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Trophic variability of stream macroinvertebrates along an altitudinal gradient and among size groups in the Oyacachi River Basin

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Resumen

Los macroinvertebrados acuáticos desempeñan un rol fundamental en la función ecosistémica de los ríos a través de sus hábitos alimenticios. Sin embargo, poco se conoce sobre la alimentación de estos organismos en el Neotrópico. En este estudio buscamos caracterizar la dieta de cuatro morfoespecies de macroinvertebrados acuáticos (*Anacroneuria* sp.1, *Baetodes* sp. 5, *Leptohyphes* sp. 3 y *Smicridea* sp. 3). Buscamos además evaluar el efecto de la altitud y del tamaño sobre la alimentación de estos individuos. Investigamos también una posible relación entre la composición alimenticia de las morfoespecies y variables ambientales. Realizamos nuestro estudio en once ríos tributarios del Oyacachi entre los 1705 y 3867 msnm. Hicimos análisis de contenido intestinal en los macroinvertebrados y clasificamos su composición alimenticia utilizando códigos difusos y el índice de diversidad alimenticia de Simpson.

Encontramos que *Smicridea* sp. 3 y *Anacroneuria* sp. 1 tienen una alimentación más generalista que *Leptohyphes* sp. 3 y *Baetodes* sp. 5. Además tanto en *Leptohyphes* sp. 3 como en *Smicridea* sp. 3, la diversidad alimenticia aumenta con el tamaño de los individuos. En las morfoespecies más generalistas también encontramos que la composición alimenticia está asociada al tamaño de los individuos de manera que los de mayor tamaño consumen progresivamente más alimentos de mayor tamaño y menos material orgánico particulado fino (FPOM, por sus siglas en inglés). Específicamente encontramos que tanto en *Anacroneuria* sp. 1 como en *Leptohyphes* sp. 3 y *Smicridea* sp.3 la proporción de FPOM de la dieta disminuyó al aumentar el tamaño del cuerpo de los individuos. Por otro lado, encontramos que existe una variación en la proporción de los alimentos a lo largo de la gradiente altitudinal consumidos por las especies más generalistas. También encontramos que la diversidad alimenticia de cada especie varió entre los sitios de estudio (a excepción de *Baetodes* sp. 5). Esta variación en la composición alimenticia y la diversidad alimenticia nos indica que existe plasticidad trófica en estas especies. Sin embargo, la relación entre la alimentación y las variables ambientales no es clara. Esto sugiere que las diferencias alimenticias están posiblemente más relacionadas a distintas dinámicas comunitarias. La composición alimenticia de los macroinvertebrados parece ser, entonces, una característica dinámica; y a futuro se deberían estudiar otros factores ambientales para explicar su variación. Específicamente debería analizarse el efecto de la abundancia de distintas fuentes de alimento así posibles interacciones con factores a nivel de la comunidad como la depredación y la competencia para iluminar los procesos que determinan la variación alimenticia en los macroinvertebrados de río.

Abstract

Stream macroinvertebrates have a fundamental role in river ecosystem function through their feeding habits. However, little is known about the diet of these organisms in the Neotropic. In the present study we characterized the diet of four stream macroinvertebrate morphospecies (*Anacroneuria* sp.1, *Baetodes* sp. 5, *Leptohyphes* sp. 3 y *Smicridea* sp. 3). We also studied the effect of altitude and individual size over the feeding habits of these macroinvertebrates and investigated the possible relationship between dietary composition and environmental variables for the mentioned species. We carried out our investigation in tributary streams of the Oyacachi River between 1805-3867 masl. We did a gut content analysis on the macroinvertebrates and classified their diet composition with fuzzy codes and Simpson's food diversity index.

We found that *Smicridea* sp. 3 and *Anacroneuria* sp.1 have a more generalist diet than *Leptohyphes* sp. 3 and *Baetodes* sp. 5. Furthermore, both in *Leptohyphes* sp. 3 and in *Smicridea* sp. 3, trophic diversity increases with increasing individual body size. In the generalist species we also found that diet composition is associated with individual body size so that larger individuals consume progressively more of larger food types and less of fine particulate organic matter (FPOM). Specifically, we found that in *Anacroneuria* sp. 1, *Leptohyphes* sp. 3, and *Smicridea* sp. 3 the proportion of FPOM in their diet decreased with increasing individual body size. On the other hand, we found that there is a variation in the proportion of food types along the elevation gradient for the more generalist species. We also found that diet diversity varied among elevation sites (except for *Baetodes* sp. 5). This variation in the diet composition and in diet diversity along the elevation gradient is an indicator of trophic plasticity among the species where it occurred. Finally, we found that the relationship between feeding habits and environmental variables is not straightforward. This suggests that dietary differences within a species are associated with different community level dynamics. The diet composition of macroinvertebrates is thus a dynamic characteristic and in the future other factors should be accounted for in the description of its variation. Specifically, data on the abundance of food sources and on possible interactions with biological factors such as predation and competition could shed light on the processes that lie behind trophic plasticity and diversity of stream macroinvertebrates.

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Introduction

Streams are unidirectional, dynamic and complex ecosystems in which several ecological processes are pivotal for their functioning (Allan & Castillo, 2007). Of particular importance is the processing of organic and inorganic matter, the lateral, longitudinal transport of nutrients, sediments and other materials throughout their areas of influence (Meybeck, 1982).

Ecosystem function is defined as the biological, physical and chemical characteristics and processes of an ecosystem (Solan et al., 2012). These processes include primary and secondary production as well as nutrient cycling (Solan et al., 2012). Stream macroinvertebrates have direct impacts over the primary production of a stream (Lamberti & Resh, 1983), the decomposition of allochthonous and autochthonous matter (Gonçalves Jr et al., 2009) and the amount of organic and inorganic particles in the benthos through filtering and collecting (Wallace & Webster, 1996). Overall, invertebrate community composition, interspecific trophic relations (Covich et al., 1999) and flow conditions influence heavily the rates at which these materials and nutrients are processed and transported downstream (Wallace & Webster, 1996). Therefore, aquatic macroinvertebrates are fundamental players in the rates at which these processes are performed (Covich et al., 1999; Wallace & Webster, 1996). Trophic habits of stream invertebrates not only regulate ecosystem function but, because these trophic traits result from evolutionary processes, they may be an indicator of their specific vulnerability towards environmental changes in the future (Luciano et al., 2009).

Given the relationship that exists between stream ecosystem function and trophic dynamics of aquatic macroinvertebrates, significant progress has been made in the description of the feeding habits of the river biota (Wallace & Webster, 1996). Merrit and Cummins (2006) have proposed a system of dietary classification that has been used widely for macroinvertebrates in United States. However, the system has been extrapolated to other countries and areas (e.g. Encalada et al. 2010). This classification is based in the morphology of feeding parts as well as on the ingested items of species (Merrit & Cummins, 2006). Based on these criteria, Merrit and Cummins (2006) suggest that there are four basic Functional Feeding Groups (FFG). Organisms that feed on plant detritus or coarse particulate organic matter (CPOM) are classified as *shredders*. Those that feed primarily on algae that grow on rocks or other substrates are classified as *scrapers*.

Collectors filter fine particulate organic matter (FPOM) or harvest it from the benthos. *Predators* feed on other macroinvertebrates (Cummins et al., 2005). Besides this classification system, invertebrates can be grouped based solely on the materials they ingest into trophic guilds (Allan & Castillo, 2007). Algivores, detritivores, omnivores, carnivores and omnivores are some of the proposed trophic guilds used to describe stream macroinvertebrates (Allan & Castillo, 2007).

Even though these categories have been used to describe dietary habits of stream taxa in diverse geographic regions, both FFGs and guild assignment are based on the feeding habits of temperate organisms from the northern hemisphere (Tomanova et al., 2006). Little is known on the feeding habits of Neotropical aquatic organisms, but some studies suggest they may be highly flexible and significantly different from their temperate pairs (Tomanova et al., 2006; Covich, 1988). In a study conducted in Bolivia for instance, it was noted that most aquatic insect genera could be placed in two or more trophic levels and that their feeding habits varied significantly across sites (Tomanova et al., 2006).

The same study found that out of 49 analyzed taxa, only 24 could be classified accordingly into a single FFG (Tomanova et al., 2006). Out of those 24 taxa, four did not match the usual FFG assigned to their genus (Tomanova et al., 2006). It is thought that this level of trophic breadth may aid in avoiding niche overlap among species and in coping with the highly variably nature of Neotropical streams (Tomanova et al., 2006).

Besides a tendency for higher trophic breadth in the tropics, feeding habits within taxa are not consistent in regions within this area (Tomanova et al., 2006). This highlights the importance of studying the feeding habits of tropical macroinvertebrates and to extend this analysis beyond the genus level. Going beyond the genus level may be valuable because the apparent high level of trophic plasticity in the tropics could be an artifact of high species cryptic diversity in this region.

A broad trophic breadth or trophic plasticity is defined as the variation in the feeding habits of a species (referencia?). Trophic plasticity is an adaptation that is favored in environments where changes occur within a single generation and is generally achieved though traits that can be induced by the environment (Ruehl & DeWitt, 2007). A clear example of this is found in the different feeding compositions of the gastropod *Hydrobia ulvae* (Riera, 2010). In habitats with high sand and mud presence, *H. ulvae* feeds on

allochthonous detritus while in *Spartina maritima* marshes it feeds mostly on autochthonous detritus (Riera, 2010). Opposite to trophic plasticity is trophic specificity, which describes species that feed only on one type of resource (Singer, 1994). This trait seems to be a result of evolutionary processes that help avoid predation or that provide some physiological advantage from consuming only one species of a resource (Singer, 1994). The components of dietary habits that may vary include the type of ingested foods as well as their proportion in the diet of a species (Riera, 2010). In organisms classified as plastic, food choice may respond to the abundance of food sources which in turn results from differences in primary and secondary production. In specialist organisms, the consumption of items does not depend on said item's abundance in the ecosystem (Singer, 1994). Both trophic plasticity and specificity can be found in all taxonomic groups including vertebrates and invertebrates (Ruehl & DeWitt, 2007; Singer, 1994). Wherever a species falls in this spectrum of trophic variability can be a useful indicator of the sensitivity that a species can have towards environmental changes.

One important source of environmental variation that is common in stream ecosystems is altitude. Changes throughout an altitudinal range in a stream include variations in temperature, land-cover, oxygen concentration and light availability (Jacobsen, 2003; Tomanova et al., 2007). A common way of conceptualizing environmental and community level changes across a stream gradient is the River Continuum Concept. This template was proposed by Vannote et al. in 1980 to describe functional changes of a stream across a longitudinal gradient. It predicts levels of primary production in a stream based on variations of riparian vegetation as well as on the relative abundance of functional feeding groups (Vannote et al, 1980). It is also commonly used to describe the nature, origin and size of organic matter found in different orders of a stream (Allan & Castillo, 2007; Vannote et al., 1980). While it is mostly used to describe FFG composition and ecosystem processes through different stream orders, it may illustrate the dynamics that occur through an altitudinal gradient (Tomanova et al., 2007).

In general elevation changes have been shown to have significant variations at the ecosystem, community and species levels. Of particular importance to our study is the variation in riparian vegetation that occurs along the altitudinal gradient (Rosenheim, 1918). In the Andes, these changes are mostly associated with different photic environments, temperatures and humidity across the gradient (Molina-Montenegro &

Cavieres, 2010). The association between types of riparian vegetation and food sources in a stream (Monzón et al., 1991) generate different FFG compositions within stream ecosystems along the altitudinal gradient (Tomanova et al., 2007 and Monzón et al., 1991). Within the stream ecosystem, primary productivity changes with altitude although the relationship is not constant across studies (Villa et al., 2010). At the community level, species richness and community composition has been shown to vary significantly with altitude (Jacobsen, 2003; Villa et al., 2010). This is also true for Neotropical Andean rivers (Jacobsen, 2003; Molina et al., 2008). Metabolic rates of macroinvertebrate communities may also be affected by varying oxygen availability (Jacobsen, 2000). Outside of the stream system, foraging behavior in single species has also been found to be affected by altitude through differing temperatures and food availability (Grindal et al., 1999). Here it is important to notice that changes related to altitude don't respond directly to single abiotic factors but rather to complex interactions of components (Kuhn et al., 2011).

