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MOUTHPART DEFORMITIES AND COMMUNITY COMPOSITION OF CHIRONOMIDAE (DIPTERA) LARVAE DOWNSTREAM OF METAL MINES IN NEW BRUNSWICK, CANADA

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Abstract—The effect of metal enrichment on chironomid communities was examined in streams receiving mine drainage from metal mining operations in New Brunswick, Canada. At five sites receiving mine drainage, metal concentrations were significantly ($p < 0.05$) elevated in water (Zn), periphyton (Cd, Co, Cu, and Zn), and chironomid tissue (Cu, Cd, and Zn) relative to five paired reference locations. Metal concentrations in chironomid larvae were significantly correlated with concentrations in both water and periphyton. Chironomid communities were severely affected at sites receiving mine drainage as demonstrated by reduced genera richness and altered community composition. Sites receiving mine drainage exhibited an increased abundance of metal-tolerant Orthocladiinae and a reduced abundance of metal-sensitive Tanytarsini relative to reference sites. The incidence of mentum deformities was significantly elevated at sites receiving mine drainage (1.43 ± 0.24%), with the mean percentage approaching a doubling of that observed at reference sites (0.79 ± 0.22%). Trace metal concentrations at mine-associated streams in New Brunswick significantly affected the benthic community and have the potential to alter the structure and function of these aquatic ecosystems.

Keywords—Chironomid larvae Metal mining Mouthpart deformities Community composition

INTRODUCTION

Benthic invertebrates are frequently used to assess the effects of contaminants in aquatic ecosystems. The limited mobility of invertebrates, their documented sensitivity to a variety of contaminants, and their close association with contaminant-accumulating substrates (sediment and periphyton) make them excellent biomonitoring organisms that are capable of reflecting the location and severity of contamination [1].

Midge (Diptera: Chironomidae) larvae 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. Chironomidae genera exhibit a range of sensitivities to contaminants, which permits the detection of subtle effects in metal-enriched environments [2,3]. Consequently, even in the most severely contaminated aquatic environments, certain chironomid assemblages persist and can continue to be studied. In addition, chironomids are an important diet item for predatory insects and benthivorous fish. As a result, chironomids represent a significant transfer mechanism for the movement of contaminants to higher trophic levels [4].

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 [2]. Exposure to lower concentrations of metals may elicit stress in an individual organism, which may be undetectable at the community assessment level. At the level of the individual, cellular, physiological, or morphological changes that may ultimately influence growth or reproductive success may be better indicators of impairment [5].

Hamilton and Saether [6] 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 [7]. Mouthpart deformities (mentum, ligula, mandibles, and maxillary palps) have been observed in many genera inhabiting a variety of environments. The reported incidence of mentum deformities ranges from 0 to 83% [8,9] in metal-enriched environments and 0 to 48% [9–11] in non–metal-enriched environments. As a result of the range of deformity frequencies, significantly elevated incidences in metal-enriched environments frequently are not observed [12]. Small sample sizes and a range of operational definitions of deformities contribute to the lack of significant differences observed [12–14]. Frequently, the severity of metal contamination and environmental degradation are assessed by examining the incidence of deformities in fewer than 10 larvae per site [8,15].

The objectives of this study were to compare the richness, community composition, and incidence of mouthpart (mentum) deformities in chironomids collected from streams receiving mine drainage with paired reference streams. Sites receiving mine drainage are expected to have higher trace metal concentrations in water, periphyton, and chironomid tissue. Expected effects on the biota were lower chironomid gen-

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Table 1. Location of sampling sites (reference and receiving mine drainage) in New Brunswick, Canada, from which chemical and biological samples were collected in June and August 1999

River	Latitude	Longitude
Reference sites		
Northwest Miramichi River	47°11.184′N	65°53.671′W
McCormack Brook	47°17.574′N	66°06.512′W
South Branch Pabineau River	47°26.474′N	65°49.784′W
Nepisiquit River	47°23.502′N	66°11.104′W
44 Mile Brook	47°23.545′N	66°10.959′W
Sites receiving mine drainage		
Little South Branch Tomogonops River	47°17.538′N	66°03.123′W
Mosquito Brook	47°19.012′N	66°06.470′W
Austin Brook	47°23.823′N	65°49.165′W
Nepisiquit River	47°23.652′N	66°07.799′W
40 Mile Brook	47°24.064′N	66°07.885′W

era richness, altered community composition, and an increased incidence of deformed larvae than observed at reference sites.

MATERIALS AND METHODS

Study sites

Rich ore deposits in northeastern New Brunswick have sustained a century-old metal mining industry, which has contributed to elevated levels of metals in many cobble streams and rivers of this area. Chemical and biological samples were collected downstream of five metal mining facilities (Heath Steele Mine, Stratmat Mine, Brunswick 6 Mine, Caribou Mine, and Wedge Mine; Table 1). At each mine, with the exception of Caribou Mine, a site suspected of receiving untreated mine drainage (i.e., percolation of surface water through excavated waste rock, but not mine tailings process water) was selected as a replicate of metal enrichment. At Caribou Mine, a suitable metal enrichment site could not be found immediately downstream of mine drainage and therefore a site downstream of effluent release was selected. A paired reference, or non–metal-enriched, site was chosen for each site receiving mine drainage, paired according to similarity in substrate characteristics, canopy cover, stream size, and water velocity.

