SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 28, No. 2, pp. 354–363, 2009

Printed in the USA
0730-7268/09 $12.00 + .00

EFFECTS OF HEAVY METALS ON RIVERINE BENTHIC MACROINVERTEBRATE ASSEMBLAGES WITH REFERENCE TO POTENTIAL FOOD AVAILABILITY FOR DRIFT-FEEDING FISHES

YUICHI IWASAKI,*† TAKASHI KAGAYA,‡ KEN-ICHI MIYAMOTO,§ and HIROYUKI MATSUDA†

†Graduate School of Environment and Information Sciences, Yokohama National University, 79-7, Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan

‡Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

§Research Center for Chemical Risk Management, National Institute of Advanced Industrial Science and Technology, 16-1, Onogawa, Tsukuba 305-8569, Japan

(*Received* 29 *April* 2008; *Accepted* 14 *August* 2008)

Abstract—We examined the influence of heavy metal pollution from an abandoned mine on benthic macroinvertebrates, at population and community levels, and the potential amount of food available for drift-feeding fish in northern Japanese streams. We studied multiple polluted and unpolluted sites with similar longitudinal positions to avoid problems caused by upstream–downstream comparisons. The ranges of zinc, copper, cadmium, and lead concentrations among the study sites were 5 to 812 μg/L, less than 0.12 to 5.2 μg/L, less than 0.0026 to 4.9 μg/L, and 0.1 to 18.6 μg/L, respectively. The abundance of several populations and community metrics showed a significant negative response to heavy metal pollution. Mayfly diversity and abundance was relatively sensitive to heavy metal pollution. In addition, the biomass of groups of macroinvertebrate taxa that are highly available for salmonids were significantly reduced at metal-polluted sites; this decrease in the most highly available group was noticeable (99% at the heavily polluted upper sites and 69% at the moderately polluted lower sites in spring). These results suggest that we should consider the indirect effect of pollution on food availability for the conservation of fish populations that depend on drifting macroinvertebrates.

Keywords—Zinc Pollution Ecological risk assessment Ecotoxicology Water quality standard

INTRODUCTION

Metal pollution is of widespread concern for ecological management of streams and rivers. Mining and industrial activities, effluent from sewage treatment plants, and urban runoff may be major sources of heavy metals in riverine habitats. Heavy metal pollution is well recognized to affect many lotic organisms [1]. The purpose of ecological risk assessment is to contribute to the protection and management of the environment through scientifically credible evaluation of ecological effects due to human activities [2]. Ecological risk assessment for chemicals in particular can provide environmental criteria or standards. Several studies have reported on the acute and chronic toxicity of heavy metals for fish and aquatic invertebrate species [3–5]. Although single-species toxicity tests in the laboratory can estimate toxicity thresholds for individual organisms and endpoints for survival, growth, or reproductive measures, they do not necessarily assess the impact to natural populations of the target species [6]. Nakanishi [7] noted that, except in special cases, protecting every organism should not be the objective of chemical risk management. In addition, many ecologists have pointed out the importance of evaluating the ecological risks at population and higher levels [8].

Benthic macroinvertebrate assemblages contain species with various sensitivities to contaminants and have been widely used to evaluate the ecological impacts of metal contamination in streams [9–11]. They play vital roles in lotic food webs by forming a major link between primary producers and higher trophic levels and in lotic ecosystems by regulating organic matter decomposition and nutrient cycling [12]. Some studies have investigated the effects of heavy metal pollution on leaf-litter breakdown [13] and secondary production of macroinvertebrates [14]; however, the impact of heavy metals on macroinvertebrates has not been evaluated in terms of their food value for fish, even though invertebrates are an important food source for many moving-water fish species. It is of particular importance to evaluate the effects of heavy metal pollution on drift-prone macroinvertebrates, on which most commercially or recreationally important salmonid species depend.

In Japan, an environmental water quality standard for total zinc (Zn) was established at a recommended value to prevent population extinctions of aquatic organisms [15; www.env.go.jp/council/toshin/t094-h1504.html]. The standard Zn concentration in the freshwater environment is 0.03 mg/L and was determined by the nonobserved effect concentration of chronic toxicity for the mayfly *Epeorus latifolium* [4] based on results of laboratory single-species toxicity tests for a variety of species. However, this value may be too conservative for the prevention of population extinctions because it is not based on population-level risk for any species. It is necessary to investigate macroinvertebrate populations in rivers with widely varying degrees of Zn contamination to assess whether the existing standard Zn concentration limit is accurate. In Japan, little accurate data is available regarding the effects of heavy metals on riverine organisms under natural conditions. Although some research has been conducted in areas with mining activity [16,17], most have compared only upstream unpolluted sites with downstream polluted sites and their results

* To whom correspondence may be addressed (d06tf019@ynu.ac.jp).

Published on the Web 8/28/2008.

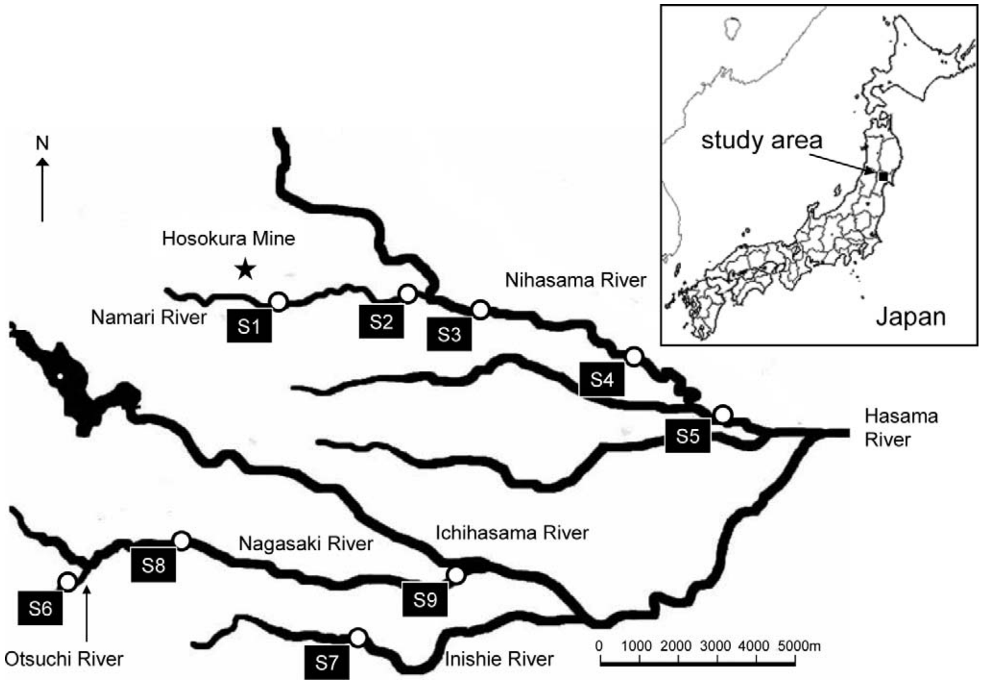

Fig. 1. Map of study sites (S) and the abandoned Hosokura Mine.

have suffered from the confounding effects of indigenous longitudinal variation [18,19].

