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

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Chapter 1

General Introduction
Mountain regions cover about 27% of the Earth’s surface, and are dominated by the Andes, the Rocky Mountains, the Himalayas, the Alps and the Atlas ranges (Figure 1). These regions encompass glacial stocks and freshwater reservoirs that play a valuable role providing ecosystem services to large human populations living downstream (e.g. drinking water, food, and electric power, or purifying water). Mountain regions are unique through the heterogeneity of ecosystems and diversity of climates arising from their sharp altitude gradients. When approaching the summits, these factors create exceptional harsh conditions for life, which may be challenged even further by man-made disturbances: life at the edge.

Figure 1. Profile of the latitudinal position of altitude belts in mountains across the globe. Grey is montane; black is alpine; white is the nival belt (Körner, 2003).

Life in high altitude streams

Alpine streams are perhaps our most important water sources, but are at the same time one of the least studied ecosystems in the world. They are located above the tree line, which ranges in elevation from near sea level at high latitudes, to almost 4000 m above sea level (a.s.l.) in tropical mountains. High altitude streams show particular environmental characteristics, such as banks consisting of bedrock or mineral sediments, devoid of dense riparian vegetation; little wood debris and a low organic matter content due to the presence of only herbaceous plants and small shrubs; and a limited autotrophic production governed by low temperatures and nutrient levels. With increasing altitude environmental conditions,
such as water temperatures, oxygen levels and nutrient concentrations become more extreme and consequently, the diversity of aquatic communities in mountain streams exhibits a decline towards the summits (Vinebrooke and Leavitt, 1999; Miserendino, 2001; Jacobsen, 2004; Rostgaard and Jacobsen, 2005; Jacobsen and Marin, 2007; Jacobsen 2008a). Under these extreme conditions, invertebrates such as mites, collembolans, dipterans, coleopterans, ephemeropterans and trichopterans, an array of selected traits including small body size, clinger habits, short life cycles, photoprotective pigmentation, sediment dwelling and case-protection confer different adaptative strategies to cope with the stressful habitats typical of high altitude streams (Townsend and Hildrew, 1994; Snook and Milner, 2002).

Altitude governs several environmental parameters known to be crucial for biota. Temperature regimes tend to be extreme at high altitude and indirectly affect water discharge rates in streams. Low air pressure diminishes the oxygen concentrations in water, while more ultraviolet (UV) radiation penetrates the thinner atmosphere above high mountains. These correlated factors act together on alpine biota and the integrated effect may be more important than the effects of the single factors. Accordingly, vertical zonations of species distribution and variations in community structure have been better explained by altitude than by small-scale factors associated with the specific habitat (Jacobsen, 2003; Finn and Poff 2005). Yet, life at the highest altitudes (> 4000 m) has seldomly been studied. Because of the unique suite of environmental conditions at such heights, the present project selected high altitude as potential driver of the composition of benthic invertebrate assemblages.

A blistering solar radiation

The Tropical Andes is the only large mountain range on the equator, covering approximately 1 542 644 km$^2$ across Venezuela, Colombia, Ecuador, Peru and Bolivia (Figure 1). Andean streams exhibit all the aspects of high altitude streams discussed above, like low water temperatures, oxygen levels and nutrient concentrations, but the unique striking feature is the very intense solar irradiance due to the thinner ozone layer over low latitudes and its proximity to the equator. Above 4000 m, irradiance is up to 50% higher than that at sea level for an equivalent atmospheric moisture regime (Jacobsen, 2008b; Sevink, 2009), the highest levels being recorded between May and August, coinciding with a reduced cloudiness and rainfall. These values (4.2 W/m$^2$, Zaratti, 2003) stand out exceeding those registered in temperate and high latitude alpine areas (0.1 W/m$^2$, Cabrera et al., 1997; 0.17 W/m$^2$, Vinebrooke and Leavitt, 1999; 0.5 W/m$^2$, Kiffney et al., 1997b; 1.6 W/m$^2$, Kelly et al., 2003), and are among the highest irradiances reaching the Earth’s crust.
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Solar radiation, especially UV-B radiation (280–320 nm), may shape the structure and function of aquatic communities by inhibiting primary production (Kinzie et al., 1998), altering the abundance and richness (Kiffney et al., 1997a), and the distribution of sensitive species, influencing trophic interactions (Kelly et al., 2003) and inducing DNA damage (Macfadyen et al., 2004). In high altitude Andean streams, macrobenthic communities are constantly exposed to blistering levels of UV-B, further accentuated by the lack of shaded areas and dissolved organic matter, which are two major factors attenuating the penetration of solar radiation (Cabrera et al., 1997; Clements et al., 2008). This exposure may become most intense during dry season low-water flow conditions, when radiation levels reaching the Earth's surface peak (Kiffney et al., 1997a). Thus macrofauna thriving in high altitude Andean streams must be especially adapted to counteract the potential adverse effects of the highest UV-B radiation levels on Earth.

