Life at the edge: Benthic invertebrates in high altitude Andean streams

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Chapter 2

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. Since environmental pollution may add further stress to life at high altitude, the present study explored the effect of metal pollution on macroinvertebrate community composition in Andean streams between 3500 to 4500 meters above sea level (m a.s.l) 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 up to 3500 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. It is 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.

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 (Jacobsen et al., 2003; Jacobsen and Marín, 2007; Jacobsen, 2008a). It is generally believed that cold high altitude streams are more oxygen rich than warm low land 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 (Jacobsen, 2008b). Also the regime of UV exposure and temperature fluctuation tends to create challenging conditions for aquatic life (Jacobsen, 2008a; Tartarotti et al., 1999). 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 (Romero et al., 2008). 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 (Ministerio de Energía y Minas, 1998). Moreover, since 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 (UNEP, 2003).

Studies on the effects of increased acidity and dissolved metal concentrations (cadmium, copper, zinc) 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 (Kiffney and Clements, 1994; Gerhardt et al., 2004). 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 (Clements, 1999; Courtney and Clements, 2002; O'Halloran et al., 2008). In addition, it has been reported that related species from elevated temperate streams (2500 m a.s.l.) are more sensitive to metals than those from low land streams (Kiffney and Clements, 1994; Kiffney and Clements, 1996). 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 (3500 to 4500 m a.s.l.) in the Cordillera Blanca and Cordillera Negra (Peruvian Andes). 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 5500 m a.s.l along the Santa River. Below the permanent snow-line, between 3700 to 4200 m a.s.l, 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 3500 to 4500 m a.s.l. (Figure 1). Four reference sites were selected. Three reference sites (Honda, Aquilpo and Ishinca) were located in the Cordillera Blanca at 3500 m a.s.l. in three different streams and one reference site (Paclla) was in the Cordillera Negra at 3800 m a.s.l. Two polluted sites were selected, both in the Santiago stream in the Cordillera Negra. The first one was located at 4500 m a.s.l. where the geological formations contain high concentrations of metals which 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 3800 m a.s.l, downstream 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.

Physical chemical characteristics

Measurements of pH, temperature (C°), conductivity and dissolved oxygen were performed at each sampling site using a multi-parameter instrument equipped with SenTix® 41-3, TetraCon® 325-3 and CellOx® 325-3 probes (WTW Multi 340i, Weilheim, Germany). Transparency was measured with a 120-cm polycarbonate turbidity tube (Wildlife Supply Company, Buffalo, NY, USA). 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.

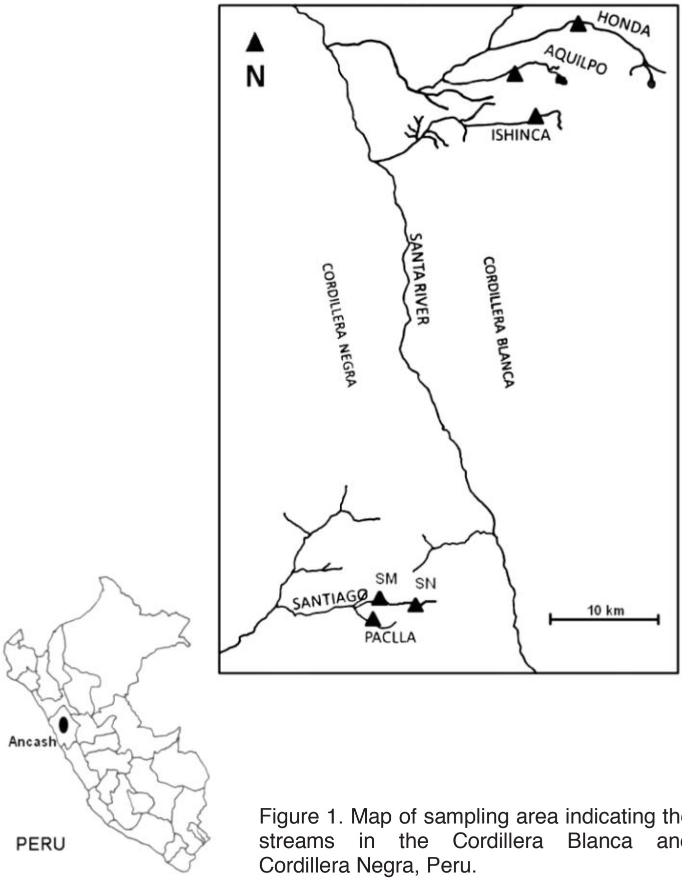

Figure 1. Map of sampling area indicating the streams in the Cordillera Blanca and Cordillera Negra, Peru.

Discharge was calculated as the average of the three products of mean current velocity, mean depth and stream width at three cross-sections (Miserendino and Pizzolón, 2003). 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 (Clesceri et al., 1998). Water samples for total metals were preserved in 10 N HNO_3 and analyzed by ICP-ES (induced-coupled plasma emission spectroscopy) (USEPA, 1994). Samples for determining hardness, phosphates, ammonia and metals were taken in triplicate at each sampling time.