The objective of the present study was to examine the influence of heavy metal pollution on riverine benthic macroinvertebrates at population and community levels. In particular, we evaluated heavy metal effects on potential food availability for drift-feeding fish. We conducted field surveys in northern Japanese streams, one of which is polluted by heavy metals originating from an abandoned mine.

MATERIALS AND METHODS

Study sites

We conducted the present study in the Nihasama River, its tributary the Namari River, and tributaries of the Ichihasama River, all of which are located in the Hasama River catchment in the Tohoku region, Japan (Fig. 1). An abandoned mine (the Hosokura Mine) exists in the upstream area of the Namari River. This mine produced the highest levels of lead (Pb) and Zn in Japan but was abandoned in 1987. Mine wastewater is still discharged into the Namari River after being neutralized, and sedimentation is treated using calcium hydroxide. Springwater from the bed of the Namari River near the mine contains high concentrations of Zn, cadmium (Cd), copper (Cu), and Pb [20]. Preliminary research revealed relatively high concentrations of these heavy metals in the Nihasama River at the downstream confluence with the Namari River. We thus selected five sites in the Nihasama River catchment as metal-polluted sites and four sites in the Ichihasama River catchment as unpolluted sites (Fig. 1), where the polluted and unpolluted sites showed similar longitudinal positions. This approach avoids problems caused by upstream–downstream comparisons [18,19]. All study sites were cobble-dominated, well-defined riffles.

Benthic macroinvertebrates

We sampled benthic macroinvertebrates in summer (September 2004) and spring (April 2005) at all sites except site 6, which was surveyed only in spring. At each site, we collected macroinvertebrates using a Surber net (mesh size = 0.25 mm) from five stones (maximum diameter = 15–20 cm) that were chosen randomly. Samples were preserved in 10% formalin in the field. In the laboratory, we rinsed the samples through a 0.5-mm sieve. The retained invertebrates were sorted and generally identified to genus or species. Biomass (wet wt) of each taxon was determined by weighing all individuals of that taxon together for each sample.

Benthic macroinvertebrate assemblages were analyzed using abundances (individual numbers per stone) of dominant taxa (mostly genus or species), abundances of dominant families, and benthic community metrics, including total taxon richness, total abundance, total taxon richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT), total EPT abundance, and total abundance and taxon richness of three major aquatic insects groups (mayflies, caddisflies, and chironomids). The dominant taxa and families were defined separately in each season as those that accounted for more than 5% of total abundance at any site.

We also analyzed invertebrate assemblages using biomass metrics in terms of food availability for drift-feeding fishes. All taxa found in the present study were categorized into one of six groups showing different drift propensity according to Rader [21], in which many aquatic invertebrate species were ranked and classified using their traits (e.g., propensity to intentionally drift, habitat, mobility, and body size) to evaluate their importance as a food resource for salmonids. For taxa that were not examined in Rader [21], we ranked and categorized them by following his procedure, as shown in the Table S1. Groups with smaller numbers were composed of more available taxa for salmonids. We calculated the biomass of each of six groups and the total invertebrates. Because the biomass metrics may be greatly affected by rare occurrences of large-bodied taxa, we excluded taxa that occurred on less than 5% of stones (*Semisulcospira* and *Protohermes grandis*) for this calculation.

Table 1. Result of principal components (PC) analysis on physicochemical variables, followed by varimax rotation in summer and spring[a]

	Factor loading[b]											
	Summer (2004)						Spring (2005)					
Variables	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Cu	NA	NA	NA	NA	NA	NA	0.88	−0.07	0.24	−0.22	−0.18	−0.29
Zn	0.96						0.99					
Cd	0.99						0.96					
Pb	0.90				−0.23		0.95					
Temperature	0.22		−0.42	0.53	0.33	0.62	0.78		−0.32	0.22		−0.27
pH			0.99						0.94			0.22
DO	0.68	−0.26	0.64				−0.89		0.28			
BOD	−0.72			0.40		0.53	−0.23	−0.30	0.32	0.29		0.82
TOC		−0.26	−0.28	−0.74	0.38	−0.35		−0.42	−0.61	−0.26	−0.47	
Conductivity	0.95				0.20		0.99					
Hardness	0.94				0.20		0.99					
Catchment area		0.60	−0.32	0.36	0.28	0.56		0.35		0.70	0.59	
Stream width		0.31				0.95			0.22	0.93		
Riffle width		0.32				0.92		0.22		0.95		
Stream depth	0.20				0.93	0.22	−0.26	0.56	0.26	0.33	0.27	
Stream velocity		0.89				0.39		0.89		0.30		−0.32
Stone depth		0.77			0.53						0.99	
Stone velocity		0.93				0.25	−0.24	0.36	0.29	0.45	0.71	
Stone size	0.26			0.91	0.27		−0.22	−0.74	−0.33	−0.36	−0.36	
Contribution (%)	31.5	16.9	10.5	11.2	10.0	17.5	38.1	12.3	10.4	16.2	12.4	5.8

[a] NA = data not available; DO = dissolved oxygen; BOD = biochemical oxygen demand; TOC = total organic carbon.
[b] Factor loading is omitted if the absolute value is smaller than 0.2.

For all of these invertebrate metrics, means of five stones at each site were calculated and used for analyses. Abundance and biomass of invertebrates per stone was log-transformed (x + 1) before calculation of the site means to satisfy assumptions of later analyses.

Water quality and physical environment

Water temperature, dissolved oxygen, pH, and electrical conductivity were measured on site, and water samples were collected when benthic invertebrates were surveyed. Heavy metals (Cu, Zn, Cd, and Pb), biochemical oxygen demand, total organic carbon, and water hardness were analyzed in the laboratory. All measurements and analyses were carried out according to the Japanese Industrial Standard [22; www.jisc.go.jp/]. Catchment area was determined for each site using a digital map 50-m grid (Geographical Survey Institute of Japan, www.gsi.go.jp/ENGLISH/index.html) and geographic information system (ArcGIS 9, Environmental System Research Institute, Redlands, CA, USA), and these areas were log-transformed for later analyses. Stream width (representative width of the reach containing the study riffle) and riffle width (representative width of the study riffle) were evaluated on site. Maximum velocity and depth were estimated based on measurements at dozens of places at each site; current velocity at 60% of water depth was measured using an electromagnetic velocity meter (SF5551-1; Tokyo Keisoku, Tokyo, Japan). For each stone from which macroinvertebrates were collected, water depth at and current velocity (at 60% depth) above its upper

Table 2. Total heavy metal concentrations (μg/L) and first principal component score (PC 1) derived from physicochemical data from the study sites in summer and spring[a]