Water bodies examined in this study consisted of coldwater, forested, headwater streams and medium-sized streams with open canopies. Mean summer water temperatures in these streams seldom exceed 25°C. Generally, stream morphology consisted of riffles and runs, with few pools observed. Substrate at all sampling locations consisted of loose cobbles, with occasional bedrock outcroppings and limited fine sediment. Cobbles and other hard substrates were coated with a light film of periphyton. Streams were fast-flowing and well oxygenated, with circumneutral pH and low conductivity (<0.10 mS/cm).

Water sampling and analysis

Water samples were collected in June 1999. With the use of 500-ml acid-washed polyethylene bottles, samples were collected at a depth equidistant from the surface of the water and the surface of the substrate. Water samples were acidified to a 2% HNO_3 (v/v) solution within 48 h of collection and stored at 4°C before analysis. Total concentrations of 25 trace elements were analyzed by inductively coupled plasma equipped with an optical emission spectrophotometer at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory (Windsor, ON, Canada) [16,17].

Periphyton collection and analysis

Periphyton samples were collected during August 1999. Palm- to hand-sized cobbles were removed from the steam bed and periphyton was scrubbed off with nylon brushes. Periphyton was rinsed off the cobble surface 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.

Samples were centrifuged at 6,000 rpm for 15 min and remaining water was decanted. Periphyton was freeze-dried with a vacuum freeze drier for 24 h. Total concentrations of 16 trace elements were analyzed by inductively coupled plasma equipped with an optical emission spectrophotometer at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory [16–18].

Chironomid collection

Benthic samples were collected during June and August 1999, with a 0.1-m^2 modified Hess sampler (253-μm mesh size) and a D-shaped kick net (1-mm mesh size; Wildlife Supply, Saginaw, MI, USA). Chironomids were handpicked from shallow pans in the field to ensure the collection of at least 200 individuals at each site. Initially, 200 individuals per site was deemed adequate, considering that at least 125 individuals of one genus is recommended to detect a doubling over background levels of deformities of 3% as statistically significant at $p < 0.05$ [13]. Larvae for identification and deformity analysis where preserved in chilled Carnoy's solution (anhydrous ethanol:glacial acetic acid, 3:1, v/v) and stored at 4°C. Remainders of benthic samples were preserved in Kahle's solution (water:95% ethanol:100% formalin:glacial acetic acid, 30:15:6:1, v/v/v) and later sorted in the laboratory.

Benthic samples were partitioned into size fractions by rinsing through a series of brass sieves (4-mm to 250-μm mesh). Under a dissecting microscope, a portion of each size fraction was sorted. Approximately 300 additional chironomids per site were sorted from these composite samples, to augment the number of chironomids examined for deformity analysis to approximately 500 individuals per site.

Chironomid metal analysis

For metal analysis, chironomid larvae were picked from trays in the field and placed on dry ice. Approximately 60 chironomid larvae were collected at each site and combined as a composite sample for metal analysis. Because of the small available sample size (2–10 mg dry wt), chironomid samples were analyzed by inductively coupled plasma equipped with a mass spectrophotometer at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory [16–18]. Samples were maintained at −80°C before analysis. Chironomids were dried on a hot plate at 110°C for 60 min in screw-top perfluoroalkoxy vials. Weight and weight loss (i.e., % moisture) were recorded. Two milliliters of high-purity 50% HNO_3 was pipetted into each vial. The vials were sealed and placed on a hot plate at 85°C for at least 2 h. The solution was allowed to cool and then 2 ml of H_2O_2 was added. Solutions stood for 60 min and then were resealed and placed on a hot plate at 85°C for 2 h. Solutions were allowed to cool, the caps were removed, and then the solutions were evaporated to dryness on the hot plate at 85°C. The samples were finally taken to 12-g final solution mass with 1% HNO_3 containing the inductively coupled plasma–mass spectrometry internal standards Be, In, and Tl, at concentrations of 5, 0.5, and 1 ngm/g, respectively.

Chironomid identification and deformities

Heads of individual chironomid larvae were severed and mounted ventral side up beside the body on microscope slides in CMC-9AF® aqueous mounting medium (Masters, Bensenville, IL, USA). Chironomid larvae were identified to the level of genus [19]. Ten percent of the identified larvae were randomly selected and reexamined to verify correct designation.

Mouthparts of each larva were examined for deformities of the mentum (one or more missing or additional teeth) during taxonomic identification. Deformed larvae were later reexamined to ensure correct designation. 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, which indicates breakage. Other structures were not examined for deformities. Data were expressed as percentage of individuals deformed, with standard error determined according to the binomial distribution.

Statistical analysis

Principal component analyses (Statistica®, StatSoft, Tulsa, OK, USA) were performed to identify trends in metal concentration (water, periphyton, and chironomid tissue), and relative abundance of dominant genera data according to sites receiving mine drainage and reference sites. Data for several elements were excluded from all statistical analyses because their concentrations were undetectable in most samples. When concentrations of other elements in individual samples were below detectable limits, the detection value was used in statistical analyses. Mean relative abundance (percentage) of genera was examined across all 10 sites, and the nine most abundant taxa were selected. All data were logarithmically transformed before analysis (log 10 for metal concentration data and log 2 for abundance data).

Principal component analysis was performed from a correlation matrix. Factor loadings were varimax rotated and metal concentrations or relative abundances of individual genera were said to be associated with a specific principal component when the loading of that genus on a component was >0.600. Factor scores for each site generated from principal component analysis were then analyzed with a one-tailed paired comparison t-test to test for differences between reference sites ($n = 5$) and sites receiving mine drainage ($n = 5$) (with Bonferroni-adjusted p values).