In the Ecuadorian Andes, a close relationship has been established between vegetation types and defined elevation ranges (Baquero et al., 2004). Variations of species composition and diversity with altitude have also been confirmed not only in aquatic macroinvertebrates of the Andes, but also in amphibians in the South Oriental Andes and Coleoptera of the Amazonian Cutucú Range (Celi et al., 2004; Jacobsen, 2003; Ramírez et a., 2009). However, it has been found that oxygen concentration is not directly associated with altitude in the Andes (Rostgaard & Jacobsen, 2005). This is due to the paired effect of temperature and partial pressure of oxygen over the oxygen concentration in water (Rostgaard & Jacobsen, 2005). This underlies the importance of considering several environmental factors when inferring about ecosystem changes through an elevation gradient.

Additional to environmental factors that affect trophic composition, intrinsic characteristics of consumers also influence food consumption. The size of consumers has often been shown to affect dietary preferences (Godinho et al., 1997). In general, larger organisms of a species tend to choose larger food items or prey due to increased handling abilities with increasing size (Godinho et al., 1997). Increased size has been shown to also confer greater swimming abilities and visual capacity to predatory marine fish, which results in higher predation success (Scharf et al., 2000). Larger individuals may also ingest a greater variety

of item sizes (Scharf et al., 2000). This represents a significant competitive advantage for larger sized consumers over smaller ones whose diet is size limited (Scharf et al., 2000).

The elevation gradient occurring in the Oyacachi River watershed located in the North Oriental Andes provides an excellent opportunity to study potential trophic variation of macroinvertebrates along this gradient. So does the insect size diversity that we have found among the invertebrate taxa we are studying. Given the relationship that exists between the trophic dynamics and ecosystem function, learning about variations in macroinvertebrate diets can provide information about the changes in ecosystem processes across a gradient and within a community. Furthermore, the spatial temperature variation along the altitudinal gradient can be used to model the consequences in the temporal temperature variation predicted in the context of climate change (Boyero et al., 2012).

In the present study we (1) analyzed the diet composition of aquatic insects along an elevation gradient in the Napo watershed. We also (2) quantified the levels of dietary diversity and trophic plasticity of different insect species at different size classes and (3) identified the relationship between environmental variables and the dietary composition of the studied species across sites. We analyzed the most abundant morphospecies of four genera corresponding to the traditionally assigned major FFGs (*Perlidae, Leptohyphes, Smicridea, and Baetodes*). We used the morphospecies level because, in the absence of genetic analysis it is virtually impossible to differentiate species of aquatic nymphs.

Justification

With the present study, we hope to significantly contribute to the body of knowledge on the factors that affect dietary habits of widely distributed species and their evolution. This is because the feeding strategy of a species, including diet diversity and composition is "an adaptive trait" and as such can help reflect upon the evolutionary pressures to which a species has been subjected (Tomanova et al., 2007). Considering that the species we chose for these analysis have a wide distribution along the elevation gradient, information regarding the diversity and plasticity of each species' diet provides information about the different trophic strategies that widely distributed species may have.

On the other hand, in the applied sciences field, trophic habits of stream invertebrates can be used to monitor stream quality. Specifically, the diversity of trophic guilds in a stream can be used to qualify the level of human disturbance that it has experienced (bij de Vaate & Pavluk, 2004). This is done through the calculation of the Index of Trophic Completeness in which streams where more trophic guilds are present are thought have experienced lower degrees of anthropogenic disturbance (bij de Vaate & Pavluk, 2004 and Tomanova et al., 2007). Given the simplicity in calculating this index and its overall effectiveness in reflecting levels of human disturbance, this index is commonly used in tropical streams (bij de Vaate & Pavluk, 2004 and Tomanova et al., 2007). However, the scant studies that have been conducted on diet composition of tropical stream invertebrates suggest that the same feeding groups cannot be assigned between tropical and temperate congeners (Tomanova et al., 2007). By describing the diets of some highly abundant macroinvertebrate species, we will contribute to increase the accuracy with which tropical genera are assigned to trophic guilds. This is an important step towards constructing a region- specific system of trophic classification.

Thus, we hope that the present study can significantly contribute to the knowledge of the ecology and evolution in the feeding habits of stream Andean macroinvertebrates and in the quality assessment of Andean streams.

Methods

Study Area

We collected invertebrate samples in twelve first or second order tributary streams along the Oyacachi basin during February of 2012. The samples were collected in an altitude range of 1705-3863 masl, between sites there was an altitude interval of 136-229m (Fig. 1 & Table 1). There is a mean annual precipitation in the Oyacachi area of 3000-3500 mm and a mean temperature of 5˚C to 17˚C along the altitudinal gradient (Skov, 1997). The studied streams vary in several physical and chemical characteristics, but all share typical characteristics of mountain streams. Primarily, they are fast flowing pristine waters with very high oxygen saturation levels (92-100%) and a significant slope (Table 2). All have pebble gravel bottoms and are relatively shallow (<30 cm) but mean width varies among stream sites (0.92-5.03 m).

The Oyacachi basin expands from the highlands of the Cayambe-Coca National Park in the Northern Andes of Ecuador (UTM zones 18S and 17S) to the lowlands of the town El Chaco, Napo Province (Fig. 1). Along the gradient of our study sites the vegetation changes severely. At the highest site (3623 masl)is the Andean shrub ecosystem or dwarf forest with representatives like the Andean Alder *Alnus acuminata* and *Gynoxis* sp. (Quintanilla, 1983). This vegetation is a transition between the páramo and the lower highland evergreen montane forest (Quintanilla, 1983). In the next altitudinal range (3417-2838 masl) there is highland evergreen montane forest (HEMF), which is characterized by representatives like *Valea estipularis*, *Croton elegans,* and *Freziera canescens* (Baquero et al., 2004). High epiphyte and moss concentration on trees and presence of moss on the ground are characteristic of the HEMF (Baquero et al., 2004). This type of forest is followed by the montane forest (3609-1867 masl) which is characterized by a high concentration of mosses, orchids, ferns, bromeliads, and bamboo and by tree – tops between 20 – 25 m high(Baquero et al., 2004). The lowest site (1705 masl) is found in a low montane forest with characteristics vegetation like trees of *Tibouchina* sp., *Inga* sp., and *Fuchsia macrostigma* (Báez et al., 1999). Treetops in this area reach up to 25-30 m (Baquero et al., 2004). The low montane forest is also characterized by the presence of bromeliads, mosses and orchids (Baquero et al., 2004).

Data collection

We measured several physico-chemical variables (Table 2) to characterize the sites and to use as possible environmental factors along the altitude gradient. Altitude and geographic position were measured at each site using a Garmin Etrex vista H © GPS. Physical and chemical parameters were all measured *in situ* in the stream sites. Temperature, pH, and conductivity were measured using the Orion 4 Star multi-parameter device from Thermo ©. We measured dissolved oxygen placing the 550A oxygen probe from YSI into running waters. We measured flow velocity using global water FP11 Global Water flow meter. Discharge was calculated by doing a discharge curve as suggested by Hauer & Lamberti's protocol (2007). For this measure, three replicates were taken along a single transversal section across the stream. We divided the transversal section into equal sized cells for which we measured depth and water velocity.

For the characterization of the surrounding vegetation we calculated riparian vegetation cover average at 20m by selecting a point 20 m from the stream. For this we used a spherical densitometer held at knee's height to calculate the light density (non-covered area). We performed four replicates in each point and later we averaged for each stream site.

Coarse benthic organic matter (CBOM), which is a retained form of CPOM (Pozo & Elosegi, 2005), was measured by collecting coarse particles using a surber stream bottom sampler (30cm x 30 cm). The sampler was located on three different transects in each site across the stream. The collected samples were stored in Ziploc© bags, labeled, and transferred to the laboratory where each was placed in a labeled aluminum tray. These were then dried in the muffle oven at 70˚C for 24 hours. Samples were weighed in a four decimal balance and then each was placed in a crucible where all organic matter was incinerated at 500˚C for four hours. Ashes were weighed and the CBOM mass was calculated from the difference between dried matter and ash matter. This method is based on that of Pozo & Elosegi (2005).

We also included two indexes of stream ecosystem quality which were the fluvial habitat quality measure (IFH) and vegetation quality of riparian quality index (QBR). IFH is a numerical index that describes the heterogeneity of stream habitats. It is represented by a percentage and is constructed with seven physical and biological parameters that describe

the stream ecosystem (Barquín et al., 2011). QBR takes into account riparian vegetation cover, vegetation quality, vegetation structure and modifications of the channel and results in 0-100 values (Munné et al., 2003).

Invertebrate sampling

Additionally, in each stream site, we sampled aquatic invertebrates. Invertebrates were collected using five qualitative surber sampler nets (30x30cm) and a semi-quantitative Kicknet for 5 minutes. Unfiltered samples were placed in plastic containers and preserved in the field with alcohol (96%). Once in the laboratory, invertebrates were separated from stream debris and placed in glass vials with 96% alcohol. We identified the individuals using the key of Fernández and Domínguez (2009) to the morphospecies level. To assign morphospecies we considered consistent differences in pigmentation patterns, gill and setae disposition, among others. After classification we calculated the invertebrate community composition of invertebrates of all sites (Encalada et al. in press) and different species were selected according to their abundance and prevalence along the gradient. Specifically, we chose the morphospecies that were the least absent along the gradient and that had the most individuals of the selected genera.

We selected *Smicridea* sp. 3 (Hydropsychidae)*, Anacroneuria* sp. 1 (Perlidae)*, Baetodes* sp.5 (Baetidae) y *Leptohyphes* sp. 3 (Leptohyphidae) for our analyses. We purposefully chose genera that are usually assigned to one of four different FFG. The *Baetodes* genus is usually described as a scraper in temperate regions while in the tropics it is described as a collector (Merrit & Cummins, 1996; Tomanova et al., 2006). The families from the order Plecoptera are usually classified as predators (Peckarsky, 2006), however there are several families that are considered shredders (Merrit & Cummins, 2006). Specifically, the genus *Anacroneuria* in neotropical streams has been shown to be predatory (Tomanova et al., 2006). *Smicridea* is usually classified as a filterer collector of FPOM (Merrit & Cummins, 2006; Callisto et al., 2001). Finally, *Leptohyphes* is usually classified as a scraper (Baptista et al., 2006; Merrit & Cummins, 2006).

Gut content analysis

We analyzed 12 individuals in each site and for each taxa. We used individuals collected in both the surber and kick samples. First, we selected individuals of the desired taxa from the surber samples and if less than 12 individuals were present in these samples, we used

individuals from the Kicknet samples. Still, in some cases we were not able to dissect 12 individuals. In total, we analyzed 2690 fields corresponding to different species at different sites (Table 3).

Previous to dissection, we measured each individual under the Olympus SZX16 optic ruler. All measurements were made at 1.25X magnification and then converted to mm. Each individual was dissected using fine point forceps in the stereoscope to isolate the intestine of each one. We saved the remaining body parts in glass vials with 96% with individual labels to conduct molecular analysis in the future. We placed individual intestines in a microscope slide and spread their contents in a homogeneous manner. To preserve the gut content sample we added one drop of Canada balm over the sample and covered it with a coverslip.

We then observed each slide in a ZEIZZ© Scope.A1 microscope under 400x magnification. We performed a sweep over the slide and stopped at 10 microscope fields at random. For each field we visually estimated the percentage that was covered by either fine particular organic matter (FPOM), dead coarse particulate organic matter (dCPOM), live coarse particulate organic matter (lCPOM), algae, and chitin. We considered elements of <1mm (Tomanova et al., 2006) with indistinct borders and inner structures (such as cell walls) as FPOM (Fig. 2a). We considered elements >1mm to be CPOM (Tomanova et al., 2006). We divided CPOM items into dead CPOM when they had a brown hue (Fig. 2b) and as live CPOM when they had a light green color and conspicuous cell walls (Fig. 2c). Algae were all the elements with evident cell walls that had filamentous, single-cellular or bi-cellular, or budding structures (Fig. 2d). Elements that were fragments from other insects or had chaetae were classified as chitin (Fig. 2e). We avoided fields that were mostly empty (less than 1% covered by food matter) to include the greatest fraction if ingested items in each individual. The estimations of the percentage of the field covered by each food type were all made by a single observer to minimize errors due to observer bias.

Data analysis

Invertebrate percentage data

To calculate the individual diet composition proportions we first added all the values of each optic field for each food type for every individual. Then we converted the sums of each food type per individual so that together all food types added up to 100% coverage (Fig. 3). To calculate the proportion of each food type at the species level, we averaged the proportion of food types among individuals of the same species.

