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METAL-INDUCED SHIFTS IN BENTHIC MACROINVERTEBRATE COMMUNITY COMPOSITION IN ANDEAN HIGH ALTITUDE STREAMS

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Abstract—High altitude creates unique challenging conditions to biota that limit the diversity of benthic communities. Because environmental pollution may add further stress to life at high altitude, the present study explored the effect of metal pollution on the macroinvertebrate community composition in Andean streams between 3,500 to 4,500 meters above sea level (masl) during wet and dry seasons. At polluted sites, showing a high conductivity and a low pH, metal concentrations (e.g., Al, 13.07 mg/L; As, 3.49 mg/L; Mn, 19.65 mg/L; Pb, 0.876 mg/L; Zn, 16.08 mg/L) ranged from 8-fold up to 3,500-fold higher than at reference sites. The cumulative criterion unit allowed quantifying the potential toxicity of metal mixtures at the contaminated sites. Principal component analysis of physical chemical variables showed that reference sites were more likely to be structured by transparency, water discharge, and current velocity, while polluted sites appeared to be determined by metals and conductivity. Canonical correspondence analysis indicated a strong influence of highly correlated metals in structuring invertebrate communities, which were dominated by dipterans, coleopterans, collembolans, and mites at polluted sites. At reference sites crustaceans, ephemeropterans, plecopterans, and trichopterans were the most representative taxa. We concluded that severe metal pollution induced changes in macroinvertebrate community composition in high-altitude Andean streams, with a replacement of sensitive taxa by more tolerant taxa. Yet relatively species-rich communities persisted under harsh conditions. Environ. Toxicol. Chem. 2010;29:2761–2768. © 2010 SETAC

Keywords—Andean high altitude streams   Macroinvertebrates   Community composition   Taxa replacement   Metal pollution

INTRODUCTION

Andean high-altitude streams are among the least studied freshwater ecosystems, although they represent unique challenging conditions, mainly due to low water temperature and low dissolved oxygen concentrations that limit the diversity of benthic communities [1–3]. It is generally believed that cold high-altitude streams are more oxygen-rich than warm lowland streams because of better oxygen solubility at lower temperatures and better aeration generated by fast-flowing, turbulent waters. This is not the case, however, since oxygen pressure also decreases with altitude, rendering these streams close to a critical oxygen saturation level [4]. Also, the regime of ultraviolet (UV) exposure and temperature fluctuation tends to create challenging conditions for aquatic life [3,5]. Environmental pollution may add further stress to life at high altitude and these combined stressors may have a strong impact on local communities.

Mining exploitation has been one of the most important economic activities developed at high altitudes in Andean countries and is still growing. In the past, mining practices were performed without environmental protection and mineral waste was stored in large piles exposed to rainfall [6]. Currently, these abandoned dumps and mine tailings represent a standing threat for Andean rivers and streams due to the continuous mobilization of metals and acid drainage, changing water chemistry and biotic communities [7]. Moreover, because in several cases metal levels exceed the permissible limits for human or agricultural water use, it is deemed that such toxic contaminants have critically deteriorated important freshwater sources in the region over decades of exposure. [8].

Studies on the effects of increased acidity and dissolved metal concentrations (Cd, Cu, Zn) in natural and artificial streams in the Rocky Mountains (USA) have shown a significant reduction of invertebrate abundance and species richness, due to loss of sensitive taxa and a shift in community composition towards more tolerant species [9,10]. Indirect effects include smothering of the streambed substrate by metal precipitates, reducing the habitat availability for stream fauna, decreasing food quality, and modifying interactions between functional feeding groups [11–13]. In addition, it has been reported that related species from elevated temperate streams (2,500 meters above sea level [masl]) are more sensitive to metals than those from lowland streams [10,14]. However, in spite of the complex geology of the Andes and the presence of an active mining industry, little attention has been devoted to the effects of metals and acid drainage on Andean high-altitude streams. Hence, the aim of the present study was to determine if elevated metal concentrations represent a stress factor shaping benthic invertebrate community composition, comparing reference and metal polluted streams at high altitude. To this purpose, benthic macroinvertebrate communities in reference waters and those exposed to natural and anthropogenic metal contamination were sampled during four consecutive seasons in 2006 and 2008 at six high altitude sites (3,500–4,500 masl) in the Cordillera Blanca and Cordillera Negra (Peruvian Andes).

All Supplemental Data may be found in the online version of this article.
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Multivariate analysis was used to identify those environmental factors that most strongly influenced biodiversity and composition of macroinvertebrate assemblages.

MATERIALS AND METHODS

Study sites

In Central-Northern Peru (Ancash region), the Cordillera de los Andes comprise two parallel mountain ranges, the eastern Cordillera Blanca and the western Cordillera Negra, both extending beyond 5,500 masl along the Santa River. Below the permanent snow line, between 3,700 to 4,200 masl, slopes have been modified for small agriculture and cattle rearing, and also mining activities take place. Streams in this area are fast-flowing, with substrate consisting of gravel, pebble, and cobbles in runs and riffles. They show a very sparse macrophyte growth and are almost completely unshaded.

Six sampling sites were selected, all located between 3,500 to 4,500 masl (Fig. 1). Four reference sites were selected. Three reference sites (Honda, Aquilpo, and Ishinca) were located in the Cordillera Blanca at 3,500 masl in three different streams and one reference site (Paclla) was in the Cordillera Negra at 3,800 masl. Two polluted sites were selected, both in the Santiago stream in the Cordillera Negra. The first one was located at 4,500 masl, where the geological formations contain high concentrations of metals that cause the water to be polluted, even though there were no mining activities upstream of this site (Santiago, natural pollution) (http://intranet2.minem.gob.pe/web/archivos/dgm/publicaciones/public03/mapas/12.jpg). The second polluted site was located at 3,800 masl, downstream of abandoned mines (Santiago mine pollution). All sites were sampled in March, June, September, and December. The Santiago natural pollution site was not sampled in September because it became dry.

