit mining at Brunswick No. 6 began in 1966 and continued until 1983 (Fig. 1.6). Over 12 million t of Cu, Zn, Pb and silver ores were produced during its period of operation (Environment Canada 1995).

Mine water was discharged to Knight Brook (Environmental Protection Service 1974). Acid mine drainage from on-site waste rock piles enter both Knight and Austin brooks (Environmental Protection Service 1974). Historical effects at Knight Brook have included the elimination of a resident fish population and survival of only a very limited benthic community (Environment Canada 1995). More recently (1993), Atlantic

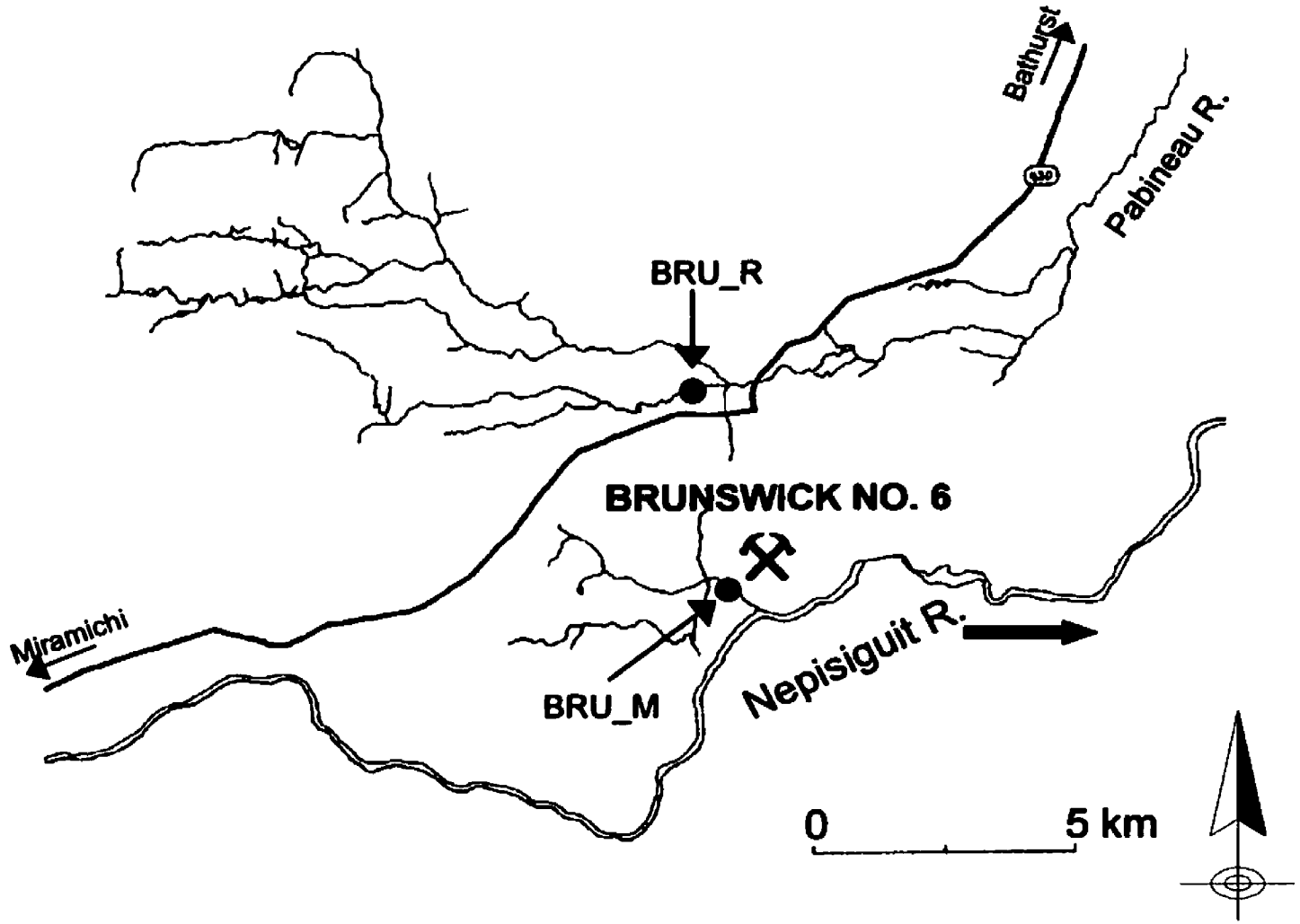

Figure 1.7: Location of Brunswick No. 6 Mine site and corresponding sampling sites (BRU_R, BRU_M).

salmon have been observed spawning to a limited degree in the lower section of Knight Brook (Environment Canada 1995). No information on the effects of AMD on Austin Brook is available. Notably, the Austin Brook Fe mine was also located in the Austin Brook drainage basin. It operated from the early to mid-1900s (Environment Canada 1995).

Wedge Mine

Wedge Mine (Cominco) is located on the northern bank of the Nepisiguit R., approximately 11 km NW of Heath Steele Mine and 26 km NW of Brunswick No. 6 (Fig. 1.8) (NBGIC 1997). Mining began at this site in 1962 and continued until 1968 (Fig. 1.6). At peak production, ore was extracted from an underground shaft at a rate of 750 t/d (Montreal Engineering Company 1971; Environmental Protection Service 1974).

Mine related effects on receiving waters were minimal at this site. This was due in part to the extraction process employed, the brief length of operation, and the fact that no ore processing was done on site. However, in an attempt to improve safety at the closed/abandoned site the underground shaft was collapsed unsuccessfully. Dynamite blew a hole in the side of the shaft, releasing water being held in the shaft into the Nepisiguit R. Fish kills were reported. However, the extent of damage incurred by the benthic communities of the Nepisiguit River was unknown. Limited quantities of AMD continue to leave the site and drain into the Nepisiguit River (George Lindsay, Environment Canada, pers. comm.). No information is available on effects in the receiving stream.

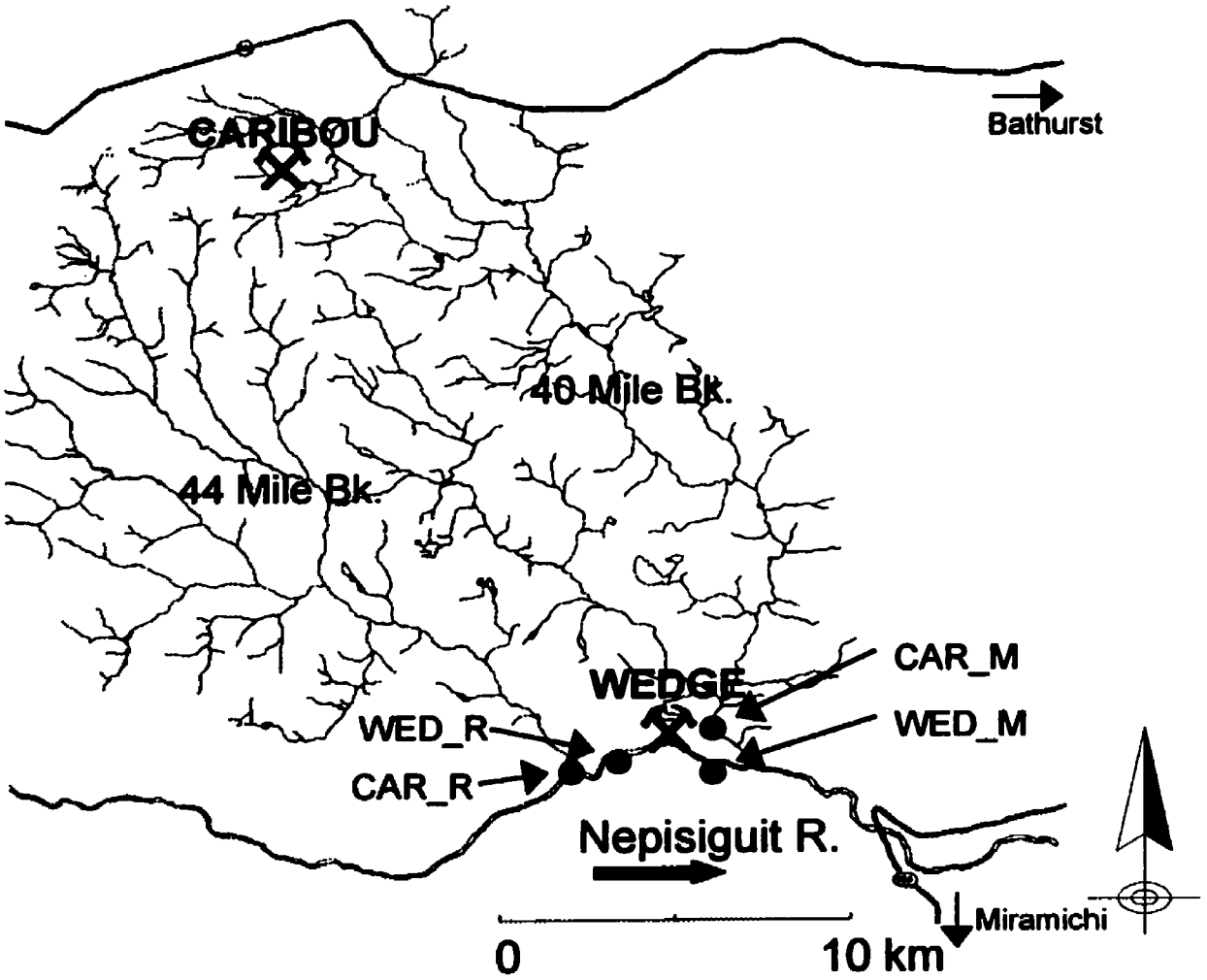

Figure 1.8: Location of Caribou and Wedge Mines and corresponding sampling sites (CAR_R, CAR_M; WED_R, WED_M).

Caribou Mine

Caribou Mine (Breakwater Resources Ltd.) is located 48 km W of Bathurst, NB (NBGIC 1997), and lies in the watershed of 40 Mile brook (Fig. 1.8). Forty Mile brook enters the Nepisiguit R. 35-km downstream of Caribou Mine. Caribou Mine actively mined Pb and Zn ores for a few years in the 1970's, again in the late 1980's, and re-opened recently (1995) (Fig. 1.6). Ore extraction occurred through both open pit and underground shaft (Montreal Engineering Company 1971; Environmental Protection Service 1974).

Tailings pond effluent, contaminated site run-off, and seepage from waste rock piles enters 40 Mile brook. Water quality is seriously degraded downstream of the mine, with Zn concentrations in water elevated more than 300 times the upstream concentration (Environment Canada 1995). Water quality 35 km downstream still does not meet Canadian Water Quality Guidelines for the protection of aquatic life (Environment Canada 1995). No information is available on the impacts on fish and benthic communities.

Experimental Design

This study was designed to examine the effects of metal enrichment from metal mining facilities on the chironomid community. Field biomonitoring studies frequently examine community composition upstream and downstream of contaminant input. Communities at upstream and downstream sites, prior to contaminant input, are assumed to be quite similar, considering they are living in similar habitats (Kiffney and Clements 1993). Therefore, differences in community composition downstream of a contaminant introduction are attributed to the contaminant. However, biological processes occurring at an upstream site can influence the distribution and abundance

of organisms at downstream sites, making these sites spatially dependent (Hurlbert 1984).

This study employed a paired comparison approach. Five different mine facilities on four different rivers (Heath Steele and Stratmat Mines are both on the Tomonogops R.) were identified. At each mine, a site suspected of receiving mine drainage was selected as a replicate of metal enrichment (Table 1.3). A paired reference, or non-metal enriched, site was chosen for each mine drainage receiving site. The Wedge Mine site was the only location sampled, whose reference site was upstream on the same water body. Reference sites were paired to a particular mine drainage receiving site according to characteristics of substrate, canopy cover, stream size, and water velocity. Reference sites were chosen from a pool of approximately 15 sites, identified from previous biomonitoring reports (Environmental Protection Service 1974; BEAK 1994; BEAK 1998) or from topographic maps (NBGIC 1997).

All mine drainage receiving sites were upstream of tailings effluent release, with the exception of the Caribou mine drainage receiving site (CAR_M). I attempted to sample Caribou Mine upstream of the current tailings pond effluent. However, the stream there was devoid of benthic invertebrates. Tailings pond effluent appeared to dilute the presumed effect of AMD. I sampled approximately 25 km downstream of the mine. This was the nearest accessible location at which I could collect benthic invertebrates.

Ecological Implications

Metal enrichment of streams chironomids may elicit changes in chironomid individuals, populations and communities of chironomids. Reduced survival could reduce population size, while slowed growth and development may impede emergence

Table 1.3: Location, sampling dates, and abbreviation codes for reference and mine drainage receiving sites

RIVER	CODE	LATITUDE, LONGITUDE	WATER AND CHIRONOMID	PERIPHYTON
Reference Sites				
Northwest Miramichi River	HEA_R	47°11.184 N, 65°53.671 W	04 June 1999	25 August 1999
McCormack Brook	STR_R	47°17.574 N, 66°06.512 W	09 June 1999	25 August 1999
South Branch Pabineau River	BRU_R	47°26.474 N, 65°49.784 W	14 June 1999	27 August 1999
Nepisiquit River	WED_R	47°23.502 N, 66°11.104 W	12 June 1999	30 August 1999
44 Mile Brook	CAR_R	47°23.545 N, 66°10.959 W	17 June 1999	27 August 1999
Metal Receiving Sites				
Little South Branch Tomogonops River	HEA_M	47°17.538 N, 66°03.123 W	10 June 1999	25 August 1999
Mosquito Brook	STR_M	47°19.012 N, 66°06.470 W	08 June 1999	25 August 1999
Austin Brook	BRU_M	47°23.823 N, 65°49.165 W	11 June 1999	24 August 1999
Nepisiquit River	WED_M	47°23.652 N, 66°07.799 W	12 June 1999	30 August 1999
40 Mile Brook	CAR_M	47°24.064 N, 66°07.885 W	16 June 1999	27 August 1999

and reduce reproductive success. Adult chironomids live only short periods of time, with a limited number of generations occurring in many Canadian environments. Delayed emergence may result in fewer adults being present simultaneously, reducing swarming activity and the probability of successful mating. Reduced population size may lead to altered community composition. Differences in the metal sensitivity of chironomid taxa may also alter community structure.

Due to the importance of chironomids in most aquatic food webs, toxic effects of metal enrichment can have implications at both lower and higher trophic levels. Reduced abundance of chironomids may result in reduced nutrient recycling, accumulation of basal food resources (i.e., periphyton), and reduced abundance of food items for predatory invertebrates, fish, and birds. At higher trophic levels, bioaccumulation of metals in chironomids may lead to biomagnification, contributing to elevated levels of metal in biota of aquatic and terrestrial ecosystems.

Examination of periphyton as a source of metal uptake for primary consumers could increase our understanding of metal bioavailability in aquatic environments. This could lead to the development of more sensitive monitoring practices, resulting in better protection of aquatic organisms living in environments susceptible to metal contamination.

CHAPTER 2: MENTUM DEFORMITIES AND COMMUNITY COMPOSITION OF CHIRONOMIDAE LARVAE DOWNSTREAM OF NEW BRUNSWICK METAL MINES

INTRODUCTION

Benthic invertebrates are frequently used to assess the effects of contaminants in aquatic ecosystems (Wiederholm 1984a; Kiffney and Clements 1993; Ciborowski et al. 1995). Invertebrates' limited mobility, documented sensitivity to a variety of contaminants, and their close association with contaminant accumulating substrates (sediment, periphyton) makes them excellent biomonitoring organisms capable of reflecting the location and severity of contamination (Resh and Rosenberg 1984).

Midge larvae (Diptera: Chironomidae) typically comprise a significant portion of the benthic invertebrate biomass and much of the diversity in aquatic systems, and are thus commonly used in biomonitoring (Pinder 1986). Chironomidae genera exhibit a range of sensitivities to contaminants, with Tanytarsini larvae being most sensitive and Orthocladiinae larvae most tolerant (Armitage 1980; La Point et al. 1984; Chadwick et al. 1986; Clements et al. 1988; Clements et al. 1989; Clements 1994; Clements and Kiffney 1994). Consequently, certain chironomid assemblages persisting in degraded environments can continue to be studied in even the most severely contaminated aquatic environments (Hare and Carter 1976; Warwick et al. 1987). Chironomids represent an important diet item for predatory insects and benthivorous fish (Pinder 1986; Woodward et al. 1994). As a result, they represent an important transfer mechanism for the movement of contaminants to higher trophic levels (Woodward et al. 1994; Reinfelder et al. 1998).

Exposure to high concentrations of metals can elicit changes in the benthic invertebrate community, such as reduced abundance, lower diversity and increased dominance of benthic communities by metal tolerant taxa (Leland et al. 1989; Clements 1991; Hoiland and Rabe 1992; Clements et al. 1988; Kiffney and Clements 1994a,b; Beltman et al. 1999). Exposure to lower concentrations of metals may elicit stress in an individual organism, which may be undetectable at the community assessment level. Thus, measurable community parameters (abundance, diversity, dominance) may be relatively insensitive measures of contaminant exposure (Chadwick and Canton 1984; Clements et al. 1988), as well as being difficult to interpret, in terms of change in community structure and function (Petersen and Petersen 1983; Wiederholm 1984a). At the level of the individual, cellular, physiological, and/or morphological changes that may ultimately influence growth and/or reproductive success may be better indicators of impairment (Ciborowski *et al.* 1995). Various studies have been done to develop biomarkers at the individual level that can be examined in disturbed and undisturbed natural environments (Hudson and Ciborowski 1996a,b).

Hamilton and Saether (1971) first proposed the examination of chironomid deformity levels as an indication of environmental degradation. A deformity is defined as any morphological feature that departs from normal configuration (Warwick 1988). Deformities in the mentum, ligula, mandibles, maxillary palps, and antennae have been observed in many genera inhabiting a variety of environments (Warwick 1988). In metal contaminated environments, the reported incidence of mentum deformities ranges from 0.0-82.5% (Canfield et al. 1994; Warwick et al. 1987; Burt 1998; Diggins and Stewart 1998; Groenendijk et al. 1998; Janssens de Bisthoven et al. 1998a,b; Vermeulen et al. 1998). At reference sites, the reported incidence of mentum deformities ranges from 0.0-48.0% (Dickman et al. 1992; Janssens de Bisthoven et al.

1992; Lenat 1993; Wiederholm 1984b; Bird et al. 1995; Groenendijk et al. 1998; Janssens de Bisthoven et al. 1998b; Vermeulen et al. 1998). Due to the range deformity frequencies observed at reference sites, significantly elevated incidences at metal contaminated environments are frequently not observed (Burt 1998). Small sample sizes and a range of operational definitions of deformities also contribute to the lack of significant differences observed (Burt 1998; Hamalainen 1999).

The objectives of this study were to compare the richness, community composition, and incidence of mentum deformities in chironomids collected from streams receiving mine drainage with paired reference streams. Mine drainage receiving sites are expected to have higher trace metal concentrations in water and periphyton, lower chironomid taxa richness, altered community composition, and an increased incidence of deformed larvae than observed at reference sites.

MATERIALS AND METHODS

Sampling Sites

To examine the effects of metal contamination on chironomid larvae, five mines in northeastern New Brunswick were chosen (Heath Steele Mine, Stratmat Mine, Brunswick No. 6, Caribou Mine and Wedge Mine; see chapter 1). Chironomid larvae were sampled from 5 streams receiving drainage from metal mining facilities and five reference sites (Figures 1.6 – 1.9). In all but one case, reference sites were chosen in separate streams. Each reference site was paired with one of the mine drainage receiving sites, each chosen according to similarity in characteristics of substrate, canopy cover, stream size, and water velocity specific to the mine drainage receiving sites.

Qualitative estimates of stream width, stream morphology, stream canopy, channel type, bank stability, instream cover, and substrate type were made at each site (Appendix 3, Table A3.1). Stream order was determined from topographic maps (1: 150,000 NBGIC 1997). Streams examined in this study ranged from forested headwater streams to medium sized streams with open canopies (Vannote et al. 1980). Generally, stream morphology consisted of riffles and runs, with few pools observed. Substrate at all sampling locations consisted of loose cobbles (64 – 128 mm), with occasional bedrock outcroppings and limited soft sediment. At all streams examined in this study, cobble and other hard substrates were coated with a light film of periphyton. Filamentous algae were absent at most sites, except at both the Stratmat mine drainage receiving and reference sites.

Water Sampling and Analysis

Water quality characteristics (pH, water temperature, dissolved oxygen, conductivity, turbidity, and salinity) were measured at each sampling site using a hand held water meter (Horiba U-10). Measurements were taken at the time of sampling.

Water samples (two per site) were collected in June 1999. Samples were taken at a depth equidistant from the surface of the water and the surface of the substrate using 500-mL acid-washed polyethylene bottles. One water sample from each site was filtered through a 0.45-µm membrane filter to remove particulate matter. All water samples were acidified to a 2% HNO_3 (analytical grade) solution within 48 h of collection and stored at 4 °C prior to analysis. Total and dissolved concentrations of 25 trace elements were analyzed by inductively coupled plasma equipped with an optical emission spectrophotometer (ICP-OES) at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory (Environment Canada 1979,

1989).

Periphyton Collection and Analysis

Periphyton was collected during the period 24-30 August, 1999 at all 10 sites. Palm to hand-sized cobbles (diameter 64 – 128 mm) were removed from the steam bed and periphyton scrubbed off using nylon brushes. Periphyton was rinsed with stream water into acid-washed plastic pails. The suspension was allowed to settle overnight at 4° C. Stream water was decanted off the surface of the settling periphyton, and all visible macroinvertebrates were removed.

To remove the remaining water, samples were centrifuged at 6,000 rpm for 15 min. Water was decanted and periphyton was freeze-dried using a vacuum freeze drier for 24-h. Dried periphyton was stored in pre-cleaned acid-washed containers. Total concentrations of 16 trace elements were analyzed by ICP-OES at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory (Environment Canada 1979; Agemian et al. 1980; Environment Canada 1989).

Chironomid Collection

Chironomid larvae were collected throughout June 1999. Benthic samples were collected using a 0.1 m^2 modified Hess sampler (253-µm mesh size) and a D-shaped kick net (1-mm mesh size) and placed in shallow pans. Chironomids were hand-picked from the pans in the field to ensure the collection of at least 200 individuals at each site. Initially, the collection of 200 individuals per site was considered to be an adequate sample. At least 125 individuals of one genus are recommended to detect a doubling over background levels of deformities of 3% as statistically significant at alpha < 0.05 (Hudson and Ciborowski 1996b). Larvae were individually blotted on paper toweling

and then preserved in chilled Carnoy's solution (3 parts anhydrous ethanol: 1 part glacial acetic acid). Larvae were stored at 4 °C prior to identification and deformity analysis.

Remainders of benthic samples were preserved in Kahle's solution and sorted in the laboratory. Benthic samples were partitioned into size fractions by rinsing through a series of brass sieves (4 mm – 250 µm mesh). Under a dissecting microscope, a portion of each size fraction was sorted (Appendix 3, Table A3.10). Approximately 300 additional chironomids were sorted from these composite samples, in order to augment the number of chironomids examined for deformity analysis to approximately 500 individuals per site.

Chironomid Identification and Deformity Screening

In the laboratory, the heads of individual chironomid larvae were severed and microscope slide-mounted ventral side up beside the body in CMC-9AF® aqueous mounting medium (Masters Company Inc., Bensenville, IL). Chironomid larvae were identified to the generic level according to the keys of Oliver and Roussel (1983), Wiederholm (1983), and Ferrington and Coffman (1996). Ten percent of the identified larvae were randomly selected and re-examined to verify correct designation.

Mentum deformities (a missing or additional tooth (Hudson and Ciborowski 1996b)) were scored during larval identification, and deformed larvae were later re-examined to ensure correct designation. I performed all examinations for deformities. Individuals displaying broken, chipped or worn teeth were classified as damaged, not deformed. Gaps in the mentum were classified as deformities if their surface was smooth, rather than jagged indicating breakage. Deformities in other structures were not examined. Data were expressed as percentage of individuals deformed, with

standard error determined according to the binomial distribution.

Statistical Analysis

Principal component analysis was used to summarize each of three data sets; trace element concentrations in water, trace element concentrations in periphyton, and the relative abundance (percentage) of dominant chironomid genera. Each analysis characterized the original variable in the data set as a smaller number of independent factors (principal components), each characterized by a suite of correlated variables. Individual variables (metals, genera) are associated with a component (factor loadings). Factor scores reflect the association of a particular case (i.e. site) with the principal components. The number of cases limited the numbers of variables that could be used in each principal component analysis.

Factor scores generated for each principal component, were used to compare reference and mine drainage receiving sites using paired comparison t-tests. Because multiple tests were performed, the critical value required to reject a null hypothesis of no difference was adjusted using Bonferroni correction to give an experiment wise Type I error of 0.05. p values were corrected. Analyses were performed using the factor analysis and dependent t-test modules of Statistica® (StatSoft 1998, 1998 Edition).

Water

Water quality data were $\log_{10}$ transformed, except for pH. Mean values for pH, conductivity, dissolved oxygen and water temperature were compared at reference ($n = 5$) and mine drainage receiving ($n = 5$) sites by paired comparison t-tests ($p<0.05$).

Principal component analysis was performed from a variance-covariance matrix generated from 9 elements and 10 cases. All data were $\log_{10}$ transformed prior to

analysis. Water concentrations of trace elements undetected at most sites (n > 6) were excluded from analysis (i.e., Ag, Al, As, B, Be, Bi, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sn, Ti, and V). Factor loadings were varimax rotated and the principal component scores associated with loadings of >0.600 were including in the analysis. Factor scores generated from PCA were then analyzed using a one-tailed paired comparison t-test ($p<0.05$) to test for differences between reference (n=5) and mine drainage receiving (n=5) sites.