Correlations among metal concentrations in water, periphyton, and chironomid tissue at sites receiving mine drainage and reference sites were determined with Pearson's correlation coefficients (Statistica). Metal concentrations that were below detection limits were replaced by detection values. Data were logarithmically transformed (log 10) before analysis.

The composition of chironomid communities at sites was estimated from both handpicked 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 site in the field. Absolute estimates of abundance were not determined because of differences in the sampling effort expended among sites. Cluster analysis (Euclidean distances grouped by Ward's method) was used to group sites on the basis of community composition (log 2 [relative abundances of genera]). Genera were included in the cluster analysis if their mean relative abundance averaged across all 10 sites was $>1.0\%$ and if they were present in at least 4 of the 10 sites.

Differences in genera richness (number of chironomid genera) and mean incidence of deformities between sites receiving mine drainage and their paired reference site were assessed with one-tailed paired-comparison t tests ($p < 0.05$). Richness and deformity data (percent) were logarithmically transformed (log 10) before analysis.

RESULTS

Trace element analysis of water, periphyton, and chironomid larvae

Metal concentrations in water, periphyton, and chironomid larvae (Tables 2 to 4) generally were greater at sites receiving mine drainage than at their paired reference site (Fig. 1). At sites receiving mine drainage, concentrations of some metals in water (Mn and Zn), periphyton (Cd, Co, Mn, Pb, and Zn), and chironomid larvae (Cd, Pb and Zn) were at least five times greater than concentrations at reference sites. Cadmium, Co, Cr, Cu, Ni, Pb, and V were undetected in water but were at detectable levels in periphyton and chironomid larvae (Fig. 1).

Three principal component (PC) factors were extracted from metal concentrations in water, accounting for 84.8% of the variation in the original data (Table 5). Concentrations of Ca, Mg, Na, and Sr were strongly associated with values of PC-I, which did not significantly differ between sites receiving mine drainage and reference sites ($p < 0.97$; Table 5). Iron, Mn, and Zn concentrations were associated with values of PC-II, and the mean value of scores for this factor was significantly higher at sites receiving mine drainage than at reference sites ($p < 0.05$; Table 5). Concentrations of Fe, Mn, and Zn were most different between mine and reference locations at Heath Steele and Caribou. Barium and K concentrations were strongly associated with values of the PC-III (Table 5) and the score for PC-III was significantly higher at sites receiving mine drainage than at reference sites ($p < 0.05$).

Two principal component factors described metal concentrations in periphyton, accounting for 84.1% of the variation in the original data (Table 5). Concentrations of Cd, Co, Cu, Mn, Pb, and Zn were strongly associated with values of PC-I, and the mean value of scores for this factor was significantly higher at sites receiving mine drainage than at paired reference sites ($p < 0.05$; Table 5). Trace metal concentrations of these elements were most different between mine and reference locations at Heath Steele and Caribou. Concentrations of Cr, Ni, and V were strongly associated with values of PC-II, which did not differ significantly between sites receiving mine drainage and reference sites ($p < 0.60$; Table 5).

Two principal component factors were extracted from metal concentrations in chironomid tissue, accounting for 76.0% of the variation in the original data (Table 5). Concentrations of Fe, Mn, and Pb were strongly associated with PC-I, scores of which did not differ significantly between sites receiving mine drainage and reference sites ($p > 0.07$; Table 5). Concentrations of Cu, Cd, and Zn were positively associated with PC-II, but Ba concentration was negatively associated with this factor (Table 5). The scores for PC-II were significantly different at sites receiving mine drainage and reference sites ($p < 0.05$).

Generally, metal concentrations were highest in periphyton, followed by chironomid larvae, and lowest in water (Fig. 1).

Table 2. Total concentrations of trace elements (μg/L) in water at reference sites (Ref.) and sites receiving mine drainage (Mine) at five metal mining facilities (Heath Steele, Stratmat, Brunswick 6, Wedge, and Caribou mines) in New Brunswick, Canada

	Heath Steele		Stratmat		Brunswick 6		Wedge		Caribou	
Metal	Ref.	Mine	Ref.	Mine	Ref.	Mine	Ref.	Mine	Ref.	Mine
Ag	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Al	ND	233.4	ND	466.8	ND	154.3	ND	ND	ND	ND
As	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ba	2.53	2.8	3.29	6.8	5.58	5.83	3.23	3.76	5.13	3.59
Be	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bi	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ca	4,173	2,428	2,239	1,802	6,026	4,857	3,763	4,118	5,203	13,281
Cd	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Co	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cu	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fe	105	337.5	79.86	150.7	137.2	180.7	63.57	78.66	38.9	62.18
K	352.2	544.9	468.5	627.8	421.1	410.4	365.7	446.7	406.1	556.9
Mg	734.4	809.4	808.2	780.4	1,230	1,170	871.6	922	877.2	1,577
Mn	6.32	87.72	8.69	38.01	57.81	241.2	3.48	9.86	2.44	55.88
Mo	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Na	1,550	1,466	1,363	1,442	4,615	1,527	1,646	1,620	1,268	2,287
Ni	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sn	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sr	17.24	12.9	10.54	11.75	31.05	19.79	17.35	18.8	20.99	34.95
Ti	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
V	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Zn	2.56	108.7	5.2	75.25	18.57	237.3	ND	14.72	3.03	151.3

[a] ND = not detected.