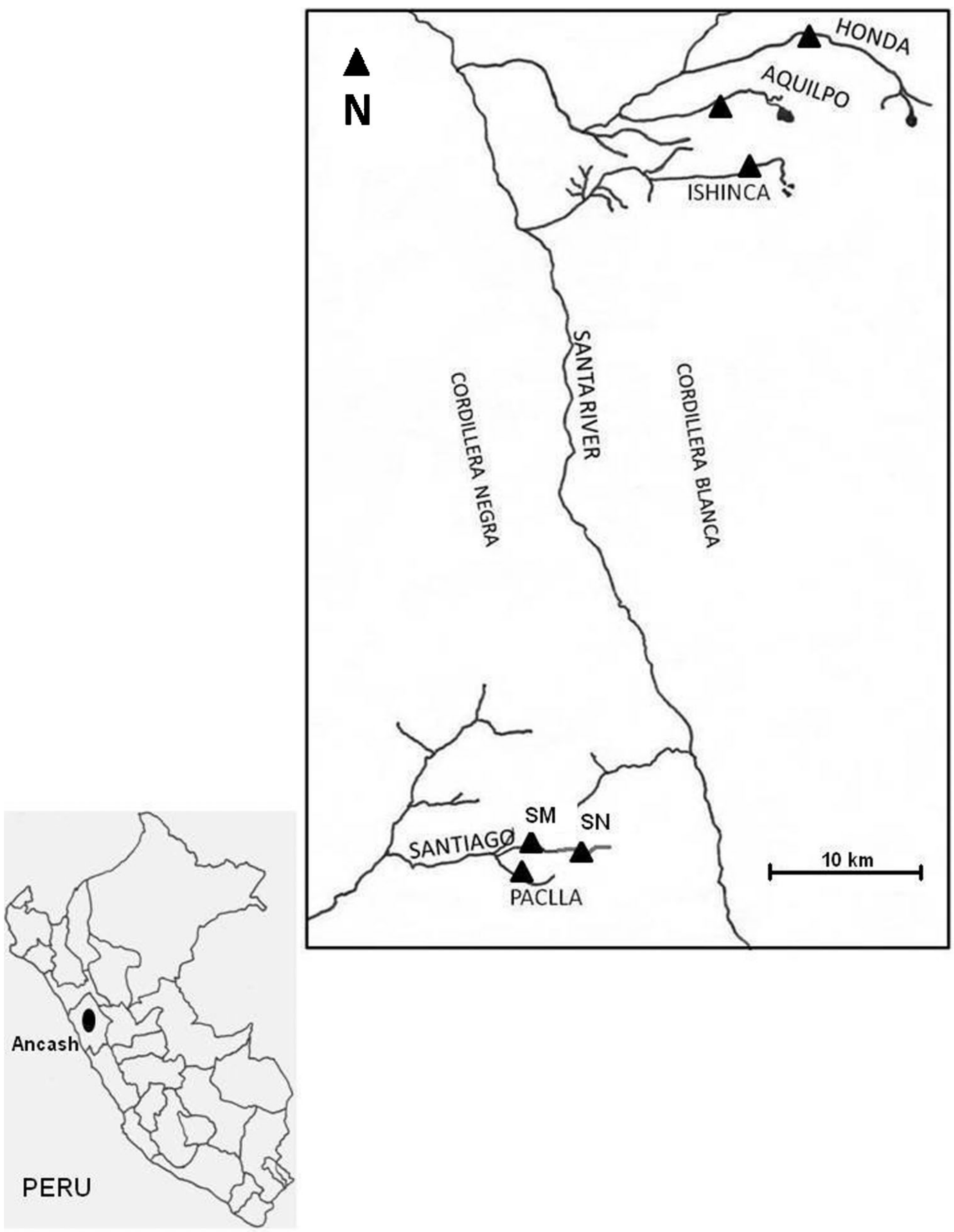

Fig. 1. Map of sampling area indicating the streams in the Cordillera Blanca and Cordillera Negra, Peru.

Physical chemical characteristics

Measurements of pH, temperature (°C), conductivity, and dissolved oxygen were performed at each sampling site using a multiparameter instrument equipped with SenTix® 41-3, TetraCon® 325-3, and CellOx® 325-3 probes (WTW Multi 340i). Transparency was measured with a 120-cm polycarbonate turbidity tube (Wildlife Supply). Stream depth was calculated from four measurements at each of three parallel cross-sections with a calibrated stick. Mean current velocity was obtained by timing a float three times as it moved over a distance of 10 m. Discharge was calculated as the average of the three products of mean current velocity, mean depth, and stream width at three cross-sections [15]. For determining hardness, phosphates, and ammonia, water samples were taken below the water surface, kept at 4°C in a Styrofoam box, and analyzed using standard methods [16]. Water samples for total metals were preserved in 10 N HNO_3 and analyzed by induced-coupled plasma emission spectroscopy [17]. Samples for determining hardness, phosphates, ammonia, and metals were taken in triplicate at each sampling time.