Periphyton

Principal component analysis was performed from a variance-covariance matrix generated from 9 elements and 10 cases. All data were $\log_{10}$ transformed prior to analysis. Elements that were suspected to be of anthropogenic origin were included in the principal component analysis (i.e., Cd, Co, Cr, Cu, Mn, Ni, Pb, V, and Zn). Arsenic was excluded from the analysis because it was found to vary independently of the other elements among sites (data not presented). Factor loadings were varimax rotated and the principal component scores associated with loadings of >0.600 were including in the analysis. Factor scores generated from PCA were then analyzed using a one-tailed paired comparison t-test ($p<0.05$) to test for differences between reference (n=5) and mine drainage receiving (n=5) sites.

Chironomid Genera

The composition of chironomid communities at mine drainage receiving and reference sites was estimated from both hand-picked and composite chironomid samples. The total number of chironomids sorted from composite samples in the laboratory was added to the number of chironomids that had been picked from the

whole sample in the field. Absolute estimates of abundance were not determined due differences in the number of samples taken at each site.

Generic richness was the number of chironomid genera present at each sampling site. Data were $\log_{10}$ transformed prior to analysis. Differences in mean richness between mine drainage receiving and reference sites were analyzed using a one-tailed paired comparison t-test on $\log_{10}$ transformed data.

The relative abundance (percentage) of dominant genera was characterized by principal component analysis, performed from a covariance matrix of 9 genera and 10 cases. All data was $\log_2$ transformed prior to analysis. Mean relative abundance (percentage) of genera was examined across all 10 sites, and the nine most abundant taxa selected. Factors were varimax rotated and the principal component scores associated with loadings of >0.600 were including in the analysis. Factor scores generated from PCA were then analyzed using a one-tailed paired comparison t-test ($p<0.05$) to test for differences between reference (n=5) and mine drainage receiving (n=5) sites.

Cluster analysis (Ward's method) was used to compare chironomid community composition at mine drainage receiving and reference sites. Unlike PCA, the number of variables used in cluster analysis is not limited by the number of cases employed (StatSoft 1998, 1998 Edition). Cluster analysis groups sites according to similarity of the variables specified for analysis. Linkage distances describes site similarity. For example, sites with small linkage distances are more similar. All data were $\log_2$ transformed prior to analysis. Genera were included in the analysis if their mean relative abundance across sites was > 1.0 % and if they were present in at least 4 of the 10 sites. All statistical analysis was performed using the cluster analysis module of Statistica® (StatSoft 1998, 1998 Edition).

Chironomid Deformities

Deformity data were $\log_{10}$ transformed prior to analysis. Differences in the total incidence of deformities at mine drainage receiving and reference sites were assessed using a one-tailed paired comparison t-test ($p<0.05$).

RESULTS

Water Quality

Generally, streams surveyed in this study were slightly acidic, had low conductivity and were well oxygenated (Table 2.1). Water quality parameters did not differ significantly between mine drainage receiving sites and reference sites.

Trace Element Analysis of Water

Except for sodium, metal concentrations were generally greater at mine drainage receiving sites than at their paired reference sites (Table 2.2). Mean differences in trace element concentrations between mine drainage receiving and references sites were largest for Al, Fe, Mn and Zn.

Principal component analysis was performed for 9 elements. Three factors were extracted from total trace element concentrations at each site by PCA, accounting for 84.8 % of the variation in the original data (Table 2.3). Concentrations of Ca, Mg, Na, and Sr were strongly associated with values of the first factor, which did not significantly differ between mine drainage receiving and reference sites ($p<0.97$). Concentration of Ca, Mg, Na and Sr differed markedly between Caribou sites (CAR_R and CAR_M), Brunswick sites (BRU_R and BRU_M), and Heath Steele sites (HEA_R and HEA_M). Little difference in metal concentrations were observed between Wedge sites (WED_R and WED_M) (Appendix 3, Table A3.3). Iron, Mn, and Zn were associated with values

Table 2.1: Mean (± 1 SE, n = 5) pH, conductivity, dissolved oxygen and water temperature at mine drainage receiving and reference sites measured during June 1999. Reference and mine drainage receiving sites compared using a paired comparison t-test ($p<0.05$).

WATER QUALITY PARAMETER	MEAN (± 1 SE)		t value	p value
	Reference Sites	Mine Drainage Receiving Sites		
pH	6.87 (0.14)	6.84 (0.15)	0.0932	NS[1]
Conductivity (mS/cm^2)	0.042 (0.008)	0.049 (0.014)	-0.4708	NS
Dissolved Oxygen (mg/L)	8.74 (0.37)	8.95 (0.39)	-0.5944	NS
Temperature (°C)	15.3 (2.6)	16.6 (1.4)	-0.7001	NS

[1]not significant ($p>0.05$)

Table 2.2: Mean (± 1 SE, n = 5) total concentrations of trace elements (µg/L) in water at metal drainage receiving and reference sites

TRACE ELEMENT	PRINCIPAL COMPONENT ASSOCIATION	CONCENTRATION (µg/L)		t value
		Reference Sites	Mine Drainage Receiving Sites	
Aluminum (Al)	NA[1]	ND[2]	**283.4 (92.4, n=3)**	
Calcium (Ca)	I	4,281 (646)	5,298 (2,070)	
Magnesium (Mg)	I	904 (85)	1,051 (148)	
Sodium (Na)	I	2,088 (635)	1,869 (157)	
Strontium (Sr)	I	19.43 (3.36)	19.59 (4.16)	
Iron (Fe)	II	84.9 (16.9)	152.9 (50.4)	
Manganese (Mn)	II	15.7 (10.6)	86.6 (40.7)	
Zinc (Zn)	II	7.3 (3.3)	**117.7 (37.3, n=4)**	
Barium (Ba)	III	3.95 (0.59)	4.55 (0.75)	
Potassium (K)	III	402.7 (20.7)	516.1 (38.5)	
PC - I		0.012 (0.413)	-0.012 (0.528)	0.047
PC - II		-0.549 (0.369)	0.549 (0.404)	-3.115[a]
PC - III		-0.467 (0.384)	0.467 (0.429)	-2.957[a]

[1] not included in principal component analysis
[2] not detected
[3] bolded values are above Canadian Water Quality Guidelines for the protection of aquatic life (CCREM 1987)
[a] significantly different at $p<0.05$. Statistical analysis was performed on principal component scores factor for each site using a paired comparison t-test.

Table 2.3: Identification of the metals from total concentrations in water loading most heavily on the first three principal components and the cumulative proportion of total variance explained by each component

ELEMENT	PC-I	PC-II	PC-III
Strontium	**0.98**	-0.092	0.018
Calcium	**0.94**	-0.15	-0.029
Magnesium	**0.90**	0.18	0.29
Sodium	**0.76**	0.21	-0.039
Iron	-0.18	**0.95**	-0.14
Manganese	0.27	**0.87**	0.38
Zinc	0.21	**0.76**	0.56
Barium	0.14	-0.034	**0.82**
Potassium	-0.18	0.41	**0.69**
Cumulative Proportion of Total Variance Explained	0.38	0.65	0.84

of the second factor, and their concentrations were significantly higher at mine drainage receiving sites ($p<0.036$) than at reference sites. Trace metal concentrations of Fe, Mn and Zn were most different between mine and reference locations at Heath Steele (HEA_R and HEA_M) and at Caribou (CAR_R and CAR_M) (Fig. 2.1). Barium and K were strongly associated with values of the third factor, and their concentrations were significantly higher at mine drainage receiving than at reference sites ($p<0.042$).

Trace Element Analysis of Periphyton

Periphyton abundance at mine drainage receiving and reference sites was not quantitatively assessed. However, periphyton was estimated to be most abundant at mine drainage receiving sites of Heath Steele, Brunswick, and Caribou Mines.

For most elements, concentrations in periphyton were higher at mine drainage receiving sites than at paired reference sites (Table 2.4). However, concentrations of Al, Ca and Na were higher at reference sites. Mean concentrations of Zn were 100 x higher and Al, Cd, Co, Cu, Mn, and Pb 10 x higher at mine drainage receiving sites than at reference sites.

Principal component analysis was performed for 9 elements. Two factors were extracted from trace element concentrations at each site by PCA, accounting for 84.1% of the variation in the original data (Table 2.5). Concentrations of Cd, Co, Cu, Mn, Pb, and Zn were strongly associated with values of the first factor, and their concentrations were significantly higher at mine drainage receiving sites than at paired reference sites ($p<0.002$). Trace metal concentrations of Cd, Co, Cu, Mn, Pb, and Zn were most different between mine and reference locations at Heath Steele (HEA_R and HEA_M) and at Caribou (CAR_R and CAR_M) (Fig. 2.2). Chromium, Ni and V were strongly associated with values of the second factor. Concentration of these elements differed

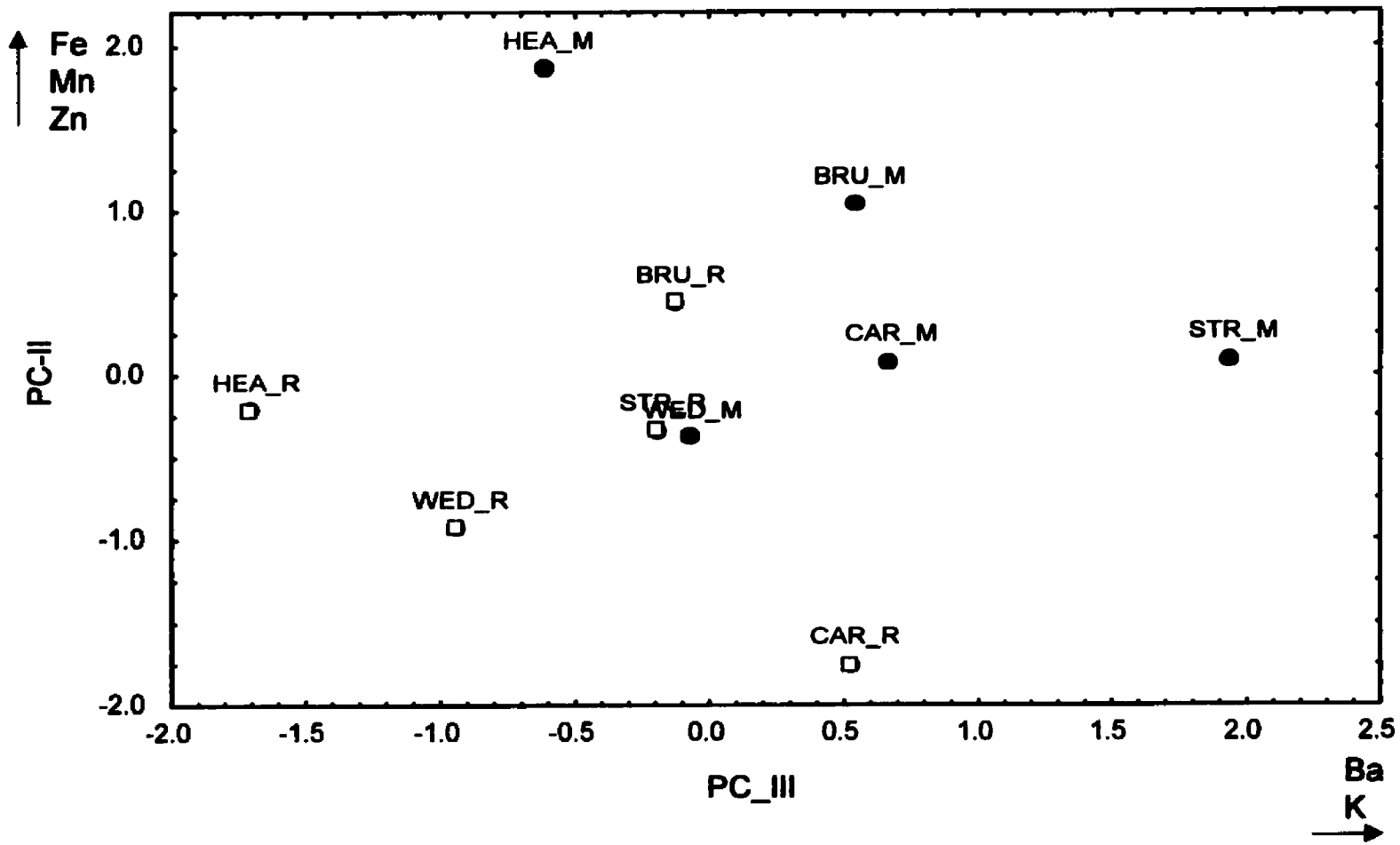

Figure 2.1: Total trace element concentrations in water at mine drainage receiving and reference sites as plotted in two principal components of the principal components analysis (PCA). Open squares represent reference sites while solid circles represent mine drainage receiving sites.

Table 2.4 Mean (± 1 SE, n = 5) total concentrations of trace elements (µg/g) in periphyton at mine drainage receiving and reference sites

ELEMENT	PRINCIPAL COMPONENT ASSOCIATION	CONCENTRATION (µg/L)		t value
		Reference Sites	Mine Drainage Receiving Sites	
Cadmium (Cd)	I	3.40 (1.29)	25.17 (9.91)	
Cobalt (Co)	I	42.65 (29.36)	222.9 (109.0)	
Copper (Cu)	I	14.25 (1.80)	763.4 (249.4)	
Manganese (Mn)	I	7,837 (4,832)	41,920 (29,747)	
Lead (Pb)	I	15.40 (4.54)	546.4 (241.0)	
Zinc (Zn)	I	589.3 (365.7)	10,249 (5,000)	
Chromium (Cr)	II	19.47 (3.82)	30.45 (9.89)	
Nickel (Ni)	II	20.71 (8.92)	45.60 (21.74)	
Vanadium (V)	II	28.29 (7.87)	33.38 (9.05)	
Aluminum (Al)	NA[1]	10,788 (2,520)	24,800 (7,160)	
Arsenic (As)	NA	26.77 (10.26)	72.47 (26.14)	
Calcium (Ca)	NA	6,515 (1,035)	5,548 (1,243)	
Iron (Fe)	NA	14,011 (3,303)	42,067 (14,639)	
Potassium (K)	NA	4,500 (826)	4,796 (1,221)	
Magnesium (Mg)	NA	3,471 (923)	3952 (711)	
Sodium (Na)	NA	891.5 (436.6)	399.5 (90.8)	
PC - I		-0.797 (0.279)	0.797 (0.235)	-7.222[a]
PC - II		0.099 (0.456)	-0.099 (0.487)	0.567

[1] not included in principal component analysis

[a] significantly different at $p<0.01$. Statistical analysis was performed on principal component scores factor for each site using a paired comparison t-test.

Table 2.5: Identification of the metals from total concentrations in periphyton loading most heavily on the first two principal components and the cumulative proportion of total variance explained by each component

ELEMENT	PC-I	PC-II
Zinc	**0.93**	0.25
Cadmium	**0.89**	0.36
Lead	**0.81**	0.018
Copper	**0.79**	0.26
Cobalt	**0.75**	0.60
Manganese	**0.73**	0.43
Chromium	0.31	**0.93**
Vanadium	0.057	**0.91**
Nickel	0.51	**0.80**
Cumulative Proportion of Total Variance Explained	0.49	0.84

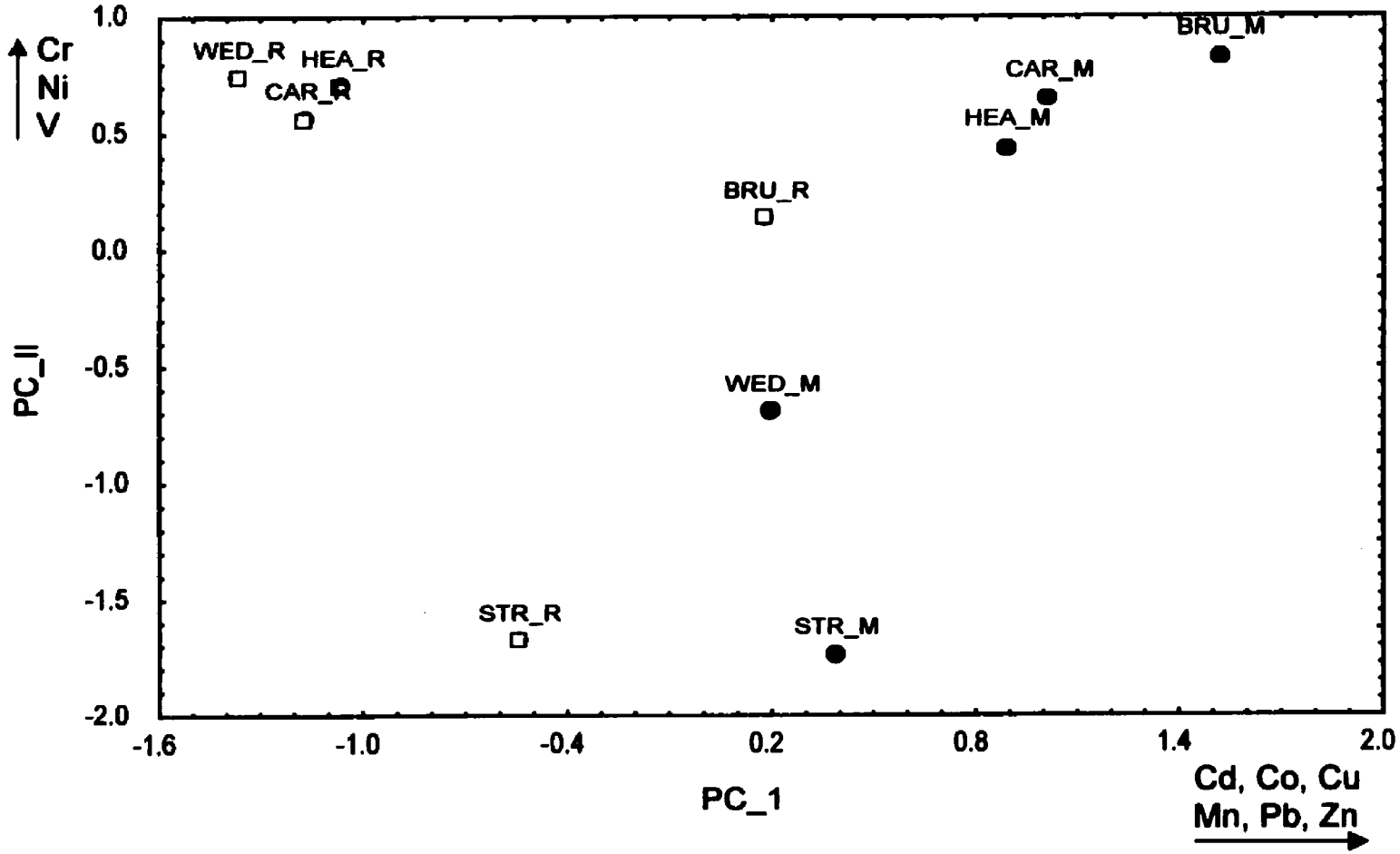

Figure 2.2: Total trace element concentrations in periphyton at mine drainage receiving and reference sites as plotted in two principal components of the principal components analysis (PCA). Open squares represent reference sites while solid circles represent mine drainage receiving sites.

between mine and reference locations at Brunswick (BRU_R and BRU_M) and at Wedge (WED_R and WED_M) but not at the other three mines (Fig. 2.2). Consequently, concentrations of Cr, Ni, and V did not significantly differ between mine drainage receiving and reference sites ($p<0.60$).

Chironomid Community Composition

A total of approximately 4.1×10^4 chironomid larvae were collected at various samples. Identification of a subset of approximately 5,000 slide-mounted larvae yielded 48 genera within 5 subfamilies from 10 sites (Table 2.6). Chironominae comprised 49 %, Orthocladiinae 39%, Tanypodinae 11 %, Diamesinae <1 % and Pseudochironominae <1% of collected larvae. *Cricotopus/Orthocladius sp.* was the most abundant taxon, representing 23% of chironomid larvae collected. *Cricotopus* and *Orthocladius* genera are difficult to distinguish (Ferrington and Coffman 1996) and thus were classed jointly (i.e. *Cricotopus/ Orthocladius*).

Chironomid richness among reference and mine drainage receiving sites ranged from 12 to 27 genera (Table 2.6). Difference in generic richness between mine drainage and reference sites was largest at Heath Steele (HEA_R and HEA_M) and smallest at Stratmat (STR_R and STR_M). Mine drainage receiving sites had a mean generic richness of 16 (± 2), which was significantly lower ($p<0.025$) than the mean number of taxa at reference sites [23 (± 1)] [mean ± 1 SE, n = 5, paired comparison t-test].

Community composition differed between mine drainage receiving and reference sites differed at both the subfamily and generic levels. Tanypodinae, Chironomini and Tanytarsini larvae were more abundant at reference sites, while Orthocladiinae larvae were more abundant at mine drainage receiving sites (Fig. 2.3).

Table 2.6: Numbers of chironomids (number of deformities in parentheses) collected at mine drainage receiving and reference sites. At each site, approximately 200 larvae were hand-picked in the field. Additional 300 larvae per site were sorted from composite samples in the laboratory

	REFERENCE SITES					MINE DRAINAGE RECEIVING SITES				
	HEA_R	STR_R	BRU_R	WED_R	CAR_R	HEA_M	STR_M	BRU_M	WED_M	CAR_M
Tanypodinae										
Ablabesmyia			26		20			2		1
Larsia		3	116	69	35	1	2		2	69
Paramerina			2		1					
Thienemannimyia	19	13	32 (1)[1]	59 (2)	165 (1)	8	12 (1)	6	16	73 (2)
Macropelopia			1	1			1			1
Nilotanypus	1	5	8		2		14 (2)	1		
Procladius			2 (1)							
Diamesinae										
Diamesa		1				6	5	1		24
Pagastia	1									
Potthastia	12			19						19
Chironominae										
Chironomini										
Chironomus			2							
Cryptochironomus	1		6	3		3				
Demicryptochironomus	1		1	1				1		
Glyptotendipes			1							
Microtendipes	32	111	31 (1)	5	3			1	2	
Nilothauma	3									
Parachironomus		11								
Paralauterborniella	1			2						
Paratendipes	24	17	44 (1)	12	2		2		5	1
Phaenopsectra			8 (1)	38						1
Polypedilum	131	133 (2)	52	151 (2)	21	17	208 (4)	63 (3)	307 (3)	6
Saetheria				1						
Tribelos				1						
Xenochironomus										1
Tanytarsini										
Cladotanytarsus			1							

[1]Values in parentheses represent the number of deformed larvae

Table 2.6: Cont'd

	REFERENCE SITES					MINE DRAINAGE RECEIVING SITES				
	HEA_R	STR_R	BRU_R	WED_R	CAR_R	HEA_M	STR_M	BRU_M	WED_M	CAR_M
Heterotanytarsus										1
Micropsectra	61 (1)	58 (1)	32	17 (1)	129		98 (1)	8	82 (1)	6
Paratanytarsus			3							
Rheotanytarsus	88	36	56		1		1	1	5	
Stempellina	1		7	2					4 (2)	
Stempellinella		28	6	15	52		2		2	2
Tanytarsus	13	7	22 (2)	50	17		35 (1)	3	4	2
Pseudochironominae										
Pseudochironomus	2									
Orthocladiinae										
Brillia						1	10			
Corynoneura	3	2	1	1	9		1	2		
Cricotopus/Orthocladius	65	2	7	42	5	375 (5)	20	260 (4)	16	225
Eukiefferiella	12	22	22	2	9	118 (4)	33	90	29	3 (1)
Heterotrissocladius				2						
Krenosmittia					1		1	1		
Lopescladius	1				2					
Parakiefferiella	1									
Parametriocnemus	7	14	10	1	12	2	27	9 (1)	21	1
Psectrocladius	5				1			8 (1)		78
Pseudosmittia								1		
Rheocricotopus	1	2				2				
Symposiocladius		3								
Synorthocladius		7			13		1			
Thienemanniella	3	37 (2)	2	21	7	1	46	6		
Sum	489	502	501	515	507	534	519	466	495	514
Generic Richness	25	20	27	23	21	12	19	20	13	18
Proportion Deformed	0.20	1.00	1.40	0.97	0.39	1.69	1.73	1.81	1.21	0.58

[1]Values in parentheses represent the number of deformed larvae

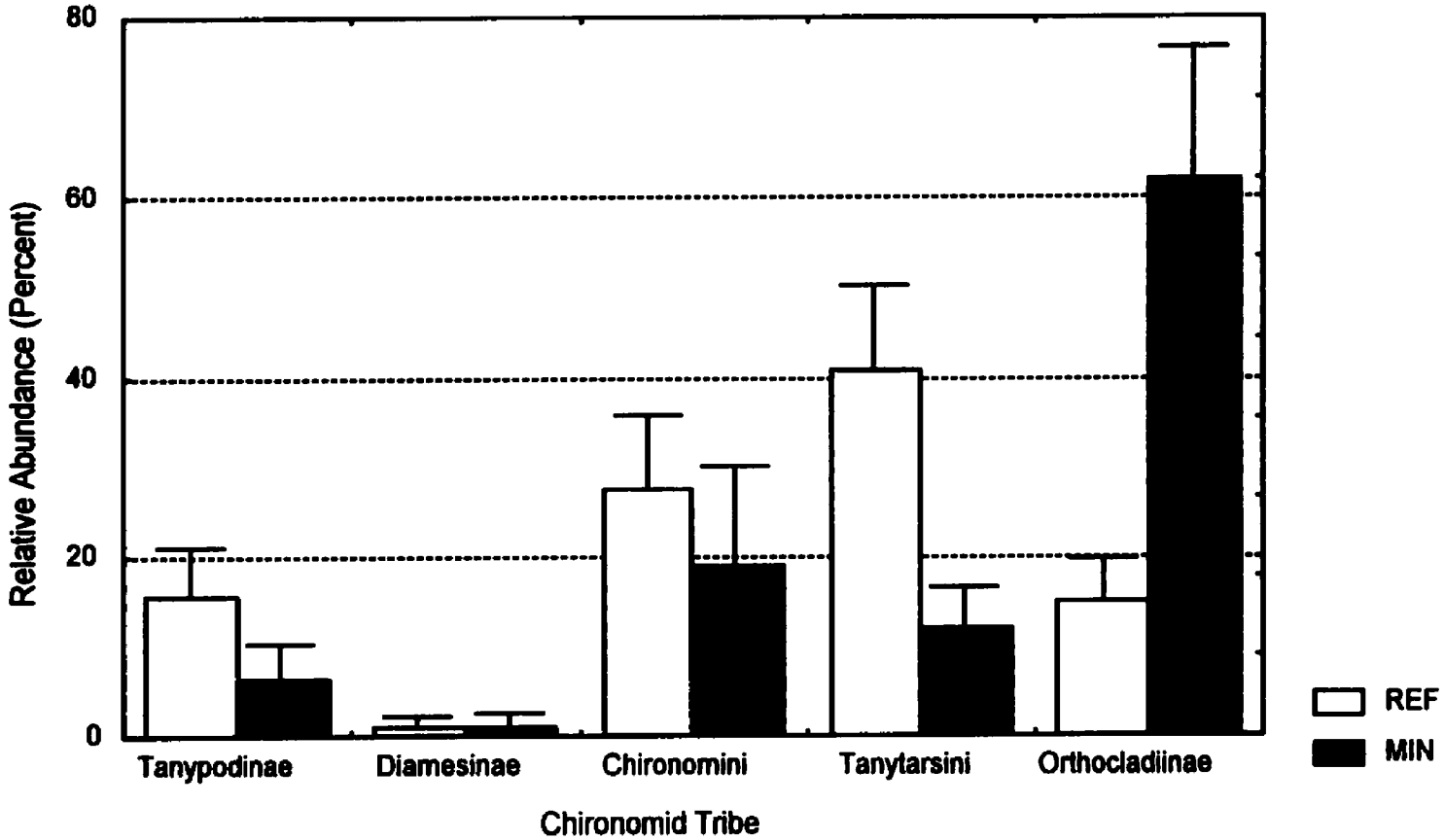

Figure 2.3: Mean (± SE, n=5) relative abundance of chironomid tribes at mine drainage receiving and reference sites.