Quantification of trophic specificity/generalism

Using the percentages calculated, we calculated corresponding fuzzy codes for each morphospecies (Tomanova et al., 2006 and Usseglio-Polatera, 1994). Fuzzy codes are a numeric classification system that incorporate various levels of affinity towards each food category (Tomanova et al., 2006). They represent a useful method for visualizing and quantifying trophic breadth in non-specialist species. We applied the fuzzy coding proposed by Tomanova et al. (2006) in which a score of 0 is assigned when there is no affinity towards a food type, 1 when there is a weak affinity, 2 when there is moderate affinity and 3 when there is a strong affinity. An affinity of 0 is assigned when a food type is present in <5% of the observed fields. An affinity of 1 is assigned when a food type is present in 5-20% of the observed fields, 2 when a food type is present in 20-35% of the fields, and 3 when a food type is present in more than 35% of the observed fields. Presence is considered when an item occupies at least 15% of the optic field that is being analyzed (Acosta et al., in press). To calculate the percentage of fields where a food item was present, we counted all fields in a species where a given food item occupied >15% of the field. We then divided this count by the total number of fields for each morphospecies. Each morphospecies was thus assigned a score for all of the food types analyzed.

To identify the principal differences between the diets of the studied morphospecies, we applied a Principal Component Analysis (PCA) to the proportion of food items ingested by all individuals of all species. We colored coded the resulting coordinate points by morphospecies.

To characterize the degree of diet diversity/ homogeneity for each morphospecies we applied numerical indexes. To determine the degree of diet generalism we applied the

Simpson diet diversity index (Equation 1) (Felten et al., 2008). We calculated Simpson's diversity index for each individual based on the proportion of each food item and the total number of food items found in its intestine.

$$D = 1 - \sum_{k=1}^{n} \frac{P_k^2}{i}$$

Equation 1

P_k = proportion of k food type in a given individual

i = number of food types ingested by a certain individual

Effect of size over trophic specificity/diversity

We also analyzed the effect of size over dietary diversity using Spearman's Rank correlation test. We used the non-parametric test of correlation in this case because we were not able to find normality or homoscedasticity in raw data or through conventional transformations.

Feeding variability along the altitudinal gradient

To quantify trophic plasticity throughout the altitudinal gradient we applied Kruskal-Wallis tests (one for each species) for each food type. Specifically, we compared the proportion of each food type among all individuals of a single species among stream sites. We chose a non-parametric test because normality and homoscedasticity could not be achieved through any conventional transformation.

We also compared mean Simpson's diversity index for each morphospecies along the elevation gradient. For this ran a regression analysis using excel to find the best fitting elevation – diversity index model. Because our data was not normal, this regression should be interpreted as a description of the general tendency of change in diet diversity along the altitudinal gradient and not as a predictive model.

Feeding variability among size groups

We compared diet composition among different sizes using Spearman's Rank correlation tests.

Influence of environmental factors

We conducted a Principal Component Analysis (PCA) using environmental variables to identify the main drivers of possible differences among stream sites. The environmental variables we tested were temperature (˚C), slope (%), mean depth (m), mean width (m), IHF, QBR and mean % of riparian cover at 20 m. Using Primer v6 we first standardized and normalized our data. We took all variables that had >30% resemblance to Principal Component 1 to run a stepwise regression test along with the proportion of each food type for each species that showed some degree of trophic plasticity along the gradient. Additionally, we ran simple linear regressions for each CPOM concentration and mean river velocity and food proportion for each species for which we had found significant variations in food proportion along the elevation gradient. We could not perform this analysis for river velocity and FPOM in *Anacroneuria* sp. 1 because we only had values for this variable on 2 of the sites where this species was present. We did this test separately because we only had data for five stream sites along our gradient. We normalized the data using Primer v6 software. However, this results should be taken with caution because they do not account for the role of the remaining environmental variables on diet composition.

Results

Diet composition

Overall, we found that the most predominant food type among the four analyzed morphospecies was FPOM (Fig. 4). This food item represented at least 23,94% ± 2,452 of the *Anacroneuria sp. 1* diet and up to 96,401% ± 0,872 in that of *Baetodes sp. 5.* This food type was the dominant ingested item in all morphospecies (*Baetodes* sp. 5, *Leptohyphes* sp.3, and *Smicridea* sp. 3) except for *Anacroneuria* sp.1. In this morphospecies chitin was the most abundant food item (Fig. 4a).

While in all cases we found one clearly dominant food type, some species held strong affinities to other food types (Table 4). Along with chitin, we found *Anacroneuria* sp.1 to also consume considerable amounts of FPOM and algae and, to a lesser degree dead CPOM. *Leptohyphes* sp. 3 also consumed algae as a compliment to FPOM. We found *Smicridea* sp. 3 to hold some degree of affinity to all the food types we studied. In this morphospecies we mostly found FPOM along with considerable amounts of algae and some dead CPOM, live CPOM, and chitin. Only *Baetodes* sp. 5 held a strong affinity only to one food type (FPOM). In fact, in the intestines of this morphospecies we found FPOM almost exclusively (Fig. 4b).

Most of the differences in feeding habits among morphospecies were explained by the proportion of chitin and FPOM (close to Component 1= 37.1%) and the proportion of dead CPOM, live CPOM and algae (close to Component 2 = 25%) (Fig. 5). Here *Baetodes* sp. 5 and *Leptohyphes* sp. 3 are are tightly grouped in the direction of higher FPOM proportion and are found far from the direction of high chitin proportion. This results are consistent to the lower affinities to non-dominant food types that we found on *Baetodes* sp. 3 and *Leptoyphes* sp.3. On the other hand, *Smicridea* sp. 3 has highly dispersed dots in the direction of high FPOM proportion as well as in the direction of high dead CPOM, live CPOM, and algae proportions. *Anacroneuria* sp. 1 also has highly dispersed dots in the high dead CPOM, live CPOM and algae proportions, but its dots are more abundant in the direction of high chitin proportion. The dispersed distribution of these two species dots are also consistent with the high levels of affinity to more than one food group found that they exhibit.

Degree of specialization/generalism

Smicridea sp. 3 and *Anacroneuria* sp. were the most generalist morphospecies, as suggested by the high values of the Simpson dietary index (Fig. 6). We found *Leptohyphes* sp. 3. and *Baetodes* sp. 5 to be the most specialist (Fig. 6).

In our analysis of trophic diversity by body size we found a significant correlation for *Leptohyphes* sp. 3 and *Smicridea* sp. 3 (Table 5). For these morphospecies, dietary diversity increased with increasing body size (Figs. 7& 8). Specifically, we observed that in smaller individuals, the diet almost exclusively consisted of FPOM while in larger individuals the proportion of this food type dropped significantly (Fig.9). In the larger Leptohyphers insects, there is a significant inclusion of live CPOM in the diet which is particularly noticeable in the largest size group (Fig. 9).

Diet composition variability with individual body size

In regards to diet composition, we found weak significant correlations with individual body size, differences of FPOM and algae proportion in *Anacroneuria sp. 1* (Table 6). We found that smaller individuals of this morphospecies consume more FPOM than those in the largest size group (Fig. 10). On the other hand, we found a higher consumption of algae in the largest group size than in the other three size classes (Fig. 10).

 In *Leptohyphes* sp. 3 we found a significantly higher consumption of live CPOM in the largest size group compared to that of other three smaller size groups (Fig. 9). Conversely, we found that the largest individuals consumed considerably less FPOM than the smaller size classes (Fig. 9).

In *Smicridea sp.* 3 we found variation in the proportion of dead CPOM; it increases gradually with size group for the first three size classes (Fig. 11). The largest size class consumed a considerably higher percentage of this food item (more than twice as much as the second largest size class). We also found this species to gradually decrease the percentage of FPOM consumed in larger insects.

In *Baetodes sp.* 5 we found no significant differences in diet composition for the analyzed size groups (Table 6).

In general, we found that for the three morphospecies that showed diet composition variation with size, FPOM consumption was higher in smaller individuals than in larger

ones. We also found that larger individuals had a higher percentage of larger food items (such as live or dead CPOM and algae) than did smaller individuals.

Diet variability along the altitudinal gradient

We found variable results of the effect of altitude on diet composition in the analyzed morphospecies (Table 7). In *Anacroneuria* sp. 1 we found a significant altitudinal variation in the percentage of FPOM, with the highest proportions occurring at intermediate altitudes (2008-2609 masl), and lowest proportions at low and high altitudes (1867 and 2838-3623 masl, respectively) (Fig. 12).

In *Baetodes* sp. 5 we found that FPOM and algae proportions were significantly different across elevations (Fig. 13). However, the differences observed are not biologically significant. This is because the differences for both mean FPOM and algae concentration among stream sites are no greater than 10 percentage points. More significantly, the mean FPOM concentration for all sites is always >94% and the mean concentration of algae is always <6% (Fig. 13). This means that levels of affinity towards each food type are not affected by altitude in *Baetodes* sp. 5.

For *Leptohyphes* sp. 3, we also found significant variations for the proportions of both FPOM and algae (Fig. 14). We found higher concentrations of FPOM in the lowest and highest altitudes where this morphospecies was present (1705-1867 masl and 2609-3417 masl, respectively). Lower concentrations occurred at intermediate altitudes (2008-2236 masl). On the other hand, the percentage of algae in *Leptohyphes* sp. 3 doubled from 1705 masl to 2236 masl (Fig. 14). Then, between 2609-3012 masl, the proportion of this food type decreased continuously until it reached low representativity (<5%). At 3199 masl we found another doubling of the algae proportion and finally at 3417 masl the percentage dropped to <2% of diet contents.

In *Smicridea* sp. 3 we found slight significant variations in the proportion of dead CPOM (Fig. 15) and chitin (Fig. 15). Overall, we found higher portions of CPOM at higher altitudes (3838-3012 masl and 3623 masl) with the exception of the 3199 masl stream site where we did not find this food source. Conversely, we found continuously higher proportions of chitin in *Smicridea* sp.3's diet at low-intermediate altitudes (1867-2372 masl), very low proportions at the lowest stream site (1705 masl) and the 2838 masl and none at the highest sites (3012-3623 masl).

We also found that diet diversity varied along the elevation gradient in different ways for each morphospecies (Fig. 16). In *Anacroneuria* sp.1 we observed that diet diversity changes in a lump-shaped fashion so that intermediate elevations have higher mean diversity indexes. This is consistent with the higher FPOM proportions that we found at intermediate elevations, which suggests that the increase in diet diversity in *Anacroneuria* sp. 1 is associated with an increased representation of FPOM in the diet. In *Baetodes* sp. 5 we found no apparent tendency of change in diet diversity across the elevation gradient. Although we did find different mean diet diversity index values across the gradient, all values were low which reiterates the specialist nature of *Baetodes* sp. 5's feeding habits. In *Leptohyphes* sp.3 we found a similar pattern as in *Anacroneuria* sp. 1 where higher mean trophic diversity values were found at intermediate elevations. In this case however, the increase in diversity coincides with increased proportions of algae in the diet of *Leptohyphes* sp. 3 at intermediate elevations. Finally, in *Smicridea* sp. 3 we found that the diet diversity decreased with increasing elevation even if slightly. This decrease in diet diversity coincides with the disappearance of chitin from *Smicridea* sp. 3's diet and with a significant increase in the proportion of dead CPOM. Overall, we find that even if there seems to be changes in the degree of diet diversity across the elevation gradient for each species, the degree of diet diversity between morphospecies remains constant. This means that *Smicridea* sp. 3 always has the highest diet diversity values, followed by *Anacroneuria* sp. 1, *Leptohyphes* sp. 3, and finally, *Baetodes* sp.5.

In general, we found that food type proportion changes along the altitudinal gradient had a lump-shaped pattern where either maximum or minimum values were almost always found at intermediate altitudes.

Response of dietary composition to environmental variables

Across elevation sites, we found that pH, temperature, dissolved oxygen concentration, IHF, and QBR were most closely associated with the first principal component the PCA (Table 8 & Fig.17). Alone, this component accounted for 45.2% of the variation among streams. Principal Component 2 accounted for 27.4% of the variation and was most closely associated to standard conductivity, slope %, depth, and flow. We conducted our regression analysis based only on the variables associated with PC1. We also found that sites were not grouped by elevation. Thus there seems to be no relationship between altitude and the measured environmental variables. Additionally, we found that the 2008

masl stream site to be found farthest from the rest in the PCA – biplot graph. However, we found that no single variable explained this site's extraneousness with respect to the other sample sites.

We found linear regression models for multiple environmental variables and varying food items that were statistically significant in all cases except for the proportion of chitin of *Smicridea* sp. 3 (Table 9). However, none of the linear models that were produced explained more than 15% of the variation in food item proportion of a species' diet. This may suggest that a linear model of variation in food proportion by environmental variables is not the most accurate.