Because the polluted sites were expected to contain a mixture of metals with potential additive effects, the cumulative criterion unit (CCU) [18] was calculated. The CCU is defined as the ratio between the stream metal concentration and the U.S. Environmental Protection Agency (U.S. EPA) criterion value for toxicity, summing the ratios for all metals measured at a specific site [19]: $CCU = \sum m_i/c_i$, where m_i is the total recoverable metal concentration and c_i is the criterion value for the ith metal. The criterion value is based on U.S. EPA guidelines on critical concentrations, which may harm aquatic organisms when exceeded. Cumulative criterion unit values are scaled as follows: <1.0, no adverse effects; 1.0 to 2.0, adverse effects; 2.0 to 10.0, significant mortality to sensitive species and altered benthic community composition are expected; >10.0, highest toxicity [18]. Because water hardness affects the toxicity and bioavailability of some metals, criterion values for Cd, Cu, Pb, and Zn were modified to account for variation in water hardness between streams. For Al, Fe, and Mn no adjustment was needed and we followed the U.S. EPA criterion values [19].

Invertebrate sampling

At each sampling site three Surber samples (each 20 cm^2, mesh size 250 μm) were collected randomly from gravel-pebble substratum. In addition, a qualitative sample was collected for about 20 to 30 min, including all other possible microhabitats over representative sections, such as stones and stagnant water along the banks, using forceps and a white plastic tray. All samples were preserved in 70% (v/v) ethanol, and sorted in the laboratory with the use of a stereomicroscope Zeiss Stemi DV4. Insects were identified to the family level and most noninsects to order or class, using taxonomical keys [20,21]. The relative abundance of Ephemeroptera, Plecoptera, and Trichoptera (%EPT) was calculated, because these groups are generally considered to contain species sensitive to environmental pollution.

Statistical analysis

A principal components analysis (PCA) based on a correlation matrix was used to describe the main variation in physical chemical variables between all samples. Prior to this analysis, environmental variables were checked for normality, and those not meeting a normal distribution were log transformed. All variables were transformed except temperature, dissolved oxygen, current velocity, water discharge, and potassium concentration. Because the PCA revealed that most samples from the same site could not be considered independent, the seasonal samples for each environmental variable were averaged. These averages were used in a one-way analysis of variance (ANOVA) to test for differences between the reference and polluted sites. Analyses were done in SPSS 16.0.

Canonical correspondence analysis (CCA) [22] was done to examine the effects of the principal variation in physical chemical variables (as extracted by the PCA, see above) on the faunal assemblage of all samples. In the CCA, we focused the scaling on so-called interspecies distances and applied Hill's scaling type [21]. Prior to the CCA, the family abundances were log transformed. The significance of the first canonical axis and the two canonical axes together was assessed with a permutation tests using 499 permutations under a reduced model [23]. This analysis was done with CANOCO 4.5 (Microcomputer Power).

RESULTS

Physical chemical differences

Mean conductivity was lower and mean pH was higher in the reference streams compared to the polluted streams (Table 1). Except for Co, Fe, K, Mg, and Na, the mean concentrations of all metals were higher in the polluted streams than in the reference streams (Table 2). The mean metal concentrations at the polluted sites (e.g., Al, 13.07 mg/L; As, 3.49 mg/L; Cd, 0.5 mg/L; Mn, 19.65 mg/L; Pb, 0.876 mg/L; Zn, 16.08 mg/L) ranged from 8 (Sr) to 3,500 (As) times those at the reference sites, indicating a high degree of contamination with metals. The mean CCU ranged from 1.37 to 239.38, being significantly higher at the polluted sites. Although the CCU at reference sites exceeded 1.0, indicating metal pollution, the large and significant differences with CCU values at polluted sites allowed separating the two categories.

The loadings along the first PCA axis indicated that the principal variation in physical chemical variables was based on a positive high correlation between all metals and conductivity (Fig. 2). These variables were, in turn, highly negatively correlated with pH, transparency, and, to a lesser degree, with current velocity, water discharge, and phosphates. As such, the first PCA axis clearly arranged the samples along a gradient of contamination: to the right the polluted sites and to the left the reference sites (Fig. 2). The second PCA axis was positively loaded by phosphates, stream velocity, water discharge, Co, and K, and negatively by N-ammonium and dissolved oxygen. Because most samples from each stream were located near each other in the ordination diagram, the PCA suggested that the physical chemical properties of the streams did not substantially differ between the seasons.

Physical-chemical characteristics

A total of 45 families of aquatic insects and 10 other taxa were identified (Supplemental Data, Table S1). Among the insects, Diptera (15), Coleoptera (8), Trichoptera (7), and Collembola (7) were represented by the highest number of families. At the reference sites Honda, Aquilpo, and Ishinca, Perlidae (Plecoptera) and Simuliidae (Diptera) had the highest number of individuals, while at both polluted sites in the Santiago stream chironomids were most abundant. Reference and polluted sites did not differ in mean abundance or family richness (Table 3) and the seasonal variation in faunal

Table 1. Means (± standard deviation; *n* samples per site) for physical chemical variables at six sites in the Cordillera Blanca (*) and Cordillera Negra (+) area, Peru