The nine most relatively abundant chironomid genera accounted for 60-93% of total chironomids at the different sites. Relative abundance of *Larsia*, *Thienemannimyia*, *Micropsectra*, *Stempellinella*, and *Microtendipes* was higher at reference sites. Relative abundances of *Polypedilum*, *Cricotopus/ Orthocladius*, *Eukiefferiella*, and *Thienemanniella* were higher at mine drainage receiving sites (Table 2.7). Principal component analysis extracted three factors, accounting for 77.6% of the variation in the original data (Table 2.8). Relative abundance of *Larsia*, *Thienemannimyia*, *Eukiefferiella*, and *Polypedilum* were most associated with the values of the first factor, which accounted for 33 % of the variation. The predatory tanypodines *Larsia* and *Thienemannimyia* were positively associated with each other but negatively associated with the relative abundances of *Eukiefferiella*, and *Polypedilum*. *Micropsectra*, *Stempellinella*, *Microtendipes* and *Cricotopus/Orthocladius* were most strongly associated with the second factor, although the association of *Microtendipes* with factor 2 was not statistically significant ($p<0.10$). Relative abundances of the Tanytarsini taxa (*Micropsectra* and *Stempellinella*) were positively associated with factor 2, whereas the relative abundances of orthoclads (*Cricotopus/Orthocladius*) were negatively associated with values of factor 2. Relative abundance of *Thienemanniella* was associated with values of the third factor.

No significant difference was observed in relative abundance of taxa associated with PC-I and III (*Larsia*, *Thienemannimyia*, *Eukiefferiella*, *Polypedilum*, and *Thienemanniella*) between mine drainage receiving and reference sites (one-tailed paired-comparison t-tests). Relative abundances of these taxa were only substantially different between two of the five groups of sites; Brunswick sites (BRU_R and BRU_M) and Wedge sites (WED_R and WED_M) (Fig. 2.4). Relative abundances of *Micropsectra*, *Stempellinella*, and *Cricotopus/Orthocladius* differed greatly between

Table 2.7: Mean (± 1 SE, n = 5) relative abundance of dominant chironomid genera at reference and mine drainage receiving sites

GENERA	PRINCIPAL COMPONENT ASSOCIATION	RELATIVE ABUNDANCE (%)		t value
		Reference Sites	Mine Drainage Receiving Sites	
Eukiefferiella	I	3.05 (1.31)	8.11 (3.27)	
Larsia	I	7.52 (4.35)	1.47 (1.45)	
Polypedilum	I	15.11 (4.61)	18.69 (10.69)	
Thienemannimyia	I	5.10 (1.77)	3.17 (1.92)	
Cricotopus/Orthocladius	II	3.12 (2.01)	37.55 (13.87)	
Micropsectra	II	16.76 (5.45)	9.41 (4.73)	
Microtendipes	II[1]	8.24 (4.01)	0.04 (0.03)	
Stempellinella	II	10.94 (6.53)	0.71 (0.51)	
Thienemanniella	III	5.13 (3.03)	7.03 (6.43)	
PC - I		0.210 (0.485)	-0.210 (0.439)	1.073
PC - II		0.702 (0.136)	-0.702 (0.430)	3.341[a]
PC - III		-0.168 (0.502)	0.168 (0.429)	-0.489

[1]Genera not significantly associated with PC - II

[a] significantly different at $p<0.03$. Statistical analysis was performed on principal component scores factor for each site using a paired comparison t-test.

Table 2.8: Identification of the relative abundance of chironomid taxa loading most heavily on the first three principal components and the cumulative proportion of total variance explained by each component

ELEMENT	PC-I	PC-II	PC-III
Thienemannimyia	**0.96**	0.17	0.0099
Polypedilum	**-0.77**	0.40	0.030
Larsia	**0.72**	0.18	-0.018
Eukiefferiella	**-0.68**	-0.42	0.32
Cricotopus/Orthocladius	-0.098	**-0.94**	-0.090
Micropsectra	0.13	**0.87**	0.096
Stempellinella	0.51	**0.66**	0.40
Thienemanniella	-0.24	0.28	**0.82**
Microtendipes	-0.37	0.52	-0.49
Cumulative Proportion of Total Variance Explained	0.33	0.64	0.77

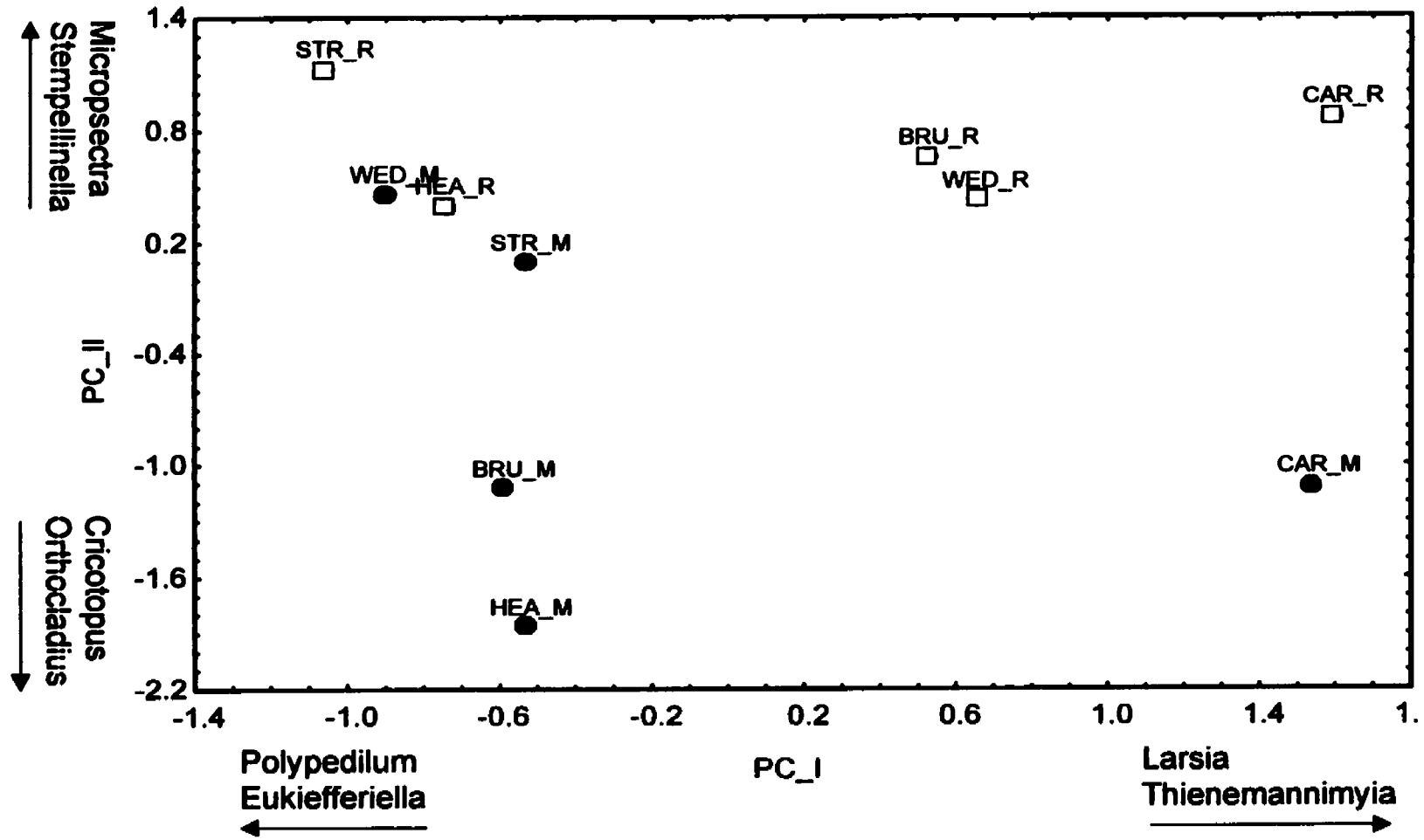

Figure 2.4: Relative abundance of 9 dominant chironomid genera at mine drainage receiving and reference sites as plotted in two principal components of the principal components analysis (PCA). Open squares represent reference sites while solid circles represent mine drainage receiving sites.

reference and mine sites for Heath (HEA_R and HEA_M), Brunswick (BRU_R and BRU_M) and Caribou sites (CAR_R and CAR_M) (Fig. 2.4). Consequently, relative abundance of taxa associated with PC-II differed significantly between mine drainage receiving and reference sites (one-tailed paired-comparison t-test, $p<0.03$).

Eighteen chironomid genera, examined by cluster analysis, accounted for >92 % of the chironomids identified at all 10 sites. Cluster analysis identified two relatively distinct clusters of sites (Fig. 2.5). Mine drainage receiving sites of HEA_M, BRU_M, and CAR_M had similar chironomid communities, characterized by higher relative abundance of *Cricotopus/Orthocladius* and *Psectrocladius*. Chironomid communities at all reference sites, and 2 mine drainage receiving sites (WED_M and STR_M) were characterized by higher relative abundance of *Microtendipes*, *Paratendipes*, *Rheotanytarsus*, and *Stempellinella* (Table 2.9).

Chironomid Deformities

The menta of 5,042 chironomid larvae were examined for deformities (~500 per site). A total of 56 deformed individuals were observed in samples, belonging to 15 different genera (Table 2.6). The difference in the incidence of mentum deformities between mine drainage receiving and references sites were largest at Heath Steele and smallest at Caribou Mines. Arithmetic mean percentage (± 1 SE, n = 5) of total deformed individuals was 1.43 (± 0.24) in larvae from mine drainage receiving sites. This was significantly higher ($p<0.05$, one-tailed paired comparison t-test) than the mean incidence observed in larvae from reference sites [0.79 (± 0.22)]. The differences in incidence of deformities between reference and mine drainage receiving sites were not statistically significant at the subfamily or generic level, owing to small sample sizes.

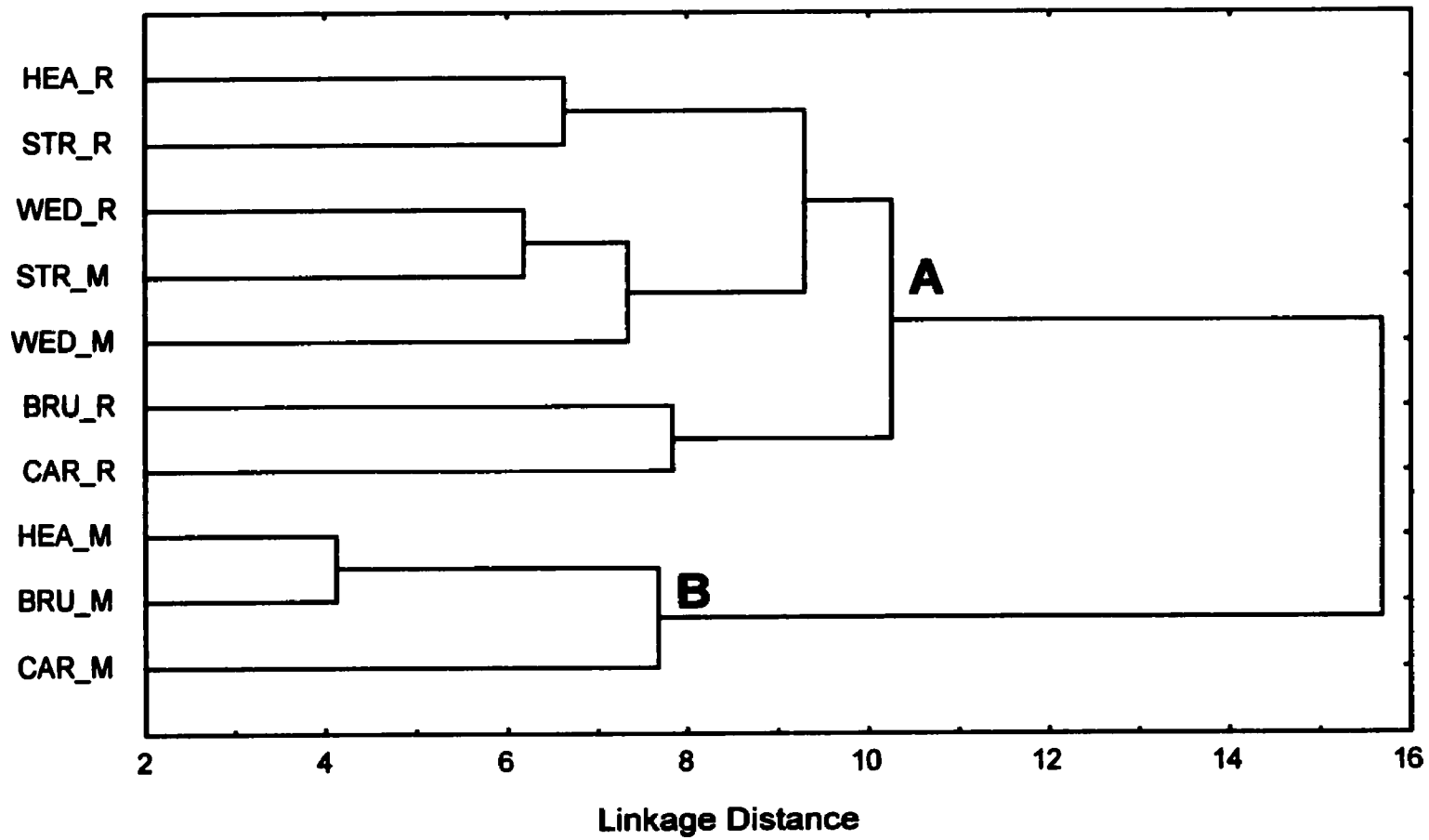

Figure 2.5: Cluster analysis of chironomid generic relative abundance at mine drainage receiving and reference (Ward's Method).

Table 2.9: Mean relative abundance (± 1 SE, n=7 cluster A, n = 3 cluster B) of chironomid taxa in Clusters A and B, as determined by k-mean cluster analysis. Membership in Cluster A included all reference sites and the mine drainage receiving site of STR_M and WED_M. Membership in Cluster B included the mine drainage receiving site of HEA_M, BRU_M and CAR_M

CHIRONOMID TAXON	CLUSTER A	CLUSTER B
Micropsectra	4.04 (0.27)	1.64 (0.78)
Polypedilum	4.02 (0.58)	2.34 (0.58)
Stempellinella	2.33 (0.64)	0.31 (0.17)
Thienemannimyia	2.08 (0.36)	1.92 (0.84)
Thienemanniella	2.00 (0.79)	0.71 (0.56)
Eukiefferiella	1.95 (0.32)	2.96 (1.13)
Cricotopus/Orthocladius	1.73 (0.48)	5.91 (0.14)
Microtendipes	1.72 (0.74)	0.20 (0.06)
Rheotanytarsus	1.66 (0.67)	0.15 (0.01)
Tanytarsus	1.65 (0.43)	0.91 (0.54)
Larsia	1.57 (0.71)	1.11 (0.98)
Paratendipes	1.31 (0.37)	0.16 (0.02)
Parametriocnemus	1.15 (0.28)	0.42 (0.16)
Nilotanypus	1.06 (0.42)	0.25 (0.11)
Ablabesmyia	0.86 (0.47)	0.33 (0.09)
Corynoneura	0.84 (0.29)	0.15 (0.01)
Stempellina	0.79 (0.47)	0.14 (0.00)
Psectrocladius	0.40 (0.24)	2.11 (1.22)

DISCUSSION

Water Quality

At sites receiving untreated mine drainage, pH is commonly depressed, altering the bioavailability of metals (Foster and Junt 1978; Boult et al. 1994; Courtney and Clements 2000). However, pH and other water quality parameters (conductivity, dissolved oxygen and temperature) did not differ significantly at mine drainage receiving and reference sites. Streams of this area generally exhibit low conductivity (BEAK 1994; 1998), elevated only at sites receiving limed tailings effluent.

Trace Element Analysis of Water and Periphyton

Water chemistry data confirm that elevated concentrations of metals are associated with metal mining operations, even upstream of effluent release locations. Leaching of metals from waste rock scattered across mine sites contributes significantly to elevated concentrations. Metal concentrations in water were most elevated at mine drainage receiving sites of mines in operation at the time of sampling (i.e., HEA_M and CAR_M).

Concentrations of Cd, Cu, and Pb observed in periphyton indicated that while these metals were not detectable in water samples, they were present at elevated levels downstream of metal mining facilities. Concentrations of Cd, Cu, Pb, and Zn in periphyton were similar to concentrations reported in other metal contaminated environments (Table 1.1). Metal concentrations in periphyton were highest at mine drainage receiving sites of mines in operation at the time of sampling, reflecting the higher water concentrations at these sites. Concentrations of trace metals in periphyton were between 10 and 100 times higher than observed in water, suggesting that periphyton provides a time integrated assessment of environmental metal levels.

Considering the importance of periphyton in lotic food webs (Ledger and Hildrew 1998), the presence of elevated metal concentrations in periphyton may have important implications. Periphyton is an important food source for many aquatic organisms, which ingest predominately algae (scrapers, grazers) and detritus associated with algae (collectors, gatherers). Invertebrates associated with periphyton may ingest and suffer deleterious effects of consuming the accumulated metals. Metals accumulated by primary consumers may be transferred to higher trophic levels (Clements 1991).

Chironomid Genera

Metal accumulation in streams reduces the abundance and species richness of aquatic insects and alters the proportional abundance of different groups (Winner et al. 1980; Canfield et al. 1994; Wiederholm 1984a; Hickey and Clements 1998; Beltman et al. 1999). Few studies have examined the effects of metals on the composition of chironomid communities (Waterhouse and Farrell 1985). Due to their taxonomic complexity, many studies have not identified chironomids past the level of family (Winner et al. 1975; Occhiogrosso et al. 1979; Roline 1988), obscuring the potential for chironomid larvae to reflect metal exposure through loss of richness. In addition, the assumption that chironomids as a group are tolerant to eutrophication, has permeated metal mining literature, resulting in the classification of this group as metal-tolerant (Yasuno et al. 1985; Clements et al. 1988; Clements 1994).

In this study, sites with elevated metal concentrations had significantly reduced chironomid generic richness relative to their reference pair, confirming the sensitivity of chironomids to metal contamination. Chironomid generic richness at mine drainage receiving sites was comparable to generic richness observed at other metal contaminated sites, ranging from 2 – 22 (Winner et al. 1980; Waterhouse and Farrell

1985; Diggins and Stewart 1998). Most studies at metal contaminated sites have not estimated generic richness at reference sites. Canfield et al. (1994) observed three and seven chironomid genera at two reference sites, while Clements et al. (2000) observed 14 genera. Generic richness at reference sites in this study were 2 to 9 times higher than previously reported in the literature (Canfield et al. 1994; Clements et al. 2000).

Lower generic richness at mine drainage receiving sites may result directly from the loss of sensitive taxa due to metal toxicity. For example, Zn concentrations in water at mine drainage receiving sites of this study were similar to concentrations causing a 50 % reduction in survival of *Tanytarsus* larvae in laboratory tests (Anderson et al. 1990). A lack of high quality food resources at mine drainage receiving sites may result in lower chironomid generic richness. For example, the replacement of palatable species of algae (e.g., diatoms) by metal-tolerant algal taxa (green algae) may reduce available food sources for grazing chironomid taxa (Gower et al. 1994). Similarly, food resources of predatory chironomids may be limited due to the loss of sensitive chironomid taxa. The consequences of taxa loss are influenced by their role and abundance in the community. Loss of taxa representing a unique niche or whose presence influences the abundance of other species will adversely affect ecosystem processes (Luoma and Carter 1991).

At mine drainage receiving sites, community composition shifted towards a greater relative abundance of individuals reported as tolerant and a decreased abundance of sensitive taxa. Winberg (1978) suggested that uncontaminated waters are dominated by larvae of the subfamily Orthocladiinae. However, increased relative abundance of orthoclad larvae has often been observed downstream of metal mining and electroplating industries (Armitage 1980; Winner et al. 1980; La Point et al. 1984; Yasuno et al. 1985; Chadwick et al. 1986; Clements et al. 1988; Leland et al. 1989;

Clements 1994; Clements and Kiffney 1994; Kiffney and Clements 1994a; Hickey and Clements 1998). At the mine drainage receiving sites of this study, the relative abundance of *Cricotopus/Orthocladius* was significantly higher than at paired reference sites. Trends of increasing relative abundance of *Eukiefferiella* and *Psectrocladius* were also observed at mine drainage receiving sites. *Cricotopus*, *Orthocladius*, and *Eukiefferiella* spp. have previously been reported as metal tolerant (Surber 1959; Winner et al. 1980; Chadwick et al. 1986; Hoiland and Rabe 1992; Gower et al. 1994). The mechanism involved in the superior ability of Orthocladiinae larvae to tolerate metals is unknown. Surber (1959) suggested that perhaps the ability to tolerate metals was indirectly responsible for their dominance at metal contaminated sites. Rather, their dietary preference for metal resistant blue-green algae, found dominating these sites, could explain their presence.

Larvae of the subfamily Tanytarsini frequently become less abundant at metal contaminated sites (Clements et al. 1988, 1989; Gower et al. 1994). Reduced relative abundance may result from enhanced metal sensitivity. Chadwick et al. (1986) found that although *Micropsectra* sp. was abundant upstream of a metal mining facility, larvae were rare downstream. Kiffney and Clements (1994a), however, observed increased abundance of Tanytarsini larvae in conditions of low (0.001 mg Cd/L, 0.010 mg Cu/L, 0.108 mg Zn/L) metal concentrations, suggesting that they may exhibit "moderate" metal tolerance. Clements et al. (2000) also found that the abundance of *Micropsectra* and *Stempellinella* spp. were only reduced at highly-metal-contaminated sites. At mine drainage receiving sites of this study, the relative abundances of *Micropsectra* and *Stempellinella* spp. were reduced by a factor of two and ten respectively, supporting their designation by some as sensitive to metal contamination.

A trend of decreasing abundance due to metal contamination was also apparent in *Microtendipes* (Chironomini) and *Larsia* (Tanypodinae) larvae. This is contrary to the assertion that contaminated waters are dominated by larvae of the subfamily Tanypodinae (Winberg 1978; Winner et al. 1980). The lower relative abundance of the predator *Larsia* at mine drainage receiving sites than at paired reference sites, could be explained by their sensitivity to metal exposure through trophic transfer or the loss of metal sensitive food sources.

Chironomid community composition at mine drainage sites below Heath Steele, Brunswick and Caribou Mines was similar, being dominated by Orthocladiinae larvae (*Cricotopus/Orthocladius*, *Eukiefferiella*, *and Psectrocladius*). Heath Steele and Caribou Mines, exhibited the largest differences in metal concentrations in both water and periphyton between reference and mine sites, and thus a more significant effect on chironomid communities could be expected. Brunswick No. 6, although closed since 1983, was an intensive open-pit operation. The grouping of this site with the two active mines suggests that this mine is still significantly affecting receiving water quality.

In contrast, Wedge and Stratmat Mines had similar chironomid communities as observed at paired reference sites. These sites were dominated by *Polypedilum* (Chironomini), *Micropsectra*, *Stempellinella* (Tanytarsini), and *Thienemanniella* (Orthocladiinae). Owing to low trace metal concentrations in water and periphyton and the brief history of metal mining at Wedge and Stratmat mines, less significant effects on the downstream chironomid community would be expected. Wedge, an underground mine, did not have an extensive operation and has been decommissioned for over 30 years. In addition, the Nepisiguit River is large, potentially diluting mine drainage effluents. Stratmat Mine was in operation less than 10 years ago, however, it was not an intensive operation. Nearby Heath Steele Mine site handled much of the waste

generated during the operation of Stratmat, potentially diminishing the effects observed at the Stratmat site.