Additionally, individual B-coefficients in all cases indicate that there are only weak associations between the environmental variables considered and the proportions of types in the diet of a *Anacroneuria* sp. 1, *Leptohyphes* sp.3, *Smicridea* sp. 3. These weak associations include a negative relation between pH and FPOM proportion in *Anacroneuria* sp. 1, a negative relation between IHF and the proportion of FPOM in *Leptohyphes* sp. 3, a positive relation between IHF and the proportion of algae in *Leptohyphes* sp. 3, and a negative relation between QBR and the proportion of dead CPOM in *Smicridea* sp.3.

When looking for a linear association between food proportions and stream velocity, we found only one case in which this model significantly described the variation in the food proportion of a species. This was the case for proportion of algae in *Leptohyphes.* Sp. 3's diet. We found the proportion of this food item to be moderately negatively explained by stream velocity (Table 10). As for the concentration of CPOM, we only found a significant linear model for the proportion of FPOM in *Anacroneuria* sp. 1's diet. Here we found a moderate positive association between the proportion of FPOM in the diet of this morphospecies (Table 10). In both cases however, little of the variation in food proportions are explained by the resulting linear models (Table 10).

Discussion

Our findings show that the diet composition of Andean stream macroinvertebrates can be characterized by different degrees of trophic generalism or specificity. *Smicridea* sp. 3 and *Anacroneuria* sp. 1 are generalist species even though they consume predominantly one food type. *Baetodes* sp. 5 and *Leptohyphes* sp .3 lie in the more specialist end of the diet diversity spectrum due to their primarily FPOM-based diet. However, we found *Leptohyphes* sp .3's degree of trophic diversity to be dependent on individual size so that larger individuals have a higher degree of generalism. Body size is also an important component determining the diet composition of the three more generalist species. Larger organisms ate larger food types (CPOM) than smaller individuals for which FPOM was a more significant food source.

Although we found that most species consumed more than one food source, the high representation of FPOM in the diets of three morphospecies can't be ignored. The high prevalence of FPOM in macroinvertebrate we found is consistent with other results for the tropics (Tomanova et al., 2006 and Campbell, 1985). For instance, one study in Australia found that 13 out of 19 species had more than 69% FPOM in their guts (Campbell, 1985). It is thought that tropical streams may hold high amounts of FPOM given the constant input of leafs into the streams throughout the year and a high rate of decomposition and fragmentation of such input (Dudgeon et al., 1982 in Covich, 1988 in Tomanova et al., 2006). Additionally, the gathering collector strategy requires less mobility than other feeding modes (scraping, grazing, and predation) (Tomanova et al., 2006). A low mobility feeding strategy may be favored in tropical streams given the high degree of variability and disturbance (2006).

It is suggested that the predominance of this food category may also be due to the fact that it includes a large array of food types (Palmer et al., 1993), in other words; it is a category that is more inclusive than other food categories generally used. It is thought that particulate matter is mostly composed of fungi, bacteria, algal fragments and non-living organic matter but the relative contribution of each type of food source is unknown (Campbell, 1985). This is why some authors suggest that the term algal grazers be replaced by the more general "browser" category which encompasses those species that feed on the rocks and sand of a stream bed(Campbell, 1985).

Additionally, we found that the most significant difference among the diet of studied morphospecies was the proportion of chitin and FPOM consumed. These results are also consistent with Tomanova et al.'s (2006) findings in Bolivia. This seems to indicate that there exists a real separation between species that are predominantly predatory, those that consume FPOM and those that eat other types of non-animal coarse matter.

The importance of body size in trophic diversity and diet composition has also been previously reported. Increased dietary diversity was found previously for gammarids (Amphipoda) and certain fish (Felten et al., 2008; Shcarf, 2000). Increased trophic breadth in larger individuals is likely due to larger mouthparts which allow for a greater size variety in items consumed and also to increased mobility (Felten et al., 2008 and Scharff, 2000).

The effect of size on dietary composition (rather than diversity) is more consistent. Here, we found that FPOM proportion varied according to size in most taxa. It has been proposed that larger organisms tend to eat less FPOM and choose higher proportions of different large materials (Felten et al., 2005). This is true in our study for *Smicridea sp.3* in which we found larger proportions of dead CPOM in larger individuals and for *Leptohyphes sp. 3* where we found greater proportions of live CPOM in larger individuals. This shift in the diet may also be explained by growing mouthparts that, as mentioned before, allow the consumption of larger food items (Felten et al., 2008). A diet shift at different individual sizes may also contribute in reducing intraspecific competition between larger and smaller individuals (Scharf, 2000). Additionally, the preference of larger individuals for larger food particles may indicate that these food sources are of higher quality.

We also found that food composition varies throughout altitudes for some species. This variation occurred in the proportion of FPOM in *Anacroneuria* sp. 1, the proportion of FPOM and algae in *Leptohyphes* sp. 3, and the proportion of dead CPOM and chitin in *Smicridea* sp. 3. We take this variation to be a sign of trophic plasticity, which we found to be a characteristic of some but not all species. This results are also supported by the change in trophic diversity that we found across the elevation gradient for the more generalist morphospecies. Additionally, we found that there are different degrees of trophic plasticity but that there exists a constant dominant food type in all morphospecies across sites. This means that there is room for plasticity only in secondary food sources. Furthermore, we found that the more specialist morphospecies (*Baetodes* sp. 5 and

Leptohyphes sp. 3) were also the least plastic and vice-versa. This suggests that trophic generalism may be important for allowing trophic plasticity through a spatial range.

In the tropics, it has been suggested that organisms have evolved a lower tolerance for environmental variation due to more constant climatic conditions throughout the year (Janzen, 1967). This would imply that there are evolutionary constrains for tropical organisms to adapt to a great range of environmental conditions. However, the species that we analyzed seem to have a wide range of tolerance for different physicochemical variables along the elevation gradient which allows them to be present in a wide distribution. Thus there seems to exist a degree of physiological plasticity which is often but not always accompanied by trophic plasticity. We find this to be the case of *Baetodes* sp. 5 which not only throughout its distribution but also throughout its lifespan maintain an almost identical diet based virtually solely on FPOM. The wide distribution of this species across the altitudinal gradient along with its diet specificity suggests that FPOM is a reliable and abundant food source in the Andean streams that we analyzed. This shows that, food-wise, there is not a strong selection pressure in this species for the ability to ingest more than one food type. A similar situation is that of *Leptohyphes* sp. 3 which predominantly consumes FPOM and rarely more than 30% algae.

Smicridea sp. 3 and *Anacroneuria* sp. 1 exhibit a wider trophic breadth and a higher degree of trophic plasticity than the other two analyzed morphospecies. We suggest that there is a phylogenetic basis for the ability of these two morphospecies to consume more food types than the other two. However, even this dietary variability seems to be limited so that, on average, *Smicridea* sp. 3 always feeds mostly on FPOM and *Anacroneuria* sp. 1 mostly feeds on other macroinvertebrates. This would be consistent with Janzen's theory of evolutionary constrains to broad temperature tolerance in the tropics. However, this may not be a conclusive evidence for a narrow niche breadth in either of the four morphospecies analyzed given the broad nature of the food categories we used in this study.

An important consideration that must be made is that the morphospecies we chose were purposefully those with broader altitudinal distributions. This represents an important bias towards species adapted to wide-ranging environmental conditions. From our results, we propose that such a broad distribution may be obtained either by specialists that consume a highly reliable food source (*Baetodes* sp. 5 and *Leptohyphes* sp. 3) or by generalists that

may or may not have trophic plasticity (*Smicridea* sp. 3 and *Anacroneuria* sp. 1) (we make a distinction between generalist and plastic feeding strategies in that the first implies a broad array of food items consumed and the second implies a variation in the proportion of food types consumed throughout a gradient). It is likely that analyzing more species with narrower altitudinal distributions would produce a wider range in the specificity – generalism spectrum. Specifically, a more representative sample of the macroinvertebrate community could also produce a more moderate FPOM abundance across taxa. We speculate that FPOM is a convenient food choice for largely distributed species given its constant presence along the elevation gradient. However, it may be a more marginal food item in narrowly distributed taxa. Instead, it may be that narrow – range species consume items that vary significantly along the altitudinal gradient (such as different species of algae or leaf litter) which would in part account for their inability to survive in a wide range of habitats.

Additionally, an important consideration is that our data are based on a single collection of macroinvertebrates all collected throughout 3 months. It is likely that variations in diet also exist throughout the year. Seasonal variations have been reported for stream macroinvertebrates in temperate regions (Palmer et al., 1993) but no research has been conducted on this topic in the tropical Andes and along elevation gradients.

Beyond the degree of trophic plasticity of a species, diet variability through the altitudinal range may provide information towards each species' feeding strategies. This can be illustrated by a comparison between the chitin consumption levels of *Smicridea* sp. 3 and *Anacroneuria* sp. 1. While the first morphospecies did not consume chitin above 2800 masl, the second did not significantly vary in its consumption of this food source. This may result from the different feeding behavior of both species. While *Smicridea* sp. 3 is a generalist feeder that collects its food from the water column, *Anacroneuria* sp. 1 is an active predator. *Anacroneuria*, being most specialized in hunting, is likely to have a competitive advantage over opportunistic predators such as *Smicridea* sp.3 where prey are sparse. Furthermore, *Smicridea* sp. 3 may be less adapted to high altitude conditions which may be expressed as a reduced ability to feed on other macroinvertebrates. This may force individuals of this species to feed on less mobile items such as CPOM. However, given that plant tissue has a lower protein and energy content than animal tissue (Bowen et al., 1995), we take *Smicridea* sp. 3's chitin deprived diet to be a sign of sub-optimal conditions

for this species at higher altitudes. On the other hand, in *Leptohyphes* sp. 3 higher consumption of algae at intermediate altitude streams is likely to result from increased abundance of this food source at said altitude. It would be valuable to have direct measurements of algal abundances among sites to determine if the proportion of algae in *Leptohyphes* sp. 3 depends on the abundance of this food source or if it is related to other dynamics.

Although we find that there are differences in the diet composition of most species across the altitudinal gradient, associating these differences with environmental variables was not straightforward. We found IHF, QBR, pH and dissolved oxygen concentration to explain most of the variation among sites. However, these variable were found to be very weakly associated with the proportion of food types of the analyzed morphospecies. The precise mechanism through which these associations occur seems unclear. In *Anacroneuria* sp. 1 for instance we found that FPOM proportion seems to decrease with increasing pH. Studies have shown that at lower pH levels there is less microbial decomposition of leaf litter (Chamier, 1987), wich should mean that less FPOM is produced locally. However, we found that there was a higher abundance of this food source in *Anacroneuria* sp. 1 at lower pH levels. In the case of *Leptohyphes* sp. 3 we found IHF to be negatively associated with FPOM consumption and positively associated with algal consumption. The algal consumption for stream macroinvertebrates has been shown to be associated with habitat type (Moore, 1977) and it is likely that a stream with higher diversity of stream habitats (as expressed by higher a higher IHF) may contain a habitat where algal consumption is favored. We could find no possible mechanism through which FPOM consumption may be negatively associetaed with IHF. Similarly, we found that the proportion of dead CPOM in *Smicridea* sp. 3 diet seems to decrease where there are lower QBR indexes. This seems contradictory given that a higher QBR index is associated with higher riparian vegetation cover and that this vegetation should be a main source of CPOM. Overall, we find that the weakness of the associations between environmental variables and food item proportions paired with the lack of an explanation of how these associations come to be suggests that these are not direct associations. Specifically, the lack of a consistent association between environmental variables and diet composition seems to indicate that there are more complex dynamics that influence food ingestion patterns in stream macroinvertebrates. Specifically, we hypothesize that community interactions may be more important in determining food consumption in different species.

Additionally, the effect of environmental variables may be exerted not as much in the proportion of ingested food items but in their specific composition. For example, algae species composition in macroinvertebrate diet has been shown to change across sites and seasons (Moore, 1977). Likewise, it has been shown that predatory Plecoptera have low prey selectivity and that they mostly feed on the most abundant prey species present (Siegfried & Knight, 1976 and Malmqvist & Sjöström, 1980).

Conclusions

Throughout this investigation, we have shown that stream macroinvertebrate diet composition is not a static characteristic of a species but rather a complex and dynamic trait. Food item consumption responds to intrinsic characteristics that may vary in the lifespan of an individual (i.e. size) or to extrinsic environmental patterns. The mobile nature of macroinvertebrate diet contests traditional attempts to classify a species into a single feeding guild. It also implies the existence of poorly understood biological relations that span across individual, population, community, and ecosystem level. However, the mobility in diet diversity seems to be constrained by the evolutionary history of tropical ecosystems which are considered to be more stable than their temperate counterparts.

The drivers of diet variability across the altitudinal range are likely associated with complex community dynamics that depend not only on elevation, but also on stream origin and history.