Site group	*n*	Name	Conductivity (μS/cm)	Hardness (mg $CaCO_3$/L)	pH	Temperature (°C)	PO_4-P (mg P/L)	NH_4-N (mg N/L)	Oxygen (mg/L)	Transparency (cm)	Velocity (cm/s)	Discharge (L/s)
Reference	4	Honda (*)	137.8 (32.9)	105.1 (29.3)	7.4 (0.4)	10.8 (2.3)	0.64 (0.02)	0.023 (0.016)	5.08 (0.64)	100 (0)	63.0 (15.0)	867.5 (291.4)
Reference	4	Aquilpo (*)	45.0 (12.7)	85.5 (21.5)	7.6 (0.4)	11.6 (1.5)	0.53 (0.06)	0.020 (0.011)	5.13 (0.48)	120 (0)	52.8 (13.3)	748.8 (263.1)
Reference	4	Ishinca (*)	45.8 (7.7)	102.3 (13.3)	7.7 (0.4)	10.9 (1.6)	0.63 (0.10)	0.030 (0.024)	5.16 (0.76)	120 (0)	60.3 (14.8)	680.0 (236.2)
Reference	4	Paclla (+)	118.9 (51.5)	95.0 (26.7)	7.2 (0.4)	12.8 (1.4)	0.50 (0)	0.045 (0.070)	5.18 (0.95)	120 (0)	31.5 (10.3)	125.0 (86.9)
Polluted	3	Santiago natural pollution (+)	410.2 (292.8)	130.0 (45.6)	4.2 (0.2)	9.3 (0.6)	0.50 (0)	0.057 (0.081)	5.62 (1.22)	120 (0)	9.3 (5.5)	20.3 (9.1)
Polluted	4	Santiago mine pollution (+)	1776.0 (760)	1026.5 (377.8)	3.4 (0.2)	11.5 (1.3)	0.50 (0)	0.025 (0.030)	5.00 (0.74)	35 (44)	29.0 (10.4)	152.5 (70.4)
F			14.4	6.3	102.1	1.5	2.2	1.1	0.8	2.2	7.0	5.5
p			0.019	0.084	0.001	0.290	0.216	0.349	0.433	0.211	0.057	0.078

F and *p* denote the results of the analysis of variance (ANOVA) test between the mean of the four reference sites and the mean of the two polluted sites. PO_4-P, phosphates; NH_4-N, ammonium nitrogen.

Table 2. Means (± standard deviation; *n* samples per site) of metal concentrations and cumulative criterion unit (CCU) at six sites in the Cordillera Blanca (*) and Cordillera Negra (+) area, Peru

Site group	*n*	Site	Al	As	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Na	Pb	Sr	Zn	CCU
			mg/L														
Reference	4	Honda	0.92	0.009	11.15	0.001	0.028	0.003	1.50	0.97	3.15	0.36	1.95	0.010	0.06	0.12	10.77
		(*)	(0.05)	(0.009)	(2.06)	(0)	(0.039)	(0.002)	(0.45)	(0.16)	(0.68)	(0.12)	(0.24)	(0.01)	(0.01)	(0.06)	(3.54)
Reference	4	Aquilpo	0.11	0.001	4.55	0.001	0.004	0.001	0.12	0.36	0.56	0.01	2.05	0.001	0.05	0.01	1.37
		(*)	(0.04)	(0)	(1.41)	(0)	(0.005)	(0)	(0.02)	(0.11)	(0.30)	(0.002)	(1.40)	(0)	(0.02)	(0.003)	(0.06)
Reference	4	Ishinca	0.59	0.001	5.43	0.001	0.003	0.001	0.45	0.59	0.63	0.03	1.50	0.001	0.06	0.02	4.95
		(*)	(0.18)	(0)	(0.57)	(0)	(0.004)	(0)	(0.10)	(0.19)	(0.30)	(0.002)	(0.41)	(0)	(0.01)	(0.006)	(0.38)
Reference	4	Paclla	0.16	0.006	8.78	0.003	0.001	0.004	0.13	0.63	1.03	0.03	2.78	0.004	0.06	0.36	5.84
		(+)	(0.01)	(0.001)	(2.61)	(0.004)	(0)	(0.002)	(0.05)	(0.59)	(0.51)	(0.004)	(1.33)	(0)	(0.02)	(0.34)	(0.92)
polluted	3	Santiago natural	3.89	0.027	33.80	0.074	0.002	0.108	0.57	0.40	1.50	2.07	2.63	0.051	0.13	7.68	103.7
		pollution (+)	(3.08)	(0.016)	(19.4)	(0.023)	(0.001)	(0.038)	(0.47)	(0.1)	(0.62)	(1.41)	(1.36)	(0.032)	(0.07)	(2.32)	(23.9)
polluted	4	Santiago mine	13.07	3.490	59.76	0.501	0.092	1.011	37.48	1.33	17.13	19.65	5.88	0.876	0.39	16.08	239.38
		pollution (+)	(8.49)	(4.44)	(20.49)	(0.302)	(0.091)	(0.384)	(23.55)	(0.43)	(12.51)	(9.94)	(3.50)	(0.79)	(0.27)	(4.52)	(65.21)
Highest/lowest metal concn.			118	3490	13	501	92	1011	312	4	31	1965	4	876	8	1608	
F			13.6	7.5	27.1	51.7	0.6	36.2	2.9	0.2	2.8	12.7	4.6	13.1	17.7	15.7	33.5
p			0.021	0.05	0.007	0.002	0.484	0.004	0.163	0.701	0.170	0.024	0.980	0.022	0.014	0.017	0.004

F and *p* denote the results of the analysis of variance (ANOVA) test between the mean of the four reference sites and the mean of the two polluted sites. Highest/lowest metal concentration indicates the ratio of the highest to the lowest mean metal concentration.