Chironomid Deformities

This study documents one of the lowest deformity frequencies at both reference and contaminant receiving sites in the published literature. We were able to document significant differences in deformities despite the low frequency because we examined more individuals than is typically done in deformity analysis. The background incidence of deformities observed at our reference sites (0.79 %) falls in the low end of the range (0 – 5 %) of mentum deformities frequently observed at reference locations (Dickman et al. 1992; Lenat 1993; Groenendijk et al. 1998; Janssens de Bisthoven et al. 1998b; Vermeulen et al. 1998). Natural incidences of deformities have previously been observed to be < 1 % based on subfossil records (Warwick 1980a, Wiederholm 1984b) and thus sites with a frequency of > 1% are considered contaminated (Bird 1994).

The incidence of deformities at mine drainage receiving sites also falls within the range routinely observed by others at reference sites. Low levels of deformities could be due to several factors. Firstly, different chironomid genera are reputed to exhibit differential sensitivity to deformity expression (Hudson and Ciborowski 1996a; Burt 1998; Diggins and Stewart 1998). Our estimate of the incidence of deformities could therefore be conservative considering that this metric was expressed as a percentage pooled across all genera. Similarly, tolerance to metal exposure could also contribute to lower incidence of deformities at mine drainage receiving sites (Janssens de Bisthoven 1998a). Metal tolerance has been observed in midge larvae (Wentsel et al. 1978, Krantzberg and Stokes 1989, Gerhardt and Janssens de Bisthoven 1995) and has resulted in altered responses to biomonitoring indices (Postma et al. 1995a), and

lower deformity frequencies (Janssens de Bisthoven et al. 1998a). The comparison of the incidence of deformities observed in this study to that observed in others may also be inappropriate, considering the dissimilarity in habitats. Most deformity analysis studies have focused on chironomid communities of soft-sediment lotic and lentic environments. This is one of the few to report chironomid deformity levels in cobble streams and rivers. Lastly, differences in the classification of deformities are apparent in the literature (Burt 1998), contributing to observed variation in the incidence of deformities (Vermeulen 1995).

Regardless of the low incidence of deformities measured, there was a consistent increase at mine drainage receiving sites, ranging from 25 to over 800% above background. Incidences observed at mine drainage receiving sites approached a doubling of that observed at reference sites, implying that elevated metal concentrations were indeed associated with higher levels of deformities than normally encountered in these populations. While the manifestation of deformities has been used as an indicator of contaminant exposure, deformities may also indicate a lower fitness of the deformed individuals (Hudson and Ciborowski 1996b). Higher body burdens of contaminants (Dickman et al. 1992, Janssens de Bisthoven 1992), slower growth and development (Van Urk and Kerkum 1986 in Janssens de Bisthoven et al. 1998b), and lower emergence rates (Cervi 1996; Janssens de Bisthoven et al. 1998b) have been observed in deformed individuals than normal individuals.

Conclusions

The presence of metal mining facilities was significantly associated with elevated metal concentrations in water and periphyton in receiving streams. Effects in chironomids seen at both the community and individual level provide indications of

metal contamination. Mine-affected sites have significantly less diverse chironomid communities, with Tanytarsini larvae appearing to most sensitive and Orthocladiinae larvae least sensitive to metals. The incidence of deformities at mine drainage receiving sites was double that observed at reference sites.

Considering the importance of chironomids in lotic food webs, significant alterations in community composition could affect other trophic levels, particularly in the transfer of contaminants. These results imply that trace metal concentrations at mine-associated streams in New Brunswick affect the benthic community and thus have the potential to alter the structure and function of these aquatic ecosystems.

This is the first study examining the incidence of deformities in chironomids of cobble-bottomed streams. A significant difference in mentum deformities was detected using a conservative definition of deformity and a large sample size. It is recommended, due to the low incidence of background deformity incidences observed in this study that subsequent study employed larger sample sizes than currently employed. The analysis of metals in periphyton is recommended in biomonitoring studies, due to its ease of collection and ability to accurately reflect metal contamination of aquatic environments. Future studies should investigate the role of periphyton in the trophic transfer of metals to primary consumers.

CHAPTER 3: EFFECTS OF METAL ENRICHED PERIPHYTON ON THE MIDGE *CHIRONOMUS RIPARIUS* (DIPTERA: CHIRONOMIDAE)

INTRODUCTION

In aquatic environments enriched in trace metals, biota may experience exposure to metals through water, sediment and/or diet (Hare 1992; Schlekat and Luoma 2000). Elevated concentrations of metals in water tend to be transient in most systems, so that aqueous measurements are inadequate to assess the history of metal contamination in aquatic environments (Woodward et al. 1994; Genter and Lehman 2000). Metals tend to bind to and accumulate on inorganic and organic matter (Florence 1977; Allen et al. 1980). Periphyton, an assemblage of algae, bacteria, fungi and detritus attached to substrates, exhibit high metal concentrations (Clements and Kiffney 1994; Hickey and Clements 1998). Metals undetected in water samples are bioconcentrated in periphyton (Cd, Co, Cr, Cu, Ni, Pb, and V: see chapter 2) and provide a time-integrated assessment of metal contamination (Newman and McIntosh 1991; Woodward et al. 1994; Lee et al. 2000).

In streams and rivers with a relatively open canopy, autochthonous organic matter drives aquatic food webs (Allan 1995; Benke 1998). Fast flowing environments marginalize any aquatic macrophyte growth and limit the residence time of phytoplankton (Allan 1995). Thus, periphyton comprises the major source of autochthonous organic matter in these systems (Newman and McIntosh 1989; Hart and Robinson 1990). Benthic invertebrates scrape and collect periphyton and also live within its complex matrix (Power 1990). In metal stressed environments, periphyton

represents a significant source of contaminants, transferable to primary consumers (Newman and McIntosh 1989) (Table 1.1). These contaminants may subsequently be transferred to higher trophic levels in aquatic and terrestrial ecosystems (Luoma et al. 1992; Fisher and Reinfelder 1995; Wang et al. 1996; Munger and Hare 1997).

I used a novel approach was used to examine the effects of metal-enriched periphyton on chironomid growth and development. Metal contaminated periphyton was collected downstream of untreated mine drainage inflows from metal mining facilities in northeastern New Brunswick. In the laboratory, chironomids were fed either metal-enriched or a non-metal enriched periphyton. Chironomids fed mine drainage receiving stream periphyton were expected to accumulate metals, possibly in toxic quantities. Larvae associated with metal enriched periphyton were expected to exhibit lower survival and growth, and an increased incidence of deformities than chironomids fed diets of periphyton that were not metal-enriched.

MATERIALS AND METHODS

Periphyton Collection and Study Region

To examine the effects of metal enriched periphyton on chironomid larvae, five mines in northeastern New Brunswick were chosen (Heath Steele Mine, Stratmat Mine, Brunswick No. 6, Caribou Mine and Wedge Mine; see Chapter 1). Periphyton was sampled from five streams receiving untreated drainage from metal mining facilities and from five paired reference sites (Fig. 1.5, 1.7, 1.8). In all but one case, reference sites were located on separate streams. Each reference site was chosen to match the aquatic environment at a paired mine drainage receiving site in size, flow, substrate composition, and periphyton characteristics.

Periphyton was collected throughout 24-30 August, 1999 at 10 sites. One hundred and fifty to three hundred palm to hand-sized cobbles (diameter 64 – 128 mm) were removed from the steam bed at each site and periphyton scrubbed off using nylon brushes. Periphyton was rinsed into acid-washed plastic pails with stream water. The periphyton suspension was allowed to settle in the pails overnight at 4° C. Water was decanted off the surface of the settling periphyton, and all visible macroinvertebrates were removed. To remove the remaining water, samples were centrifuged at 6,000 rpm for 15 min. Water was decanted and periphyton was freeze-dried using a vacuum freeze drier for 24 h. Dried periphyton was stored in pre-cleaned acid washed containers.

A subsample of periphyton from each site was ashed at 550° C for 1-h to determine organic matter content (Table 3.1). Total concentrations of 16 trace elements were analyzed by ICP- OES at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory (Environment Canada 1979; Agemian et al. 1980; Environment Canada 1989).

Experimental Design

Tests were conducted in covered 2-L (12 x 12 x 15 cm) glass jars filled to a depth of 2 cm (approximately 500 g) with washed silica sand (particle size approximately 200 µm). Dechlorinated tap water (1-L) was added to each jar and gently aerated throughout the experiment by a branching, capillary tube system (Corkum and Hanes 1989). Experimental conditions for the laboratory bioassay were static, with replacement of evaporative water loss. Experiments were conducted at a constant air temperature of 22° C, with a 16:8 h L:D photoperiod maintained. Water quality parameters (dissolved oxygen, pH, water temperature) were measured on days

Table 3.1: Organic matter content of periphyton collected from mine drainage receiving and reference sites

SITE IDENTIFICATION	ORGANIC MATTER CONTENT (%)
Reference Sites	
HEA_R	53.2
BRU_R	52.8
STR_R	93.8
WED_R	46.7
CAR_R	50.4
Mine Drainage Receiving Sites	
HEA_M	21.5
BRU_M	27.6
STR_M	96.4
WED_M	57.7
CAR_M	57.1

zero, 6, and 10. Measurements of pH and water temperature were also taken on day 3. All materials were acid washed in 10% HNO_3 (analytical grade) and triple-rinsed in distilled water.

A single-factor ANOVA design was used, with n = 5 replicates of each treatment Three dietary treatments were tested, one metal enriched and two non-metal enriched diets. Chironomids were fed reference stream periphyton (REF), mine drainage receiving stream periphyton (MIN), or Nutrafin® (NUT) (Rolf C. Hagen, Mansfield, MA). Each periphyton replicate represented samples collected at a different mine site and its paired reference location.

Fifty first-instar *Chironomus riparius* (Diptera: Chironomidae) larvae, reared from laboratory cultures (Appendix 1), were randomly allocated to each jar. Larvae were hatched from multiple egg masses. Fifty larvae were counted under a dissecting microscope and pipetted below the water surface of the experimental jars within 24 h of hatching. Dried periphyton was ground, and Nutrafin® was blended and fed daily to larvae in suspension. Larvae were fed 1.0 mg of organic matter/ind/d. In a series of feeding studies, this feeding rate resulted in high survival and biomass of chironomid larvae (Appendix 2). Chironomids were harvested 10 d later.

At the conclusion of the study, jar contents were collected by gently sieving the sediment through a 180-µm mesh sieve. Individual larvae were hand-picked from the sieve, counted, and blotted to remove excess water. The majority of larvae were preserved in cold Carnoy's solution (3 parts anhydrous ethanol : 1 part glacial acetic acid), while several were set aside for metal analysis. Total concentrations of 25 trace elements in water from bioassay jars were analyzed by ICP-OES at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory (Environment Canada 1979, 1989).

Determination of Biomass

Chironomids were examined under a dissecting microscope, equipped with a Hitachi VK-C370 video camera. Images were captured with a Targa 64 digitizing card. Larval length, defined as the line measured along the dorsum from the anterior margin of the head capsule to the distal end of the procercus, was measured using Mocha Image Analysis Software, Version 1.2 (Jandel Scientific, San Rafael, CA). Length measurements were converted to biomass according to the regression equation of Nolte (1990). Regression constants a and b were adapted from the mean a and b values derived for *Microtendipes* and *Polypedilum* sp. (Diptera: Chironomidae: Chironomini). The equation is as follows:

$$\ln M = \ln(-7.3175) + 2.5925 \ln L$$

where: M = Mass (ug AFDW)

L = Length (mm)

Chironomid Deformities

The heads of individual *Chironomus* larvae were severed and slide-mounted ventral side up beside the body in CMC-9AF® aqueous mounting medium (Masters Company Inc., Bensenville, IL). The mentum of *Chironomus* larvae have a trifid median tooth, and 6 pairs of lateral teeth. Absolute mentum deformities (a missing or additional tooth only (Hudson and Ciborowski 1996b)) were scored, and re-examined to ensure correct designation. I performed all examinations for deformities. Individuals displaying broken, chipped or worn teeth were classified as damaged, not deformed. Gaps in the mentum were classified as deformities if their surface was smooth, rather than jagged, indicating breakage. Deformities in other structures were not examined. Data were

expressed as percentage of individuals deformed, with standard error determined according to the binomial distribution.

Statistical Analysis

Trace Element Analysis of Water and Chironomids

Principal component analysis was used to summarize trace metal concentrations in water from bioassay jars. Each analysis characterized the original variables in the data set as a smaller number of independent factors (principal components), each characterized by a suite of correlated variables. Individual variables (metals) are associated with a principal component (factor loadings). Factor scores reflect the association of a particular case (i.e. replicate) with the principal components. The number of replicates limited the number of variables used in the principal component analysis.

Factor scores, generated for each principal component, were used to compared Nutrafin, reference periphyton and mine drainage receiving periphyton treatments using a single factor ANOVA with *a posteriori* tests. Because multiple tests were performed, the critical value required to reject a null hypothesis of no difference was adjusted using Bonferroni correction to give an experiment wise Type I error of 0.05. Analyses were performed using the factor analysis and one-way ANOVA modules of Statistica® (StatSoft 1998, 1998 Edition).

Principal component analysis was performed using 12 elements and 15 cases. All data were $\log_{10}$ transformed prior to analysis. Concentrations of Ag, Al, As, Be, Bi, Cd, Co, Cr, Mo, Pb, Sn, Ti, and V in water were undetected in all replicates. Twelve elements, detected in most replicates were summarized by principal component analysis. Elements that were undetected in replicates were given values of the limits of

detection. Factors were varimax rotated and the principal component scores associated with loadings of >0.700 were including in the analysis. Mean factor scores for each treatment generated from PCA were then analyzed using a one-way ANOVA. Tukey's least significant difference tests were used to detect differences between dietary treatments.

Analysis of Survival, Biomass and Incidence of Deformities

Mean survival, biomass and incidence of deformities data were $\log_{10}$ transformed prior to analysis, then analyzed using a one-way ANOVA with planned comparisons. Non-metal enriched treatments (Nutrafin + reference periphyton) were compared against the metal enriched treatment (mine drainage receiving periphyton), followed by comparison of the two non-metal enriched treatments (Nutrafin vs. reference periphyton).

All statistical analysis was performed using Statistica (StatSoft '98 Edition, Tulsa, OK).

RESULTS

Trace Element Analysis of Water

Concentrations of Ca, Cu, K, Mg, Ni, Na and Sr in water from bioassay jars were higher in Nutrafin treatments than in either the reference stream or metal enriched stream periphyton treatments (Table 3.2). Concentrations of Ba, Mn, and Ti were higher in the bioassay water from reference stream periphyton treatment than other treatments. Iron and Zn concentrations were higher in bioassay water from the metal contaminated periphyton treatment than in water from other dietary treatments. Mean

Table 3.2: Mean (± 1 SE, n = 5) total concentrations of trace elements (µg/L) in water from bioassay jars of Nutrafin, reference stream periphyton and mine drainage receiving stream periphyton treatments

TRACE ELEMENT	PRINCIPAL COMPONENT ASSOCIATION	CONCENTRATION (ug/L)		
		Nutrafin	Reference Sites	Mine Drainage Receiving Sites
Copper (Cu)	I	**76.76 (6.33)**	nd[1]	**41.03 (15.25, n=2)**
Iron (Fe)	I	22.15 (n=1)	104.8 (27.8)	196.0 (50.4)
Manganese (Mn)	I	1.19 (0.28, n=2)	353.8 (216.4)	212.6 (87.8)
Sodium (Na)	I	8,552 (270)	6,754 (587)	7,429 (1,205)
Calcium (Ca)	II	31,499 (306)	30,696.00 (541)	28,564.00 (810)
Potassium (K)	II	2,829 (87)	2,115.00 (105)	1,592 (81)
Magnesium (Mg)	II	9,159 (118)	8,489 (193)	8,327 (37)
Barium (Ba)	III	13.40 (0.20)	58.15 (23.20)	17.94 (8.09, n=3)
Nickel (Ni)	III[3]	44.97(1.25)	nd	nd
Strontium (Sr)	II[3]	137.3 (1.9)	119.9 (2.7)	94.4 (16.7)
Titanium (Ti)	III[3]	nd	3.64 (0.76, n=3)	1.66 (0.05, n=2)
Zinc (Zn)	III[3]	14.07 (0.95)	11.98 (3.77, n=3)	**52.97 (8.75)**
PC - I		1.300 (0.054)[a]	-0.518 (0.111)[b]	-0.782 (0.217)[b]
PC - II		0.326 (0.165)	-0.005 (0.367)	-0.321 (0.697)
PC - III		0.160 (0.068)[b]	-0.925 (0.421)[a]	0.765 (0.389)[b]

[1]not detected
[2]boldface values are above Canadian Water Quality Guidelines for the protection of aquatic life (CCREM 1987)
[3]Metal not significantly associated with principal component
[a,b]different letters indicate significant differences at $p<0.001$. Statistical analysis was performed on principal component scores factor for each site using a single factor ANOVA and LSD comparisons.

differences in aqueous trace element concentrations between treatments were largest for Fe, Ni and Mn.

Principal component analysis was performed for 12 elements. Three factors were extracted from total metal concentrations in bioassay water by PCA, accounting for 80.0 % of the variation in the original data (Table 3.3). Sodium, Sr, Ti and Zn were not associated with any of the factors. Copper, Fe, Mn and Ni loaded were most associated with the values of the first factor, and their concentrations were significantly different among dietary treatments (one-way ANOVA, F_s = 66.60, $p<0.001$) (Table 3.4). Concentrations of Cu and Ni were significantly higher, and concentrations of Fe and Mn significantly lower in NUT treatment water than in REF (Tukey's test, $p<0.001$) or MIN treatment water (Tukey's test, $p<0.001$). In treatments containing mine drainage receiving stream periphyton and reference stream periphyton, concentrations of Cu, Fe, Mn, and Ni in water from bioassay jars differed only slightly, with largest differences observed among Stratmat (STR_R and STR_M) and Wedge (WED_R and WED_M) replicates (Fig. 3.1). As a result, concentrations of Cu, Fe, Mn, and Ni in water did not differ significantly among REF and MIN treatments (Tukey's test, $p<0.21$).

Concentrations of Ca, K and Mg were most associated with the values of the second factor, which did not significantly differ among dietary treatments (one-way ANOVA, Fs = 0.48, $p<0.49$) (Table 3.5).

Barium was most associated with values of the third factor, and its concentrations were significantly different among treatments (one-way ANOVA, Fs = 6.60, $p<0.012$) (Table 3.6). Concentrations of Ba were significantly higher in water from the REF than water from NUT treatments (Tukey's test, $p< 0.04$). Barium concentrations in water from treatments containing mine drainage receiving stream periphyton and reference stream periphyton were consistently different, with the largest

Table 3.3: Identification of the metals from total concentrations in water from bioassay jars loading most heavily on the first three principal components and the cumulative proportion of total variance explained by each component

ELEMENT	PC-I	PC-II	PC-III
Sodium (Na)	**0.95**	0.23	0.12
Copper (Cu)	**0.90**	0.089	0.16
Manganese (Mn)	**-0.86**	-0.27	-0.069
Iron (Fe)	**-0.83**	-0.38	0.055
Calcium (Ca)	0.086	**0.94**	-0.23
Magnesium (Mg)	0.24	**0.92**	0.094
Potassium (K)	0.50	**0.81**	-0.15
Barium (Ba)	-0.075	0.29	**-0.81**
Nickel (Ni)	0.44	0.044	0.68
Strontium (Sr)	0.30	0.66	-0.49
Titanium (Ti)	-0.43	-0.12	-0.62
Zinc (Zn)	-0.35	-0.23	0.66
Cumulative Proportion of Total Variance Explained	0.34	0.61	0.80

Table 3.4: One-way analysis of variance (ANOVA) of factor scores generated for principal component I (derived from mean concentrations of Cu, Fe, Mn, and Ni in water from bioassay jars) from treatments fed a Nutrafin suspension, reference stream periphyton, and mine drainage receiving stream periphyton (data log_{10} transformed)

VARIABLE	df	SUM OF SQUARES	MEAN SQUARE	F STATISTIC	p VALUE
Dietary Treatment	2	12.843	6.421	66.600	<0.001
Within Treatment Error	10	1.157	0.096		
Total	12	13.000	6.517		

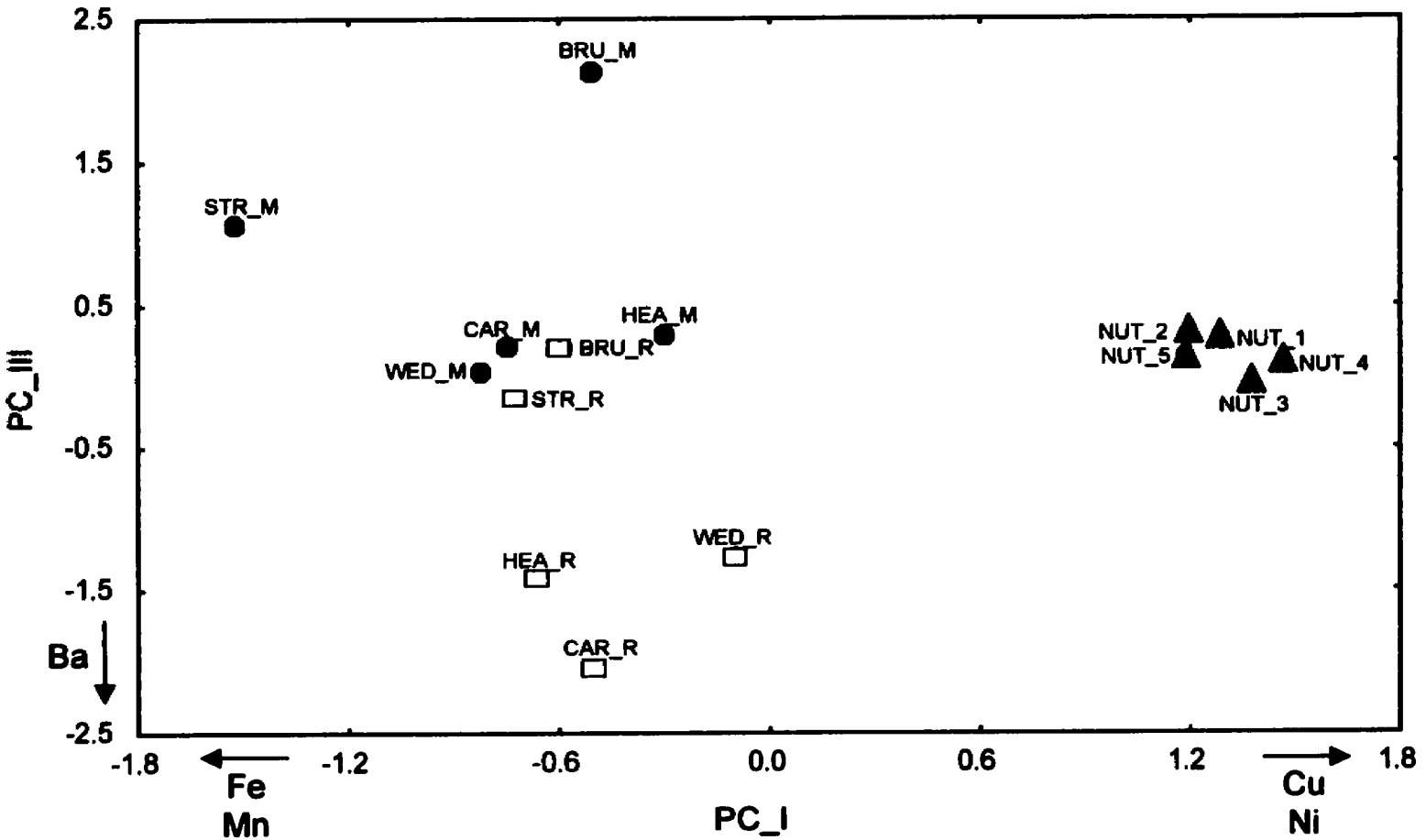

Figure 3.1: Total concentrations of Ba, Cu, Fe, Mn, and Ni in water in treatments fed nutrafin, periphyton from reference streams, and periphyton from mine drainage receiving streams as plotted in two principal components of the principal components analysis (PCA). Triangles represent NUT, open squares represent REF and solid circles represent MIN.

Table 3.5: One-way analysis of variance (ANOVA) of factor scores generated for principal component II (derived from mean concentrations of Ca, K, and Mg in water from bioassay jars) from treatments fed a Nutrafin suspension, reference stream periphyton, and mine drainage receiving stream periphyton (data $\log_{10}$ transformed)

VARIABLE	df	SUM OF SQUARES	MEAN SQUARE	F STATISTIC	p VALUE
Dietary Treatment	2	1.045	0.523	0.484	<0.628
Within Treatment Error	10	12.955	1.080		
Total	12	13.000	1.603		

Table 3.6: One-way analysis of variance (ANOVA) of factor scores generated for principal component III (derived from mean concentration of Ba in water from bioassay jars) from treatments fed a Nutrafin suspension, reference stream periphyton, and mine drainage receiving stream periphyton (data $\log_{10}$ transformed)

VARIABLE	df	SUM OF SQUARES	MEAN SQUARE	F STATISTIC	p VALUE
Dietary Treatment	2	7.335	3.667	6.603	<0.012
Within Treatment Error	10	6.665	0.555		
Total	12	14.000	4.222		

difference observed among Caribou replicates (CAR_ R and CAR_M) (Fig. 3.1). Barium concentrations were significantly higher in water from the REF than MIN treatments (Tukey's test, $p<0.04$). Barium concentrations were not significantly different between NUT and MIN treatments (Tukey's test, $p<0.23$).

Survival

Chironomid survival ranged from 0 – 68 % among dietary treatments. One set of replicates (STR_R and STR_M) fed *Fontinalis* (aquatic moss) rather than periphyton had 94 and 100% mortality respectively. These replicates were identified as outliers and removed from further analysis. Mean (± 1 SE) chironomid survival was 59% (± 4, n=5) for Nutrafin fed, 42% (± 8, n=4) for reference stream periphyton fed, and 16% (± 6, n=4) for mine drainage receiving stream periphyton fed (Fig. 3.2). Survival differed significantly among treatments (Table 3.7) (one-way ANOVA, $Fs = 7.45$, $p<0.01$). Chironomid survival in the mine drainage receiving periphyton treatment was significantly lower than survival of chironomids fed other diets (planned comparison, $p<0.005$). There was no significant difference in survival between the reference periphyton and Nutrafin treatments (planned comparison, $p>0.50$).