The diet compositions that we analyzed in this study also provide valuable information about the Andean stream ecosystem in regards to the transport of organic matter that takes place in this area. A higher diversity of taxa should be analyzed in order to produce more concluding results, but the prevalence of FPOM in the diets of three of four of our taxa suggests that this is a predominant form of organic matter in the system.

Furthermore, while in this study we concentrated on the spatial sources of diet variation, the seasonal (temporal) component may also play a fundamental role in diet composition of stream macroinvertebrates. Similarly, different degrees of human intervention on stream ecosystems and riparian vegetation may also impact macroinvertebrate diet composition. Ultimately, if knowledge around this subject continues to grow, the diet of stream macroinvertebrates could even serve as indicators of ecosystem function. That is, the dominance of certain food items among a representative sample of community taxa may provide valuable information regarding the relative importance of specific ecological processes (such as primary production and leaf litter decomposition) along the elevation gradient. Specifically, this information speaks of which ecosystem products are more constant along the elevation gradient and which are more restricted in their distribution.

Ultimately, it must be emphasized that the species we analyzed are all plastic in their tolerance to the environmental variables that change throughout the elevation gradient,

which is why they can have a wide range of distribution. However, we find that this physiological plasticity may not always be coupled with trophic plasticity. The selective forces that lead a species to be plastic in its feeding behavior are unclear but are fundamental in understanding the different degrees of plasticity found in equally distributed species.

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Annex 1 – Tables

Table 1. Stream sites altitude, coordinates, and main riparian vegetation type along tributary streams of the Oyacachi watershed, Napo Province, Ecuador

Stream site	Altitude (masl)	Latitude	Longitude	Main riparian vegetation type
8	3623	9958415	820408	Andean shrubbland/Andean brow
11	3417	9952796	167377	Evergreen Highland Montane Forest
9	3199	9958343	825646	Evergreen Highland Montane Forest
12	3012	9951735	170631	Evergreen Highland Montane Forest
10	2838	9955243	828947	Evergreen Highland Montane Forest
4	2609	9957092	811232	Montane Forest
1	2372	9943717	813658	Montane Forest
3	2236	9961649	811714	Montane Forest
2	2008	9944292	807337	Montane Forest
5	1867	9963186	817574	Montane Forest
6	1705	9957091	817481	Lowland Montane Forest

Table 2. Measured physical-chemical-biological data for stream sites in the Oyacachi watershed, Napo Province, Ecuador

Stream site	Elevation (masl)	Coarse Benthic Organic matter (g/m2)	Temperature ˚C	pH	Dissolved Oxygen %	Temperature standardized conductivity (µS/cm)	Mean Width(m)	Mean depth (m)	Flow (L/s)	Slope (%)	IHF	QBR	Riparian vegetation cover 20m (average%)
7	3863	N/A	7	7.81	94	75.23	0.92	0.27	131.9	2.10	84	100	83
8	3623	N/A	6.95	7.82	96	51.40	1.50	0.25	105.2	15	90	75	6.25
11	3417	5,25	7.95	7.68	100	41.26	1.70	0.3	119.5	5	82	95	77.00
9	3199	N/A	7.42	8.02	95	100.35	2.77	0.18	36.3	15	85	80	9.52
12	3012	N/A	9.12	7.85	96	60.00	1.97	0.13	42	5	65	85	0.94
10	2838	15,34	9.30	7.84	96	76.34	5.03	0.17	154.1	15.23	75	95	11.34
4	2609	N/A	12.44	7.45	94	56.06	1.67	0.14	25.8	15.22	84	65	4.06
1	2372	58,52	12.68	7.83	93	153.12	2.05	0.11	42.3	35	77	95	12.38
3	2236	N/A	12.81	7.78	92	97.18	2.09	0.22	191.7	15	86	95	5.36
2	2008	N/A	13.39	7.48	95	35.82	4.67	0.26	813	10	85	100	55.80
5	1867	9,46	14.1	8.12	95	73.38	3.03	0.24	173.2	17.40	83	100	4.58
6	1705	14,01	16.2	8.19	94	167.52	3.13	0.29	159.9	18.22	78	100	6.66

Table 3. Microscope optic fields (40x) and individual number for *Anacroneuria* sp.1, *Baetodes* sp.5, *Leptohyphes* sp. 3, and *Smicridea* sp. 3 collected from tributary stream of the Oyacachi watershed, Napo Province, Ecuador

Species	Site name	Altitude (masl)	No. Individuals	No. fields analyzed
Anacroneuria sp. 1	7	3863	-	-
Anacroneuria sp. 1	8	3623	13	127
Anacroneuria sp. 1	11	3417	-	-

Anacroneuria sp. 1	9	3199	-	-
Anacroneuria sp. 1	12	3012	-	-
Anacroneuria sp. 1	10	2838	13	124
Anacroneuria sp. 1	4	2609	10	100
Anacroneuria sp. 1	1	2372	11	110
Anacroneuria sp. 1	3	2236	12	120
Anacroneuria sp. 1	2	2008	11	110
Anacroneuria sp. 1	5	1867	12	108
Anacroneuria sp. 1	6	1705	1	10
Anacroneuria sp. 1	total		82	691
Baetodes sp. 5	7	3863	-	-
Baetodes sp. 5	8	3623	-	-
Baetodes sp. 5	11	3417	12	117
Baetodes sp. 5	9	3199	11	107
Baetodes sp. 5	12	3012	12	118
Baetodes sp. 5	10	2838	12	115
Baetodes sp. 5	4	2609	-	-
Baetodes sp. 5	1	2372	-	-
Baetodes sp. 5	3	2236	-	-
Baetodes sp. 5	2	2008	9	67
Baetodes sp. 5	5	1867	11	101
Baetodes sp. 5	6	1705	-	-
Baetodes sp. 5	total		67	625
Leptohyphes sp. 3	7	3863	-	-
Leptohyphes sp. 3	8	3623	-	-
Leptohyphes sp. 3	11	3417	4	40
Leptohyphes sp. 3	9	3199	3	30
Leptohyphes sp. 3	12	3012	13	125
Leptohyphes sp. 3	10	2838	11	107
Leptohyphes sp. 3	4	2609	12	121
Leptohyphes sp. 3	1	2372	-	-
Leptohyphes sp. 3	3	2236	12	119
Leptohyphes sp. 3	2	2008	6	60
Leptohyphes sp. 3	5	1867	12	120
Leptohyphes sp. 3	6	1705	3	30
Leptohyphes sp. 3	total		76	752
Smicridea sp. 3	7	3863	-	-
Smicridea sp. 3	8	3623	2	20
Smicridea sp. 3	11	3417	-	-
Smicridea sp. 3	9	3199	1	10
Smicridea sp. 3	12	3012	3	30
Smicridea sp. 3	10	2838	7	70
Smicridea sp. 3	4	2609	-	-
Smicridea sp. 3	1	2372	12	116
Smicridea sp. 3	3	2236	12	120
Smicridea sp. 3	2	2008	2	20
Smicridea sp. 3	5	1867	11	109
Smicridea sp. 3	6	1705	5	50
Smicridea sp. 3	total		55	545

Table 4. Fuzzy code scores for food affinity to dead coarse particulate organic matter (dead CPOM), live particulate organic matter (live CPOM), fine particulate organic matter (FPOM), algae and chitin according to % of microscope optic fields where each food item is present for *Anacroneuria* sp.1, *Baetodes* sp. 5, *Leptohyphes* sp.3 and *Smicridea* sp. 3 collected from tributary streams of the Oyacachi watershed, Napo Province, Ecuador

	Anacroneuria sp.1	*Baetodes* sp.5	*Leptohyphes* sp.3	*Smicridea* sp.3
Dead CPOM	1	0	0	1
Live CPOM	0	0	0	1
FPOM	2	3	3	3
Algae	2	0	2	2
Chitin	3	0	0	1

Table 5. Spearman's Rank Correlation test result summary for size and Simpson's diet diversity index by morphospecies (*Anacroneuria* sp. 1, *Baetodes* sp.5, *Leptohyphes* sp. 3 and *Smicridea* sp. 3) collected from tributary streams of the the Oyacachi watershed, Napo Province, Ecuador

	S		**rho**	**p**
Anacroneuria **sp. 1**	100370.4	-	0.0924	0.409
Baetodes **sp. 5**	41578.65	+	0.0914	0.469
Leptohyphes **sp. 3**	39465.3	+	0.4605	<0.0001
Smicridea **sp. 3**	1879.66	+	0.3236	0.016

Table 6. Spearman's Rank Correlation test result summary for body size and food item proportion by morphospecies (dead particulate organic matter (dCPOM), live particulate organic matter (lCPOM), fine particulate organic matter (FPOM), algae and chitin) for *Anacroneuria* sp. 1, *Baetodes* sp.5, *Leptohyphes* sp, 3 and *Smicridea* sp. 3 collected from tributary streams of the Oyacachi watershed, Napo Province, Ecuador

size on diet composition	**food item**	**S**		**rho**	**p**
Anacroneuria sp. 1	dead CPOM	102079.7	-	0.111	0.3208
	live CPOM	83103.85	+	0.096	0.3933
	FPOM	121284.1	-	0.320	0.0034
	algae	70762.07	+	0.230	0.0377
	chitin	82725.01	+	0.010	0.3731

Baetodes **sp. 5**	dead CPOM	40637.4	+	0.112	0.375
	live CPOM	35961.77	+	0.214	0.087
	FPOM	51443.04	-	0.124	0.324
	algae	46138.73	-	0.008	0.948
	chitin	36631.77	+	0.199	0.111
Leptohyphes **sp. 3**	dead CPOM	51779.52	+	0.292	0.010
	live CPOM	46031.36	+	0.371	0.001
	FPOM	108414.1	-	0.482	<0.0001
	algae	48455.26	+	0.338	0.003
	chitin	50423.3	+	0.311	0.006
Smicridea **sp. 3**	dead CPOM	13981.3	+	0.496	0.0001
	live CPOM	26891.12	+	0.030	0.848
	FPOM	43182.49	-	0.558	<0.0001
	algae	33841.64	-	0.221	0.105
	chitin	24119.08	+	0.130	0.345

Table 7. Summary of results for Kruskal-Wallis test for size group and food category proportion (dead coarse particulate organic matter (dCPOM), live coarse particulate organic matter (lCPOM), fine particulate organic matter (FPOM), algae and chitin) for *Anacroneuria* sp. 1, *Baetodes* sp.5, *Leptohyphes* sp, 3 and *Smicridea* sp. 3 collected from tributary streams of the Oyacachi watershed, Napo Province, Ecuador

| Morphospecies	Food category	Kruskal-Wallis Chi^2	Degrees of freedom	p
Anacroneuria **sp. 1**	Dead CPOM	4,6907	3	0,1959
Anacroneuria **sp. 1**	Live CPOM	1,8987	3	0,5937
Anacroneuria **sp. 1**	FPOM	16,6918	3	0,0008
Anacroneuria **sp. 1**	algae	8,6237	3	0,0347
Anacroneuria **sp. 1**	chitin	2,7003	3	0,4402
Baetodes **sp. 5**	Dead CPOM	3,3655	3	0,3386
Baetodes **sp. 5**	Live CPOM	n.a.	3	n.a.
Baetodes **sp. 5**	FPOM	5,1877	3	0,1586
Baetodes **sp. 5**	algae	3,9679	3	0,2649
Baetodes **sp. 5**	chitin	4,0895	3	0,2550
Leptohyphes **sp. 3**	Dead CPOM	5,7790	3	0,1228
Leptohyphes **sp. 3**	Live CPOM	13,4876	3	0,0037
Leptohyphes **sp. 3**	FPOM	20,2991	3	0,0001
Leptohyphes **sp. 3**	algae	11,2821	3	0,1090
Leptohyphes **sp. 3**	chitin	6,2986	3	0,0980
Smicridea **sp. 3**	Dead CPOM	16,9404	3	0,0007
Smicridea **sp. 3**	Live CPOM	6,5725	3	0,0869

Smicridea **sp. 3**	FPOM	19,1852	3	0,0003
Smicridea **sp. 3**	algae	3,2012	3	0,3616
Smicridea **sp. 3**	chitin	0,3160	3	0,9570

Table 8. Summary results for Principal Component Analysis (PCA) of environmental factors (pH,temperature, dissolved oxygen concentration (mg/L), standard conductivity, slope %, mean width, mean depth, flow, IHF, QBR and mean riparian cover % at 20 m) across eleven stream sites along the Oyacachi watershed, Napo Province, Ecuador