		Cu		Zn		Cd		Pb		PC 1	
Site	Category[b]	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring
1	HU	BD	5.2	812	447	4.9	2.79	18.6	11.4	1.59	1.75
2	HU	BD	4.4	457	377	4.7	3.97	8.8	6.2	1.19	1.56
3	ML	BD	1.9	301	136	2.7	1.23	3.4	2.1	0.21	−0.11
4	ML	BD	1.4	326	152	2.6	1.12	2.9	1.9	0.13	−0.11
5	ML	BD	1.3	269	126	2.0	0.90	1.8	2.4	−0.24	−0.02
6	NU	BD	3.3	53	64	0.4	0.49	0.8	0.6	−0.69	−0.60
7	NU	NA	0.3	NA	5	NA	BD	NA	0.2	NA	−0.73
8	NL	BD	BD	5	6	BD	0.03	0.2	0.1	−1.19	−1.02
9	NL	BD	0.3	7	6	BD	0.01	0.3	0.2	−1.02	−0.73
Result of a priori contrasts[c]											
	HU vs NU		*		*		*		*		*
	ML vs NL		NS		*		*		NS		*

[a] BD = below detection limit (Cu: 1.4 μg/L, summer; 0.12 μg/L, spring; Cd: 0.0026 μg/L); * = $p < 0.05$; NA = data not available; NS = not significant.
[b] Study sites were divided into four categories: HU = heavily polluted upper sites; ML = moderately polluted lower sites; NU = unpolluted upper sites; NL = unpolluted lower sites.
[c] A priori contrasts of HU versus NU and ML versus NL were done after one-way analysis of variance in spring survey (see *Data analysis* section for further details).

Table 3. Physical characteristics of study sites in summer and spring[ab]

Site	Category[c]	Catchment area (km^2)	Stream width (m)		Riffle width (m)		Maximum depth (cm)		Maximum velocity (cm/s)		Stone depth (cm)		Stone velocity (cm/s)		Relative stone surface area (cm^2)	
			Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring
1	HU	11.5	8	8.5	8	8.5	15	12	114.0	66.8	2 (0.6)	3 (0.6)	46 (3)	33 (3)	1,244 (165)	1,106 (98)
2	HU	17.6	7	7.5	10	8.9	30	13	88.8	143.0	2 (0.2)	3 (0.7)	53 (6)	42 (8)	1,273 (85)	1,063 (42)
3	ML	100.6	16	15.9	23	16.9	22	15	167.6	139.9	5 (0.4)	4 (0.2)	93 (6)	60 (10)	922 (117)	1,028 (125)
4	ML	104.1	7	6.8	8	7.4	20	16	152.5	116.4	8 (1.9)	6 (1.0)	104 (3)	65 (4)	1,198 (240)	993 (77)
5	ML	128.0	11	11.5	15	17.4	39	20	110.0	158.5	8 (1.4)	3 (1.1)	68 (6)	52 (6)	1,287 (108)	1,010 (48)
6	NU	3.3	3	1.9	3	1.9	15	15	73.6	115.0	2 (0.4)	2 (0.2)	36 (4)	37 (1)	900 (88)	1,236 (116)
7	NU	8.1	NA	3.8	NA	6.5	NA	10	NA	68.1	NA	2 (0.5)	NA	25 (2)	NA	1,339 (86)
8	NL	35.2	9	10.0	12	9.3	19	16	140.3	134.1	4 (1.1)	3 (0.0)	62 (7)	54 (8)	1,180 (107)	976 (50)
9	NL	41.1	9	9.7	10	10.5	12	15	78.8	65.2	1 (0.2)	5 (1.0)	41 (3)	54 (2)	1,212 (132)	1,229 (189)
Result of a priori contrasts[d]																
HU vs NU		*		NS		NS		NS		NS		NS		NS		NS
ML vs NL		*		NS		NS		NS		NS		NS		NS		NS

[a] * = $p < 0.05$; NA = data not available; NS = not significant.

[b] Stone depth, stone velocity, and relative stone surface area are the means (± standard error) of five sampled stones.

[c] Study sites were divided into four categories: HU = heavily polluted upper sites; ML = moderately polluted lower sites; NU = unpolluted upper sites; NL = unpolluted lower sites.

[d] A priori contrasts of HU versus NU and ML versus NL were done after one-way analysis of variance in spring survey (see *Data analysis* section for further details).

surface were measured before macroinvertebrate sampling. The relative surface area of each stone was evaluated as a product of maximum diameter and maximum boundary length. These stone variables were averaged at each site for later analyses. The relative surface area of each stone was log-transformed before the calculation of the site mean.

Data analysis

Data collected in summer and spring were analyzed separately because of seasonal changes in taxa occurrence. We intended to determine the effects of heavy metals on benthic invertebrates after removing other physicochemical characteristics and to assess each metal effect separately if possible. However, many high correlations were found between heavy metal concentrations and other physicochemical variables and among heavy metal concentrations. To avoid multicollinearity, we first conducted a principal components analysis on all physicochemical variables and then performed a principal components regression. We treated below-detection values of heavy metals as zero for this analysis. The principal components analysis was performed with varimax rotation. As a result, we obtained a first principal component (PC 1) that was positively correlated with concentrations of all heavy metals for either the summer or the spring survey (Table 1). Water temperature, dissolved oxygen, biochemical oxygen demand, conductivity, and hardness also showed high loadings to PC 1 in at least one season (Table 1). However, most variation in water temperature could be attributed to the difference in the time of measurement on a specific day, and dissolved oxygen in spring (>9.1 mg/L at all sites) and biochemical oxygen demand in summer (<1.1 mg/L at all sites) were unlikely to have large effects on macroinvertebrates in the study sites. Variation in conductivity and hardness would be caused by calcium hydroxide derived from the mine water treatment; therefore, we interpreted PC 1 as the influence of heavy metal pollution (Table 2). Principal components regressions were performed using a forward stepwise procedure ($F_{in} \geq 2.0$ to add, $F_{out} \leq 1.9$ to remove) using the first six PCs (cumulative contribution > 90%) as independent variables. We focused only on the significance of the partial regression coefficient of PC 1.

In addition to regression analysis, we performed analysis of variance (ANOVA) to compare polluted sites with unpolluted sites on each of the upper and lower reaches. Based on heavy metal concentrations, catchment area, and stream width (Tables 2 and 3), we placed all nine sites into one of four categories: heavily polluted upper sites (HU; sites 1 and 2), moderately polluted lower sites (ML; sites 3–5), unpolluted upper sites (NU; sites 6 and 7), and unpolluted lower sites (NL; sites 8 and 9). We performed one-way ANOVA as a factor of the site category, with a priori contrasts [23] of HU versus NU and ML versus NL. Because the upper and lower reaches have different potential fauna and suffer from different levels of heavy metal pollution, this analysis is complementary to the regression analysis, despite the small number of replications. This analysis was also performed for physicochemical data. As the NU category included only one site in summer, this analysis was performed for spring data alone. All statistical analyses were performed using Statistica 6.1 (StatSoft, Tulsa, OK, USA). We chose a significance level of α = 0.05 for all analyses.