Biomass

Biomass among treatments ranged from 0.037 – 1.144 ug AFDW. Mean biomass of chironomids differed significantly among treatments (Table 3.8) (one-way ANOVA, $Fs = 13.984$, $p<0.001$). Chironomids fed Nutrafin were the largest (mean ± 1 SE, 0.81 ± 0.14 µg AFDW, n=5), followed by chironomids fed reference stream periphyton (mean ± 1 SE, 0.42 ± 0.11 µg AFDW, n=4) and chironomids fed mine drainage receiving stream periphyton (mean ± 1 SE, 0.07 ± 0.01 µg AFDW, n=4) (Fig.

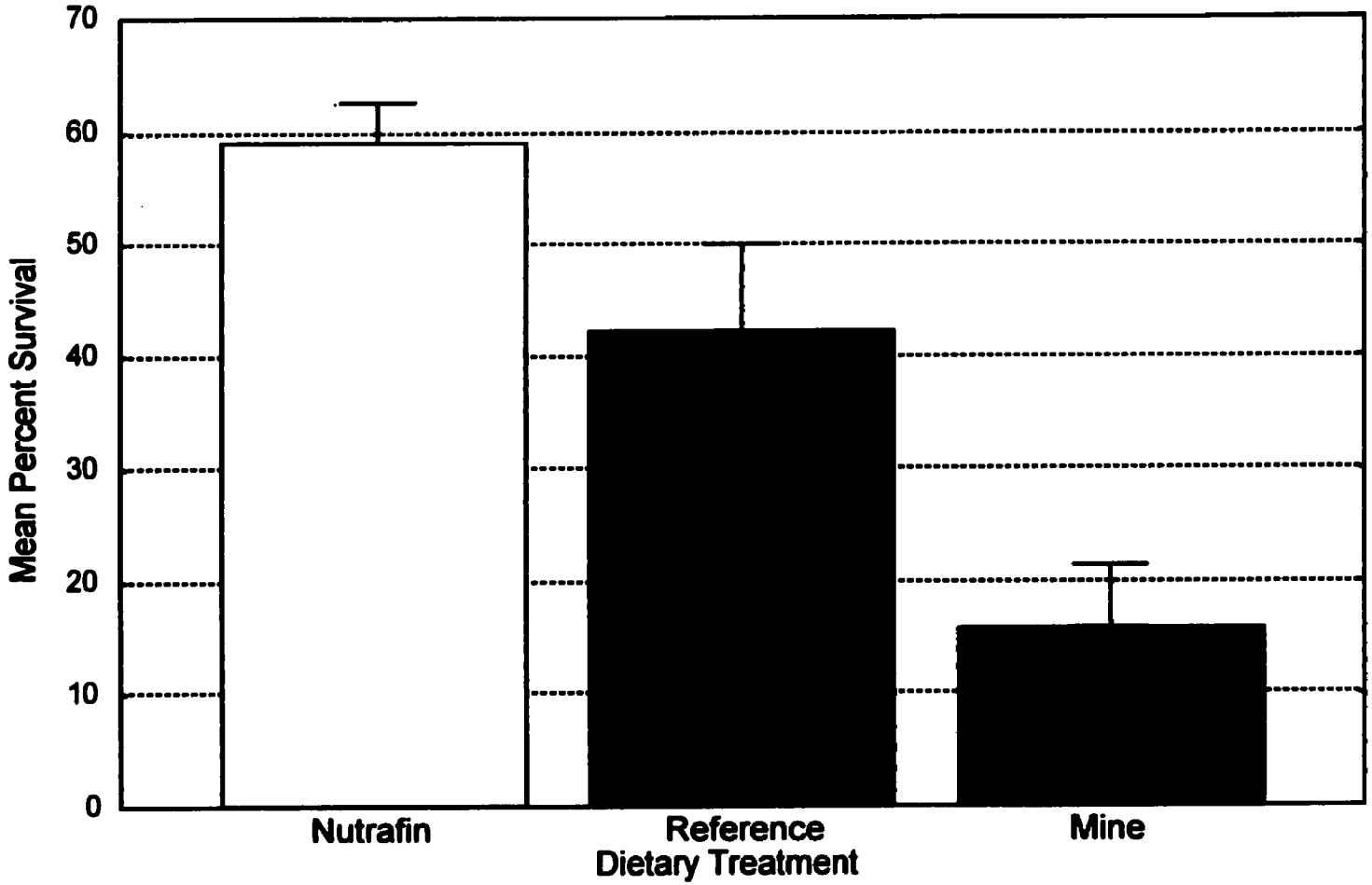

Figure 3.2: Mean survival (percent ± 1SE, n = 5) of chironomid larvae fed Nutrafin, reference stream periphyton, and mine drainage receiving stream periphyton

Table 3.7: One-way analysis of variance (ANOVA) of mean chironomid survival (%) fed a Nutrafin suspension, reference periphyton, and metal contaminated periphyton (data log_{10} transformed)

VARIABLE	df	SUM OF SQUARES	MEAN SQUARE	F STATISTIC	p VALUE
Dietary Treatment	2	1.041	0.520	7.449	<0.010
NUT vs. REF	1	0.068	0.068	0.973	<0.5
(NUT + REF) vs. MIN	1	0.972	0.972	13.906	<0.005
Within Treatment Error	10	0.699	0.070		
Total	12	2. 640	0.590		

Table 3.8: One-way analysis of variance (ANOVA) of mean chironomid biomass (µg AFDW of larvae) fed suspensions of Nutrafin, reference periphyton, and mine drainage receiving periphyton (data $\log_{10}$ transformed)

VARIABLE	df	SUM OF SQUARES	MEAN SQUARE	F STATISTIC	p VALUE
Dietary Treatment	2	0.0953	0.0477	13.984	<0.001
NUT vs. REF	1	0.021	0.021	6.158	<0.05
(NUT + REF) vs. MIN	1	0.0739	0.0739	21.672	<0.001
Within Treatment Error	10	0.0341	0.00341		
Total	12	0.1294	0.05111		

3.3). Chironomids fed periphyton from mine drainage receiving sites were significantly smaller than larvae from two control treatments ($p<0.001$). Chironomids fed periphyton from reference streams were significantly smaller that those fed a Nutrafin suspension ($p<0.05$).

Mentum Deformities

Overall incidence of mentum deformities was low. A slightly higher percentage (4.55 ± 2.22 %, n = 88) of deformed larvae were observed in Nutrafin fed chironomid larvae. Incidence of deformities in larvae fed reference stream periphyton was 1.89 ± 1.89 % (n = 53) and was 3.70 ± 3.70 % (n = 27) in larvae fed mine drainage receiving stream periphyton (Fig. 3.4). Sample sizes were too small for meaningful comparisons of deformity incidence to be made among treatments.

DISCUSSION

Trace Element Analysis of Water

Aqueous concentrations of some metals differed significantly among dietary treatments. Significantly elevated levels of Fe and Mn were detected in water from both reference stream periphyton and mine drainage receiving stream periphyton treatments. Aqueous Ba concentrations were significantly elevated in only the reference stream periphyton treatment. Apparently, some metals initially bound to the interior or exterior surface of periphyton may have been released due to sample preparation (i.e. cell lysis), or processes such as leaching or release from the periphyton surface.

Contamination of the Nutrafin treatment is apparent in that elevated concentrations of Cu and Ni were observed. Concentrations of both of these metals exceeded the Canadian Water Quality Guidelines (CWQG) for the protection of aquatic

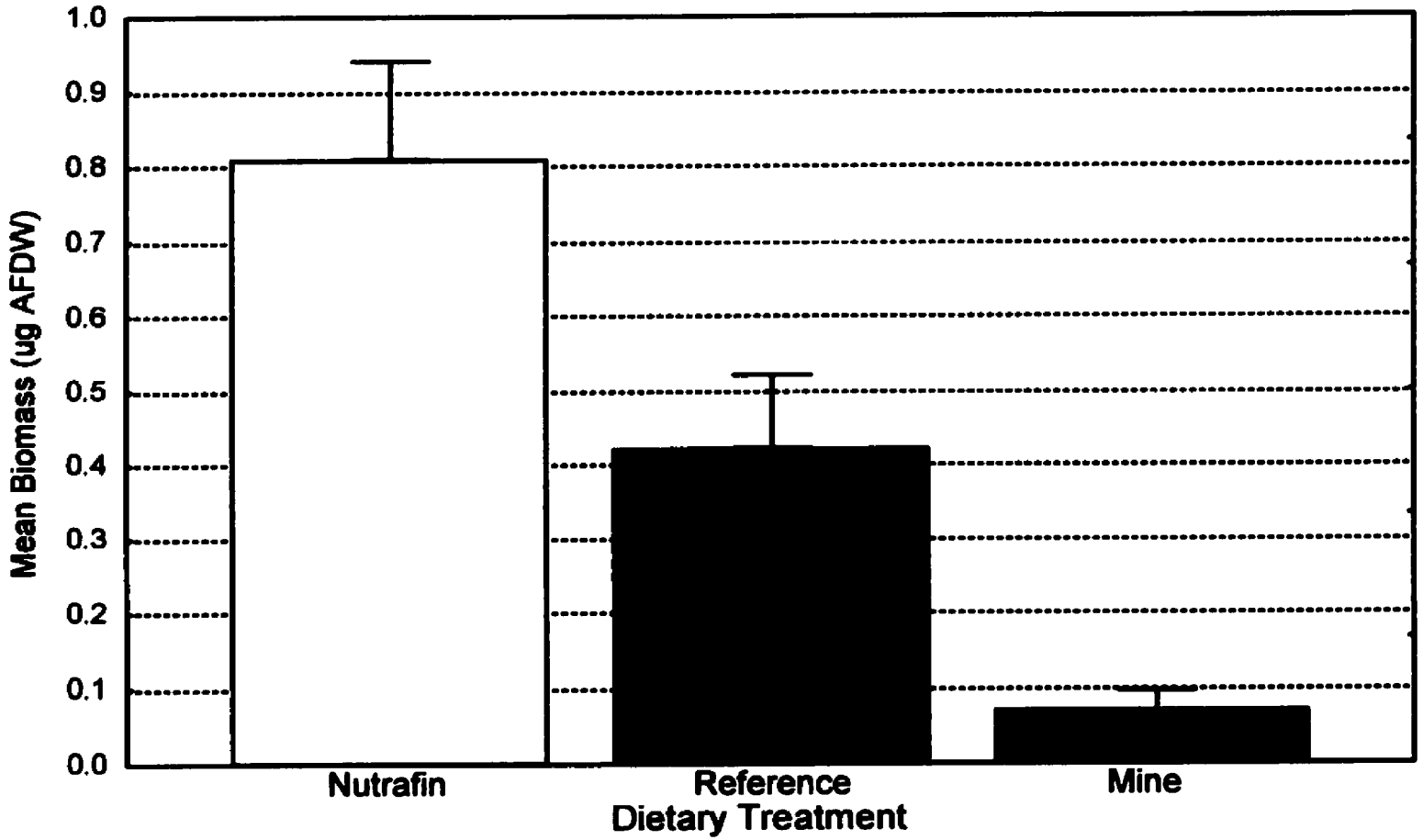

Figure 3.3: Mean biomass (ug AFDW (± 1SE, n = 5)) of chironomid larvae fed Nutrafin, reference stream periphyton, and mine drainage receiving stream periphyton

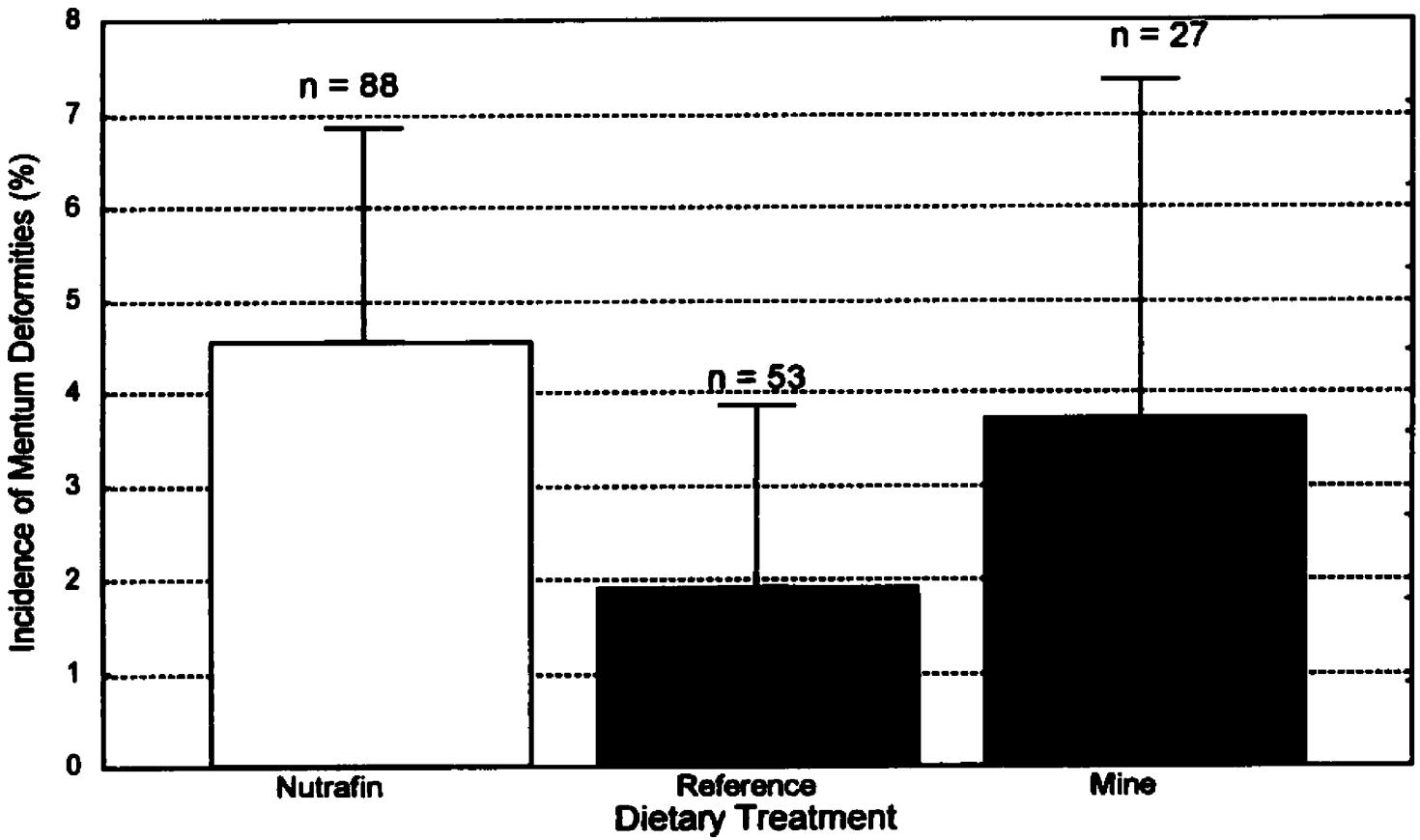

Figure 3.4: Incidence of mentum deformities (± 1SE) in chironomid larvae fed Nutrafin, reference stream periphyton, and mine drainage receiving stream periphyton

life (Cu: <2 µg/L; Ni: <25 µg/L CCREM 1987). Copper and Ni contamination may have resulted from high Cu and Ni concentrations in the Nutrafin itself, or may have arisen due to release of dissolved metals following acid washing of the blender. While the blender was subsequently triple rinsed with distilled water following acid washing, Cu and Ni from the metal blender blades and bearings may have contributed to the food suspension. Only the Nutrafin suspension was blended, eliminating the possibility of contamination of the other treatments from this source.

Survival

Survival was relatively poor among all dietary treatments. Concentrations of Ba, Fe and Mn in water were elevated in periphyton treatments. However, little work has examined the effects of these metals on chironomid survival. Manganese (Mn^{5+}), at concentrations lower than observed in the mine drainage receiving periphyton treatment, caused 43 % mortality of *C. plumosus* larvae (Fargasova 1997). However, the absence of differences in survival between NUT (low Mn) and REF (high Mn) treatments, suggests that Mn may not have been bioavailable to chironomid larvae in REF and MIN treatments. Similarly, the lack of difference in survival of larvae in NUT and REF treatments, also suggests that aqueous Ba and Fe did not contribute to reduced survival in periphyton treatments. Therefore, significant reductions in survival of larvae fed metal enriched periphyton is a result of the periphyton itself.

Survival in non-metal enriched diets was less than 70 %, the accepted standard for control survival in aquatic toxicity testing (Environment Canada 1997). This experiment was performed on multiple occasions in an effort to increase the survival of control larvae. And although 70% control survival was not achieved, all trends were consistent (Table A3.17). Reduced survival of chironomid larvae fed a Nutrafin

suspension may have resulted due to elevated Cu and Ni concentrations in water. Aqueous Ni concentrations can reduce survival of chironomid larvae. However, effective concentrations were orders of magnitude greater than concentrations measured in this experiment (Khangarot and Ray 1989; Fargasova 1997). Alternatively, aqueous concentrations of Cu in the range observed in the Nutrafin treatment, have been demonstrated to cause 50 % reductions in survival of chironomid larvae (Anderson et al. 1980; Phipps et al. 1995; Fargasova 1997).

Biomass

Significant differences in the size of larvae fed Nutrafin and reference stream periphyton can likely be attributed to different nutritional quality of the food. Chironomid larvae grow to much larger size and more quickly when fed a diet of animal origin compared to a diet of plant origin (Vos et al. 2000). Higher proportions of N, P, and lipids and lower levels of carbohydrates in food of animal origin contribute to higher growth rates (Vos et al. 2000). In the present study, the smaller size of larvae fed a periphyton diet relative to the animal rich Nutrafin diet, is consistent with the findings of Vos et al. (2000).

Reductions in chironomid biomass have been observed with exposure to both aqueous and dietary metal concentrations. The effect of Ba, Mn, and Fe concentrations in water on biomass of chironomid larvae has not been investigated. Copper exposure has elicited significant reductions in biomass of *Paratanytarsus* larvae. However, effective aqueous concentrations were much higher than observed in this study (Hatakeyama and Yasuno 1981). In all treatments, metal concentrations in water were not sufficient to reduce chironomid biomass (Hatakeyama and Yasuno 1981; Heinis et al. 1990).

Few studies have investigated the effects of metal enriched diets on chironomid larvae. Trace metals are thought to be strongly associated with food particles and thus unavailable for uptake by gut tissues (Hare 1992). Few of these studies that incorporate dietary metal exposure, have been able to differentiate between effects due to uptake through gill surfaces from water or uptake from diet. For example, Hatakeyama and Yasuno (1981) found that the combination of Cu concentrations in algae and water resulted in reduced growth of *Paratanytarsus parthenogeneticus*. In the present study, significant reductions in chironomid larval biomass were observed upon introduction of metal enriched periphyton. Copper concentrations in algae detected by Hatakeyama and Yasuno (1981) were comparable to levels observed in metal enriched periphyton of this study. This would suggest that significant reductions in biomass of chironomid larvae fed metal enriched periphyton may have been due to its metal content.

Effects of reduced growth and survival may be attributable to differences in the periphyton assemblage in mine drainage receiving streams. In metal contaminated environments, the loss of sensitive algae and dominance of more tolerant taxa characterize periphyton assemblages, contributing to reduced species diversity (Austin and Deniseger 1985; Leland and Carter 1985; Patrick 1988; Takamura et al. 1990; Genter and Lehman 2000). Generally, metal contamination results in decreased abundance of diatoms (*Asterionella formosa*, *Diatoma vulgare*, *Melosira varians*, *Tabellaria* sp.) (Patrick 1978; Rushforth et al. 1981; Austin and Deniseger 1985; Genter et al. 1987; Medley and Clements 1998) and moderate increases in abundance of green algae (*Stigeoclonium tenue*) and cyanobacteria, with unicellular green algae dominating at high metal levels (Harding and Whitton 1976; Leland and Carter 1984; Genter et al. 1987; Genter and Amyot 1994; Soldo and Behra 2000). Metal tolerant

taxa may be unpalatable to chironomids (Surber 1959; Gower et al. 1994). Reduced growth and survival may actually be in response to starvation, as a result of reduced availability of nutritional diets (Gower et al. 1994).

Deformities

Few deformities were found in chironomid larvae in any of the dietary treatments. Small sample sizes contributed to the low incidence of deformed individuals observed and the lack of significant differences among treatments.

Conclusion

The introduction of metal enriched periphyton had significant effects on growth and survival of chironomid larvae. In aquatic ecosystems, chironomid populations may be seriously affected by metal bioavailability from periphyton or by a lack of palatable food resources. Both mechanisms could contribute to reduced chironomid population productivity and consequently a reduction in the number of chironomids available to invertebrate predators. If metal toxicity represents a substantial selection pressure in these environments, chironomids tolerant to metals could persist in metal-enriched environments. However, if food availability limits chironomid survival, one would expect to find either impoverished communities or chironomid populations that preferentially feed on metal tolerant taxa. Surber (1959) suggested that the dietary preference of orthoclads for cyanobacteria, which are metal-resistant, explained their dominance at metal contaminated sites. This study is the first to feed field-collected periphyton to chironomid larvae in laboratory bioassays. This method approximated actual exposure of primary consumers to metal enriched diets in aquatic environments. Increases in control survival would significantly enhance the use of this bioassay in biomonitoring

studies. Further studies should examine the actual mechanisms (metal toxicity vs. palatability) contributing to reduced survival and growth demonstrated here. Elucidating the relative role of these two factors in determining effects on chironomid growth and survival may aid in the understanding of the persistence of populations in metal enriched environments.

CHAPTER 4: GENERAL DISCUSSION AND RECOMMENDATIONS

GENERAL DISCUSSION

Trace metals frequently enter aquatic environments, adversely affecting aquatic communities (Hare 1992; Clements 1994; Clements et al. 1988; Pacyna et al. 1995). This study examined the affect of untreated mine drainage on chironomid assemblages. The incidence of deformities, generic richness and community composition were compared at mine drainage receiving and paired reference sites. Trace metals were significantly higher in water and periphyton at mine drainage receiving sites than at paired reference sites. Chironomids exhibited increased incidence of deformities, reduced generic richness and altered community composition compared to chironomid assemblages at reference sites.

At mine drainage receiving sites, metal enrichment was associated with adverse biological responses on individual, population and community characteristics. Only Al and Zn concentrations in water at mine drainage receiving sites were significantly elevated, exceeding Canadian Water Quality Guidelines (CWQG's). Periphyton metal concentrations, however, were much higher than concentrations in water. Consequently, periphyton was suspected to be the major source of metals to primary consumers. Periphyton collected from mine drainage receiving and reference sites was fed to *Chironomus riparius* in the laboratory. Larvae fed periphyton from mine drainage receiving streams were significantly smaller, and survival was reduced compared to larvae fed periphyton from reference streams.

Metal mining in northeastern New Brunswick clearly contributes trace metals to receiving streams. Biological responses to metal mining are evident at the level of individual (deformities), population (size, survival) and community (richness, composition). Clearly, metal mining has an adverse affect on aquatic communities,

possibly mediated through metal enrichment of periphyton.

Metal Enrichment

At metal mining facilities, waste rock removed from underground shafts and open pits are ubiquitously distributed (Environment Canada 1995). Exposure of waste rock to oxygen and water contribute to the generation of AMD (Mills 1995). Acid mine drainage enters receiving aquatic environments, resulting in bioaccumulation of metals (Kiffney and Clements 1993), reductions in abundance and richness (Waterhouse and Farrell 1985; Leland et al. 1989), altered community composition (Clements et al. 2000) and increased incidence of deformities (Janssens de Bisthoven et al. 1998a,b).

At mine sites in northeastern New Brunswick, the presence of metal mining facilities negatively affects water quality in receiving waters. Elevated concentrations of both dissolved solutes (e.g. Na, K) and trace metals (Ba, Fe, Mn and Zn) were observed below areas suspected of receiving AMD. At these mine sites, Zn concentrations are comparable to concentrations observed at mine drainage receiving sites in Colorado (Courtney and Clements 2000; Medley and Clements 1998) and Idaho rivers (Genter and Lehman 2000). Other, metals (e.g. Cd, Cu, Pb) commonly detected at other mine drainage receiving sites (Kiffney and Clements 1993; Saiki et al. 1995; Genter and Lehman 2000; Ivorra et al. 2000) were undetected in water downstream of northeastern New Brunswick mines. However, trace elements undetected in water samples, were found in periphyton (i.e. As, Cd, Co, Cr, Cu, Ni, Pb, and V) at mine drainage receiving sites, indicating that these elements are indeed entering receiving waters.

In northeastern New Brunswick streams receiving drainage from metal mining facilities, metal concentrations in periphyton were significantly elevated. Concentrations

in periphyton were generally higher than observed at other metal contaminated sites (Kiffney and Clements 1993; Gupta 1996; Hickey and Clements 1998; Medley and Clements 1998; Genter and Lehman 2000) (Table 1.1). However, few recent studies examining the metal content of periphyton at metal contaminated sites are available for comparison. Rather, studies examining periphyton community composition in degraded environments are more common (Austin and Deniseger 1985; Takamura et al. 1990; Chindah 1998; Medley and Clements 1998; Vis et al. 1998; Genter and Lehman 2000; Hill et al. 2000; Ivorra et al. 2000; Paulsson et al. 2000; Soldo and Behra 2000).