To cope with the effects of UV-B radiation, aquatic organisms commonly acquire or synthesize photoprotective compounds that function either as sunscreens or as scavengers of photo-produced reactive oxygen species (ROS; Vinebrooke and Leavitt, 1999). Among copepods, such pigments are carotenoids and mycosporine-like amino acids (MAAs; Sommaruga and García-Pichel, 1999; Hansson, 2000). For cladocerans, such as Daphnia, and isopods with a thick chitin exoskeleton, melanin is the most important pigment, and highly melanized specimens are almost exclusively found in arctic or high altitude environments (Hebert and Emery, 1990; Sommaruga, 2010). Several studies have shown that melanized organisms can tolerate high solar irradiances better than non-pigmented relatives, and that the level of pigmentation is an inducible and adjustable defense mechanism (Hessen et al., 1999; Rautio and Korhola, 2002; Hansson, 2004, Hansson et al., 2007). Hence, it is expected that macroinvertebrates inhabiting high altitude Andean streams under exceptional high solar radiation levels should show a strong expression of UV protection mechanisms, enabling specialized taxa to persist on the ‘UV boundaries of life’.

Natural and anthropogenic metal input

Metals are natural, ubiquitous but unevenly distributed constituents of the Earth's crust. In mountain areas like the Andes, metals are readily mobilized by acid conditions produced by natural oxidation of mineral layers. The tropical Andes, in particular the Cordillera Blanca in Peru, show a unique and diverse geomorphology consisting of a granodioritic batholith formed by plagioclase and biotite minerals presenting aluminum, iron, nickel, cobalt, strontium and zinc (Rivera et al., 2008; Sevink, 2009). At the upper sections in the proglacial zone of the Cordillera Blanca, metamorphic sedimentary rocks characterized by pyrite are well oxidized, generating protons that lower the pH below 4. As
a result, rocks are readily weathered resulting in high metal concentrations being mobilized into streams (Burns, 2010). Thus, the heterogeneous morphology results in a prominent spatial diversity in leaching along the Andes. However, the most important contributor to metal pollution of rivers is mining, encompassing abandoned mines, acid-mine drainage and mine tailings releasing high concentrations of metal residues into river systems. As a consequence, metals are still one of the most ubiquitous and persistent sources of environmental contamination.

Benthic macroinvertebrates are directly or indirectly impacted by metals in the water (Kiffney and Clements, 1996b), substratum and food resources (Kiffney and Clements, 1993; Farag et al., 1998; Courtney and Clements, 2002), and show different responses to metal exposure. Metals may therefore explain much of the variability in assemblage structure between polluted and unpolluted sites, and reveal species-specific sensitivities to metals (Clements et al., 2000). Studies on the effects of metals in natural and artificial polluted streams have shown a loss of sensitive species, resulting in a significant reduction of richness and a shift towards more tolerant taxa (Gerhardt et al., 2004).

Ephemeroptera are among the most sensitive group, whereas Plecoptera, Trichoptera and Diptera may survive under high metal concentrations (Clements, 1994; Kiffney and Clements, 1994; Clements et al., 2000). Indirect effects of metal pollution include smothering of the streambed by metal oxyhydroxide precipitates, restricting available habitats for benthic fauna, impoverishing food quality, and modifying interactions between functional feeding groups (Kiffney, 1996; Clements, 1999; O’Halloran et al., 2008).