Table 4. Water quality at study sites in summer and spring[a]

Site	Category[b]	Temperature (°C)		pH		DO (mg/L)		BOD (mg/L)		TOC (mg/L)		Conductivity (μS/cm)		Hardness (mg/L)	
		Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring
1	HU	22.4	20.5	8.3	7.9	9.9	9.1	0.5	1.3	1.5	1.6	130.0	141.0	690	831
2	HU	23.1	19.0	8.3	7.7	10.8	8.9	0.5	0.8	1.5	1.3	160.0	139.0	919	806
3	ML	22.9	18.5	8.2	7.8	8.8	10.5	0.9	1.0	1.5	1.1	90.0	54.0	449	262
4	ML	22.4	16.5	8.0	7.8	7.5	11.3	0.7	0.9	1.5	1.1	85.0	49.0	424	248
5	ML	23.8	17.0	8.2	7.7	7.8	10.1	0.8	1.1	1.8	1.2	76.0	46.0	370	232
6	NU	20.4	12.0	8.3	7.8	8.6	11.3	0.5	0.8	2.2	1.3	8.2	8.1	19	18
7	NU	NA	16.0	NA	7.4	NA	10.9	NA	1.1	NA	2.3	NA	11.0	NA	27
8	NL	21.7	8.3	8.6	7.9	8.9	12.9	1.1	1.4	1.1	0.9	9.1	7.7	22	23
9	NL	23.5	8.6	8.0	7.8	7.3	11.3	1.1	1.4	1.4	1.0	10.0	7.4	26	25
Result of a priori contrasts[c]															
	HU vs NU		*		NS		*		NS		NS		*		*
	ML vs NL		*		NS		NS		NS		NS		*		*

[a] * = $p < 0.05$; NA = data not available; NS = not significant; DO = dissolved oxygen; BOD = biochemical oxygen demand; TOC = total organic carbon.
[b] Study sites were divided into four categories: HU = heavily polluted upper sites; ML = moderately polluted lower sites; NU = unpolluted upper sites; NL = unpolluted lower sites.
[c] A priori contrasts of HU versus NU and ML versus NL were done after one-way analysis of variance in spring survey (see *Data analysis* section for further details).

RESULTS

Water quality and physical characteristics

Heavy metal concentrations, electrical conductivity, and hardness were higher at the polluted sites (sites 1–5) than at the unpolluted sites (sites 6–9; Tables 2 and 4). Concentrations of heavy metals, except Cu, were generally higher in summer than in spring. Concentrations of Zn at the polluted sites substantially exceeded the Japanese Zn standard concentration (0.03 mg/L). Site 6 showed relatively high metal concentrations compared to the other unpolluted sites, approximately twice the Zn concentration of the standard value. The pH, dissolved oxygen, biochemical oxygen demand, and total organic carbon differed little between the polluted and the unpolluted sites. Water temperature in spring was higher in the polluted than in the unpolluted sites, which simply reflected the timing of measurement as described in the *Materials and Methods* section.

Stream sizes of the NU sites were relatively smaller than those of the HU sites, but depth and velocity at both site categories fell within the same range (Table 3). Catchment areas of the NL sites were smaller than those of the ML sites, where a great deal of water is taken for agricultural use, while other measured physical characteristics (Table 3) and observed discharge in spring and summer did not differ much between these categories.

Responses of dominant taxa and families

Regression analysis revealed the significant negative effects of heavy metal pollution (PC 1) on the abundances of 38% of the dominant macroinvertebrate taxa in summer and 36% of those in spring and on the abundances of 40% of the dominant families in summer and 71% of those in spring (excluding Chironomidae, the results of whose abundance are described in the *Community-level responses to heavy metals* section). Abundances of total Ephemerellidae, the baetid mayflies *Baetis* sp. H, total Baetidae, and total Heptageniidae showed great negative responses to heavy metals (Fig. 2 and Tables 5 and S2). In addition, results of ANOVA showed the significant negative response of the ephemerellid mayfly *Uracanthella punctisetae* abundance to heavy metal pollution in the lower reaches (Table 5). The hydropsychid caddisflies *Cheumatopsyche* and *Hydropsyche orientalis*, total Hydropsychidae, the chironomids *Tvetenia*, *Polypedilum*, *Rheotanytarsus*, and the black fly *Simulium* also showed negative responses to heavy metals. However, the regression analysis revealed significant positive effects of heavy metals on abundances of the chironomid *Orthocladius* and total pupal Orthocladiinae. The tiplid *Antocha* and total Tipulidae showed positive or negative responses to metals, depending on the season. In addition, significant positive responses of the abundances of the chironomids *Cricotopus* and *Thienemanniella* to heavy metal pollution in the lower reaches were found by ANOVA.

Community-level responses

Except for chironomid abundance, all macroinvertebrate community metrics for richness and abundance (total taxon richness, total abundance, EPT richness, EPT abundance, mayfly richness, mayfly abundance, caddisfly richness, caddisfly abundance, and chironomid richness) had significant negative responses to heavy metal pollution in both seasons in the regression analysis (Fig. 3 and Table 6). Analysis of variance also showed that some of these metrics were significantly lower