Since the polluted sites were expected to contain a mixture of metals with potential additive effects, the cumulative criterion unit (CCU) (Clements et al., 2000) 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 (USEPA, 1986): $CCU = \sum m_i/c_i$, 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. CCU 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 (Clements et al., 2000). 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 (USEPA, 1986).

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 (Göttingen, Germany). Insects were identified to family level and most non-insects to order or class, using taxonomical keys (Merrit and Cummins, 1996; Roldán, 1996). 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. Since the PCA revealed that most samples from the same site could not be considered as 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 (SPSS Inc., Chicago, IL, USA).

Canonical correspondence analysis (CCA) (ter Braak, 1986) 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 inter-species distances and applied Hill's scaling type (ter Braak, 1986). 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 (ter Braak and Šmilauer, 2002). This analysis was done with CANOCO 4.5 (Microcomputer Power, Ithaca, NY, USA).

Results

Physical chemical differences between reference and polluted sites

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 references 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 3500 (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 (Figure 2). These variables were, in turn, highly negatively correlated with pH, transparency, and, to a lower 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 (Figure 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

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

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

Table 2. Means (± SD; *n* samples per site) of metal concentrations and Cumulative Criterion Unit (CCU) at six sites in the Cordillera Blanca (*) and Cordillera Negra (+) area, Peru. *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.

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 (0.05)	0.009 (0.009)	11.15 (2.06)	0.001 (0)	0.028 (0.039)	0.003 (0.002)	1.50 (0.45)	0.97 (0.16)	3.15 (0.68)	0.36 (0.12)	1.95 (0.24)	0.010 (0.01)	0.06 (0.01)	0.12 (0.06)	10.77 (3.54)
reference	4	Aquilpo (*)	0.11 (0.04)	0.001 (0)	4.55 (1.41)	0.001 (0)	0.004 (0.005)	0.001 (0)	0.12 (0.02)	0.36 (0.11)	0.56 (0.30)	0.01 (0.002)	2.05 (1.40)	0.001 (0)	0.05 (0.02)	0.01 (0.003)	1.37 (0.06)
reference	4	Ishinca (*)	0.59 (0.18)	0.001 (0)	5.43 (0.57)	0.001 (0)	0.003 (0.004)	0.001 (0)	0.45 (0.10)	0.59 (0.19)	0.63 (0.30)	0.03 (0.002)	1.50 (0.41)	0.001 (0)	0.06 (0.01)	0.02 (0.006)	4.95 (0.38)
reference	4	Paclla (+)	0.16 (0.01)	0.006 (0.001)	8.78 (2.61)	0.003 (0.004)	0.001 (0)	0.004 (0.002)	0.13 (0.05)	0.63 (0.59)	1.03 (0.51)	0.03 (0.004)	2.78 (1.33)	0.004 (0)	0.06 (0.02)	0.36 (0.34)	5.84 (0.92)
polluted	3	Santiago natural pollution (+)	3.89 (3.08)	0.027 (0.016)	33.80 (19.4)	0.074 (0.023)	0.002 (0.001)	0.108 (0.038)	0.57 (0.47)	0.40 (0.1)	1.50 (0.62)	2.07 (1.41)	2.63 (1.36)	0.051 (0.032)	0.13 (0.07)	7.68 (2.32)	103.7 (23.9)
polluted	4	Santiago mine pollution (+)	13.07 (8.49)	3.490 (4.44)	59.76 (20.49)	0.501 (0.302)	0.092 (0.091)	1.011 (0.384)	37.48 (23.55)	1.33 (0.43)	17.13 (12.51)	19.65 (9.94)	5.88 (3.50)	0.876 (0.79)	0.39 (0.27)	16.08 (4.52)	239.38 (65.21)
Highest/lowest metal conc.			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

stream were located near to each other in the ordination diagram, the PCA suggested that the physical chemical properties of the streams did not substantially differ between the seasons.

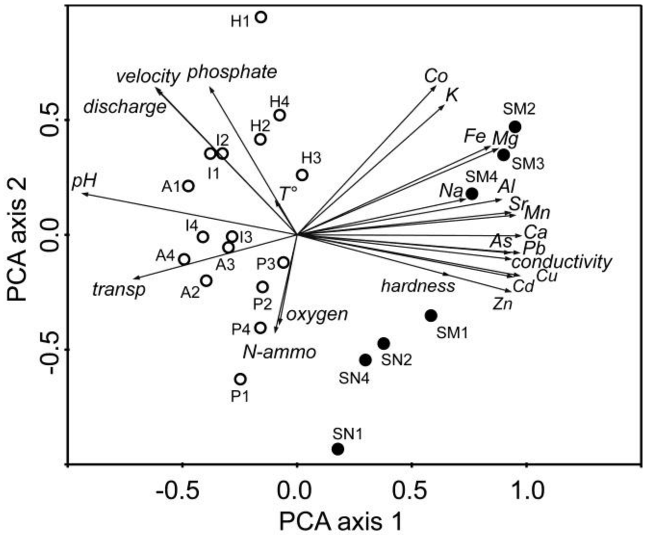

Figure 2. Principal components analysis (PCA) of physical chemical variables at six sites in the Cordillera Blanca and Cordillera Negra area in Peru. The arrows represent the loadings. Labels for stream are as follows: H = Honda, A = Aquilpo, I = Ishinca, P = Paclla, SN = Santiago natural pollution, 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.