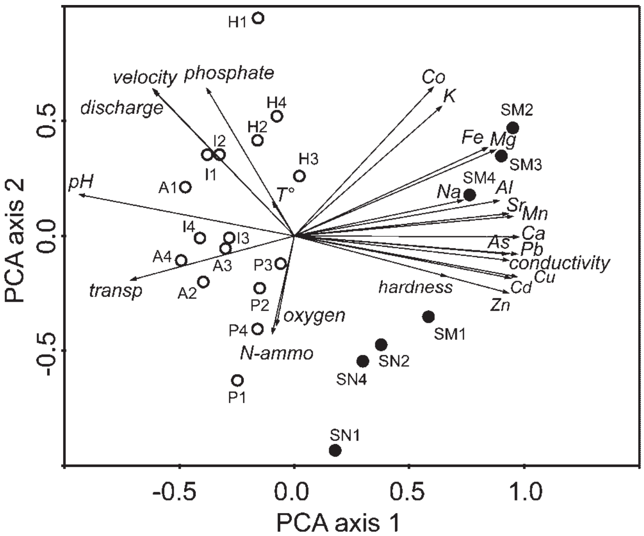

Fig. 2. Principal components analysis (PCA) of physical chemical variables at six sites in the Cordillera Blanca and Cordillera Negra area in Peru. The arrows represent the loadings. Labels for stream are as follows: H = Honda, A = Aquilpo, I = Ishinca, P = Paclla, SN = Santiago natural pollution, SM = Santiago mine pollution; Numbers indicate month: 1 = March, 2 = June, 3 = September, 4 = December. Reference sites are represented by open symbols and polluted sites by solid symbols. PCA axis 1 explained 60%, and axis 2, 12% of the variance.

composition within streams was lower than the variation between streams (Fig. 3A).

The differences in faunal assemblages between the three reference sites in the Cordillera Blanca and the reference site in the Cordillera Negra were mostly due to Chironomidae, Tabanidae (Diptera), Curculionidae, Dytiscidae, Elmidae, and Scirtidae (Coleoptera), which were more abundant in the Paclla stream. In the Cordillera Negra the faunal samples from the reference site Paclla stood out from the samples from the two polluted sites. Cladocerans, amphipods, ephemeropterans (Heptageniidae, Leptophlebiidae, Baetidae, Potamanthidae) plecopterans (Gripopterygidae, Perlidae), and trichopterans (Limnephilidae, Hydrobioscidae, Odontoceridae) dominated the reference sites and were completely absent from the polluted ones. The relative abundance of mayflies, stoneflies, and caddisflies (%EPT) was higher at the reference sites, especially in the Aquilpo stream (32–67%), whereas dipterans (Ceratopogonidae, Chironomidae, Dixidae, Empididae, Tabanidae) and coleopterans (Dytiscidae, Gyrinidae, Hydrophilidae, Staphylinidae) were abundant at the contaminated sites (76–100%).

Table 3. Mean (± standard deviation; *n* samples per site) abundance and family richness at six sites in the Cordillera Blanca (*) and Cordillera Negra (+) area, Peru

Site group	*n*	Site	Abundance	Family richness
Reference	4	Honda (*)	36.3 (10.0)	5
Reference	4	Aquilpo (*)	93.8 (41.7)	13
Reference	4	Ishinca (*)	74.8 (26.7)	14
Reference	4	Paclla (+)	1915 (1308)	36
Polluted	3	Santiago natural pollution (+)	960 (1264)	19
Polluted	4	Santiago mine pollution (+)	57.3 (70.1)	22
F			0.08	0.06
p			0.786	0.825

F and *p* denote the results of the analysis of variance (ANOVA) test between the mean of the four reference sites and the mean of the two polluted sites.