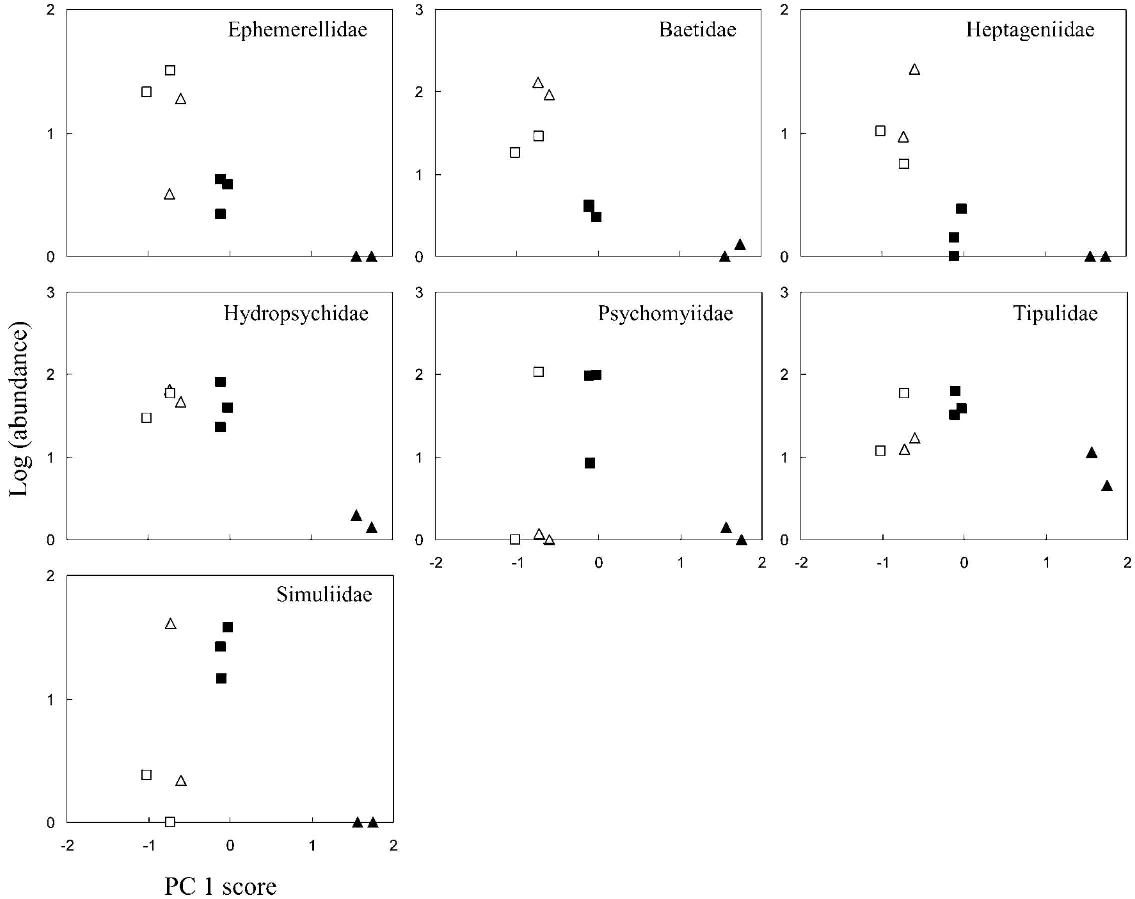

Fig. 2. Relationship between the first principal component (PC 1) score derived from physicochemical data (correlated with heavy metal concentrations) and the abundance ($\log_{10}$-transformed) of dominant families in the heavily polluted upper (▲), unpolluted upper (△), moderately polluted lower (■), and unpolluted lower (□) sites in spring surveys. Abundance of chironomids is shown in Figure 3.

at the metal-polluted sites than at the unpolluted sites in spring (Table 6). Notably, reductions in EPT richness and mayfly richness and abundance at the polluted sites compared to the unpolluted sites (NU or NL) were largest and were observed in both the upper and lower reaches (EPT richness: 85% at HU and 43% at ML, mayfly richness: 97% at HU and 67% at ML, mayfly abundance: 99% at HU and 90% at ML). Although the response of mayfly appeared to be linear with increased heavy metal pollution, several metrics such as richness and abundance of caddisfly and chironomid at the ML sites were comparable to those at the NL sites (Fig. 3). Both methods of analysis were unable to detect significant responses of chironomid abundance to heavy metal pollution.

Regression analysis detected significant negative effects of heavy metal pollution on total invertebrate biomass in spring (Fig. 4 and Table 6). When all site data were compiled, biomass composition of each drift-propensity group was as follows: in summer, group 1: 15%, group 2: 26%, group 3: 56%, group 4: 3%, group 5: less than 1%, and group 6: less than 1%, and in spring, group 1: 10%, group 2: 34%, group 3: 46%, group 4: 8%, group 5: less than 1%, and group 6: 1%. Regression analysis detected significant negative effects of heavy metal pollution on the biomass of some of these groups: on group 1 (consisting of the most highly available taxa for salmonids) in both seasons, on group 5 in summer, and on groups 2 and 3 in spring (Fig. 4 and Tables 6 and S3). Analysis of variance on the spring data revealed significant reductions in some biomass metrics at the polluted sites (Table 6): in group 1, in both the upper and the lower reaches (99% at HU and 69% at ML), and in total invertebrates and in groups 2 and 3, in the upper reaches only (92, 88, and 98% at HU, respectively). Neither method indicated that groups 4 and 6 respond significantly to heavy metal pollution.

DISCUSSION

Our results clearly show that heavy metal pollution adversely affects several macroinvertebrate populations and alters characteristics of the benthic invertebrate community in Japanese streams. In particular, the abundance of several mayfly taxa and total abundance and taxon richness of mayflies were dramatically decreased even at the moderately polluted sites. The sensitivity of mayflies to heavy metals has also been well documented in previous studies [9,10]. While negative effects of heavy metals were observed in the abundance of some caddisfly and dipteran taxa and in taxon richness of caddisflies and chironomids at heavily polluted sites, some chironomid taxa showed positive responses to heavy metal pollution. Some studies have also reported that certain orthocladine species are tolerant to heavy metals [9,24].

Heavy metals may affect benthic invertebrates indirectly through alteration of habitat conditions [25] or trophic relationships [26,27], as well as directly through water contamination. Mining activity often causes sedimentation and increases substratum embeddedness [28]. We observed thick (1–2 mm) slimy biofilm formation on the upper surface of stones at the HU sites, which were probably caused by drainage

Table 5. Results of principal components (PC) regression (summer and spring) and analysis of variance (ANOVA, spring only) for the abundance of dominant taxa and families[a]

	Influence of PC 1[b]		Results of ANOVA[c]	
Dependent variable	Summer	Spring	Upper	Lower
Dominant taxa				
Uracanthella punctisetae		NS	NS	—
Baetis sahoensis	NS			
Baetis thermicus	NS	−0.58	—	NS
Baetis sp. H	−0.73			
Epeorus nipponicus		NS	NS	NS
Cheumatopsyche	−0.65	−0.88	—	NS
Hydropsyche orientalis	NS	−0.96	—	NS
Psychomyia		NS	NS	NS
Potamomusa	NS			
Antocha	0.79	−0.45	NS	NS
Cricotopus	NS	NS	NS	+
Orthocladius	0.88	NS	NS	NS
Thienemanniella		NS	NS	+
Tvetenia	−0.77	NS	NS	NS
Orthocladiinae (pupa)	0.63	NS	NS	NS
Polypedilum	−0.45			
Rheotanytarsus		−0.73	—	NS
Simulium	−0.65	NS	NS	NS
Dominant families				
Ephemerellidae		−0.80	—	—
Baetidae	−0.96	−0.79	—	—
Heptageniidae		−0.69	—	—
Hydropsychidae	NS	−0.94	—	NS
Psychomyiidae		NS	NS	NS
Crambidae	NS			
Tipulidae	0.61	−0.48	NS	NS
Chironomidae		see Table 6		
Simuliidae	−0.65	NS	NS	NS

[a] NS = no significant difference. Result is blank when the taxon did not satisfy dominance criteria in that season (see text).
[b] The effect of PC 1 (first PC, correlated with heavy metal concentrations) represents a significant standard partial regression coefficient of PC 1.
[c] The ANOVA result represents a significantly smaller (−) or greater (+) abundance in polluted compared to unpolluted sites on each of the upper and lower reaches.

from the mine wastewater treatment using calcium hydroxide. The slimy biofilm formations would alter the physical habitat structure and food quality (reduction in organic matter and algal content) for grazing invertebrates that were dominated by mayflies at our study sites. Observed reductions in mayflies at the HU sites may be partly attributed to the biofilm formations.