Relationship between physical-chemical water characteristics and community composition

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

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

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

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 to 67%), whereas dipterans (Ceratopogonidae, Chironomidae, Dixidae, Empididae, Tabanidae) and coleopterans (Dytiscidae, Gyrinidae, Hydrophilidae, Staphylinidae) were abundant at the contaminated sites (76 to 100%).

The CCA ordination biplot (Figures 3A and 3B) 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 which 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 macroinvertrebate family composition at the polluted sites, especially downstream 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 (Figure 3B). In contrast, Baetidae, Heptageniidae, Leptophlebiidae

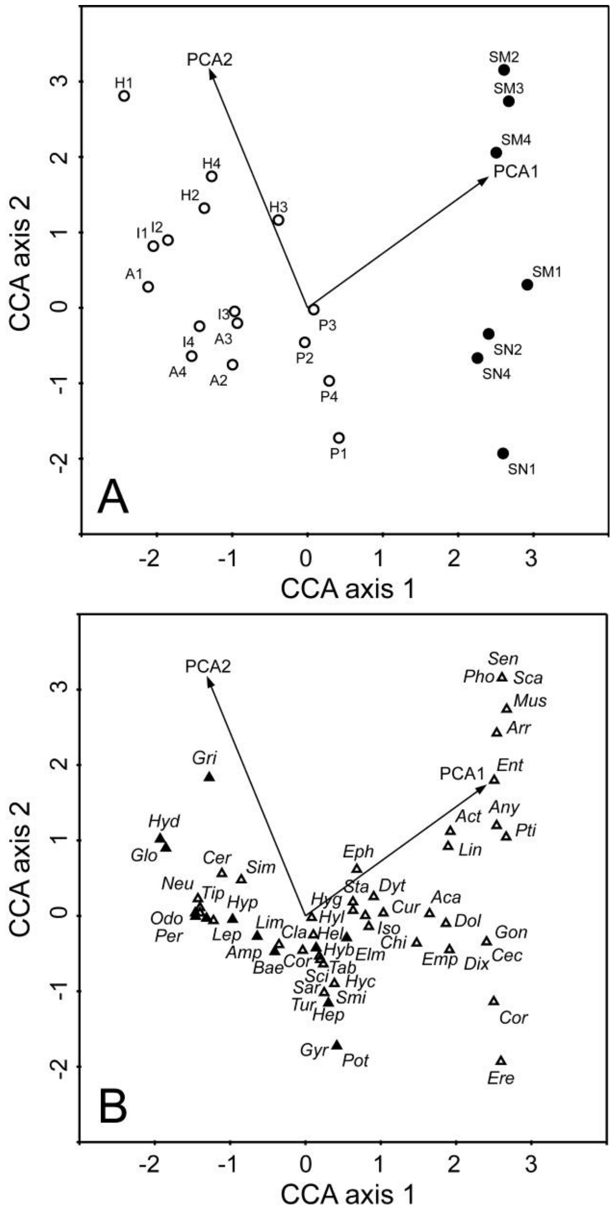

Figure 3. Canonical correspondence analysis (CCA) of faunal assemblages against the principal variation in physical environmental variables as extracted by PCA (see Figure 2), on the basis of 23 samples from six sites in the Cordillera Blanca and Cordillera Negra area in Peru. The eigenvalues of axis 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.

(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 to 60) (Monaghan et al., 2000; Jacobsen, 2008b), Peru (40) (Acosta, 2009) and Bolivia (26) (Jacobsen and Marín, 2007). Since 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 (Bournaud et al. 1996), and may allow comparative analyses of community structure and detecting effects of pollution on benthic communities (Vanderklift et al., 1996).

At reference sites a strong relationship between community composition and water discharge was revealed by the canonical ordination. Indeed, the families Perlidae, Gripopterygidae (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 (Miserendino and Pizzolon, 2003; Scheibler and Debandi, 2008). 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 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 (Courtney and Clements, 2002). 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 (Courtney and Clements, 2002; O'Halloran et al., 2008). 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 (Gerhardt, 1993; Clements, 1994). 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 9 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 cadmium concentration in these streams would affect 50%, copper 95% and zinc 85% of the species (USEPA, 2005). 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 (Clements et al., 2000). 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) may 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 (McNight and Feder, 1984; Winterbourn et al., 2000; Hirst et al., 2002; Gerhardt et al., 2004; Löhr et al., 2006).

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 (Sorensen et al., 2009), collembolans (Crouau and Pinelli, 2008) and Linyphiidae (Jung et al., 2008) 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 (Courtney and Clements, 2002). 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 low land acid mine areas and experimental microcosms (Gerhardt et al., 2004; O'Halloran et al., 2008; Peterson and van Eeckhaute, 1992). 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.

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Supplemental data available: Table S1. This information is available free of charge via the Internet at wileyonlinelibrary.com.