In periphyton, Zn concentrations were 10^4 to 10^6 times higher than in water and 10 to 100 times higher than in chironomid larvae. Compared to metal concentrations in chironomid larvae, Cu and Pb concentrations were 10 to 100 times higher in periphyton, whereas Cd concentrations were only 1 to 10 times higher in periphyton. Zinc concentrations in chironomid larvae were significantly correlated with both metal concentrations in water ($r = 0.84$; $p < 0.05$) and periphyton ($r = 0.79$; $p < 0.05$). Cadmium, Cu, and Pb concentrations in chironomid larvae were significantly correlated with metal concentrations in periphyton. Although statistically significant ($p < 0.05$), these associations were not as strong as the associations for Zn ($r = 0.68$, 0.36, and 0.52, respectively).

Chironomid community composition

Approximately 4.1×10^4 chironomid larvae were collected at the 10 study sites. Identification of a subset of approximately 5,000 slide-mounted larvae yielded 48 genera belonging to five tribes or subfamilies (Table 6). The collected larvae were comosed of 49% Chironominae, 39% Orthocladiinae, 11% Tanypodinae, <1% Diamesinae, and <1% Pseudochironominae. The genera *Cricotopus* and *Orthocladius* are difficult to

Table 3. Total concentrations of trace elements (μg/g dry wt) in periphyton at reference sites (Ref.) and sites receiving mine drainage (Mine) at five metal mining facilities (Health Steele, Stratmat, Brunswick 6, Wedge, and Caribou mines) in New Brunswick, Canada

	Heath Steele		Stratmat		Brunswick 6		Wedge		Caribou	
Metal	Ref.	Mine	Ref.	Mine	Ref.	Mine	Ref.	Mine	Ref.	Mine
Al	13,911	38,488	851.1	4,664	12,409	43,029	12,296	14,994	14,473	22,825
As	18.57	151.9	5.97	23.88	62.51	83.58	35.74	6.82	11.07	96.18
Ca	8,526	3,556	5,380	4,467	3,908	7,241	5,406	2,924	9,355	9,550
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[a]	ND	18.52	61.92	23.2	15.36	25.11	39.87
Cu	15.4	1,304	ND	21.48	17.54	475.6	ND	692.8	18.33	1,323
Fe	20,523	54,648	1,766	5,838	16,517	89,918	12,951	40,746	18,300	19,186
K	5,543	8,718	5,978	6,076	1,848	3,350	3,274	1,571	5,859	4,265
Mg	4,485	5,967	1,330	3,155	1,694	4,607	3,503	1,742	6,343	4,287
Mn	6,481	13,247	2,589	3,291	26,826	158,682	1,320	959	1,968	33,419
Na	2,561	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	1,387	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	5,377	248.3	1,328	2,048	18,213	138.9	585.9	308.7	25,740

[a] ND = not detected.

Table 4. Total concentrations of trace elements (μg/g dry wt) in chironomid larvae from reference sites (Ref.) and sites receiving mine drainage (Mine) at five metal mining facilities (Heath Steele, Stratmat, Brunswick 6, Wedge, and Caribou mines) in New Brunswick, Canada. Concentrations of Ca, K, Mg, and Na are reported as percentage of the dry mass

	Heath Steele		Stratmat		Brunswick 6		Wedge		Caribou	
Metal	Ref.	Mine	Ref.	Mine	Ref.	Mine	Ref.	Mine	Ref.	Mine
As	1.7	3.3	0.9	3.6	3.2	8.3	2.0	1.6	3.5	2.0
Ba	98.8	5.0	17.9	34.1	23.8	18.2	17.4	41.3	10.6	6.3
Bi	ND[a]	0.2	ND	0.1	ND	0.2	ND	ND	ND	ND
Ca (%)	0.1	<0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.2
Cd	0.5	4.7	3.6	20.3	3.6	5.2	0.2	1.2	0.6	37.2
Co	0.6	3.2	1.4	3.8	13.3	10.6	2.5	2.6	0.5	2.7
Cr	0.3	0.5	1.7	1.0	1.4	3.6	5.8	2.4	0.9	1.4
Cu	6	62	13	31	29	45	13	153	10	115
Fe	295	759	1,229	2,072	1,530	6,417	2,591	1,288	597	591
K (%)	0.5	0.4	0.6	1.1	0.6	0.9	0.5	0.6	1.1	0.8
Mg (%)	0.1	<0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.1
Mn	135	133	541	1,590	1,504	2,707	155	160	138	401
Na (%)	0.3	0.2	0.4	1.0	0.4	0.8	0.6	0.6	0.6	0.4
Ni	1.1	1.3	1.4	1.4	5.5	5.1	6.0	2.9	1.3	2.3
Pb	ND	13.8	6.0	81.8	10.2	131.4	3.5	23.9	2.4	1.6
Rb	1.2	1.1	2.8	3.7	3.6	4.3	11.3	8.8	7.1	1.7
Sr	1.3	0.9	6.9	8.9	3.7	5.3	7.2	4.3	4.1	3.5
V	0.2	0.6	2.4	2.0	0.9	3.3	6.8	2.4	0.7	0.5
Zn	45	436	141	1,691	308	929	81	197	113	1,813

[a] ND = not detected.

distinguish as larvae, and thus were classed jointly (i.e., *Cricotopus*/*Orthocladius*). *Cricotopus*/*Orthocladius* sp. was the most abundant taxon, representing 23% of chironomid larvae examined.