We conducted the field survey across two river catchments: one metal polluted and one unpolluted. While this approach avoids problems caused by upstream–downstream comparisons, it is impossible to exactly separate the influence of heavy metal pollution from catchment-scale effects in our study design. However, except for heavy metal concentrations, most physicochemical characteristics critical to riverine benthos did not differ much between the study sites at similar longitudinal positions in the two catchments. We selected the unpolluted catchment in contact with the polluted catchment to avoid such catchment-scale effects as much as possible. Thus, it is likely that the differences in catchment characteristics should have little importance for macroinvertebrates in the present study.

Heavy metals adversely affected the total biomass of the invertebrate assemblage in spring. Moreover, the biomass of groups of highly available taxa for salmonids was significantly reduced at the metal-polluted sites and the reduction in the most highly available group (group 1) was noticeable. The biomass of this most highly available group was dominated by baetid mayflies (72% in summer and 86% in spring), which were sensitive to heavy metals, as discussed earlier. Salmonid biomass and production are highly correlated with abundance of drifting invertebrates [29,30]. Mature rainbow and brown trout feed mainly on drifting invertebrates [31], and their diet was similar to the percentage composition of the drifting fauna during their major feeding periods [32]. Wilzbach et al. [29] observed a strong correlation between densities of drifting invertebrates and growth of cutthroat trout. In Southern Appalachian streams trout biomass were typically low because of low benthic productivity and drift densities [30]. Our results suggest, therefore, that heavy metal pollution has a negative effect on potential food availability for drift-feeding fish. We should take account of indirect effects through reduction of food, as well as direct effects for ecological risk assessment of heavy metal pollution on fish populations that largely depend on drifting macroinvertebrates. Levin et al. [6] noted that such indirect effects should be considered in ecological risk assessment for chemicals.

Heavy metal pollution also reduced the taxonomic diversity of the benthic macroinvertebrate assemblages, including EPT richness. Wallace et al. [33] reported that variation in EPT richness was related to invertebrate secondary production and detritus processing. Although we did not directly evaluate these ecosystem processes, heavy metal pollution might have affected them in our study streams. Total taxon richness of Ephemeroptera, Plecoptera, and Trichoptera may also be a good indicator of heavy metal impacts on potential food biomass for drift-feeding fish because it was highly correlated with the biomass of the most highly available group (group 1: $r = 0.94$ [summer] and $r = 0.81$ [spring]).

One of the most important concepts in ecological risk assessment is assessment endpoints, which are defined as an explicit expression of the environmental value to be protected [34]. Kamo and Naito [35] focused on population extinction as an assessment endpoint in relation to Zn concentration. They estimated that the Zn concentration to achieve 95% population protection was 0.107 mg/L based on the results of toxicity tests for various species. In spring, we detected a reduction in total taxon richness at the HU sites (Zn: 0.377–0.477 mg/L) compared to the NU sites (Zn: <0.06 mg/L) but not at the ML sites (Zn: 0.126–0.152 mg/L) compared to the NL sites (Zn: <0.01 mg/L). Ephemeroptera diversity was reduced even at the ML sites compared to the NL sites but not at one NU site (site 6; Zn: 0.064 mg/L) compared to the other NU site (site 7; Zn: <0.01 mg/L). These results do not contradict Kamo and Naito's prediction of population-level effects.

An integrated approach combining laboratory, mesocosm, and field studies, and ecological modeling is important to evaluate ecological impacts [36–40] because each type of study has specific limitations. Field studies, such as those described in the present paper, lack strict control sites that differ only in a single factor from impacted sites. Therefore, our data on whether the existing Japanese standard Zn concentration is appropriate are still limited. However, field surveys are powerful tools when observing the status of living organisms under contaminated conditions in the real world and when documenting ecological effects at population levels. Complementation of multiple approaches is essential in ecological risk assessment.

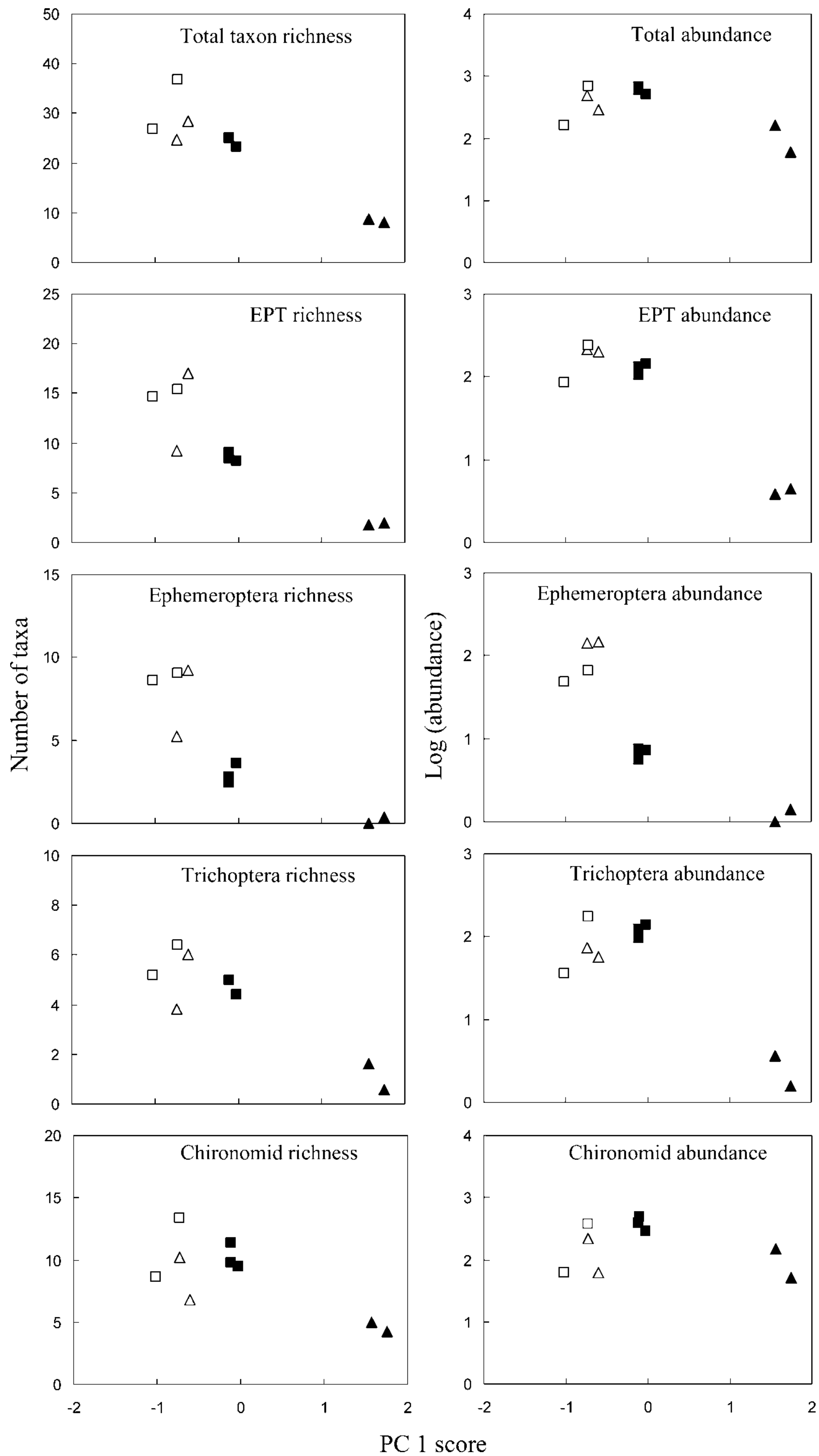

Fig. 3. Relationship between the first principal component (PC 1) score derived from physicochemical data (correlated with heavy metal concentrations) and community-level metrics (richness and abundance, log10-transformed, of major aquatic insect group) in the heavily polluted upper (▲), unpolluted upper (△), moderately polluted lower (■), and unpolluted lower (□) sites in spring surveys. EPT = Ephemeroptera, Plecoptera, and Trichoptera.