To cope with metal toxicity, aquatic invertebrates display different physiological mechanisms for detoxifying or regulating internal metal concentrations, like the expression of metal-binding metallothioneins and melanin (McGraw, 2003; Timmermans et al., 2005), changing the ion permeability of cuticle or gill epithelium (Hare, 1992), and shedding of metals during molting (Timmermans and Walker, 1989). Genetic adaptation has also been considered as a mechanism of metal tolerance in polluted environments (Groenendijk et al. 2002; van Straalen et al. 2005; Buchwalter et al. 2008), suggesting that metals can act as a selective factor structuring aquatic communities. This triggered us to study metals as a potential driving force in benthic invertebrate community composition and to answer the question if selection for stress tolerance in organisms thriving under extreme metal conditions is due to a remarkable adaptive capacity of few species or to a large diversity of species with different capacities to cope with extreme environments.

**Taxonomic status of high altitude fauna**

Investigations on the effects of high altitude conditions on the invertebrate fauna of the tropical Andes require that this fauna has been taxonomically well identified, but this
is, however, not the case. Current taxonomic keys for the Andes (Roldán, 1996; Domínguez and Fernández, 2009) have a very low resolution and the identification of individual species from remote and high altitude communities is therefore often problematic and unreliable. However, this challenge is inevitable in a study on the fauna ‘at the edge of life’. Therefore, dedicated approaches are needed and the advance brought about by genome based taxonomy invites also genomic approaches to the high Andes macrofauna. So far genomic analysis of high alpine flora have been published (Manel et al., 2012; Xavier Pico, 2012), but equivalent studies on Andean macrofauna are non existing (Scheihing et al., 2011). The present day Andean fauna has been formed through regional diversification events driven by strong selection and local speciation (Hoorn, 2010). A genomic analysis of high altitude fauna in the Andes is therefore likely to reveal unique and new taxa that are difficult to link to classical morphologically identified taxa.

**Aim and objectives**

The aim of this thesis was to identify potential drivers of diversity in poorly studied benthic invertebrate assemblages in high altitude Andean streams and to elucidate the mechanisms that enable them to cope with ‘life at the edge’. The unresolved taxonomic status of high altitude fauna required the use of genetic analysis to amend morphological identification. To this purpose, the following objectives have been set:

- To describe for the first time the benthic invertebrate assemblages in high altitude Andean streams and to relate their composition to the strong gradients in abiotic factors.
- To unravel the role of melanin as a strategy against harmful UV-B radiation and metal exposure.
- To study the genetic diversity of benthic invertebrates occurring under extreme conditions and showing specific defense strategies.

**The tropical Andes: life at the edge**

The Cordillera Blanca in the Peruvian Andes contains the highest permanently flowing streams of glacial origin (4000–5000 m a.s.l.) and ~70% of all tropical glaciers (Vuille et al., 2008). However, these are particularly sensitive to, and the most visible indicator of global climate changes, since variations in naturally high solar regimes and rising atmospheric temperatures are accelerating their melting. Simultaneously, the retreat of ice masses is accelerating the weathering of metal-rich rocks, which produces natural acid drainage and mobilizes toxic metals into lagoons and streams, deteriorating the quality
of headwaters and aquatic biota (Ministerio de Energía y Minas, 1998; Sevink, 2009). These inputs, together with those originating from abandoned mine wastes and present mining, may create extreme detrimental conditions compared to those reported in temperate and other alpine streams (Kiffney and Clements, 1996a; Clements, 1999; Courtney and Clements, 2000; Clements et al., 2000).

To meet the objectives of the present study we selected streams distributed along an altitude range (650–4200 m a.s.l.) in the Peruvian Andes, encompassing a strong gradient of abiotic factors (Figure 2). We chose reference and metal-rich streams at each altitude, showing strong differences in water quality (e.g., pH, conductivity, transparency) and habitat conditions (e.g., riverine vegetation, streambed structure, water flow).