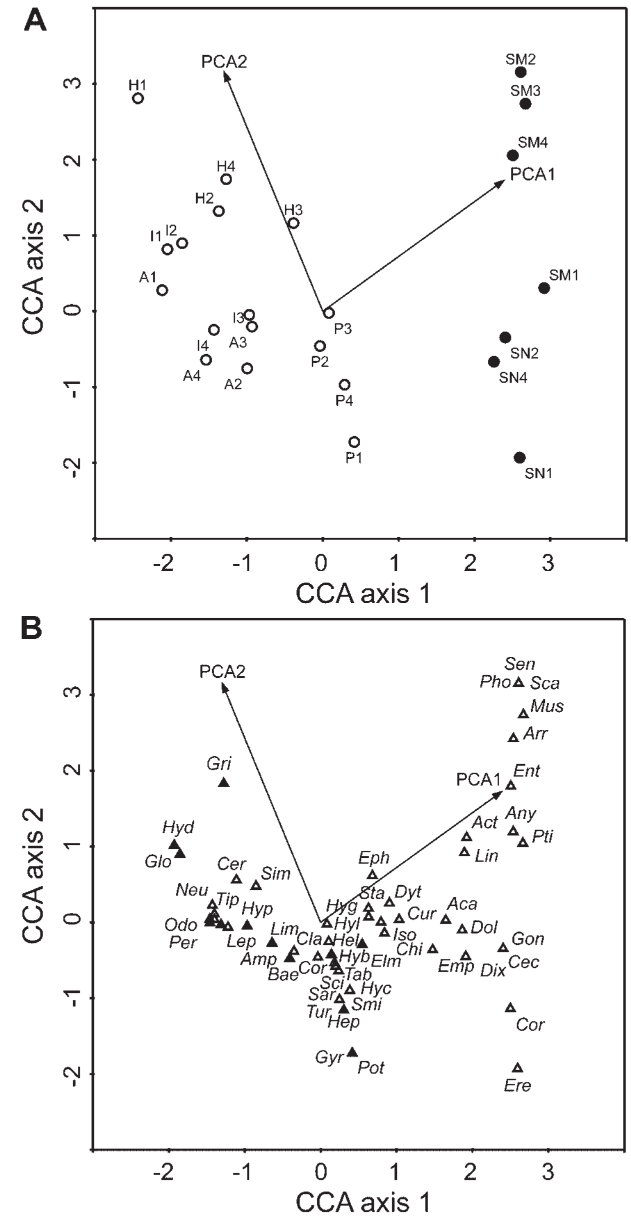

Fig. 3. Canonical correspondence analysis (CCA) of faunal assemblages against the principal variation in physical environmental variables as extracted by PCA (see Fig. 2), on the basis of 23 samples from six sites in the Cordillera Blanca and Cordillera Negra area in Peru. The eigenvalues of axis 1 and axis 2 were 0.61 and 0.46, respectively. Both axes explain 28% of the family data. Axis 1 and both axes 1 and 2 together were significant at $p < 0.01$. (**A**) Biplot of samples and explanatory variables. Sample labels correspond to the legend in Figure 2. Reference sites are represented by open symbols and polluted sites by solid symbols. (**B**) Biplot of families and explanatory variables. EPT taxa are identified as solid symbols. Family codes as in Supplemental Data, Table S1.

The CCA ordination biplot (Fig. 3A,B) shows how the macroinvertebrate family composition depended on the principal variation in physical chemical variables, as represented by the first two PCA axes. The latter are shown as arrows that point in the direction of strongest influence on the main patterns in the faunal assemblages. The longer the arrows, the stronger the influence. The CCA analysis showed that the macroinvertebrate family composition at the polluted sites, especially downstream from the mines, stood out because of their strong and positive correlation with the first PCA axis (the metal contamination factor). The optima of Ephydridae, Muscidae, Phoridae, Scatophagidae (Diptera), Actaletidae, Arrhopalitidae, Entomobrydae, Sensiphorura (Collembola), Ptiliidae (Coleoptera), Anyphaenidae, and Linyphiidae (Arachnida) were associated with high values of the first PCA axis (Fig. 3B). In contrast, Baetidae, Heptageniidae, Leptophlebiidae (Ephemeroptera), Hydroptilidae, Limnephilidae (Trichoptera), Sarcophagidae (Diptera), and Amphipoda (Crustacea) seemed more sensitive to metal contamination, because these taxa only thrived in reference streams.

DISCUSSION

The present study is among the first to describe macrofauna assemblages from high-altitude streams in the Peruvian Andes. The major groups of benthic invertebrates, such as Ephemeroptera, Plecoptera, Trichoptera, Diptera, and Coleoptera were well represented among the reference streams, with Baetidae, Perlidae, Limnephilidae, and Chironomidae being the dominant families. The total number of taxa (55) is in accordance with or even higher than data on invertebrate assemblages from other Andean streams at similar altitude ranges in Ecuador (29–60) [4,24], Peru (40) [25], and Bolivia (26) [2]. Because knowledge of Peruvian stream fauna and South American streams in general is scarce, the taxa could only be identified with certainty to the family level. This relatively coarse level of taxonomic resolution considers the high correlation between family richness of insects at individual stream sites and species richness [26], and may allow comparative analyses of community structure and detecting effects of pollution on benthic communities [27].

At reference sites a strong relationship between community composition and water discharge was revealed by the canonical ordination. Indeed, the families Perlidae, Gripoterigidae (Plecoptera), Odontoceridae (Trichoptera), and Simuliidae (Diptera) had their highest abundance in streams with steep slopes and high water flow in the Cordillera Blanca. Such conditions are common in high-altitude Andean streams, providing adequate conditions for development of these taxa. In contrast, the reference stream in the Cordillera Negra (Paclla) was dominated by dipterans and coleopterans preferring low water flow and discharge, and hence showed the highest abundance and richness towards the dry season. This observation agrees with several studies describing the influence of stream velocity on invertebrate communities [15,28]. Although the second PCA axis used in the CCA also correlated with other factors such as phosphates and ammonia, these did not show a large variation between reference and polluted sites and thus were not considered as having a relevant influence on the community composition.

The results of the physical chemical analyses revealed unprecedented high metal concentrations at polluted sites. Low pH conditions have likely increased the bioavailability of metal ions and the turbidity of the water column, both having detrimental effects on aquatic organisms [29]. Simultaneously, the formation of stable orange precipitates and encrusted layers comprising iron oxyhydroxides has completely smothered the streambed, impoverishing food and substrate quality, and restricting available habitats for benthic fauna [12,13]. The structuring role of metals was more evident during the dry season, as seen in other scenarios where increased metal levels and decreasing pH coincided with a decreased abundance of individuals and family richness [30,31]. The present study also demonstrated the importance of considering naturally occurring high metal concentrations, which had important consequences for community composition. Naturally and mine-polluted sites shared 10 families, while presenting nine and 11 site-specific families, respectively.

For some metals, their theoretical individual impact may be estimated from a species sensitivity distribution derived from individual median lethal concentration (LC50) values, revealing that the highest Cd concentration in these streams would affect 50%, copper 95%, and zinc 85% of the species [32]. However, because metal concentrations were highly correlated, an increased effect on community composition should likely be caused by all metals jointly. This was indicated by the high CCU values at the polluted sites (104 and 239), largely exceeding the upper cutoff of 10.0, which represents metal mixtures causing mortality and altering community structure [18]. Although reference sites exceeded a CCU of 1.0, indicating metal pollution, the large and significant differences of CCU values with polluted sites allowed a clear comparison of metal effects. These results suggest that elevated metal levels play a relevant role in structuring benthic macroinvertebrate assemblages in Andean high-altitude streams.