Chironomid richness ranged from 12 to 27 genera per site. Genera richness was greater at the reference site than at the site receiving mine drainage for all five pairs of sites examined. The largest difference in genera richness between mine drainage and reference sites occurred at Heath Steele (13 genera). The smallest difference was observed at Stratmat (one genus). Sites receiving mine drainage had a mean (± standard error [SE]) richness of 16 ± 2 genera, which was significantly lower than the mean at corresponding reference sites (23 ± 1; $p < 0.025$; $n = 5$, paired comparison t test).

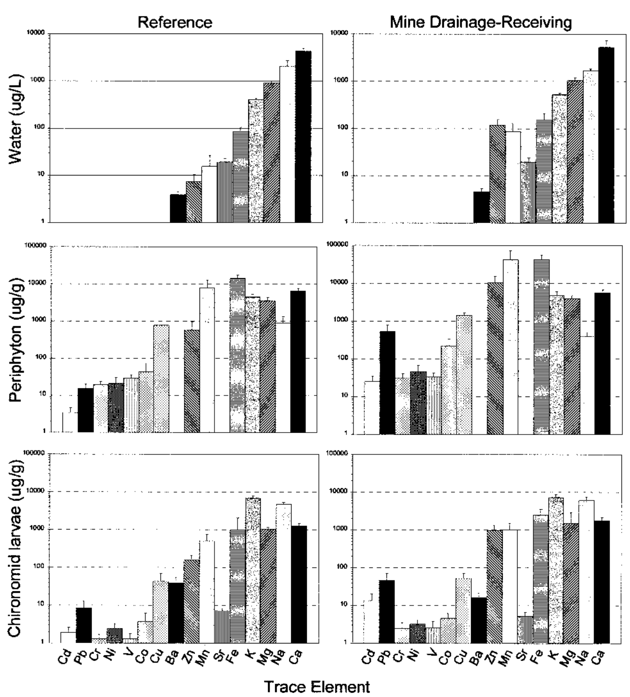

Fig. 1. Mean concentrations (± 1 standard error) of trace elements in (top) water (μg/L), (middle) periphyton (μg/g dry wt), and (bottom) chironomid larvae (μg/g dry wt) at reference sites and sites receiving mine drainage ($n = 5$) associated with metal mining facilities in New Brunswick, Canada. Metals are indicated on the x axis and were ordered according to concentrations in water at reference sites. Scales are logarithmic and scales and units of measurement differ among panels.

Community composition differed between sites receiving mine drainage and reference sites at both the tribe or subfamily level and the genera level. Larval Tanypodinae, Chironomini, and Tanytarsini were relatively more abundant at reference sites, whereas larval Orthocladiinae were relatively more abundant at sites receiving mine drainage (Fig. 2).

The nine most relatively abundant chironomid genera accounted for 60 to 93% of all chironomids at the study sites. Relative abundances of *Larsia*, *Thienemannimyia*, *Micropsectra*, *Stempellinella*, and *Microtendipes* were highest at reference sites, whereas abundances of *Polypedilum*, *Cricotopus*/*Orthocladius*, *Eukiefferiella*, and *Thienemanniella* were greatest at sites receiving mine drainage. Principal component analysis extracted three factors, accounting for 77.6% of the variation in the original data (Table 7). Relative abundances of *Larsia*, *Thienemannimyia*, *Eukiefferiella*, and *Polypedilum* were most strongly associated with the values of PC-I, which accounted for 33% of the variation. Relative abundances of the predatory tanypodines *Larsia* and *Thienemannimyia* were positively associated with each other but negatively associated with the relative abundances of *Eukiefferiella* and *Polypedilum* (Table 7).

The relative abundance of *Micropsectra*, *Stempellinella*, *Microtendipes*, and *Cricotopus*/*Orthocladius* was most strongly associated with PC-II. Relative abundances of the Tanytarsini taxa (*Micropsectra* and *Stempellinella*) were positively associated with PC-II, whereas the relative abundances of orthoclads (*Cricotopus*/*Orthocladius*) were negatively associated with values of PC-II. Relative abundance of *Thienemanniella* was positively associated with values of PC-III.

Table 5. Identification of metals from concentrations in water, periphyton, and chironomid larvae loading on principal components (PC) and the percentage of total variance explained by each component

Principal component	Parameter	Factor loadings	Variance explained (%)
	Metal concentrations in water		
PC-I	Ca	0.94	38.0
	Mg	0.90	
	Na	0.76	
	Sr	0.98	
PC-II	Fe	0.95	27.0
	Mn	0.87	
	Zn	0.76	
PC-III	Ba	0.82	19.0
	K	0.69	
	Metal concentrations in periphyton		
PC-I	Cd	0.89	49.0
	Co	0.75	
	Cu	0.79	
	Pb	0.81	
	Mn	0.73	
	Zn	0.93	
PC-II	Cr	0.93	25.0
	Ni	0.80	
	V	0.91	
	Metal concentrations in chironomid larvae		
PC-I	Fe	0.90	38.9
	Pb	0.89	
	Mn	0.86	
PC-II	Ba	−0.71	37.1
	Cd	0.85	
	Cu	0.75	
	Zn	0.84	

No significant difference was observed in mean factor scores of PC-I or PC-III between sites receiving mine drainage and reference sites (one-tailed paired-comparison *t* tests; $p > 0.05$). Relative abundances of the taxa associated with these factors (*Larsia*, *Thiennemannimyia*, *Eukiefferiella*, *Polypedilum*, and *Thienemanniella*) were only substantially different between two of the five pairs of sites, Brunswick and Wedge, respectively. Relative abundance of taxa associated with PC-II differed significantly between sites receiving mine drainage and reference sites (one-tailed paired-comparison *t* test; $p < 0.03$), with the greatest differences between mine and reference sites apparent at Heath Steele, Brunswick, and Caribou.