SUPPORTING INFORMATION

Table S1. Categorization of taxa into six drift propensity groups using categories in Rader [21], except for those taxa (*) that were not examined in his study and were categorized by following his procedure.

Table S2. Mean abundance (per stone) and biomass (mg/stone) of dominant taxa at study sites (S) in summer.

Table S3. Mean abundance (per stone) and biomass (mg/stone) of dominant taxa at study sites (S) in spring.

Table S4. Mean biomass (mg/stone) of total invertebrates and six drift propensity groups at study sites in summer and spring. The smaller numbered group is composed of more available taxa for salmonids (see text for further details).

All found at DOI: 10.1897/08-200.S1 (193 KB PDF).

Table 6. Results of principal components (PC) regression (summer and spring) and analysis of variance (ANOVA, spring only) for benthic community metrics[a]

	Influence of PC 1[b]		Results of ANOVA[c]	
Dependent variable	Summer	Spring	Upper	Lower
Total taxon richness	−0.90	−0.91	—	NS
Total abundance	−0.68	−0.61	NS	NS
EPT richness	−0.95	−0.88	—	—
EPT abundance	−0.89	−0.93	—	NS
Mayfly richness	−0.93	−0.83	—	—
Mayfly abundance	−0.97	−0.88	—	—
Caddisfly richness	−0.86	−0.89	—	NS
Caddisfly abundance	−0.64	−0.85	—	NS
Chironomid richness	−0.64	−0.73	NS	NS
Chironomid abundance	NS	NS	NS	NS
Biomass				
Total invertebrates	NS	−0.95	—	NS
Group 1[d]	−0.96	−0.90	—	—
Group 2	NS	−0.90	—	NS
Group 3	NS	−0.96	—	NS
Group 4	NS	NS	NS	NS
Group 5	−0.44	NS	NS	NS
Group 6	NS	NS	NS	NS

[a] NS = no significant difference; EPT = Ephemeroptera, Plecoptera, and Trichoptera.
[b] Effect of PC 1 (first PC, correlated with heavy metal concentrations) represents a significant standard partial regression coefficient of PC 1.
[c] The ANOVA result represents a significantly smaller (−) or larger (+) value in polluted compared to unpolluted sites on each of the upper and lower reaches.
[d] The smaller-numbered group was composed of more available taxa for salmonids (see *Benthic macroinvertebrates* section for further details).

Acknowledgement—This study was supported in part by a Japan Society for the Promotion of Science grant and the New Energy and Industrial Technology Development Organization. We thank M. Kamo, W. Naito, Y. Kameda, A. Munakata, M. Sakakibara, T. Furuya, and all professors and students who helped in our laboratory and fieldwork. We also thank two anonymous reviewers.

REFERENCES

1. Newman MC, McIntosh AW. 1991. *Metal Ecotoxicology: Concepts and Applications.* Lewis, Boca Raton, FL, USA.
2. Suter GW. 1993. *Ecological Risk Assessment.* Lewis, Boca Raton, FL, USA.
3. Paulauskis JD, Winner RW. 1988. Effects of water hardness and humic acid on zinc toxicity to *Daphnia magna* Straus. *Aquat Toxicol* 12:273–290.
4. Hatakeyama S. 1989. Effect of copper and zinc on the growth and emergence of *Epeorus latifolium* (Ephemeroptera) in an indoor model stream. *Hydrobiologia* 174:17–27.
5. Benoit DA, Holcombe GW. 1978. Toxic effects of zinc on fathead minnows *Pimephales promelas* in soft water. *J Fish Biol* 13:701–708.
6. Levin SA, Kimball KD, McDowell WH, Kimball SF. 1984. New perspectives in ecotoxicology. *Environ Manag* 8:375–442.
7. Nakanishi J. 2004. *Kankyo-risuku-gaku [Environmental Risk].* Nippon Hyoronsha, Tokyo, Japan.
8. Pastorok RA, Bartell SM, Ferson S, Ginzburg LR. 2002. *Ecological Modeling in Risk Assessment: Chemical Effects on Populations, Ecosystems, and Landscapes.* Lewis, Boca Raton, FL, USA.
9. Clements WH. 1994. Benthic invertebrate community responses to heavy metals in the upper Arkansas River basin, Colorado. *J N Am Benthol Soc* 13:30–44.
10. Clements WH, Carlisle DM, Lazorchak JM, Johnson PC. 2000. Heavy metals structure benthic communities in Colorado mountain streams. *Ecol Appl* 10:626–638.
11. Maret TR, Cain DJ, MacCoy DE, Short TM. 2003. Response of benthic invertebrate assemblages to metal exposure and bioac-