Variable	**PC1**	**PC2**
Temperature (˚C)	-0,299	-0,146
pH	-0,417	-0,119
Dissolved Oxigen (mg/L)	-0,423	-0,081
Standard conductivity (µS)	0,050	-0,472
Slope %	0,002	-0,464
Width (m)	-0,243	0,127
Depth (m)	-0,285	0,343
Flow (L/s)	0,195	0,480
IHF	-0,412	-0,071
QBR	-0,418	0,068
Mean riparian cover % at 20 m	-0,181	0,382
Eigenvalues	4.97	3.01
% Variation	45.2	27.4
Cummulative % Variation	45.2%	72.6%

Table 9. Summary Results for backwards multiple linear regression for environmental fctors (IHF, QBR, dissolved oxygen concentration (mg/L) and pH) and proportion of food types in *Anacroneuria* sp.1, *Leptohyphes* sp.3 and *Smicridea* sp. 3 collected from tributary streams of the Oyacaachi watershed, Napo Province, Ecuador

Morphospecies	Food item	Best fit model	Remaining variables	Beta - coefficients		Model R squared	d.f.	F	p
Anacroneuria **sp. 1**	*FPOM*	3	pH	-	0.350	0.101	85	4.676	0.012
			QBR	+	0.206				
Leptohyphes **sp. 3**	*FPOM*	4	IHF	-	0.329	0.108	75	8.972	0.004
	algae	4	IHF	+	0.341	0.116	75	9.729	0.003

Morphospecies	Food Item		variable		Beta-coefficient	R squared	d.f.	F	p
Smicridea **sp. 3**	*dead CPOM*	3	pH	+	0.235	0.15	54	4.587	0.015
			QBR	-	0.356				
	chitin	3	IHF	-	0.247	0.087	54	2.486	0.093
			dissolved oxygen concentration	+	0.267				

Table 10. Summary results for single regression model for environmental variables (velocity and coarse particulate organic matter (CPOM) concentration (g/m2) and food type proportions for *Anacroneuria* sp. 1, *Leptohyphes* sp. 3 and *Smicridea* sp. 3 collected from tributary streams of the Oyacachi watershed, Napo Province, Ecuador

Morphospecies	Food Item	variable	Beta - coefficient	R squared	d.f.	F	p
Anacroneuria **sp. 1**	FPOM	CPOM concentration	+ 0.409	0.168	35	6.849	0.013
Leptohyphes **sp. 3**	FPOM	CPOM concentration	- 0.328	0.108	29	3.382	0.077
		velocity	+ 0.338	0.114	29	3.614	0.068
	algae	CPOM concentration	+ 0.260	0.067	29	2.022	0.166
		velocity	- 0.343	0.118	29	3.740	0.063
Smicridea **sp. 3**	dCPOM	CPOM concentration	- 0.229	0.052	34	1.823	0.186
		velocity	- 0.434	0.188	22	4.873	0.039
	chitin	CPOM concentration	+ 0.334	0.111	34	4.140	0.05
		velocity	+ 0.218	0.047	22	1.047	0.318

Annex 2 – Figures
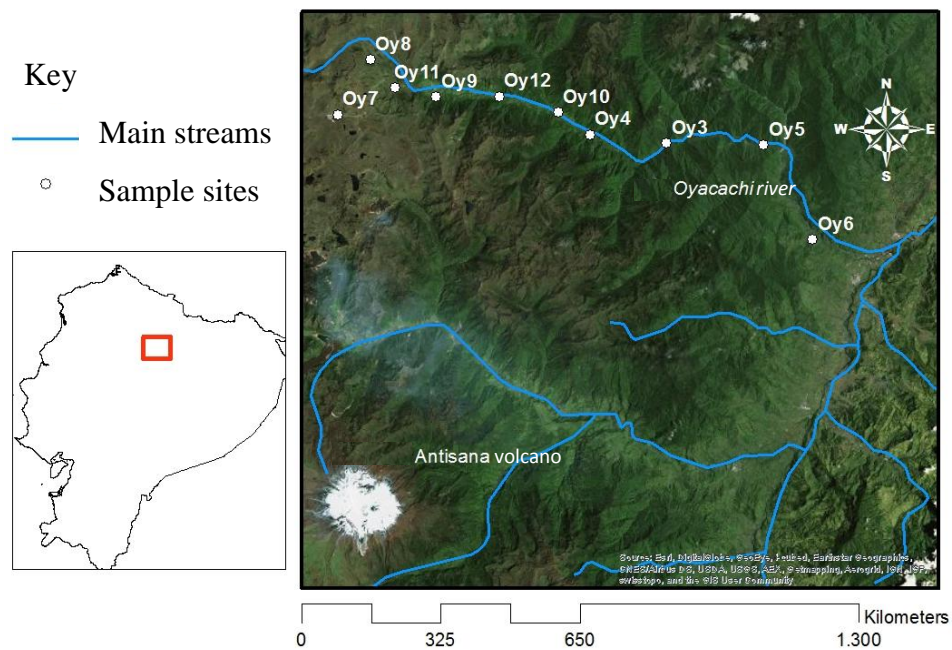
Figure 1. Map of sampled stream sites in the Oyacachi River Basin, Ecuador. Map was developed using images from Google Earth and stream site coordinates.
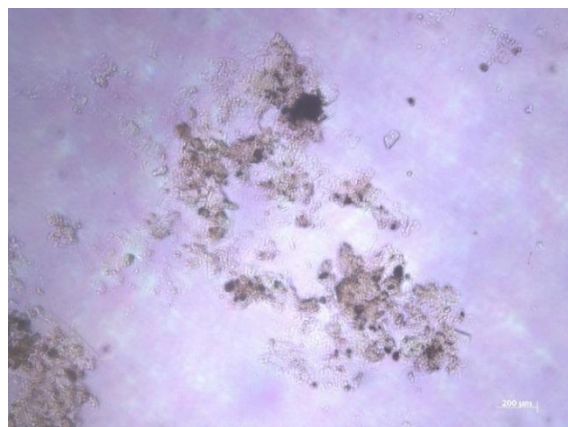
Fine particulate organic matter (FPOM)
(found in *Baetodes* sp. 1)

dead CPOM (found in *Anacroneuria* sp. 1)

Dead coarse particulate organic matter
(dCPOM) (found in *Leptohyphes* sp. 3)
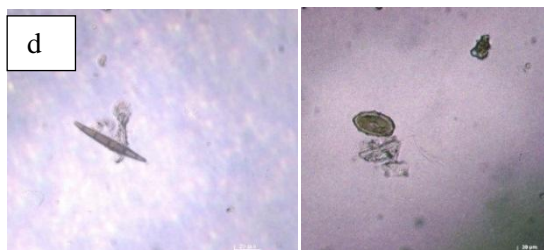
Algae (found in *Leptohyphes* sp. 3 at the top
and *Smicridea* sp. 3 at the bottom)
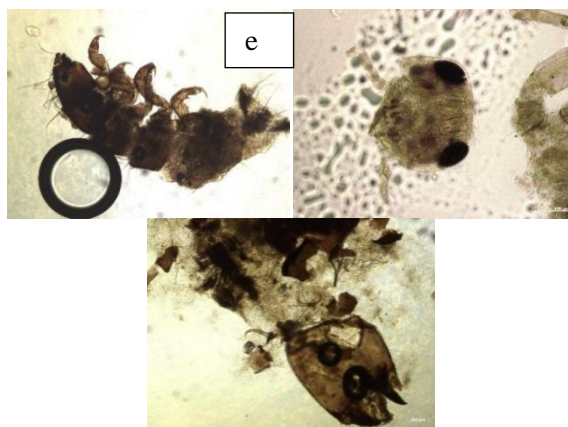
Chitin- or insect prey fragments (found in
Anacroneuria sp. 1)

Figure 2. Sample photographs of food types (a: Fine particulate organic matter (FPOM); b: dead coarse particulate organic matter (dCPOM); c:live coarse particulate organic matter (lCPOM); d: algae; e: chitin (invertebrate fragments)) found in *Anacroneuria* sp.1, *Baetodes* sp.5, *Leptohyphes* sp.3, and *Smicridea* sp.3 collected at the Oyacachi watershed, Napo Province, Ecuador
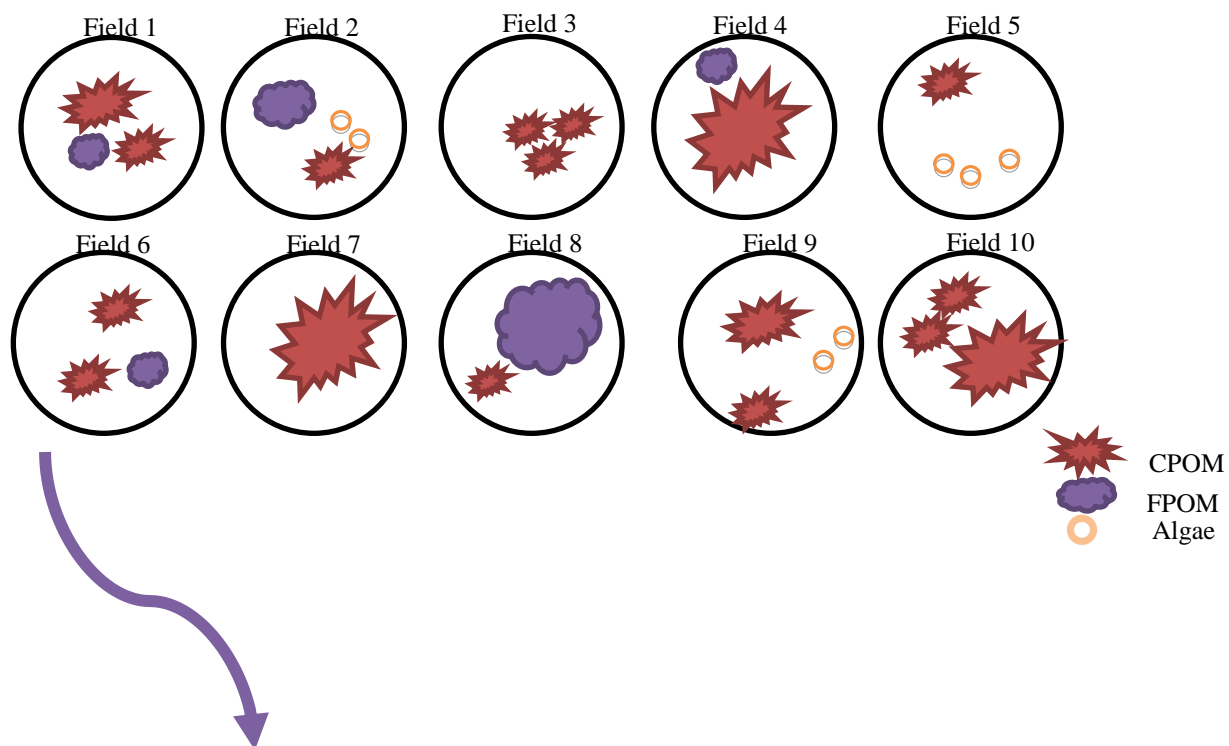
Individual number	Optic field	% CPOM	% FPOM	% Algae	
1	1	15	5	0	
	2	5	10	5	
	3	12	0	0	
	4	30	5	0	
	5	3	0	4	
	6	10	5	0	
	7	0	20	0	
	8	3	20	0	
	9	10	0	3	
	10	30	0	0	Total
	Sum	118	65	12	195
	Total individual percentage	=118*100/195 =60.51%	=65*100/195 =33.33%	=12*100/195 =6.15%	100%