The 18 chironomid genera included in the cluster analysis accounted for >92% of chironomids identified at all 10 sites. Cluster analysis generated two relatively distinct groups of sites (Fig. 3). Sites receiving mine drainage at Heath Steele, Brunswick, and Caribou mines had similar chironomid communities, characterized by higher relative abundances of *Cricotopus/Orthocladius* and *Psectrocladius*. Chironomid communities at all reference sites, and two sites receiving mine drainage (Wedge and Stratmat mines) were characterized by higher relative abundance of *Microtendipes*, *Paratendipes*, *Rheotanytarsus*, and *Stempellinella*.

Chironomid deformities

The menta of 5,042 chironomid larvae were examined for deformities (∼500 per site). A total of 56 deformed individuals belonging to 15 different genera was observed in samples (Table 6). The differences in the incidence of mentum deformities between sites receiving mine drainage and references sites were largest at Heath Steele mine (1.69% at mine drainage vs 0.20% at reference) and smallest at Caribou mine (0.58% at mine drainage vs 0.39% at reference). Mean percentage (±SE; $n = 5$) of total deformed individuals was 1.43 ± 0.24% at sites receiving mine drainage. This was significantly higher ($p < 0.05$; one-tailed paired-comparison *t* test) than the mean incidence observed in larvae at reference sites and (0.79 ± 0.22%).

DISCUSSION

Trace element analysis of water, periphyton, and chironomid larvae

Sites downstream of waste rock piles, at metal mine sites in New Brunswick, received significant metal input, even upstream of effluent release locations. Metals accumulated at the base of the food chain and in organisms feeding on these basal resources. Significantly elevated concentrations of Zn were observed in water, periphyton, and chironomid larvae. Despite concentrations being undetectable in water samples, Cd, Cu, and Pb were significantly elevated in both periphyton and chironomid larvae, indicating that these metals are indeed entering receiving waters from waste rock piles on mine property.

Metal concentrations in periphyton were 10^4 to 10^6 times higher than observed in water, suggesting that periphyton effectively bioaccumulates trace metals [20]. Considering the importance of periphyton in lotic food webs [21], the presence of elevated metal concentrations in periphyton may have important implications. Periphyton is a principal food source for many aquatic organisms, which consume predominately algae (scrapers and grazers) and detritus associated with algae (collectors and gatherers). Consequently, invertebrates associated with periphyton likely ingest and retain substantial concentrations of metals. Metals accumulated by primary consumers may be transferred to higher trophic levels, in both aquatic and terrestrial ecosystems [2].

Metals such as Cd, Cu, and Zn are readily accumulated from periphyton by benthic invertebrates [22,23]. Kiffney and Clements [23] argued that invertebrates in the Arkansas River (USA) accumulated Cd, Cu, and Zn primarily through diet, as indicated by the correlation between bioaccumulated metals and levels in periphyton. However, in this study, strong correlations between Zn concentrations in chironomid larvae and concentrations in both water and periphyton were found, making it difficult to discern the source of Zn uptake by larvae. Significant correlations between Cd, Cu, and Pb concentrations in periphyton and chironomid larvae suggest that periphyton is a source of uptake for these elements.

Chironomid community composition

According to Armitage and Blackburn [24], specific identification of chironomid larvae is as effective at distinguishing degrees of metal contamination as examining whole macroinvertebrate assemblages. However, few studies have focused on the effects of metals on chironomid communities beyond the subfamily level [3,9–11,25,26], obscuring the potential for chironomid larvae to reflect metal exposure through loss of richness and altered community composition.

In this study, sites with elevated metal concentrations supported significantly fewer chironomid genera than their paired reference site, confirming the susceptibility of chironomid diversity to reflect metal enrichment. Chironomid genera richness at sites receiving mine drainage was comparable to genera richness reported at other metal-enriched streams [9,26–28], whereras genera richness at reference sites was two to nine

Table 6. The number of chironomid genera collected at reference sites (Ref.) and sites receiving mine drainage (Mine) at five metal mining facilities (Heath Steele, Stratmat, Brunswick 6, Wedge, and Caribou mines) in New Brunswick, Canada. Values in parentheses represent the number of deformed larvae