Figure 2. Vegetation distribution in Peruvian Andes, showing the position of reference and metal-polluted streams and the solar radiation along the altitudinal gradient. (Adapted from http://wahlclassroom.blogspot.com/2012/12/the-four-zones-of-andes-mountains.html).

Because of the steep Andean slopes, the streams selected in this study were in general fast-flowing and turbulent, particularly during the rainy season. Above 4000 m streams were shallow, showing riverine herbaceous plants and small shrubs providing poorly shaded areas, while bigger shrubs and trees belonging to the Andean forest were more abundant downstream. Reference streams were characterized by transparent waters, with substrates
consisting of gravel, pebble, and stones in runs and riffles at high altitude, and larger rocks along stream banks at low altitudes. In contrast, metal polluted sites showed more turbid waters and streambeds smothered by crusts of metal precipitates (Figure 3).

Figure 3. Streams in the Cordillera Blanca and Cordillera Negra, in the Peruvian Andes. A, reference stream in the Tuco gorge (4120 m); B, metal polluted Santiago stream in the Aija gorge (4200 m); C, reference stream in the Quillcay sub-catchment (3070 m); D, polluted stream in the Aija gorge (3100 m). Photos by R. Loayza-Muro.

Outline of the thesis

The aim of this thesis was to identify potential drivers of diversity in poorly studied benthic invertebrate assemblages in high altitude Andean streams and to elucidate the mechanisms that enable them to cope with ‘life at the edge’. To meet the objectives of the present study a combined field and experimental approach was designed, meanwhile progressing from traditional taxonomy towards genetic identification.

We first explored the effect of metal pollution on benthic invertebrate community composition in Andean high altitude streams (chapter 2). Physical chemical variables and metal concentrations were measured in reference and polluted streams, and macrofauna was sampled. To determine how the sampling sites were structured by the abiotic variables
Principal Component Analysis was applied. The effects of the principal variation in physical chemical variables on the faunal assemblages was analysed by means of Canonical Correspondence Analysis. This allowed us also to identify metal sensitive and insensitive taxa.

Since the Andes represent a sharp altitude gradient the differential effects of metal pollution and altitude on benthic macroinvertebrate community composition were evaluated in chapter 3. In addition to general physical chemical parameters and metal concentrations, now also UV-B irradiance and DOC concentrations were measured. To explain the patterns in faunal composition and to identify taxa thriving under combined high metal and UV-B conditions Canonical Correspondence Analysis was applied.

Next, chapter 4 explored the adaptive responses enabling specialized macrofauna to survive under these combined high metal and UV-B conditions, hypothesizing that melanin counteracts both the adverse effects of solar radiation and of metals. Therefore, melanin was determined in chironomids from reference and metal polluted streams at 3000 and 4000 m altitude. The field observations were experimentally verified by assessing the combined effects of Cu and UV-B on the survival and melanin concentration in larvae of the model species *Chironomus riparius* (Chironomidae, Diptera).

Melanization in macroinvertebrates inhabiting high altitude Andean streams as an adaptive response to high UV-B radiation was further studied in chapter 5. To this purpose we measured UV-B radiation from 650 to 4000 m and compared body melanin concentrations from several benthic macroinvertebrate orders sampled at these altitudes. To evaluate if altitude-related differences in melanin concentration between taxa were due to a variable community composition or to population differentiation, DNA sequencing was performed. This way, five genera belonging to the mayfly family Baetidae were genetically identified, allowing comparisons of melanin concentrations at the species level.

Chironomids are among the few taxa thriving under the harshest environmental conditions, the highest metal concentrations and UV-B levels. To study if the presence of chironomids in metal polluted Andean high altitude streams is attributable to population differentiation or changed species composition, the genetic composition of chironomid communities from reference and metal polluted streams at 3000 and 4000 m was determined by mitochondrial cytochrome oxidase I (COI) gene sequencing and construction of a phylogenetic tree in chapter 6.

Finally, the concluding remarks in chapter 7 discuss the main findings of this thesis, focusing on the environmental factors that drive the diversity of benthic invertebrate assemblages in high altitude Andean streams, and on the mechanisms enabling them to cope with this harsh environment.