Figure 3. Example of the calculation of individual percentaje of level food type composition (Fine particulate organic matter (FPOM), live coarse particulate organic matter (lCPOM), dead coarse particulate organic matter (dCPOM), chitin and algae) for gut content analysis from hypothetical data
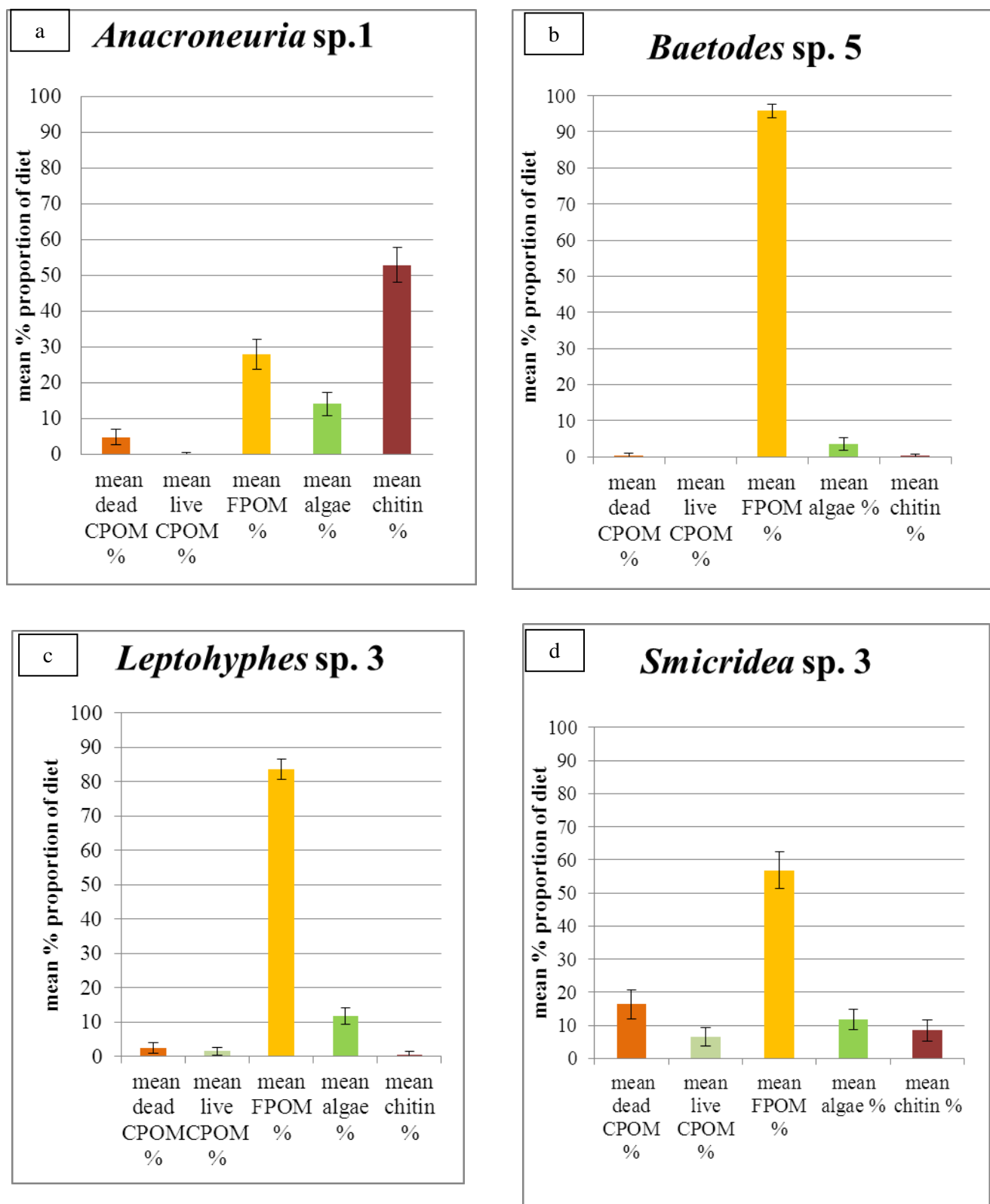
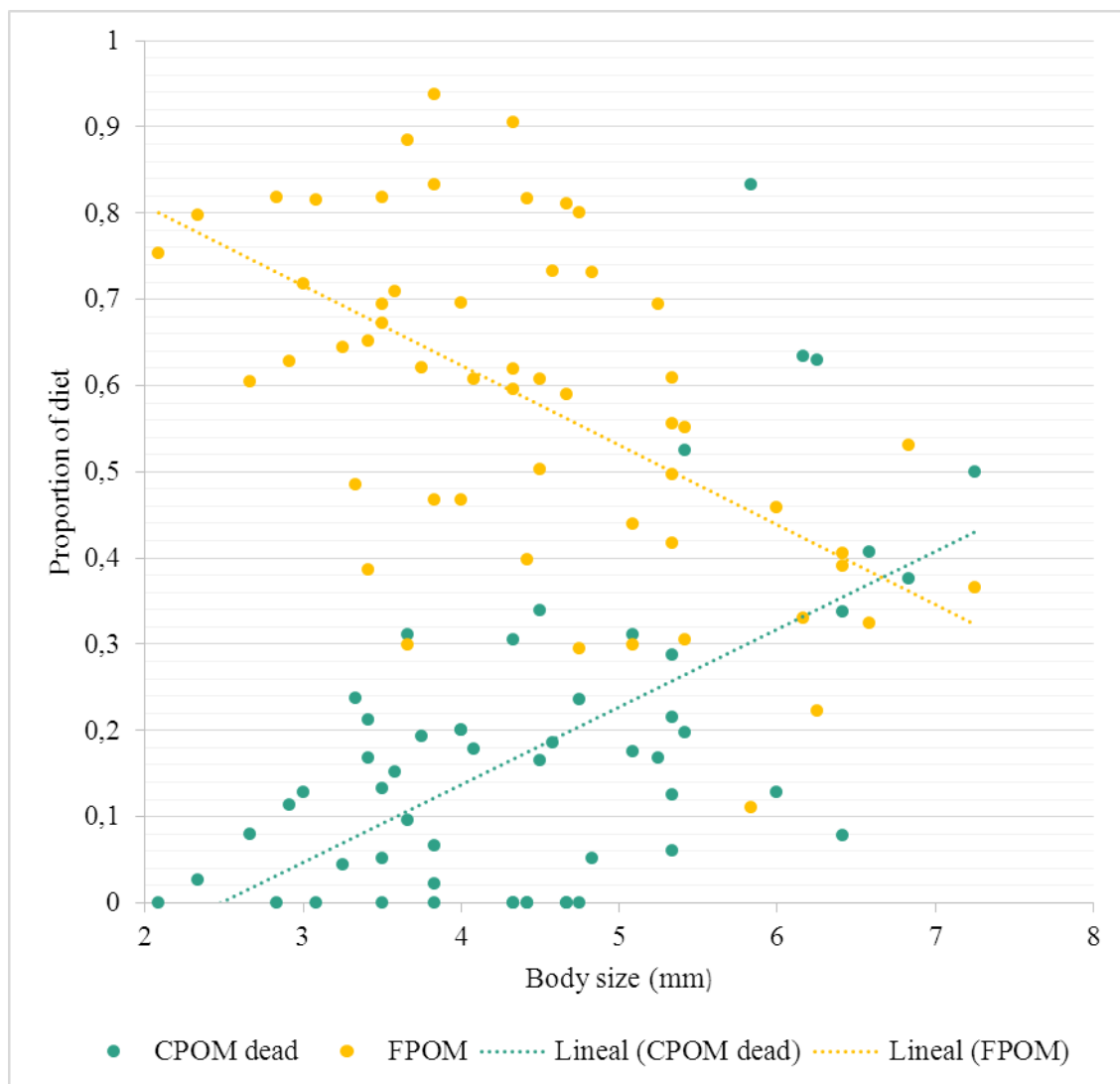*Figure 4.* Mean food type proportion (fine particulate organic matter (FPOM), live coarse particulate organic matter (FPOM), live coarse particulate organic matter (lCPOM), dead coarse particulate organic matter (dCPOM), algae and chitin) for *Anacroneuria* sp. 1, *Baetodes* sp.5, *Leptohyphes* sp. 3 and *Smicridea* sp. 3 individuals collected at the Oyacachi watershed, Napo Province, Ecuador.
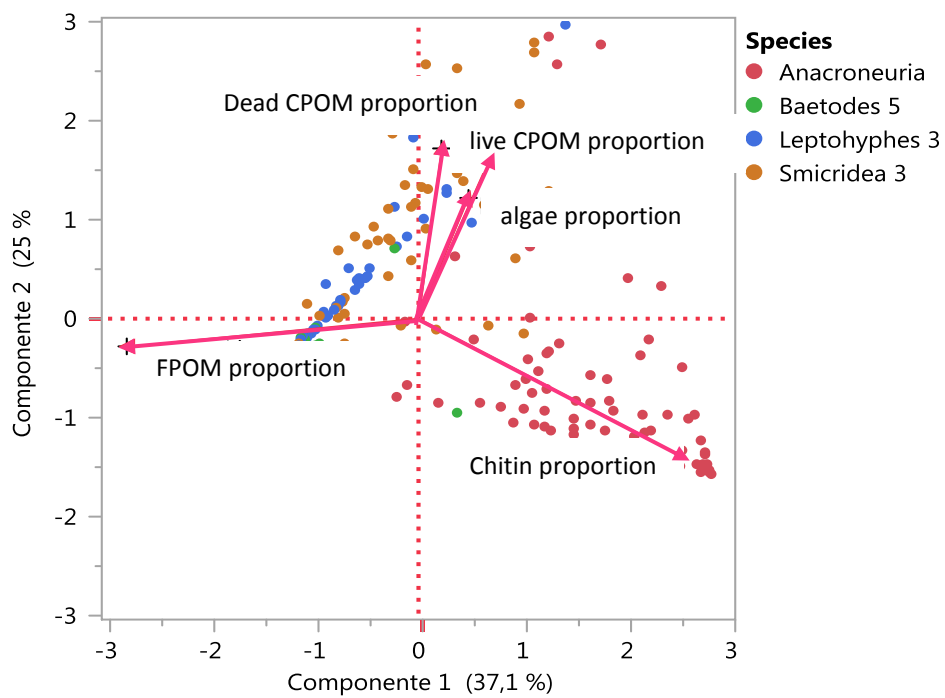
Components and relationship diagram showing... organic matter ...organic matter (dCPOM), algae a_____p. 1, *Baetodes* sp.5, *Leptohyphes* sp. 3 and *Smicridea* sp. 3 individuals collected at the Oyacachi watershed, Napo Province, Ecuador.
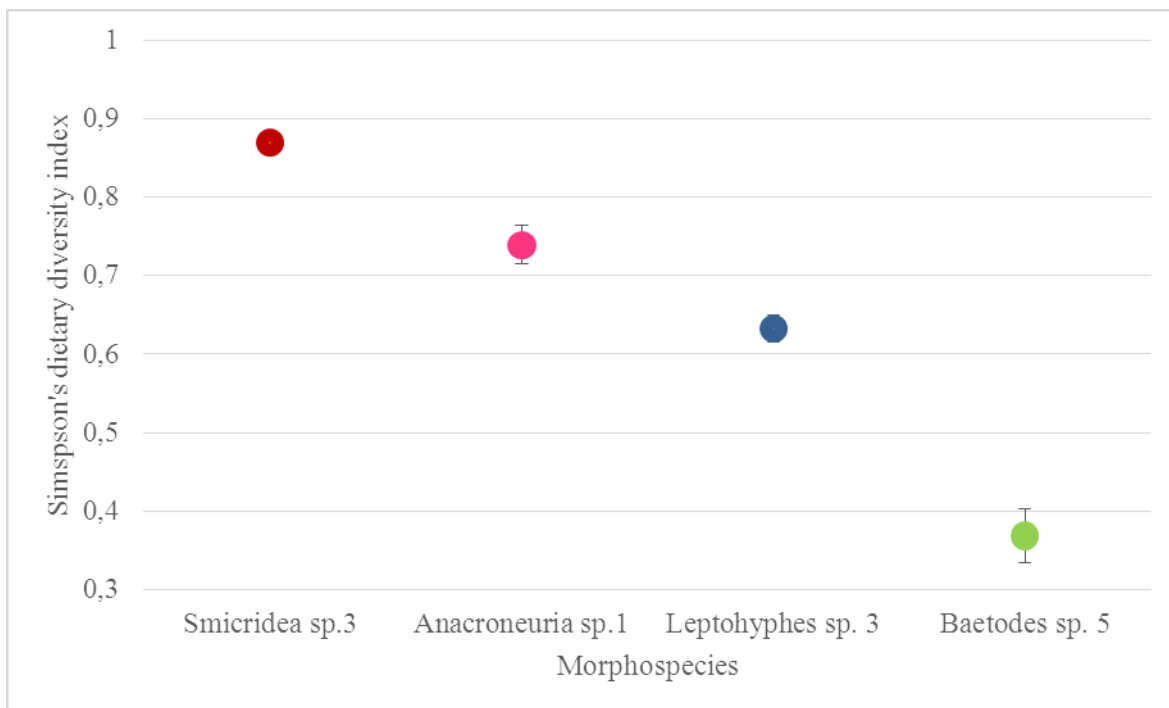
Figure 6. Simpson's dietary diversity index mean by morphospecies (+/- standard error) for *Anacroneuria* sp. 1, *Baetodes* sp. 5, *Leptohyphes* sp. 3 and *Smicridea* sp.3 collected at the Oyacachi watershed, Napo Province, Ecuador
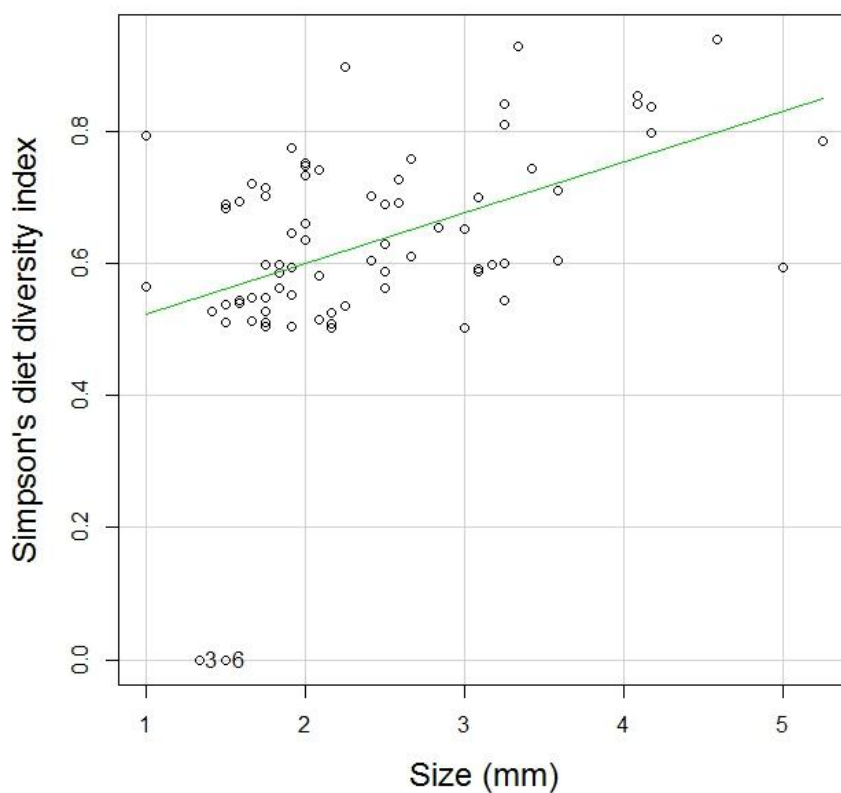
Figure 7. Simpson's diet diversity index by individual body size for *Leptohyphes* sp. 3 collected at the Oyacachi watershed, Napo Province, Ecuador
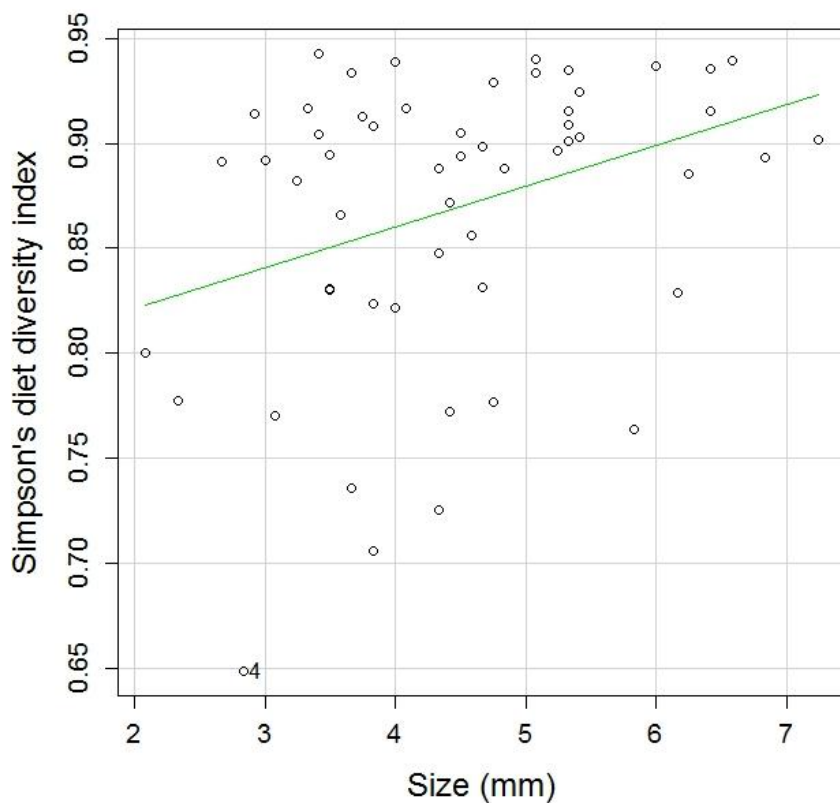
Figure 8. Simpson's diet diversity index by individual body size for *Smicridea* sp. 3 collected at the Oyacachi watershed, Napo Province, Ecuador