	Health Steele		Stratmat		Brunswick 6		Wedge		Caribou	
Taxa	Ref.	Mine	Ref.	Mine	Ref.	Mine	Ref.	Mine	Ref.	Mine
Ablabesmyia					26	2			20	1
Larsia		1	3	2	116		69	2	35	69
Paramerina					2				1	
Thienemannimyia	19	8	13	12 (1)	32 (1)	6	59 (2)	16	165 (1)	73 (2)
Macropelopia				1	1		1			1
Nilotanypus	1		5	14 (2)	8	1			2	
Procladius					2 (1)					
Diamesa		6	1	5		1				24
Pagastia	1									
Potthastia	12						19			19
Chironomus					2					
Cryptochironomus	1	3			6		3			
Demicryptochironomus	1				1	1	1			
Glyptotendipes					1					
Microtendipes	32		111		31 (1)	1	5	2	3	
Nilothauma	3									
Parachironomus			11							
Paralauterborniella	1						2			
Paratendipes	24		17	2	44 (1)		12	5	2	1
Phaenopsectra					8 (1)		38			1
Polypedilum	131	17	133 (2)	208 (4)	52	63 (3)	151 (2)	307 (3)	21	6
Saetheria							1			
Tribelos							1			
Xenochironomus										1
Cladotanytarsus					1					
Heterotanytarsus										1
Micropsectra	61 (1)		58 (1)	98 (1)	32	8	17 (1)	82 (1)	129	6
Paratanytarsus					3					
Rheotanytarsus	88		36	1	56	1		5	1	
Stempellina	1				7		2	4 (2)		
Stempellinella			28	2	6		15	2	52	2
Tanytarsus	13		7	35 (1)	22 (2)	3	50	4	17	2
Pseudochironomus	2									
Brillia		1		10						
Corynoneura	3		2	1	1	2	1		9	
Cricotopus/Orthocladius	65	375 (5)	2	20	7	260 (4)	42	16	5	225
Eukiefferiella	12	118 (4)	22	33	22	90	2	29	9	3 (1)
Heterotrissocladius							2			
Krenosmittia				1		1			1	
Lopescladius	1								2	
Parakiefferiella	1									
Parametriocnemus	7	2	14	27	10	9 (1)	1	21	12	1
Psectrocladius	5					8 (1)			1	78
Pseudosmittia						1				
Rheocricotopus	1	2	2							
Symposiocladius			3							
Synorthocladius			7	1					13	
Thienemanniella	3	1	37 (2)	46	2	6	21		7	
Sum	489	534	502	519	501	466	515	495	507	514
Generic richness	25	12	20	19	27	20	23	13	21	18
Proportion deformed	0.20	1.69	1.00	1.73	1.40	1.81	0.97	1.21	0.39	0.58

times higher than previously reported for mine-effect studies [9,28]. Undoubtedly, the sampling effort employed in this study (i.e., $n \sim 500$ per site) contributed to a greater genera richness.

Low genera richness at sites receiving mine drainage may have resulted from the loss of sensitive taxa due to both direct and indirect effects of metal toxicity. Zinc concentrations in water at sites receiving mine drainage of this study were similar to concentrations causing a 50% reduction in survival of larval *Tanytarsus* in laboratory tests [29]. Gower et al. [30] suggested that reduced richness in metal enriched environments resulted from fewer available food sources because of the replacement of palatable species of algae (e.g., diatoms) by metal-tolerant algal taxa (e.g., blue-green algae). Consequences of taxa loss are influenced by the role and abundance of metal sensitive taxa in the community. Loss of taxa representing a unique niche or whose presence influences the abundance of other species will adversely affect ecosystem processes [31].

At sites receiving mine drainage, community composition supported a greater relative abundance of genera reported as metal-tolerant, and a decreased abundance of metal-sensitive taxa. Increased relative abundances of orthoclad larvae (e.g., *Cricotopus*, *Orthocladius*, and *Eukiefferiella* spp.) have frequently been observed downstream of metal mining and electroplating industries [2,3,26–28,30,32]. At sites receiving mine drainage in this study, the relative abundance of *Cricotopus*/

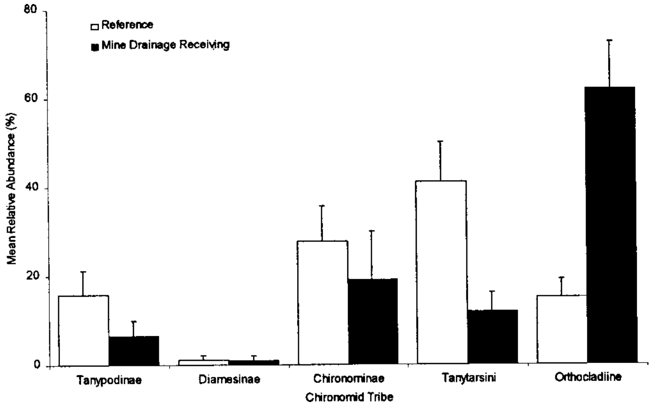

Fig. 2. Mean (± 1 standard error) percent relative abundance of chironomid tribes or subfamilies at reference sites (open) and sites receiving mine drainage (solid) (n = 5) associated with metal mining facilities in New Brunswick, Canada.

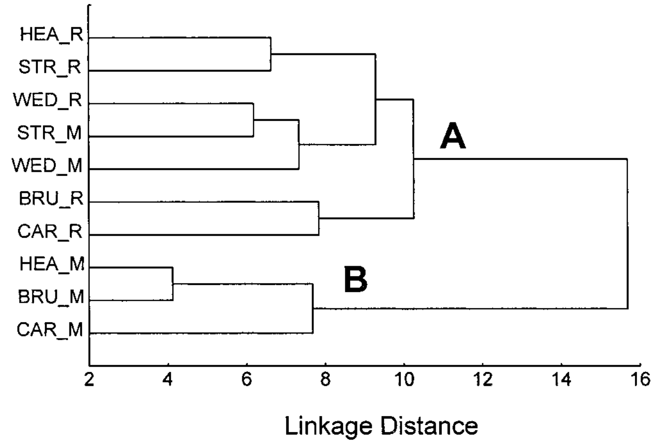

Fig. 3. Similarity of reference sites (_R) and sites receiving mine drainage (_M) associated with metal mining facilities (Heath Steele [HEA], Stratmat [STR], Brunswick 6 [BRU], Wedge [WED], and Caribou [CAR] mines) in New Brunswick, Canada, according to the percent relative abundance of chironomid genera as determined by cluster analysis. Distances between clusters were measured by Euclidean distances and clusters were grouped according to Ward's method.