This study confirms that periphyton is a significant accumulator of trace metal, exhibiting metal concentrations orders of magnitude greater than in water at mine drainage receiving sites. Periphyton analysis appears to be a suitable surrogate for sediment analysis in biomonitoring programs in aquatic environments lacking soft sediments (Newman and McIntosh 1989). According to this study, the analysis of metal concentrations in periphyton is a more sensitive indicator of contamination than chemical analysis of water. Conclusions drawn from metal concentrations in water alone would have suggested that metals such as Cd, Cu and Pb were not entering receiving environments. While more labour intensive than water and sediment collection, sufficient quantities of periphyton for metal analysis can easily be collected in lotic and lentic environments with minimal equipment and effort. Aloi (1990) has described various periphyton sampling devices.

Biomonitoring protocols would be enhanced by the addition of periphyton metal analysis, increasing their ability to assess degraded environments. In addition, periphyton metal analysis may provide an estimate of potential trophic transfer of metal to invertebrates feeding at the base of the food chain.

Metal Bioavailability

Metal contamination is apparent downstream of metal mining facilities in northeastern New Brunswick. However, biological responses are not determined by concentrations of metals, but rather by an estimate of metal exposure (Hare 1992). Metal exposure is estimated by the concentrations of metals that occur in forms readily available for uptake (Plette et al. 1999). Bioavailability of metals can be inferred from bioaccumulation in biota and/or cellular responses to metal exposure, such as the induction of metal stress proteins [e.g. metallothionein (Yamamura et al. 1983; Seidman et al. 1986) and heat shock proteins (Aziz et al. 1991; Bentivegna and Copper 1993)].

Metal bioavailability to chironomid larvae at reference and mine drainage receiving sites can not be directly inferred from the results of this study because bioaccumulation of metals in chironomids collected at reference and mine drainage receiving sites, and fed metal contaminated periphyton have not yet been analyzed. However, metal bioavailability to chironomid larvae may be inferred by the association of metal enrichment and biological responses in field and laboratory bred chironomids.

High metal concentrations in periphyton may suggest that metals are biologically available at mine drainage receiving sites. However, periphyton bioaccumulation may not be an accurate reflection of contaminant bioavailability (Newman and McIntosh 1989) to consumers. Metals bind to both organic and inorganic components of the periphyton matrix. Therefore, assuming that total metal concentrations in periphyton are available to grazing benthic invertebrates may overestimate the risk of metal exposure (Newman and McIntosh 1982; Newman et al. 1983). In a single study by Newman and McIntosh (1982), increasing metal concentrations in periphyton did not result in increased bioaccumulation in grazing gastropods, suggesting that the metals bound to periphyton were not bioavailable and hence no substantial trophic transfer

was occurring. However, in a number of recent studies, food has been demonstrated to be an important metal source (Woodward et al. 1994; Munger and Hare 1997; Lee et al. 2000). Metals bound to both organic and inorganic matter can be incorporated in aquatic organisms (Lee et al. 2000). For example, the amphipod *Hyalella azteca* bioaccumulated significant concentrations of metal from a metal contaminated periphyton diet (Ken Doe, Environment Canada, pers. comm.). The discrepancy in these results, highlights the need for further examination of the role of periphyton in trophic transfer of metals.

Biological Responses to Metal Enrichment

Biological responses to metal mining occur at all levels of organization (Reice and Wohlenberg 1993). Sub-organismal responses were not measured in this study, although chironomids were collected with the intention of examining chromosomes for evidence of stress protein synthesis. The larvae I collected in the field were too small for this type of analysis. Low survival and the small size of larvae in metal-enriched treatments of laboratory experiments also precluded chromosomal analysis.

Biological responses at the level of the individual (e.g., deformities) and population (e.g., survival, size) were clearly associated with metal enrichment in field and laboratory studies. Chironomid larvae fed metal-enriched periphyton exhibited reduced survival and growth, as evidenced by lower individual biomass. Reductions in survival and biomass have previously been observed in chironomid larvae in association with metal concentrations in water (Anderson et al. 1980; Hatakeyama 1988; Heinis et al. 1990; Phipps et al. 1995) and sediment (Wentsel et al. 1977b; Kosalwat and Knight 1987). However, this study is the first clear demonstration that metal-enriched periphyton alone produce equivalent effects. Few studies have

examined the trophic transfer of metals from algae to chironomid larvae. Hatakeyama and Yasuno (1981) demonstrated a 50 % reduction in the number of eggs produced by adult *Paratanytarsus parthenogeneticus* exposed to Cu in water and green algae (*Golenkinia radiata*). Larvae were also significantly smaller than non-metal exposed larvae (Hatakeyama and Yasuno 1981).

Chironomid larvae associated with metal enriched periphyton in the laboratory had an increased incidence of deformity compared to chironomid larvae fed periphyton from reference streams. However, this difference was not statistically significant, due to the small sample sizes available for deformity analysis. The study treatments were not expected to be lethal to chironomids, and hence, deformity analysis was expected to include more larvae per experimental unit. Approximately 400 larvae would be needed to achieve sufficient power for a doubling in the incidence of deformities (1.89 % in REF vs. 3.70 % in MIN) to be statistically significant at $p<0.05$.

To date, deformity studies have largely been conducted in lakes and large rivers (Lenat 1993). Few studies have examined the incidence of deformities in chironomid larvae in cobble-bottomed lotic environments contaminated with either metals or organic pollutants (Lenat 1993). This study is the first to document chironomid mentum deformities associated with metal contamination in cobble-bottomed streams. The incidence of deformities in field collected chironomids was consistently and significantly higher at metal contaminated sites than at paired reference sites. Fewer than 17 % of 29 studies, published in the primary literature between 1971 and 1997, examining deformities reported significant differences between contaminated and reference sites (Burt 1998). Significant results in this study required the collection and analysis of large numbers (500 per site) of chironomid larvae, making this one of the most intensive examinations of deformity levels in aquatic environments. The examination of large

numbers of chironomid larvae for deformity analysis was necessitated by the lower incidence of deformities at reference sites (<1%) compared to background levels observed in other habitats (e.g., ≤3 % in Great Lakes, Hudson and Ciborowski 1996b).

The incidence of deformities in chironomid larvae at metal contaminated sites was low compared to that observed in other metal contaminated environments (3 % - Groenendijk et al. 1998; 7.1 % - Janssens de Bisthoven et al. 1998b). Low levels of deformities observed in the current study may be attributed to a conservative definition of deformity (additional or missing teeth (Hudson and Ciborowski 1996b)). Differences in the classification of deformities are apparent in the literature (Burt 1998) and contribute to observed variation in the incidence of deformities in degraded environments (Vermeulen 1995). Sensitivity of different genera to deformity expression may also contribute to low levels of deformities observed in this study (Hudson and Ciborowski 1996a; Burt 1998; Diggins and Stewart 1998). Most studies do not compare total deformity incidences among all chironomids collected in degraded environments. Rather, comparisons at reference and metal contaminated sites are made between genera collected at both sites. However, even with the examination of 500 larvae per site, not enough common genera were collected to powerfully compare deformity incidences.

Based on this study, it is recommended that for deformity analysis with reference incidences <1 %, 500 individuals should be examined at each site. This is not unreasonably labour intensive considering that chironomids must be slide-mounted for identification, and that larvae can be examined for deformities simultaneously. The need for large samples of chironomid larvae required for powerful deformity analysis should not preclude their use as indicators of environmental degradation (Burt 1998).

Chironomid community composition was significantly altered at mine drainage receiving sites compared to paired reference sites. Fewer taxa were observed at metal-enriched sites. Similar reductions in chironomid richness have been reported in other studies of metal contaminated sites (Winner et al. 1980; Armitage and Blackburn 1985; Waterhouse and Farrell 1985; Yasuno et al. 1985; Wilson 1988; Diggins and Stewart 1998; Clements et al. 2000). I found that Tanytarsini larvae were less abundant and Orthocladiinae larvae were more abundant at metal enriched sites than at reference sites. This finding is consistent with the results of other studies (Armitage 1980; La Point et al. 1984; Chadwick et al. 1986; Clements et al. 1988; Clements et al. 1989; Clements 1994; Clements and Kiffney 1994). Sites with the highest concentrations of metals in water and periphyton exhibited the most obvious difference between sensitive and tolerant chironomid assemblages, highlighting that "dose does indeed make the poison".

Metal Association with Biological Responses

Attributing biological responses at mine sites directly and solely to metal enrichment is difficult to demonstrate in field studies, since specific cause-effect mechanisms are difficult to assess under variable conditions prevailing in nature (Luoma and Carter 1991; Kiffney and Clements 1993). However, the demonstration of clear biological responses in association with metal enrichment in both field and laboratory studies suggests that these responses are metal-related (Clements and Kiffney 1994).

The laboratory bioassay conducted in this study demonstrated that metal-enriched periphyton from mine drainage receiving sites was responsible for reductions in growth and survival of chironomid larvae. Attributing the metal content of the

periphyton as the causative factor requires one to assume that reference and metal-enriched periphyton fed to chironomids was qualitatively similar in all ways except for metal content. However, the composition of periphyton assemblages (apart from proportion of organic matter content) was not assessed in this study and may have differed between mine drainage receiving and reference sites. Typically, metal enrichment alters the algal composition of periphyton, resulting in a shift from diatom predominance to a green alga and cyanobacteria community (Patrick 1978; Austin and Deniseger 1985; Genter and Lehman 2000; Soldo and Behra 2000). Apart from metal concentrations in periphyton, metal-tolerant algal taxa (green algae and cyanobacteria) may be less palatable and have reduced nutritional quality (Gower et al. 1994). Therefore, invertebrates feeding on metal tolerant periphyton may experience reduced survival and growth (Newman and McIntosh 1989; Gower et al. 1994) due to the low nutritional quality of the algae. Previous studies have not examined the nutritional quality of periphyton in metal contaminated sites. This study was the first to feed natural periphyton assemblages from metal enriched and reference streams to chironomid larvae, with reductions in survival and growth possibly attributed to the reduced palatability of metal enriched periphyton.

Assemblages of chironomids and other invertebrates feeding on metal contaminated periphyton may be directly or indirectly affected by metal contamination. Metal toxicity, through metal accumulation in larvae, may lead to reduced richness and altered chironomid community composition (Winner et al. 1980; Armitage and Blackburn 1985; Waterhouse and Farrell 1985; Yasuno et al. 1985; Wilson 1988; Diggins and Stewart 1998; Clements et al. 2000), presumably by influencing the growth, survival and/or reproductive success of metal susceptible taxa. Metal tolerant taxa may continue to exploit resources in metal contaminated environments, with less competition

for food and space (Canfield et al. 1994). Alternatively, metal contamination may reduce the abundance of palatable algae, indirectly affecting chironomid communities. Chironomid larvae may experience reduced availability of nutritionally adequate resources, resulting in reductions in abundance and richness. Larvae capable of exploiting alternate resources may then make up a disproportionate amount of the community, resulting in altered composition (Surber 1959; Gower et al. 1994).

Attributing biological responses of chironomid larvae to metal contamination is strengthened by the strong experimental design of this study. Measurement of metal concentrations in various media and chironomid community characteristics were expected to differ greatly, due to the examination of these parameters in different streams. Despite great natural variability, I found consistent effects of metal enrichment across 8 different streams. The presence of a consistent pattern suggests that there is a widespread and strong association between mine drainage inputs and the chironomid community in northeastern New Brunswick streams. This association could only be strengthened by metal accumulation data in chironomid larvae from both bioassays and field collected larvae. Higher concentrations of metals in chironomid larvae at mine drainage receiving sites than at paired reference sites would prove that metals are indeed bioavailable to chironomid larvae. Therefore, adverse effects observed on individuals, populations and communities at mine drainage receiving sites could be more strongly ascribed to metal toxicity. No difference in metal concentrations in chironomid larvae at mine drainage receiving and reference sites could indicate that metals are not available to chironomids.

Unlike in laboratory bioassays, acute effects of growth and survival on chironomid larvae associated with metal contaminated periphyton were not studied in field collected assemblages. Differences in survival and growth are hard to detect in

field populations, particularly when examining population and community responses (Reice and Wohlenberg 1993). However, reduced survival and growth should result in reduced population size, with limited persistence in contaminated environments.

FUTURE RESEARCH

Periphyton

More research should be done on the use of periphyton in biomonitoring efforts of stony streams, examining factors that affect its abundance and composition in metal enriched environments. Periphyton has been suggested to be a suitable surrogate to sediment analysis in environments where soft sediment is limited (Newman and McIntosh 1989). In many ways, I consider it superior to sediment, due to its apparent importance as a basal resource in aquatic food webs (Lock et al. 1984; Allan 1995). Its role in the transfer of contaminants to grazing benthic invertebrates needs to be investigated if we are to better understand the fate and effects of metals in aquatic environments.

Standard toxicity tests are routinely conducted with water and sediment collected from degraded environments. This is the first published study to collect periphyton from metal-contaminated environments and feed it to laboratory-reared chironomids. This novel approach incorporates environmentally relevant metal concentrations and thus results of this bioassay can be more realistically related back to the field populations.

At sites with the highest metal concentrations in water and periphyton, periphyton was more abundant than at any of the other sites (chapter 2). Considerably less effort was required at each of these mine sites, to collect a substantial quantity of periphyton. Previous studies have reported both reductions (Leland and Carter 1985)

and increases (Butcher 1946; Armitage 1980; Gower et al. 1994; Genter and Lehman 2000) in periphyton abundance at metal contaminated sites. Periphyton abundance, measured as biomass or chlorophyll *a* content, should be assessed at mine drainage receiving sites and compared to periphyton abundances reported in the literature. According to the river continuum concept (RCC) periphyton abundance increases with stream order (Vannote et al. 1980), suggesting that increasing periphyton abundance downstream of metal mining facilities compared to upstream sites is independent of metal input. However, this study did not compare upstream and downstream sites. Rather reference and mine drainage receiving sites on different streams of similar size (stream order) were compared. Therefore, I expect that periphyton abundance will be significantly greater at mine drainage receiving sites than at paired reference sites.

The reason for expecting greater abundance of periphyton in metal contaminated environments has not been tested. Periphyton abundance may fluctuate with increased input of nutrients (Grimm and Fisher 1986; Hart and Robinson 1990), decreased abundance of grazing invertebrates (Hart 1985; Anderson et al. 1999), or decreased palatability of metal tolerant algal taxa at metal contaminated sites (Surber 1959; Gower et al. 1994). Nutrient concentrations, grazing benthic invertebrate density, and periphyton composition should be assessed at mine drainage receiving sites and compared to paired reference locations. Nutrient concentrations are not expected to differ between mine drainage receiving and reference locations, based on analyses done in this study (chapter 2). Grazing benthic invertebrate density is expected to be lower at mine drainage receiving sites than at paired reference sites. Periphyton composition is expected to differ between mine drainage receiving and reference sites. According to the literature, metal contaminated sites should be dominated by green algae and cyanobacteria, accompanied by reduced abundance of diatom algae (Patrick

1978; Austin and Deniseger 1985; Genter and Lehman 2000; Soldo and Behra 2000).

Following a general assessment of these parameters, a reciprocal transfer experiment could be performed to address each of these hypotheses. The experiment could employ a 2 x 2 x 2 factorial design. Cobbles coated with periphyton would be transferred between mine drainage receiving and reference sites. At each site, periphyton from each source would be subjected to two treatments; one where grazing benthic invertebrates are excluded and one where benthic invertebrate grazers are not excluded (for methods see Barnes and Mitchell 1983; Lamberti and Resh 1983). In grazer exclusion treatments, the role of nutrients in stimulating periphyton growth at mine drainage receiving sites would be determined. If grazer-excluded reference periphyton at mine drainage receiving sites increases in abundance relative to grazer-excluded periphyton kept at the reference site then nutrient enrichment is a contributing factor. Grazer inclusion treatments will determine the role of grazer density on reducing periphyton abundance and the palatability of metal contaminated periphyton. The reduction of metal contaminated periphyton at reference sites and the increase of reference periphyton at mine drainage receiving sites would indicate that grazer density is playing an important role in reducing periphyton abundance. However, if metal enriched periphyton placed in reference sites is not reduced, this would suggest that the periphyton is in fact unpalatable to grazing benthic invertebrates.

Chironomid Adaptation

Populations exposed to metal contaminants often evolve enhanced tolerance to metals compared to non-exposed populations (Luoma 1977; Klerks and Weis 1987; Klerks and Levinton 1989; Clements 1999; Postma and Groenendijk 1999). Survival (Wentsel et al. 1978) and biomass (Postma et al. 1995c) of chironomid larvae from

metal exposed sites are less affected by metals than chironomids from non-contaminated environments. Tolerance to contaminants may be a result of physiological acclimation or genetic adaptation (Weis and Weis 1989; Postma and Groenendijk 1999). Metal tolerant or adapted populations have greater capacities for metal detoxification and excretion than reference populations, producing higher levels of metal-binding proteins and more storage granules (Postma et al. 1996; Postma and Groenendijk 1999). However, the maintenance of tolerance mechanisms requires the expenditure of energy normally available for growth and metabolism (Holloway et al. 1990). Life history patterns of chironomid populations are affected by "costs of tolerance", as demonstrated by increased larval mortality and larval development time of metal adapted populations cultured in clean environments compared to non-metal adapted populations (Postma et al. 1995b).

Mines in northeastern New Brunswick have contributed to, in some cases, over 30 years of metal exposure to benthic invertebrate communities. During this time, I expect that significant selection for metal tolerance would have occurred, eventually resulting in metal-adapted populations. Aquatic environments with metal adapted populations may be difficult to monitor for signs of degradation, because chironomids in these environments may be less likely to display deformities (Janssens de Bisthoven et al. 1998b) than non-adapted individuals.

Metal adaptation of chironomid populations at mine drainage receiving sites should be examined. Adult chironomids can easily be collected at both metal contaminated and reference sites and the relative sensitivity of their offspring to metals assessed under laboratory conditions. Laboratory cultures can easily be started from eggs obtained from light-trapped adults, permitting controlled comparisons in the laboratory. A better understanding of the response of metal adapted populations to

metal contamination will contribute to the development of better monitoring tools that will identify degraded environments

The presence of adapted populations of chironomids is a reflection of environmental degradation (Blanck and Wangberg 1988; Dahl and Blanck 1996). Although, these adapted populations may successfully tolerate metal enrichment, their fitness may be reduced, making them less capable of tolerating predation (Clements 1999), acidity (Courtney and Clements 2000) and other novel stresses (Benton and Guttman 1990, 1992; Snyder and Hendricks 1997). The loss of other non-metal adapted populations of benthic invertebrates and fish at mine drainage receiving sites may affect the structure and function of aquatic ecosystems (Cain et al. 1992; Canfield et al. 1994; Clements 1994; Beltman et al. 1999; Clements et al. 2000).

CONCLUSION

Superficially, streams in the vicinity of metal mining facilities in northeastern New Brunswick appear to be pristine. This has contributed to an assumption that significant mine-related effects are not affecting receiving waters in this area. However, this study has demonstrated significant effects on chironomid assemblages at sites receiving drainage from waste rock piles. Mines currently in operation had the most severe effects on receiving waters, resulting in the highest metal concentrations in water and periphyton observed in this study. Mines that have been decommissioned for over 20 years, are also seriously affecting receiving environments. Limiting the input of acid mine drainage to receiving waters is difficult due to the diffuse dispersal of waste rock on mine premises. In fact, many of the mines have attempted remediation of these sites, which according to this study has not eliminated metal input to receiving

environments. Further remediation is required to identify sources of mine drainage at metal mining facilities.

Biomonitoring at mining facilities currently in operation should continue, while biomonitoring should be reinstated at decommissioned sites. Effects of metal contamination on benthic invertebrate and fish communities should be investigated, focusing on the transfer of metals to higher trophic levels.

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APPENDIX 1: *CHIRONOMUS RIPARIUS* CULTURE

GENERAL BIOLOGY

Chironomus riparius is a small nonbiting midge, with three aquatic immature stages and a brief terrestrial adult stage. This species belongs to the family Chironomidae (=Tendipedidae), subfamily Chironominae, Tribe Chironomini, and genus *Chironomus, thummi* group. Chironomids are most closely related to the family Ceratopogonidae (Diptera) (Wood and Borkent 1989).

Larvae of *C. riparius* have various features distinguishing them from other chironomid larvae. Larvae have a sclerotized head, thorax (3 segments) and abdomen (9 segments). The head capsule of *C. riparius* larvae consists of short, non-retractile antennae with a toothed mentum, and distinct paralabial plates. The mentum has a tripartite medium tooth with 6 pairs of lateral teeth. The eighth abdominal segment has two pairs of ventral tubules (Oliver and Roussel 1983).

Distribution

Chironomus riparius has a widespread European distribution (Townes 1945; Curry 1962; Credland 1973; Environment Canada 1997). This species has also been recorded in both North and South America (Townes 1945). In Canada, *C. riparius* has been collected across the country, except in British Columbia, New Brunswick, Nova Scotia, Prince Edward Island, and the Yukon (Townes 1945; Curry 1962; Rasmussen 1984).

Ecology

Chironomus riparius larvae are found inhabiting soft sediments (Rasmussen 1984) in both lentic (Parma and Krebs 1977; Jernelov et al. 1981) and lotic environments (Bendell-Young and Harvey 1991; Timmermans et al. 1992b; Postma et al. 1995a). Larvae construct silk burrows or tubes interweaved with sediment particles, detritus, and algae (Dudgeon 1994; Chaloner and Wotton 1996). *Chironomus riparius* are detritivores, feeding on resources of both plant and animal origin (Vos et al. 2000).

In natural environments, *Chironomus riparius* inhabit aquatic ecosystems experiencing both organic enrichment (Gower and Buckland 1978) and metal contamination (Postma and Groenendijk 1999). Larvae tolerate a wide range of sediment grain size (Ristola et al. 1999), temperature (ASTM 1991), pH (4 – 10) (Jernelov et al. 1981; Lohner and Fisher 1990; Bruner and Fisher 1993; Bervoets and Blust 2000), and dissolved oxygen concentrations (ASTM 1991).

Life Cycle

The life cycle of *C. riparius* has four distinct life stages; egg, larvae, pupae and adult. The first three stages of the life cycle are aquatic, while the final stage is terrestrial. Adults are most abundant during the early spring (Townes 1945; Curry 1962). Adult male chironomids are slightly smaller than their female counterparts with thinner abdomens and plumose antennae (Day et al. 1994). Mating occurs following the formation of swarms of male adults. Females are attracted to the swarms, find a mate and drop out of the swarm. Following successful mating, females deposit a single mass of eggs on the water surface. The egg mass is enveloped in a gelatinous matrix, protecting the developing eggs from desiccation and contaminants (Williams et al. 1987). The matrix also spaces eggs so that each individual receives an equal supply of

light and oxygen (Watts and Pascoe 1998). Each egg mass can hatch between 150 to 400 embryos, within 2 to 6 days, depending on water temperature (Ingersoll et al. 1990; Environment Canada 1997).

Larval chironomids hatch from the egg mass, developing through four instars or stages of development. Each instar lasts approximately 4 to 7 d (Environment Canada 1997). The first instar is planktonic, contributing towards the dispersal of individuals (Townsend et al. 1981; Oliver and Roussel 1983). Within 2 to 3 d, larvae settle into a benthivorous lifestyle. Following the second instar, larvae change from a milky white colour to the characteristic blood red, indicating the presence of an oxygen carrying pigment. Head capsule width increases with each instar, ranging from 0.09 – 0.12 mm in the first instar to 0.43 – 0.60 mm in the fourth instar (McCauley 1974). Most growth (increase in biomass) occurs during the final instar (Day et al. 1994).

After the fourth instar, larvae pupate. Following three days, pupae swim up to the water surface and emergence occurs. Male chironomids emerge approximately 2 d prior to mass female emergence (Environment Canada 1997).

The *Chironomus riparius* life cycle lasts approximately 15 to 30 d, with the adult stage lasting anywhere from 4 to 11 d (Curry 1962). *Chironomus* sp. generally complete more than one generation per year (multivoltine) in Canadian aquatic environments (Oliver 1971; Gower and Buckland 1978). Larvae overwinter as fourth instar larvae and emerge in early spring (Oliver and Roussel 1983; Rasmussen 1984).

CULTURE INITIATION AND MAINTENANCE

Initiation of Culture

Four separate cultures of *Chironomus riparius* were initiated at the University of Windsor on December 18, 1998. Egg masses were obtained from an existing culture at

Environment Canada, Canadian Centre for Inland Waters (Burlington, Ontario). Cultures were started from 20 separate egg masses.

Cultures were initiated in 20-L glass aquarium tanks. Tanks were scrubbed with a nylon brush and washed in dechlorinated tap water. Tanks were then rinsed with distilled water. Silica sand (Quarry Hill Foundry Supplies Inc., McDougall Ave., Windsor, ON), particle size approximately 180-µm, was added to each tank as a culture substrate. Sand was triple rinsed with dechlorinated water to remove any impurities (e.g. debris and silt). Three L were added to each tank to a depth of approximately 1.5-cm.

Ten L of dechlorinated tap water were added to each tank. An airstone, delivering oil-free compressed air, was placed near the surface of the sediment in each tank. Air was delivered via teflon tubing from an air pump (Rolf C. Hagen, "The Pump"®). Water was gently aerated, commencing 24-h prior to the initiation of culture.

Four to six egg masses were added to each tank. Tanks were covered with fine mesh netting to prevent the escape of adult midges. Adults remained in the tank from which they emerged, and subsequently mated and oviposited in these same tanks.

Temperature in the culture room ranged from 24.0 – 26.5 °C. Fluorescent lighting in the culture room was controlled to maintain a 16:8 h L:D photoperiod.

Maintaining Cultures

Egg masses were removed daily from culture aquariums and placed in 20 mL scintillation vials (10/vial) filled with aerated dechlorinated tap water. Surplus eggs were stored at 4 °C for a maximum of one week. Lids of vials were slightly unscrewed to prevent vials from becoming anoxic during storage. Unused egg masses were disposed of following one week of storage. Storage for longer than 1 week resulted in

decreased hatching success and increased microbial activity (Burt 1998). Egg masses were added to culture tanks to replenish culture following emergence. Occasionally, some larvae were removed from tanks when high larval density limited emergence (Stanko-Mishic et al. 1999). To minimize inbreeding of the stock, egg masses originating from more than one tank were used to replenish cultures.