Surprisingly, however, the overall richness and abundance between reference and polluted sites did not differ significantly, which suggest that these metrics may not be adequate in assessing the effects of metal pollution on community composition when many species are being replaced. This was typically the case in the present study, where the high metal concentrations clearly induced a shift towards metal-tolerant families of dipterans, coleopterans, and collembolans at contaminated sites, where dipterans (e.g., chironomidae) probably represent the prey for predatory coleopterans. Regardless of metal origin, the present study showed that the diversity of macroinvertebrates was substantial at polluted sites, in spite of the challenging conditions associated with high altitude. We considered this richness to be high, taking into account the extreme pollution and environmental conditions found here compared to similar studies [9,33–36].

The canonical ordination indicated that the abundance of specific taxa such as Ephydridae, Muscidae, Phoridae, Scatophagidae (Diptera), Actaletidae, Arrhopalitidae, Entomobrydae, Sensiphorura (Collembola), Ptiliidae (Coleoptera), Anyphaenidae, and Linyphiidae (Arachnida) was higher along the first PCA component, which represents metal pollution, being determined by almost all highly correlated metals. In agreement, it has been reported that Phoridae [37], collembolans [38], and Linyphiidae [39] are able to develop and survive in metal polluted sites, suggesting that these taxa are less sensitive to the high concentrations of metals, low pH, and altitude conditions encompassed by the present study. Although the drying of the naturally polluted site during the dry season could represent a confounding factor, the CCA indicated that the effect of metal pollution superseded any potential impacts related to this event.

Not all dipterans and coleopterans were able to thrive under high metal conditions, however. Sarcophagidae, Tabanidae (Diptera), Elmidae, Hydrophilidae, and Scirtidae (Coleoptera) only appeared in the reference Paclla stream, suggesting that unpolluted tributaries may serve as refuge for sensitive invertebrate taxa and as potential sources of colonizers following restoration [12]. The polluted sites also excluded mayflies, stoneflies, caddisflies, amphipods, and cladocerans, which only appeared in pristine waters. This trend was expected, since the sensitivity of EPT taxa towards acid pH and metals has been well described in field surveys in lowland acid mine areas and experimental microcosms [9,13,40]. The sensitivity of EPT taxa was confirmed by the CCA, which clearly identified groups relatively sensitive to metal pollution, such as Baetidae, Heptageniidae (Ephemeroptera), Limnephilidae, Hydroptilidae (Trichoptera), Sarcophagidae (Diptera), and Amphipoda, in contrast to relatively insensitive groups belonging to Diptera, Collembola, Coleoptera, and Arachnida.

It is concluded that the diversity of macroinvertebrates in high-altitude streams is substantial, despite the extreme conditions of this habitat. At reference sites water discharge and current velocity modulated macroinvertebrate assemblages. In natural and mine-related metal polluted streams highly correlated metal concentrations structured communities, changing their composition through replacement of sensitive taxa by more tolerant taxa.

SUPPLEMENTAL DATA

Table S1. (318 KB).