Orthocladius was significantly higher, whereas other orthoclad taxa (*Eukiefferiella* and *Psectrocladius*) showed trends toward increased abundance relative to reference sites. Surber [32] suggested that perhaps the ability of orthoclad taxa to tolerate metals was only indirectly responsible for their dominance at metal-contaminated sites. Rather, the dietary preference of orthoclade taxa for metal-tolerant blue-green algae, which dominate the periphyton of these sites, could explain their dominance.

Reduced abundances of larval Tanytarsini (e.g., *Micropsectra* and *Tanytarsus* spp.) are commonly observed in metal-enriched environments, resulting in their designation as metal sensitive [26,30]. However, Kiffney and Clements [33] observed increased abundance of larval Tanytarsini in conditions of low (i.e., 0.001 mg Cd/L, 0.010 mg Cu/L, and 0.108 mg Zn/L) metal contamination, suggesting that these larvae may exhibit moderate metal tolerance. Clements et al. [28] also found that the abundances of *Micropsectra* and *Stempellinella* spp. were only reduced at highly metal-contaminated sites. At sites receiving mine drainage of the present study, the relative abundances of *Micropsectra* and *Stempellinella* spp. were reduced by a factor of 2 and 10, respectively, consistent with the designation of others of Tanytarsini as sensitive to metal enrichment.

Changes in chironomid communities were consistent with metal concentrations in water and periphyton. Sites with higher metal concentrations (Heath Steele, Brunswick 6, and Caribou mines) had the lowest taxa richness and highest relative abundances of metal-tolerant taxa. Conversely, sites receiving mine drainage with lower metal concentrations (Wedge and Stratmat mines) had community assemblages resembling those at reference sites. Two of the three sites with reduced richness and dominance of metal-tolerant taxa were operating at the time of sampling, whereas mining at the third site had been terminated for a significant period of time. Presumably at this site, significant drainage from waste rock is still affecting water quality 15 years after mine closure.

Table 7. Identification of dominant chironomid genera (based on relative abundance) loading on principal components (PC) and the percentage of total variance explained by each component

Principal component	Parameter	Factor loading	Variance explained (%)
PC-I	*Thienemannimyia*	0.96	33.0
	Polypedilum	−0.77	
	Larsia	0.72	
	Eukiefferiella	−0.68	
PC-II	*Cricotopus/Orthocladius*	−0.94	31.0
	Micropsectra	0.87	
	Stempellinella	0.66	
	Microtendipes	0.52	
PC-III	*Thienemanniella*	0.82	13.0

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 elevations in incidence of 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 reported at reference locations [10,34]. Natural incidences of mentum deformities previously have been observed to be <1% based on subfossil records [35,36], and thus sites with a frequency of >1% are considered by some to be contaminated [37].

The incidence of deformities at sites receiving mine drainage also falls within the range routinely observed by others at reference sites. Low levels of deformities could be due to several factors. First, different chironomid genera are reputed to exhibit differential sensitivity to deformity expression [12,38,39]. Therefore, our estimate of the incidence of deformities could be conservative, considering that this metric was expressed as a percentage pooled across all genera. Similarly, tolerance to metal exposure also could contribute to lower incidence of deformities at sites receiving mine drainage. Metal tolerance has been observed in midge larvae [40–42] and has resulted in altered responses to biomonitoring indices [43] and lower deformity frequencies [15]. 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 [12,14], and this contributes to observed variation in the incidence of deformities.

Regardless of the low incidence of deformities measured, a consistent increase occurred at sites receiving mine drainage, ranging from 1.25 to more than 8 times above background. Incidences averaged across all sites receiving mine drainage approached a doubling of that observed across all reference sites, implying that elevated metal concentrations were indeed associated with higher levels of deformities than are normally encountered in these populations. Although the manifestation of deformities has been used as an indicator of contaminant exposure, deformities also may indicate a lower fitness of the deformed individuals [13]. Higher body burdens of contaminants, slower growth and development, and lower emergence rates have been observed in deformed individuals than in normal individuals [11,33,34,44].

A significant elevation in mentum deformities was detected when using a conservative definition of deformity and a large sample size. The necessary sample size is a function of the baseline incidence of deformities and the effect size. Considering the low baseline incidence of mentum deformities, 500 individuals, not the 200 previously assumed as sufficient, were necessary to provided adequate power to evaluate deformity frequencies.

Conclusions

The presence of metal mining facilities was significantly associated with elevated metal concentrations in water, periphyton, and chironomid larvae in receiving streams. Mine-affected sites have significantly less diverse chironomid communities, with larval Tanytarsini most sensitive and larval Orthocladiinae least sensitive to metal enrichment. The incidence of deformities at sites receiving mine drainage was double that observed at reference sites.

Considering the importance of chironomids in lotic food webs, significant alterations in community composition and metal bioaccumulation could affect other trophic levels, particularly in the transfer of contaminants through food. 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 metal-enriched, cobble-bottomed streams. A significant elevation in mentum deformities was detected when using a conservative definition of deformity and a large sample size. Because of the low incidence of background deformity incidences observed in this study, the recommendation is made that subsequent studies employ larger sample sizes than currently employed.

Finally, the biological impact of metal enrichment on these streams could not have been assessed with water quality information alone. And although the approach applied in this study effectively evaluated the impact of metal enrichment, it was quite labor intensive. As a result, preliminary work has been done on a laboratory bioassay incorporating field-collected metal-enriched periphtyon as a food source for traditional bioassay organisms (e.g. chironomids, gammarids, and others).

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