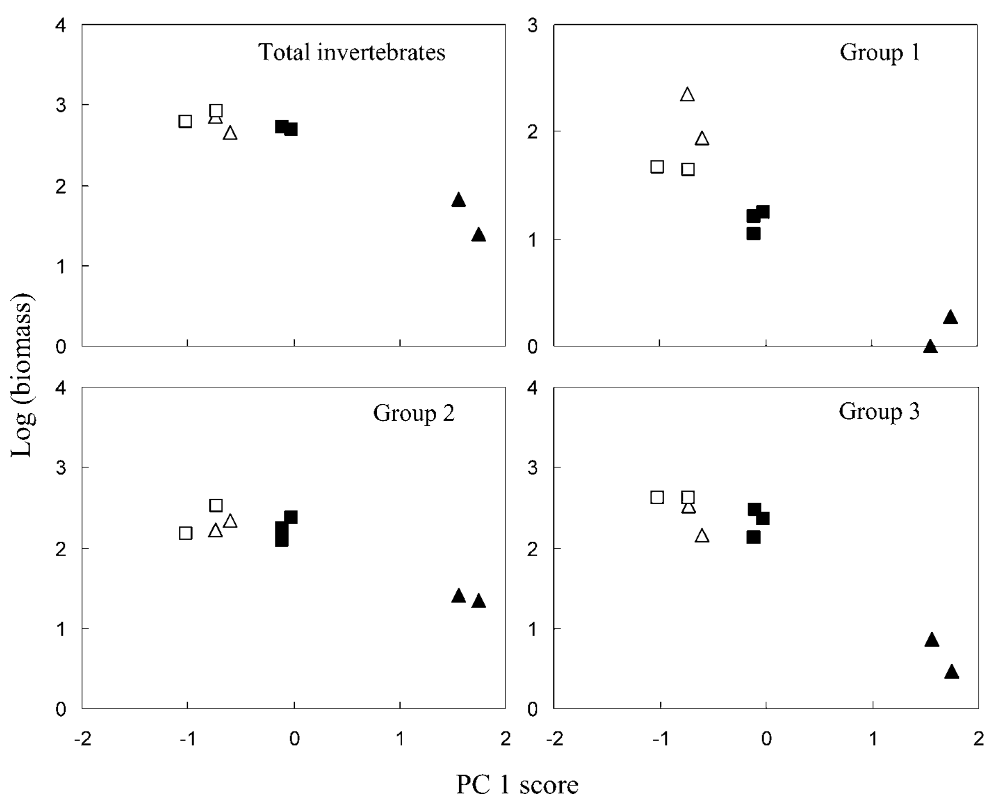

Fig. 4. Relationship between the first principal component (PC 1) score derived from physicochemical data (correlated with heavy metal concentrations) and the biomass (log10-transformed) of total invertebrates and three drift propensity groups in the heavily polluted upper (▲), unpolluted upper (△), moderately polluted lower (■), and unpolluted lower (□) sites in spring surveys. The smaller-numbered group is composed of more available taxa for salmonids (see *Benthic macroinvertebrates* section for further details).

cumulation associated with hard-rock mining in northwestern streams, USA. *J N Am Benthol Soc* 22:598–620.

12. Wallace JB, Webster JR. 1996. The role of macroinvertebrates in stream ecosystem function. *Annu Rev Entomol* 41:115–139.
13. Schultheis AS, Sanchez M, Hendricks AC. 1997. Structural and functional responses of stream insects to copper pollution. *Hydrobiologia* 346:85–93.
14. Carlisle DM, Clements WH. 2003. Growth and secondary production of aquatic insects along a gradient of Zn contamination in Rocky Mountain streams. *J N Am Benthol Soc* 22:582–597.
15. Japanese Ministry of Environment. 2003. Suiseiseibutsu no hozen ni kakawaru suishitsukankyokijun no settei nitsuite [Setting of water environmental standard for conserving aquatic life.]. Ministry of Environment, Tokyo.
16. Koyama J, Kobayashi N. 1986. Relationship between heavy metal contents of benthos and concentrations of river water. *Jap J Water Pollut Res* 9:793–797.
17. Gose K. 1961. On the influence of mine-effluents of the Kamioka Mine (Gifu Prefecture), Ogoya Mine (Ishikawa Prefecture) and Hosokura Mine (Miyagi Prefecture) on stream organisms. *Jap J Ecol* 11:111–117.
18. Clements WH, Kiffney PM. 1995. The influence of elevation on benthic community responses to heavy metals in Rocky Mountain streams. *Can J Fish Aquat Sci* 52:1966–1977.
19. Kiffney PM, Clements WH. 1996. Effects of metals on stream macroinvertebrate assemblages from different altitudes. *Ecol Appl* 6:472–481.
20. Seino S, Abe T, Fujimaki H. 2004. Assessment of high concentrations of fluorine in the Namari River system. In *Annual Report of Miyagi Prefectural Institute of Public Health and Environment,* Vol 22, pp. 109–114.
21. Rader RB. 1997. A functional classification of the drift: Traits that influence invertebrate availability to salmonids. *Can J Fish Aquat Sci* 54:1211–1234.
22. Japanese Industrial Standards Committee. 1998. Testing methods for industrial wastewater. JIS K 0102. Tokyo.
23. Sokal RR, Rohlf FJ. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research,* 3rd ed. WH Freeman, New York, NY, USA.
24. Clements W, Cherry D, Van Hassel J. 1992. Assessment of the impact of heavy metals on benthic communities at the Clinch River (Virginia): Evaluation of an index of community sensitivity. *Can J Fish Aquat Sci* 49:1686–1694.
25. Courtney LA, Clements WH. 2002. Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: An experimental approach. *Freshw Biol* 47:1766–1778.
26. Farag AM, Woodward DF, Goldstein JN, Brumbaugh W, Meyer JS. 1998. Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River basin, Idaho. *Arch Environ Contam Toxicol* 34:119–127.
27. Fleeger JW, Carman KR, Nisbet RM. 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci Total Environ* 317:207–233.
28. Church SE, Kimball BA, Fey DL, Ferderer DA, Yager TJ, Vaughn RB. 1997. Source, transport, and partitioning of metals between water, colloids, and bed sediments of the Animas River, Colorado. U.S. Geological Survey, Washington, DC.
29. Wilzbach MA, Cummins KW, Hall JD. 1986. Influence of habitat manipulations on interactions between cutthroat trout and invertebrate drift. *Ecology* 67:898–911.
30. Romaniszyn ED, Hutchens JJ, Wallace JB. 2007. Aquatic and terrestrial invertebrate drift in southern Appalachian Mountain streams: Implications for trout food resources. *Freshw Biol* 52: 1–11.
31. McLennan J, MacMillan B. 1984. The food of rainbow and brown trout in the Mohaka and other rivers of Hawk's Bay, New Zealand. *N Z J Mar Freshw Res* 18:143–158.
32. Elliott JM. 1973. The food of brown and rainbow trout (*Salmo trutta* and *S. gairdneri*) in relation to the abundance of drifting invertebrates in a mountain stream. *Oecologia* 12:329–347.
33. Wallace JB, Grubaugh JW, Whiles MR. 1996. Biotic indices and stream ecosystem processes: Results from an experimental study. *Ecol Appl* 6:140–151.
34. U.S. Environmental Protection Agency. 1998. Guidelines for ecological risk assessment. Washington, DC.
35. Kamo M, Naito W. 2008. A novel approach for determining a population-level threshold in ecological risk assessment: A case study of zinc. *Hum Ecol Risk Assess* 14:714–727.
36. Clements WH, Kiffney PM. 1994. Integrated laboratory and field approach for assessing impacts of heavy metals at the Arkansas River, Colorado. *Environ Toxicol Chem* 13:397–404.
37. Peeters E, Dewitte A, Koelmans AA, van der Velden JA, den Besten PJ. 2001. Evaluation of bioassays versus contaminant concentrations in explaining the macroinvertebrate community structure in the Rhine-Meuse delta, the Netherlands. *Environ Toxicol Chem* 20:2883–2891.
38. Clements WH, Carlisle DM, Courtney LA, Harrahy EA. 2002. Integrating observational and experimental approaches to demonstrate causation in stream biomonitoring studies. *Environ Toxicol Chem* 21:1138–1146.
39. Buchwalter DB, Cain DJ, Clements WH, Luoma SN. 2007. Using biodynamic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. *Environ Sci Technol* 41:4821–4828.
40. Suter GW. 2007. *Ecological Risk Assessment*, 2nd ed. CRC, Boca Raton, FL, USA.