Aquarium tanks were cleaned and culture water changed weekly. Walls of aquarium tanks were scraped with a razor blade to remove excess food. Water was siphoned from each tank, leaving approximately 2 cm of water in the tank. Culture water was replaced with dechlorinated tapwater, aerated for 24 h prior to use to ensure adequate oxygen content and complete dechlorination. Any larvae siphoned out with water were collected using a 250-µm sieve and returned to culture.

Sediment was changed approximately once every 3 to 4 mo. Sediment was sieved through a 250-µm sieve to collect larvae. Tanks were thoroughly cleaned and clean sediment and water added. While tanks were cleaned, larvae were held in to aerated dechlorinated water and subsequently returned to culture.

Cultures were fed ground Nutrafin fish flakes (Rolf C. Hagen), supplemented with ground alfalfa tablets to enhance dissolved organic carbon content. Alfalfa was ground with a mortar and pestle and ground in a blender with fish flakes and dechlorinated tapwater. Cultures were fed daily with a suspension of 50 mg of Nutrafin/mL water. Feeding rate ranged from 4-15 mL, depending on the density of larvae and the instar of development.

Culture Limitations: Inbreeding

Any culture originating from a finite number of egg masses will experience some inbreeding. However, the above culture technique contributed to high levels of

inbreeding and therefore, reduced genetic variability of the population. Reduction in the genetic variability of a population tends to lead to reduced fitness of individual larvae, manifested as reduced survival and growth (Charlesworth and Charlesworth 1987).

During daily maintenance of the culture, occasionally some egg masses eluded removal. These egg masses subsequently hatched, contributing enough larvae to make the addition of other egg masses unnecessary. As a result, egg masses originating from different tanks were infrequently mixed, resulting in the breeding of midges with similar parentage.

In other culture techniques, inbreeding is lessened through the use of adult mating tents (US EPA 1994; Environment Canada 1997). This involves the collection of all adult midges of the same species into a single containment unit. This decreases the probability that mating will occur between individuals originating from the same egg mass. Inbreeding can also be lessened by the infusion of egg masses generated at other facilities maintaining cultures (Environment Canada 1997). More intense monitoring of the culture (i.e. monthly testing of viability of culture), as recommended by Environment Canada (1997), could also prove useful in maintaining the viability of the culture.

APPENDIX 2: DETERMINATION OF APPROPRIATE PERIPHYTON RATION

INTRODUCTION

In aquatic environments, determining the mechanism by which metal contamination alters the structure of communities requires the examination of metal effects at a lower level of biological organization. However, determining effects of metals on individual organisms remains difficult due to problems associated with assessing survival and growth in field populations. Alternatively, laboratory bioassays are routinely conducted to determine such effects under controlled environmental conditions. Bioassays using *Chironomus riparius* begin with first instar larvae and end with third and fourth instar larvae. Over this time, feeding at a rate that promotes optimal growth and survival is crucial to the validity of the test procedure (Environment Canada 1997).

The nutritional requirements of sediment feeding invertebrates are poorly understood (Vos et al. 2000). Chironomid larvae are capable of exploiting a variety of food items, from algae to other invertebrates (Pinder 1986). However, the rate required to maintain optimal levels of survival and growth in laboratory experiments varies widely in the literature. Fish flakes are most commonly fed to chironomid larvae at rates ranging from 0.12 – 1.0 mg/ind/d (Vos et al. 2000; Ribeiro et al. 1999; Ristola et al. 1999; Sibley et al. 1998; Environment Canada 1997). Feeding rates of other diets have not been investigated extensively.

The objective of this study was to determine the appropriate periphyton-feeding rate of *Chironomus riparius* for optimal growth and survival. A logarithmic feeding scale

was testing using *Chlorella*, followed by a geometric series of rations employing field-collected periphyton.

MATERIALS AND METHODS

Experimental Set-Up

Tests were conducted in 2-L (12 x 12 x 15 cm) glass jars filled to a depth of 2 cm (approximately 500 g) with washed silica sand (particle size approximately 200 µm). De-chlorinated tap water (1L) was added to each jar and gently aerated throughout the experiment by a branching, capillary tube system (Corkum and Hanes 1989). Experimental conditions were static, with replacement of evaporative water loss. Experiments were conducted at a constant air temperature of 22° C, with a 16:8 h L:D photoperiod maintained. Water quality parameters (dissolved oxygen, pH, water temperature) were monitored. All materials were acid washed in HNO_3 (analytical grade) and triple rinsed in distilled water.

Chlorella Experimental Design

A single factor ANOVA design was used, employing 4 replicates. Eight dietary treatments were tested, one Nutrafin® (Rolf C. Hagen, Mansfield, MA) and seven *Chlorella* treatments. Nutrafin was fed at a rate of 1.0 mg/ind/d (Environment Canada 1997). *Chlorella* tablets (Nature's Herbs, American Fork, UT), obtained from a health food store, were used to administer feeding rates of 0.03, 0.10, 0.30, 1.0, 3.0, 10.0, and 30.0 mg/ind/d.

Fifty first-instar *Chironomus riparius* (Diptera: Chironomidae) larvae, reared from on-site cultures (See Appendix 1), were randomly allocated to each jar. Larvae were hatched from multiple egg masses. Fifty larvae were counted under the dissecting

microscope and pipetted below the water surface of experimental jars within 24-h of hatching. Concentrations of Nutrafin® and *Chlorella* were each ground using a mortar and pestle and fed daily to larvae in suspension. Chironomids were harvested after 14-d. Jar contents were collected by gently sieving the sediment through a 180-µm mesh sieve. Individual larvae were hand picked from the sieve, blotted to remove excess water, counted and mass determined. Larvae were preserved in cold Carnoy's solution (3 parts anhydrous ethanol, 1 part glacial acetic acid).

Periphyton Experimental Design

A single factor ANOVA design was used, employing 3 replicates. Five dietary treatments were tested, two Nutrafin® (Rolf C. Hagen, Mansfield, MA) and three periphyton treatments. Nutrafin treatments (NUT1 and NUT2) employed feeding rates of 0.5 and 1.0 mg/ind/d, while periphyton treatments (PER1 – PER3) employed feeding rates of 0.5, 1.0, and 1.5 mg organic matter/ind/d. Periphyton used in this experiment was collected during the summer of 1998 (for methods, see chapter 2) on the Northwest Miramichi River (HEA_R). Periphyton was processed as in chapter 2, except that periphyton was frozen rather than freeze-dried. The organic matter content of this periphyton was 40.0 %. Periphyton feeding rates were adjusted as to deliver 0.5, 1.0 and 1.5 mg of organic matter/ind/d.

Periphyton availability was limited in quantity in this experiment, resulting in different densities of larvae allocated to each experimental unit. First-instar *Chironomus riparius* (Diptera: Chironomidae) larvae, reared from on-site cultures (See Appendix 1), were randomly allocated to each jar as follows: 50 – NUT1, 10 – NUT2, 10 – PER1, 10 – PER2, 10 – PER3. Larvae were hatched from multiple egg masses. Larvae were counted under the dissecting microscope and pipetted below the water

surface of the experimental jars within 24-h of hatch. Preparation of dietary treatments involved the grinding of Nutrafin® in a blender. Dietary treatments were fed daily to larvae in suspension. Chironomids were harvested following a 14-d exposure. Jar contents were collected by gently sieving the sediment through a 180-µm mesh sieve. Individual larvae were hand-picked from the sieve, blotted to remove excess water and counted. Mass was not determined. Larvae were preserved in cold Carnoy's Solution (3 parts anhydrous ethanol, 1 part glacial acetic acid).

Statistical Analysis

Mean survival (%) and mean larval biomass were analyzed using a single factor ANOVA (alpha <0.05). Post-hoc comparisons were performed using Dunnett's test. All statistical analysis was performed on $\log_{10}$ transformed data using Statistica (StatSoft '98 Edition, Tulsa, OK).

RESULTS

Chlorella Experiment

Chironomid survival ranged widely across treatments, from 0 – 100 %. Larvae fed greater than 10.0 mg *Chlorella*/ind/d did not survive the 14-d test. Survival was significantly different among dietary treatments (one-way ANOVA, Fs = 269.593, p<0.001) (Table A2.1). Mean survival was significantly higher in treatments fed 1.0 mg Nutrafin/ind/d and 0.03 – 1.0 mg *Chlorella*/ind/d than in treatments fed 3.0 – 30.0 mg *Chlorella*/ind/d (Dunnett's test, p<0.0002). Larvae fed 3.0 mg *Chlorella*/ind/d had significantly higher survival than those fed 10.0 and 30.0 mg *Chlorella*/ind/d (Dunnett's test, p<0.0001) (Fig. A2.1).

Table A2.1: One-way analysis of variance (ANOVA) of mean chironomid survival (%) fed suspensions of Nutrafin (1.0 mg/ind/d) and *Chlorella* (0.03 – 30.0 mg/ind/d)

VARIABLE	df	SUM OF SQUARES	MEAN SQUARE	F STATISTIC	p VALUE
Dietary Treatment	7	19.9063	2.8438	269.592	<0.001
Within Treatment Error	24	0.2532	0.0105		
Total	31	20.1595	2.8543		

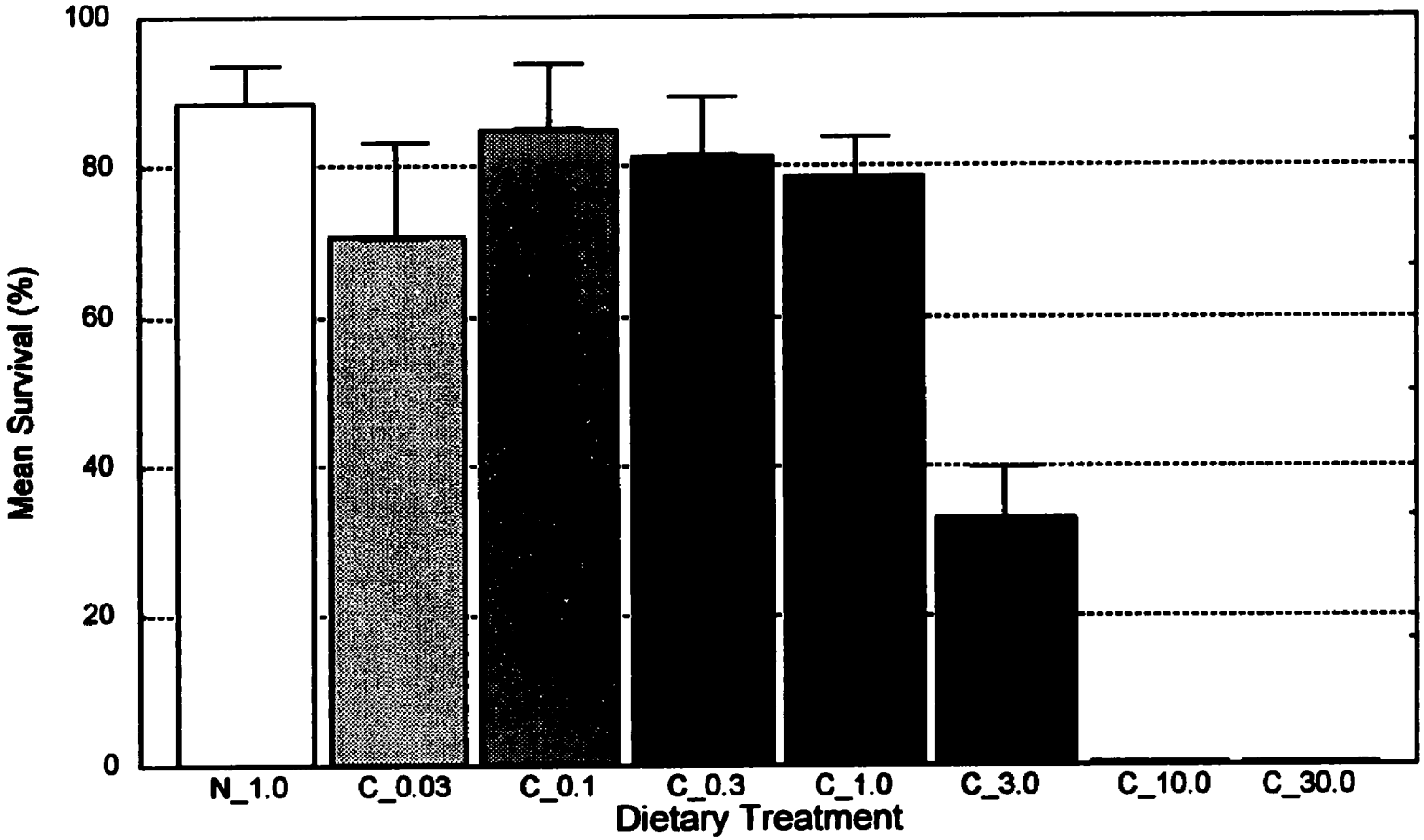

Figure A2.1: Mean survival (percent ± 1SE) of chironomid larvae fed suspensions of Nutrafin (1.0 mg/ind/day) and *Chlorella* (0.03 - 30.0 mg/ind/day).

Treatments fed 10.0 and 30.0 mg *Chlorella*/ind/d did not have any surviving larvae and were excluded from the biomass analysis. Larval biomass in treatments ranged from 0.916 – 7.934 mg (wet weight). Larvae were significantly different among dietary treatments (one-way ANOVA, Fs = 22.313, p<0.001) (Table A2.2). Significantly larger chironomid larvae were observed in treatments fed 1.0 mg Nutrafin/ind/d and 0.3 – 3.0 mg *Chlorella*/ind/d than in treatments fed 0.03 and 0.1 mg *Chlorella*/ind/d (Dunnett's test, p<0.006) (Fig. A2.2).

Periphyton Experiment

Generally, chironomid survival in treatments was low, never exceeding 62 % in any treatment. Survival was lowest in the periphyton treatment receiving 0.5 mg/ind/d and increased in both periphyton and Nutrafin treatments according to higher feeding rates (Fig. A2.3). However, survival was not significantly different among dietary treatments (one-way ANOVA, Fs = 1.930, p<0.19) (Table A2.3).

DISCUSSION

***Chlorella* Experiment**

Larvae fed a Nutrafin diet at a rate of 1.0 mg/ind/d exhibited acceptable survival (>70 %) and growth (Environment Canada 1997). Larval survival was not significantly different between Nutrafin and *Chlorella* treatments fed 0.03 – 1.0 mg/ind/d and length did not differ significantly between Nutrafin and *Chlorella* treatments fed 0.3 – 3.0 mg/ind/d. Together, acceptable survival and length of larvae was achieved at *Chlorella* feeding rates ranging from 0.30 – 1.0 mg/ind/d. This feeding rate is comparable to other levels recommended in standard bioassay procedures (Environment Canada 1997; Sibley et al. 1998; Ribeiro et al. 1999; Ristola et al. 1999; Vos et al. 2000). No

Table A2.2: One-way analysis of variance (ANOVA) of mean chironomid biomass (mg wet weight) fed suspensions of Nutrafin (1.0 mg/ind/d) and *Chlorella* (0.03 – 30.0 mg/ind/d)

VARIABLE	df	SUM OF SQUARES	MEAN SQUARE	F STATISTIC	p VALUE
Dietary Treatment	5	0.7710	0.1542	22.313	<0.001
Within Treatment Error	18	0.1244	0.0069		
Total	23	0.8954	0.1611		

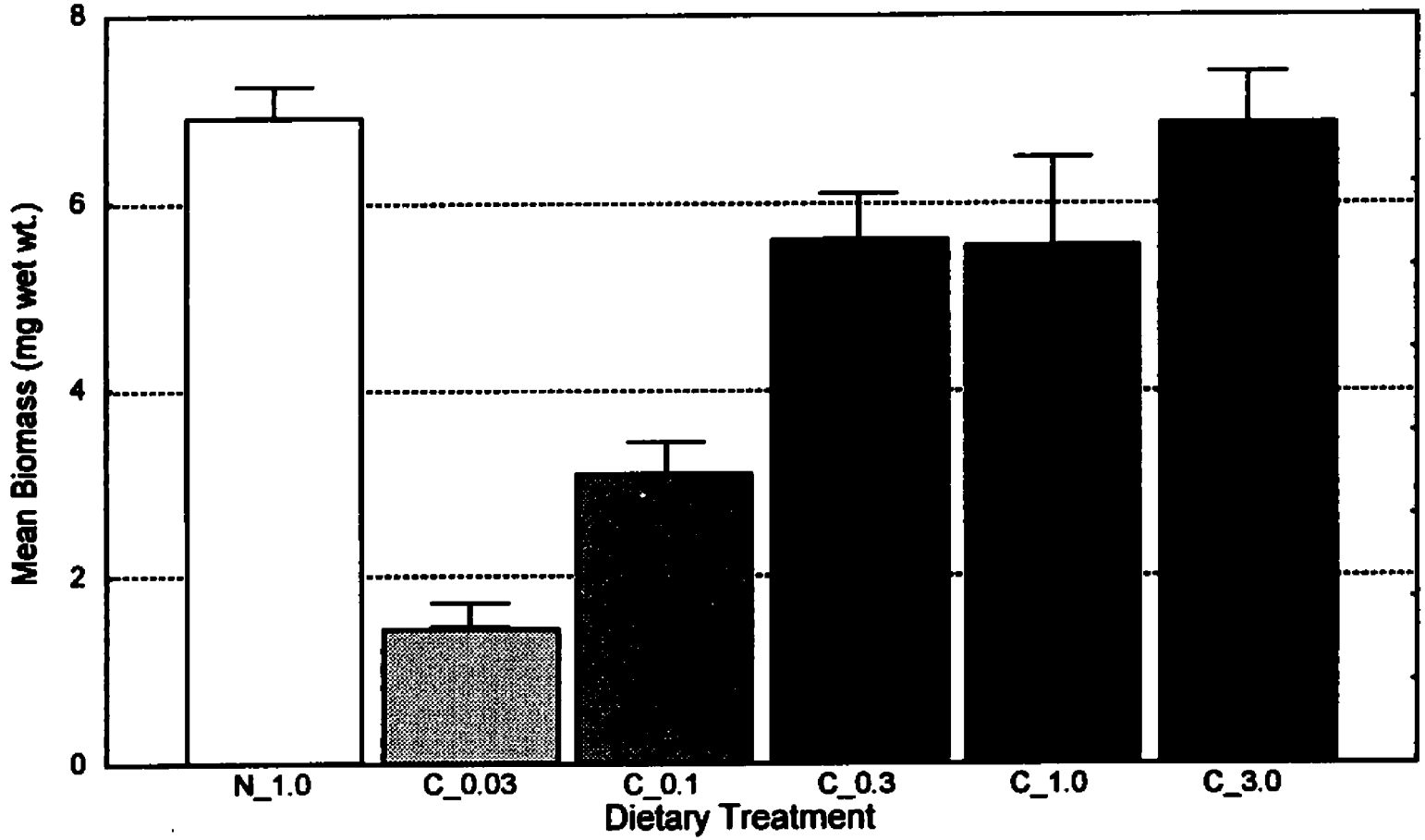

Figure A2.2: Mean biomass (± 1 SE) (mg wet mass) of chironomid larvae fed suspensions of Nutrafin (1.0 mg/ind/d) and *Chlorella* (0.03 - 3.0 mg/ind/d).

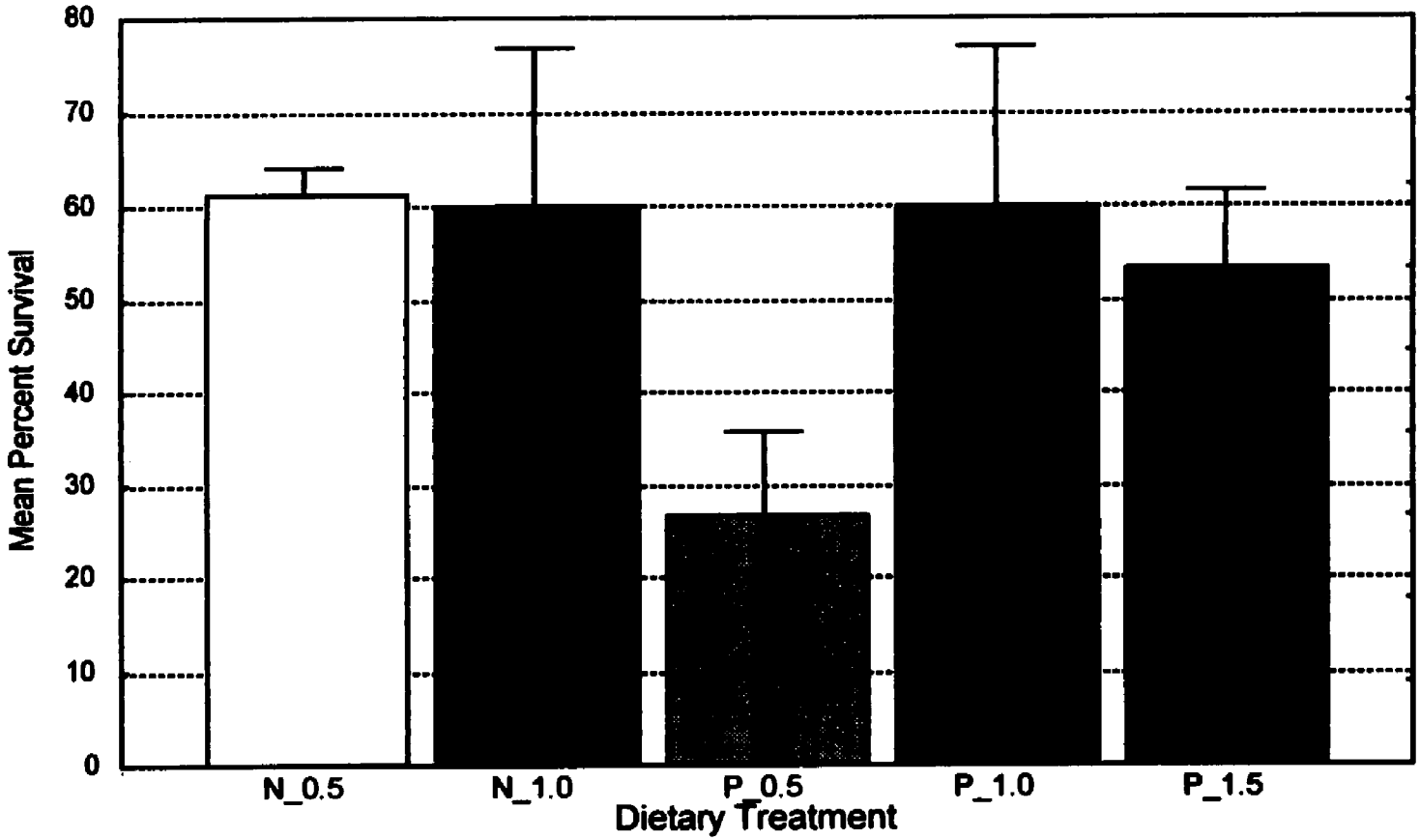

Figure A2.3: Mean survival (percent ± 1 SE) of chironomid larvae fed suspensions of Nutrafin (0.5 and 1.0 mg/ind/day) and periphyton (0.5 – 1.5 mg/ind/day).

Table A2.3: One-way analysis of variance (ANOVA) of mean chironomid survival (%) fed suspensions of Nutrafin (0.5 - 1.0 mg/ind/d) and periphyton (0.50 – 1.50 mg/ind/d)

VARIABLE	Df	SUM OF SQUARES	MEAN SQUARE	F STATISTIC	p VALUE
Dietary Treatment	4	0.3344	0.0836	1.930	<0.182
Within Treatment Error	10	0.4332	0.0433		
Total	14	0.7676	0.1269		

problems in overlying water quality were observed at these feeding rates, therefore, a *Chlorella* feeding rate of 1.0 mg/ind/d was recommended as appropriate for *C. riparius* larvae.

At comparable feeding rates (1.0 mg/ind/d), larval survival and final size did not differ significantly between Nutrafin and *Chlorella* fed larvae. Alternatively, Vos et al. (2000) found that maximum length of second instar larvae was higher in larvae fed fish food or food of animal origin compared to food items of plant origin. This difference was attributed to the reduced nutritional quality of the plant diet; namely lower levels of N, P and lipid content and higher levels of carbohydrates. Considering that most increases in larval biomass occurs in the fourth instar, it is probable that any difference in biomass of second instar larvae may not have persisted following further development (Day et al. 1994).

Periphyton Experiment

Survival of larvae fed 0.5 mg/ind/d of periphyton was reduced compared to other dietary treatments, but not significantly. Survival was highly variable within treatments with low densities of chironomid larvae (10/replicate). Larval density was reduced in periphyton treatments because only small quantities of periphyton were collected. Consequently, larvae in different treatments with the same feeding rate (1.0 mg/ind/d in NUT1 and PER2), experienced different density of food particles on the sediment surface. For example, following daily feeding, NUT1 larvae experienced food densities of 0.40 mg Nutrafin/cm^2 of sediment, while PER2 larvae experienced food densities of 0.08 mg periphyton/cm^2 of sediment. At low food availability, larvae may not collect the food spread around the sediment surface effectively, or may expend more energy foraging (Macchiusi and Baker 1992; Postma et al. 1994; Ristola et al. 1999), resulting

in reduced growth and survival. Therefore, reduced food availability may have contributed to increased variability, resulting in a lack of a significant difference between treatments.

Greater than 70% survival was not observed in this experiment. The periphyton experiment was run approximately a month following the *Chlorella* experiment. Variable water quality, common when using dechlorinated tap water, may have reduced the survival of chironomid larvae (Environment Canada 1997). Reduced survival of chironomid larvae is unlikely attributable to inbreeding, considering cultures would have only experienced one more generation.