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REFERENCES

1. Jacobsen D, Rostgaard S, Vásconez JJ. 2003. Are macroinvertebrates in high altitude streams affected by oxygen deficiency? *Freshw Biol* 43:2025–2032.
2. Jacobsen D, Marín R. 2007. Bolivian Altiplano streams with low richness of macroinvertebrates and large diel fluctuations in temperature and dissolved oxygen. *Aquat Ecol* 42:643–656.
3. Jacobsen D. 2008. Tropical high-altitude streams. In Dudgeon D, ed, *Tropical Stream Ecology*. Academic Press, San Diego, CA, USA, pp 219–256.
4. Jacobsen D. 2008. Low oxygen pressure as a driving factor for the altitudinal decline in taxon richness of stream macroinvertebrates. *Oecologia* 154:795–807.
5. Tartarotti B, Cabrera S, Psenner R, Sommaruga R. 1999. Survivorship of *Cyclops abyssorum tatricus* (Cyclopoida, Copepoda) and *Boeckella gracilipes* (Calanoida, Copepoda) under ambient levels of solar UVB radiation in two high-mountain lakes. *J Plank Res* 21:549–560.
6. Romero A, Flores S, Medina R. 2008. Estudio de los metales pesados en el relave abandonado de Ticapampa. *Rev Inst Inves FIGMMG* 11:13–16.
7. Ministerio de Energía y Minas. 1998. Estudio de evaluación ambiental territorial y de planeamiento para la reducción o eliminación de la contaminación de origen minero en la cuenca del río Santa. Dirección General de Asuntos Ambientales, Lima, Peru.
8. United Nations Environment Program. 2003. *Water resources management in Latin America and the Caribbean. UNEP/LAC-IGWG.XIV/Inf.5*. Nairobi, Kenya, p. 31.
9. Gerhardt A, Janssens de Bisthoven L, Soares AMVM. 2004. Macroinvertebrate response to acid mine drainage: Community metrics and on-line behavioural toxicity bioassay. *Environ Pollut* 130:263–274.
10. Kiffney PM, Clements WH. 1994. Structural responses of benthic macroinvertebrate communities from different stream orders to zinc. *Environ Toxicol Chem* 13:389–395.
11. Clements WH. 1999. Metal tolerance and predator–prey interactions in benthic macroinvertebrate stream communities. *Ecol Appl* 9:1073–1084.
12. 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.
13. O'Halloran K, Cavanagh J-A, Harding JS. 2008. Response of a New Zealand mayfly (*Deleatidium* spp.) to acid mine drainage: Implications for mine remediation. *Environ Toxicol Chem* 27:1135–1140.
14. Kiffney PM, Clements WH. 1996. Effects of metals on stream macroinvertebrate assemblages from different altitudes. *Ecol Appl* 6:472–481.
15. Miserendino ML, Pizzolon LA. 2003. Distribution of macroinvertebrate assemblages in the Azul-Quemquemtreu river basin, Patagonia, Argentina. *N Z J Mar Freshw Res* 37:525–539.
16. Clesceri LS, Greenberg AE, Eaton AD. eds. 1998. *Standard Methods for the Examination of Water and Wastewater* 20th ed. American Public Health Association, American Water Works Association and Water Environment Federation, Washington DC.
17. U.S. Environmental Protection Agency. 1994. Methods for the Determination of Metals in the Environmental Samples, Supplement I. EPA-600/R- 94-111. Office of Research and Development, Cincinnati, OH, USA.
18. Clements WH, Carlisle DM, Lazorchak JM, Johnson PC. 2000. Heavy metals structure benthic communities in Colorado Mountain streams. *Ecol Appl* 10:626–638.
19. U.S. Environmental Protection Agency. 1986. Quality criteria for water. EPA 440/5- 86-001. Office of Water, Washington, DC.
20. Merrit RW, Cummins KW. 1996. *An Introduction to the Aquatic Insects of North America*. Kendal/Hunt, Dubuque, IA, USA.
21. Roldán G. 1996. *Guía para el estudio de los macroinvertebrados acuáticos del departamento de Antioquia*. Editorial Presencia, Medellín, Colombia.
22. ter Braak CJF. 1986. Canonical correspondence analysis: A new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67:1167–1179.
23. ter Braak CJF, Šmilauer P. 2002. *CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5)*. Microcomputer Power, Ithaca, NY, USA.
24. Monaghan KA, Peck MR, Brewin PA, Masiero M, Zarate E, Turcotte P, Ormerod SJ. 2000. Macroinvertebrate distribution in Ecuadorian hill streams: The effects of altitude and land use. *Arch Hydrobiol* 149:421–440.
25. Acosta R. 2009. Estudio de la cuenca altoandina del río Cañete (Perú): distribución altitudinal de la comunidad de macroinvertebrados bentónicos y caracterización hidroquímica de sus cabeceras cársticas. PhD thesis. Universitat de Barcelona, Barcelona, Spain.
26. Bournaud M, Cellot B, Richoux P, Berrahou A. 1996. Macroinvertebrate community structure and environmental characteristics along a large river: Congruity of patterns for identification to species or family. *J N Am Benthol Soc* 15:232–253.
27. Vanderklift MA, Ward TJ, Jacoby CA. 1996. Effect of reducing taxonomic resolution on ordinations to detect pollution-induced gradients in macrobenthic infaunal assemblages. *Mar Ecol Prog Ser* 136:137–145.
28. Scheibler EE, Debandi GO. 2008. Spatial and temporal patterns in the aquatic insect community of a high altitude Andean stream (Mendoza, Argentina). *Aquat Insects* 30:145–161.
29. Courtney LA, Clements WH. 2000. Sensitivity to acidic pH in benthic invertebrate assemblages with different histories of exposure to metals. *J N Am Benthol Soc* 19:112–127.
30. Clements WH. 1994. Benthic invertebrate community responses to heavy metals in the Upper Arkansas River, Colorado. *J N Am Benthol Soc* 13:30–44.
31. Gerhardt A. 1993. Review of impact of heavy metals on stream invertebrates with special emphasis on acid conditions. *Water Air Soil Poll* 66:289–314.
32. U.S. Environmental Protection Agency. 2005. Methods/indicators for determining when metals are the cause of biological impairments of rivers and streams: Species sensitivity distributions and chronic exposure-response relationships from laboratory data. EPA/600/X-05/027. Washington, DC.
33. McNight DM, Feder GL. 1984. The ecological effect of acid conditions and precipitation of hydrous metal oxides in a Rocky Mountain stream. *Hydrobiol* 119:129–138.
34. Winterbourn MJ, McDiffett WF, Eppley SJ. 2000. Aluminium and iron burdens of aquatic biota in New Zealand streams contaminated by acid mine drainage: Effects of trophic level. *Sci Total Environ* 254:45–54.
35. Hirst H, Jüttner I, Ormerod SJ. 2002. Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshw Biol* 47:1752–1765.
36. Löhr AJ, Sluik R, Olaveson MM, Ivorra N, Van Gestel CAM, Van Straalen NM. 2006. Macroinvertebrate and algal communities in an

extremely acidic river and the Kawah Ijen crater lake (pH <0.3), Indonesia. *Arch Hydrobiol* 165:1–21.
37. Sorensen MA, Chase-Dunn CM, Trumble JT., 2009. Chronic exposure to elevated levels of manganese and nickel is not harmful to a cosmopolitan detritivore, *Megaselia scalaris* (Diptera: Phoridae). *Insect Sci* 16:73–79.
38. Crouau Y, Pinelli E. 2008. Comparative ecotoxicity of three polluted industrial soils for the Collembola *Folsomia candida*. *Ecotox Environ Saf* 71:643–649.
39. Jung M-P, Kim S-T, Kim H, Lee J-H. 2008. Species diversity and community structure of ground-dwelling spiders in unpolluted and moderately heavy metal-polluted habitats. *Water Air Soil Pollut* 195:15–22.
40. Peterson RH, Van Eeckhaute L. 1992. Distributions of Ephemeroptera, Plecoptera, and Trichoptera of three maritime catchments differing in pH. *Freshw Biol* 27:65–78.