Conclusion

Based on the trends observed in both *Chlorella* and periphyton experiments, a periphyton feeding rate of 1.0 mg of organic matter/ind/d is recommended for *Chironomus riparius* larvae.

APPENDIX 3: FIELD DATA SUMMARIES AND ANALYSES

Table A3.1: Physical habitat characteristics of mine drainage receiving and reference sites

SITE	STREAM ORDER	STREAM WIDTH (m)	STREAM MORPHOLOGY	CHANNEL TYPE	BANK STABILITY	STREAM CANOPY	INSTREAM COVER	SUBSTRATE TYPE
HEA_R	4	10	30 % riffle 70 % run	80 % straight 20 % meandering	90 % stable 10 % moderately unstable	100 % open	100 % boulder	5 % bedrock 5 % boulder 70 % cobble 10 % gravel 5 % sand 5 % detritus
HEA_M	3	3.5	70 % riffle 30 % run	70 % straight 30 % meandering	80 % stable 20 % moderately unstable	100 % open	100 % boulder	10 % boulder 60 % cobble 5 % gravel 5 % sand 20 % detritus
STR_R	3	2	20 % pool 60 % riffle 20 % run	100 % meandering	20 % highly unstable 50 % moderately unstable 30 % stable	90 % dense 10 % partly open	10 % boulder 30 % logs and trees 20 % deep pool 40 % aquatic macrophytes	10 % boulder 60 % cobble 20 % gravel 5 % sand 5 % detritus
STR_M	2	1	30 % pool 35 % riffle 35 % run	70 % meandering 30 % braided	70 % highly unstable 30 % moderately unstable	70 % dense 30 % partly open	50 % boulder 10 % logs and trees 40 % aquatic macrophytes	10 % boulder 50 % cobble 20 % gravel 5 % sand 5 % muck 10 % detritus
BRU_R	2	3	50 % riffle 40 % run 10 % flat	20 % straight 80 % meandering	90 % stable 10 % moderately unstable	70 % open 30 % partly open	85 % boulder 15 % logs and trees	15 % boulder 60 % cobble 15 % gravel 10 % sand
BRU_M	3	2	10 % pool 20 % riffle 60 % run 10 % flat	90 % meandering 10 % ponded	70 % stable 30 % moderately unstable	60 % open 40 % partly open	5 % undercut banks 85 % boulder 10 % deep pool	60 % cobble 10 % gravel 10 % sand 20 % detritus
WED_R	5	20	10 % riffle 90 % run	80 % straight 20 % braided	100 % stable	100 % open	95 % boulder 5 % aquatic macrophytes	5 % bedrock 10 % boulder 80 % cobble 5 % gravel
WED_M	5	20	100 % run	100 % straight	5 % moderately unstable 95 % stable	100 % open	100 % boulder	5 % bedrock 10 % boulder 75 % cobble 10 % sand

Table A3.1: Cont'd

SITE	STREAM ORDER	STREAM WIDTH (m)	STREAM MORPHOLOGY	CHANNEL TYPE	BANK STABILITY	STREAM CANOPY	INSTREAM COVER	SUBSTRATE TYPE
CAR_R	4	10	50 % riffle 40 % run 10 % flat	10 % straight 75 % meandering 15 % braided	90 % stable 10 % moderately unstable	100 % open	100 % boulder	5 % bedrock 10 % boulder 70 % cobble 5 % gravel 5 % sand
CAR_M	5	10	40 % riffle 60 % run	100 % meandering	100 % stable	100 % open	100 % boulder	5 % bedrock 10 % boulder 60 % cobble 5 % gravel 5 % sand 15 % detritus

Table A3.2: *Water quality measurements at various sampling locations in northeastern New Brunswick*

RIVER	DATE (d/m/y)	LOCATION	pH	CONDUCTIVITY (mS/cm)	TURBIDITY (NTU)[1]	DISSOLVED OXYGEN (mg/L)	WATER TEMPERATURE (°C)	SALINITY (ppt)
40 Mile Brook	16/06/1999	CAR_M	6.40	0.103	0	8.76	19.60	0.00
40 Mile Brook	27/05/1999	CAR_M	7.00	0.072	1	9.06	12.00	0.00
40 Mile Brook	30/08/1999	CAR_M	7.03	0.107	0	9.56	16.40	0.00
40 Mile Brook	16/06/1999	Mine Site	5.55	0.339	12	8.84	20.10	0.01
44 Mile Brook	17/06/1999	CAR_R	7.00	0.044	13	8.77	13.30	0.00
44 Mile Brook	27/08/1999	CAR_R	6.03	0.048	0	10.2	20.00	0.00
44 Mile Brook	27/05/1999	CAR_R	7.18	0.034	1	9.46	10.40	0.00
Austin Brook	10/06/1999	BRU_M	6.51	0.042	14	8.91	14.90	0.00
Austin Brook	11/06/1999	BRU_M	6.66	0.049	14	9.44	13.20	0.00
Austin Brook	24/08/1999	BRU_M	5.37	0.085	10	10.89	18.20	0.00
Devil's Elbow Brook	27/05/1999	At Hwy	7.22	0.018	24	11.05	7.90	0.00
Little River	21/05/1999		6.48	0.041	33	9.11	10.80	0.00
Little S Branch Tomogonops River	27/05/1999	HE-1	6.74	0.029	2	9.13	13.50	0.00
Little S Branch Tomogonops River	27/05/1999	HEA_M	6.86	0.023	2	9.31	14.20	0.00
Little S Branch Tomogonops River	10/06/1999	HEA_M	6.81	0.030	2	8.18	15.20	0.00
Little S Branch Tomogonops River	25/08/1999	HEA_M	6.06	0.036	7	10.04	22.00	0.00
McCormack Brook	31/05/1999	STR_R	6.48	0.022	29	8.69	12.70	0.00
McCormack Brook	09/06/1999	STR_R	6.31	0.027	2	7.37	8.10	0.00
McCormack Brook	25/08/1999	STR_R	5.99	0.032	0	9.12	15.40	0.00
Mosquito Brook	08/06/1999	STR_M	7.12	0.025	1	8.20	14.60	0.00
Mosquito Brook	25/08/1999	STR_M	5.81	0.04	0	8.46	14.60	0.00
Nepisiguit	12/06/1999	WED_M	7.22	0.037	10	10.20	20.40	0.00
Nepisiguit	12/06/1999	WED_R	6.95	0.034	46	9.45	21.40	0.00
Nepisiguit	30/08/1999	WED_R	6.82	0.038	3	9.02	16.30	0.00
NW Miramichi	21/05/1999	HEA_R	6.44	0.029	12	10.31	14.00	0.00
NW Miramichi	31/05/1999	HEA_R	6.99	0.032	0	10.22	18.50	0.00
NW Miramichi	04/06/1999	HEA_R	6.93	0.034	10	9.28	12.70	0.00
NW Miramichi	25/08/1999	HEA_R	6.56	0.044	3	11.06	24.00	0.00

[1]High turbidity readings are inaccurate. Sensor occasionally malfunctioned.

Table A3.2: Cont'd

RIVER	DATE	LOCATION	pH	CONDUCTIVITY (mS/cm)	TURBIDITY (NTU)[1]	DISSOLVED OXYGEN (mg/L)	WATER TEMPERATURE (°C)	SALINITY (ppt)
S. Branch Pabineau River	10/06/1999	BRU_R	6.50	0.058	0	9.21	18.10	0.00
S. Branch Pabineau River	14/06/1999	BRU_R	7.14	0.072	1	8.82	20.90	0.00
S. Branch Pabineau River	27/08/1999	BRU_R	6.55	0.089	0	8.65	17.60	0.00

[1]High turbidity readings are inaccurate. Sensor occasionally malfunctioned.

Table A3.3: Total trace metal concentrations (µg/L) in water at five mine drainage-receiving sites and five reference sites

METAL	HEA_R	HEA_M	STR_R	STR_M[a]	STR_M[b]	BRU_R	BRU_M	WED_R	WED_M	CAR_R	CAR_M[1]	CAR_M[2]
Ag	nd[3]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Al	nd	233.4	nd	462.4	466.8	nd	154.3	nd	nd	nd	2008	nd
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ba	2.53	2.8	3.29	6.76	6.8	5.58	5.83	3.23	3.76	5.13	9.61	3.59
Be	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.79	nd
Bi	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ca	4173	2428	2239	1808	1802	6026	4857	3763	4118	5203	34398	13281
Cd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.93	nd
Co	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	38.6	nd
Cr	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cu	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1039	nd
Fe	105	337.5	79.86	105.5	150.7	137.2	180.7	63.57	78.66	38.9	817.1	62.18
K	352.2	544.9	468.5	621.8	627.8	421.1	410.4	365.7	446.7	406.1	777.8	556.9
Mg	734.4	809.4	808.2	778.4	780.4	1230	1170	871.6	922	877.2	6665	1577
Mn	6.32	87.72	8.69	38.37	38.01	57.81	241.2	3.48	9.86	2.44	4292	55.88
Mo	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	13.18	nd
Na	1550	1466	1363	1446	1442	4615	1527	1646	1620	1268	3155	2287
Ni	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	26.59	nd
Pb	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sn	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	29.24	nd
Sr	17.24	12.9	10.54	11.51	11.75	31.05	19.79	17.35	18.8	20.99	95.75	34.95
Ti	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
V	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Zn	2.56	108.7	5.2	76.25	75.25	18.57	237.3	nd	14.72	3.03	10248	151.3

[a,b]duplicate samples at Mosquito Brook (STR_M)
[1]water sample just below mine on 40 Mile Brook
[2]water sample near mouth of 40 Mile Brook (CAR_M)
[3]not detected

Table A3.4: Factor scores generated from principal component analysis (PCA) of total trace metal concentrations in water at five mine drainage receiving sites and five reference sites

SITE	PC-I	PC-II	PC-III
Reference Sites			
HEA_R	-0.248	-0.204	-1.704
STR_R	-1.054	-0.331	-0.190
BRU_R	1.494	0.450	-0.125
WED_R	-0.111	-0.919	-0.946
CAR_R	-0.022	-1.742	0.523
Mine Drainage Receiving Sites			
HEA_M	-0.884	1.873	-0.621
STR_M	-1.260	0.107	1.936
BRU_M	0.383	1.050	0.541
WED_M	-0.047	-0.374	-0.081
CAR_M	1.750	0.091	0.658

Table A3.5: Dissolved trace metal concentrations (µg/L) in water at five mine drainage-receiving sites and five reference sites

METAL	HEA_R	HEA_M	STR_R	STR_M	BRU_R	BRU_M	WED_R	WED_M	CAR_R	CAR_M[1]	CAR_M[2]
Ag	nd[3]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Al	nd	154.9	nd	475.8	nd	109.1	nd	nd	nd	236.5	nd
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B	nd	nd	nd	18.9	nd	nd	nd	nd	nd	nd	nd
Ba	2.81	2.99	3.8	6.2	6.82	5.92	3.62	4.05	6.25	11.09	3.98
Be	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.55	nd
Bi	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ca	4209	2424	2413	1883	6481	4834	3782	4197	5668	38473	13513
Cd	nd	nd	nd	nd	nd	nd	nd	nd	nd	14.48	nd
Co	nd	nd	nd	nd	nd	nd	nd	nd	nd	44.6	nd
Cr	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cu	nd	nd	nd	nd	nd	nd	nd	nd	nd	1022	nd
Fe	72.29	237	62.91	49.52	103.5	134.7	29.64	52.42	19.69	308.6	28.11
K	446.5	624.4	614.2	676.8	485.5	386.5	473.2	529.6	507.7	959.9	527.9
Mg	774.3	853.8	915.1	820.7	1367	1213	919.1	961.9	1013	7426	1737
Mn	4.47	81.87	4.06	7.61	44.4	240.1	1.76	9.82	1.35	4931	54.01
Mo	nd	nd	nd	nd	nd	nd	nd	nd	nd	15.17	nd
Na	1578	1472	1563	1476	5066	1626	1695	1636	1496	3612	2520
Ni	nd	nd	nd	nd	nd	nd	nd	nd	nd	32.14	nd
Pb	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sn	nd	nd	nd	nd	nd	nd	nd	nd	nd	35.42	nd
Sr	17.53	13.94	12.28	12.58	34.54	20.78	18.45	20.02	24.93	110.2	38.75
Ti	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
V	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Zn	2.82	109.6	5.73	70.87	20.01	243.8	2.84	16.89	3.22	11274	154.1

[1]water sample near mouth of 40 Mile Brook (CAR_M)
[2]water sample just below mine on 40 Mile Brook
[3]not detected

Table A3.6: Identification of the metals from dissolved concentrations in water from mine drainage receiving and reference sites loading most heavily on the first three principal components and the cumulative proportion of variance explained by each component

ELEMENT	PC_I	PC-II	PC-III
Strontium	**0.966**	-0.025	0.171
Calcium	**0.963**	-0.062	0.001
Magnesium	**0.862**	0.252	0.331
Sodium	**0.689**	0.175	0.317
Potassium	**-0.649**	-0.011	0.507
Manganese	0.304	**0.949**	0.030
Zinc	0.123	**0.859**	0.308
Iron	-0.251	**0.844**	-0.266
Barium	0.263	0.022	**0.770**
Cumulative Proportion of Total Variance	0.415	0.687	0.826

Table A3.7: Factor scores generated from principal component analysis (PCA) of dissolved trace metal concentrations in water at five mine drainage receiving sites and five reference sites

SITE	PC-I	PC-II	PC-III
Reference Sites			
HEA_R	-0.062	-0.529	-1.888
STR_R	-1.006	-0.375	0.090
BRU_R	1.254	0.484	0.924
WED_R	0.015	-1.161	-0.725
CAR_R	0.302	-1.547	0.645
Mine Drainage Receiving Sites			
HEA_M	-1.113	1.509	-0.569
STR_M	-1.466	0.070	1.698
BRU_M	0.562	1.493	-0.440
WED_M	-0.101	-0.186	-0.181
CAR_M	1.615	0.242	0.445

Table A3.8: Total trace metal concentrations (µg/g) in periphyton at five mine drainage-receiving sites and five reference sites

METAL	HEA_R	HEA_M	STR_R	STR_M	BRU_R	BRU_M	WED_R	WED_M	CAR_R	CAR_M
Al	13911	38488	851.1	4664	12409	43029	12296	14994	14473	22825
As	18.57	151.9	5.97	23.88	62.51	83.58	35.74	6.82	11.07	96.18
Ca	8526	3556	5380	4467	3908	7241	5406	2924	9355	9550
Cd	2.11	16.57	1.35	7.47	8.36	49.77	3.4	4.02	1.77	48.04
Co	29.4	340.8	2.75	9.67	158.8	585.5	11.25	15.72	11.04	162.6
Cr	25.5	30.12	nd[1]	nd	18.52	61.92	23.2	15.36	25.11	39.87
Cu	15.4	1304	nd	21.48	17.54	475.6	nd	692.8	18.33	1323
Fe	20523	54648	1766	5838	16517	89918	12951	40746	18300	19186
K	5543	8718	5978	6076	1848	3350	3274	1571	5859	4265
Mg	4485	5967	1330	3155	1694	4607	3503	1742	6343	4287
Mn	6481	13247	2589	3291	26826	158682	1320	959	1968	33419
Na	2561	690	159.9	134.8	268	354.7	894	472.6	574.7	345.6
Ni	18.85	25.17	1.44	3.91	54.41	116.6	15.61	7.9	13.22	74.43
Pb	nd	636.4	nd	109.8	33.32	1387	nd	558.2	13.69	40.34
V	34.21	60.65	nd	6.98	15.3	41.3	47.49	22.02	39.46	35.95
Zn	202.4	5377	248.3	1328	2048	18213	138.9	585.9	308.7	25740

[1]not detected

Table A3.9: Factor scores generated from principal component analysis (PCA) of total trace metal concentrations in periphyton at five mine drainage receiving sites and five reference sites

TREATMENT	PC-I	PC-II
Reference Sites		
HEA_R	-1.068	0.710
STR_R	-0.548	-1.672
BRU_R	0.175	0.139
WED_R	-1.373	0.746
CAR_R	-1.170	0.571
Mine Drainage Receiving Sites		
HEA_M	0.883	0.446
STR_M	0.385	-1.733
BRU_M	1.514	0.831
WED_M	0.193	-0.692
CAR_M	1.009	0.654

Table A3.10: Portion of benthic sample sorted in the laboratory according to size fraction

SITE	4 mm	2 mm	1 mm	500 µm	250 µm
Reference Sites					
HEA_R	whole	whole	0.44	0.19	0.06
STR_R	whole	whole	whole	whole	0.25
BRU_R	whole	whole	0.44	0.11	0.06
WED_R	whole	whole	0.25	0.44	0.03
CAR_R	whole	whole	whole	0.25	0.02
Mine Drainage Receiving Sites					
HEA_M	0.25	0.25	0.44	0.11	0.02
STR_M	whole	whole	0.68	0.25	0.06
BRU_M	0.25	whole	whole	0.44	0.25
WED_M	whole	whole	0.25	0.11	0.06
CAR_M	whole	whole	0.68	0.25	0.06

Table A3.11: Dried mass (g) of remaining organic matter following removal of benthic invertebrates from composite benthic sample according to size fraction

SITE	4 mm	2 mm	1 mm	500 µm	250 µm	TOTAL
Reference Sites						
HEA_R	17.423	7.486	3.536	0.656	0.088	29.189
STR_R	7.923	3.016	2.396	1.095	0.108	14.538
BRU_R	2.582		1.451	0.311	0.528	4.872
WED_R	27.316	8.399	2.455	1.090	0.364	39.624
CAR_R	3.091	1.228	1.542	0.715	0.161	6.737
Mine Drainage Receiving Sites						
HEA_M	0.715	0.729	1.354	0.269	0.038	3.105
STR_M	19.597	11.603	7.224	1.310	0.153	39.887
BRU_M	11.792	10.858	11.929	3.446	1.455	39.480
WED_M	17.056	4.818	1.633	0.231	0.085	23.823
CAR_M	2.034	1.151	1.232	0.878	0.601	5.896

Table A3.12: Factor scores generated from principal component analysis (PCA) of the relative abundance of dominant chironomid genera at five mine drainage receiving sites and five reference sites

TREATMENT	PC-I	PC-II	PC-III
Reference Sites			
HEA_R	-0.724	0.438	-1.828
STR_R	-1.037	1.149	0.300
BRU_R	0.538	0.670	-0.658
WED_R	0.658	0.415	1.122
CAR_R	1.613	0.838	0.225
Mine Drainage Receiving Sites			
HEA_M	-0.572	-1.849	-0.057
STR_M	-0.532	0.089	1.664
BRU_M	-0.590	-1.116	0.493
WED_M	-0.883	0.488	-0.544
CAR_M	1.529	-1.121	-0.717

APPENDIX 4: LABORATORY DATA SUMMARIES AND ANALYSES

Table A4.1: Total trace metal concentrations (µg/L) in water from bioassay jars fed suspensions of Nutrafin, reference stream periphyton, and mine drainage receiving stream periphyton

METAL	NUT1	NUT2	NUT3	NUT4	NUT5	HEA_R	STR_R	BRU_R	WED_R	CAR_R	HEA_M	STR_M	BRU_M	WED_M	CAR_M
Ag	nd[1]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Al	nd	nd	nd	nd	nd	nd	nd	298.7	nd	432.4	304.7	nd	Nd	153.9	nd
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B	nd	nd	nd	nd	nd	nd	nd	94.02	nd	142.2	nd	nd	Nd	nd	nd
Ba	13.47	13.88	12.66	13.6	13.38	113.8	13.38	3.79	71.77	43.23	2.93	17.73	nd	30.68	20.2
Be	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Bi	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ca	32084	31498	30426	31388	32100	29977	33018	30495	30031	32282	27727	34001	26724	30219	29584
Cd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Co	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cr	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cu	94.25	70.84	63.86	82.86	72.01	nd	nd	nd	nd	nd	56.28	nd	nd	25.78	nd
Fe	22.15	nd	nd	nd	nd	32	43.33	153.6	142.6	90.98	212.3	112.5	126.4	332.1	113.1
K	3150	2843	2638	2765	2749	2196	2859	1822	2128	2312	1729	2979	1479	1733	1428
Mg	9402	9419	8833	8951	9191	8454	9381	8014	8533	8955	8239	9925	8310	8418	8339
Mn	nd	nd	1.47	nd	0.91	995.9	205.1	203	163	53.4	385.5	250.8	342.1	53.67	69
Mo	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Na	8172	9293	7882	9071	8340	7000	5997	8312	5744	5960	5920	8219	10948	7039	5810
Ni	42.24	46.96	47.37	46.65	41.65	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pb	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sn	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sr	133.3	141.5	132.4	139.4	140	118.1	127.6	113.3	122.1	125.9	96.9	141.6	46.6	119.6	114.5
Ti	nd	nd	nd	nd	nd	nd	nd	2.15	4.61	4.17	1.61	1.49	nd	1.71	nd
V	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Zn	16.98	11.41	15.01	12.87	14.09	nd	7.4	19.4	7.08	9.46	39.1	40.01	42.43	52.57	77.78

[1]not detected

Table A4.2: Factor scores generated from principal component analysis (PCA) of total trace metal concentrations in water from bioassay jars receiving suspensions of Nutrafin, reference stream periphyton, and mine drainage receiving stream periphyton

TREATMENT	PC-I	PC-II	PC-III
Nutrafin			
NUT1	1.192	0.695	0.322
NUT2	1.283	0.554	0.276
NUT3	1.368	-0.208	-0.061
NUT4	1.469	0.111	0.103
NUT5	1.186	0.477	0.159
Reference Stream Periphyton			
HEA_R	-0.103	-0.388	-1.258
STR_R	-0.725	1.281	-0.135
BRU_R	-0.590	-0.647	0.224
WED_R	-0.501	-0.609	-2.042
CAR_R	-0.672	0.338	-1.415
Mine Drainage Receiving Stream Periphyton			
HEA_M	-0.307	-1.370	0.291
STR_M	-1.523	2.315	1.095
BRU_M	-0.504	-1.574	2.149
WED_M	-0.822	-0.340	0.063
CAR_M	-0.752	-0.636	0.228

Table A4.3: Survival, chironomid biomass, chironomid length, and deformities per bioassay jar receiving suspensions of Nutrafin, reference stream periphyton, and mine drainage receiving stream periphyton

TREATMENT	SURVIVAL (%)	LENGTH (mm)	BIOMASS (ug AFDW)	DEFORMITIES (%)
Nutrafin				
NUT1	60	12.17	0.433	0.00 (n = 20)
NUT2	50	14.04	0.626	17.65 (n = 17)
NUT3	66	14.74	0.710	0.00 (n = 20)
NUT4	52	17.72	1.144	0.00 (n = 15)
NUT5	68	17.62	1.127	6.25 (n = 16)
Reference Stream Periphyton				
REF1	42	8.73	0.183	0.00 (n = 13)
REF2	56	9.95	0.269	0.00 (n = 17)
REF3	0			
REF4	48	13.98	0.619	6.67 (n = 15)
REF5	20	13.88	0.608	0.00 (n = 8)
Mine Drainage Receiving Stream Periphyton				
MIN1	2	6.87	0.098	0.00 (n = 1)
MIN2	12	4.72	0.037	0.00 (n = 4)
MIN3	6	6.35	0.080	0.00 (n = 4)
MIN4	30	6.80	0.096	0.00 (n = 10)
MIN5	20	5.57	0.057	12.50 (n = 8)

Table A4.4: Survival and chironomid biomass per bioassay jar receiving suspensions of Nutrafin (1.0 mg/ind/d) and *Chlorella* (0.03 – 30.0 mg/ind/d)

TREATMENT	SURVIVAL (%)	LENGTH (mm)
NUT1 – 1.0	106	6.98
NUT2 – 1.0	88	7.34
NUT3 – 1.0	86	7.37
NUT4 – 1.0	80	5.89
CHL1 – 0.03	74	1.39
CHL2 – 0.03	90	1.41
CHL3 – 0.03	34	2.01
CHL4 – 0.03	84	0.92
CHL1 – 0.10	98	3.06
CHL2 – 0.10	96	3.23
CHL3 – 0.10	84	2.42
CHL4 – 0.10	62	3.73
CHL1 – 0.30	92	6.45
CHL2 – 0.30	62	5.22
CHL3 – 0.30	76	4.43
CHL4 – 0.30	96	6.33
CHL1 – 1.00	84	6.92
CHL2 – 1.00	64	2.71
CHL3 – 1.00	78	5.84
CHL4 – 1.00	88	6.65
CHL1 – 3.00	34	7.55
CHL2 – 3.00	24	7.93
CHL3 – 3.00	22	6.48
CHL4 – 3.00	52	5.46

Table A4.4: Cont'd

TREATMENT	SURVIVAL (%)	LENGTH (mm)
CHL1 – 10.00	0	0.00
CHL2 – 10.00	0	0.00
CHL3 – 10.00	0	0.00
CHL4 – 10.00	0	0.00
CHL1 – 30.00	0	0.00
CHL2 – 30.00	0	0.00
CHL3 – 30.00	0	0.00
CHL3 – 30.00	0	0.00

Table A4.5: Chironomid survival in two additional trials per the laboratory bioassay employing dietary treatments feeding suspensions of Nutrafin, reference stream periphyton, and mine drainage receiving stream periphyton

TREATMENT	SURVIVAL (%) – December 1999 Trial	SURVIVAL (%) – April 2000 Trial
Nutrafin		
NUT1	52	42
NUT2	58	54
NUT3	70	40
NUT4	86	46
NUT5	74	
Reference Stream Periphyton		
REF1	4	28
REF2	0	16
REF3	0	
REF4	26	34
REF5	50	62
Mine Drainage Receiving Stream Periphyton		
MIN1	0	0
MIN2	2	0
MIN3	0	
MIN4	12	8
MIN5	0	0

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