Figure 9. Proportion of fine particulate organic matter (FPOM, live coarse particulate organic matter (lCPOM), dead coarse particulate organic matter (dCPOM), algae and chitin in diet by individual body size in *Leptohyphes* sp. 3 collected at the Oyacachi watershed, Napo Province, Ecuador

Figure 10. Proportion of fine particulate organic matter (FPOM) and algae in diet by individual body size in *Anacroneuria* sp. 1 collected at the Oyacachi watershed, Napo Province, Ecuador

Figure 11. Proportion of dead particulate organic matter (dCPOM) and fine particulate organic matter (FPOM) in diet by individual body size in *Smicridea* sp.3 collected at the Oyacachi watershed, Napo Province, Ecuador

Figure 12. Mean proportion of fine particulate organic matter (FPOM) along an elevation gradient (1705 – 3623 masl) in the diet of *Anacroneuria* sp. 1 collected from tributary streams of the Oyacachi watershed, Napo Province, Ecuador
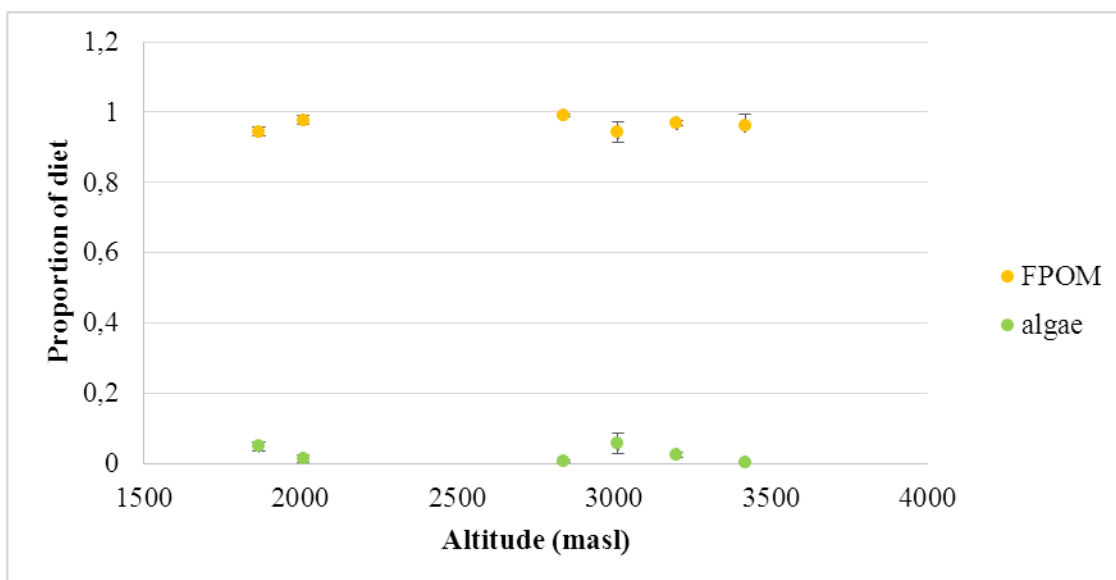
Figure 13. Mean proportion of fine particulate organic matter (FPOM) along an elevation gradient (1867 – 3417 masl) in the diet of *Baetodes* sp. 5 collected from tributary streams of the Oyacachi watershed, Napo Province, Ecuador
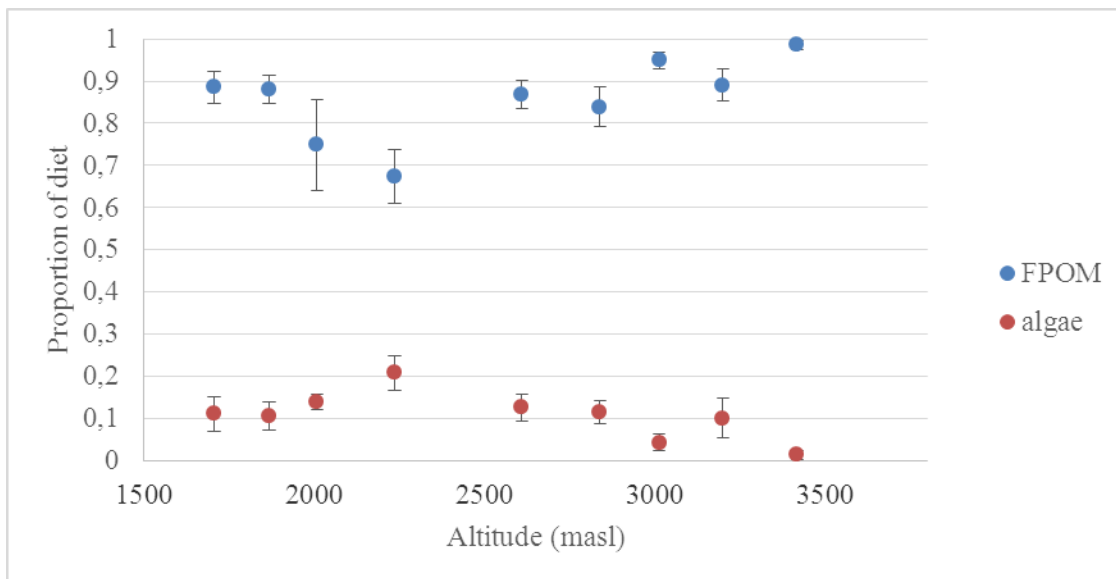
Figure 14. Mean proportion of fine particulate organic matter (FPOM) and algae along elevation gradient (1705 – 3417 masl) in the diet of *Leptohyphes* sp. 3 collected at the Oyacachi watershed, Napo Province, Ecuador
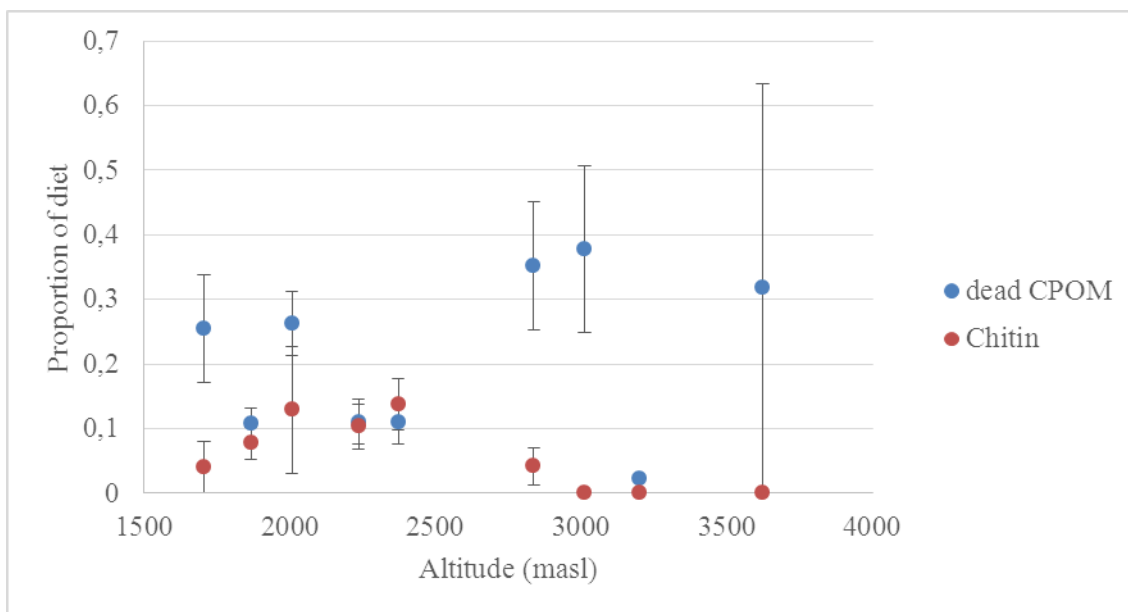

Figure 15. Mean proportion of dead coarse particulate organic matter (dCPOM) and chitin along an elevation gradient (1705 – 3623 masl) in the diet of *Smicridea* sp. 3 collected at the Oyacachi watershed, Napo Province, Ecuador
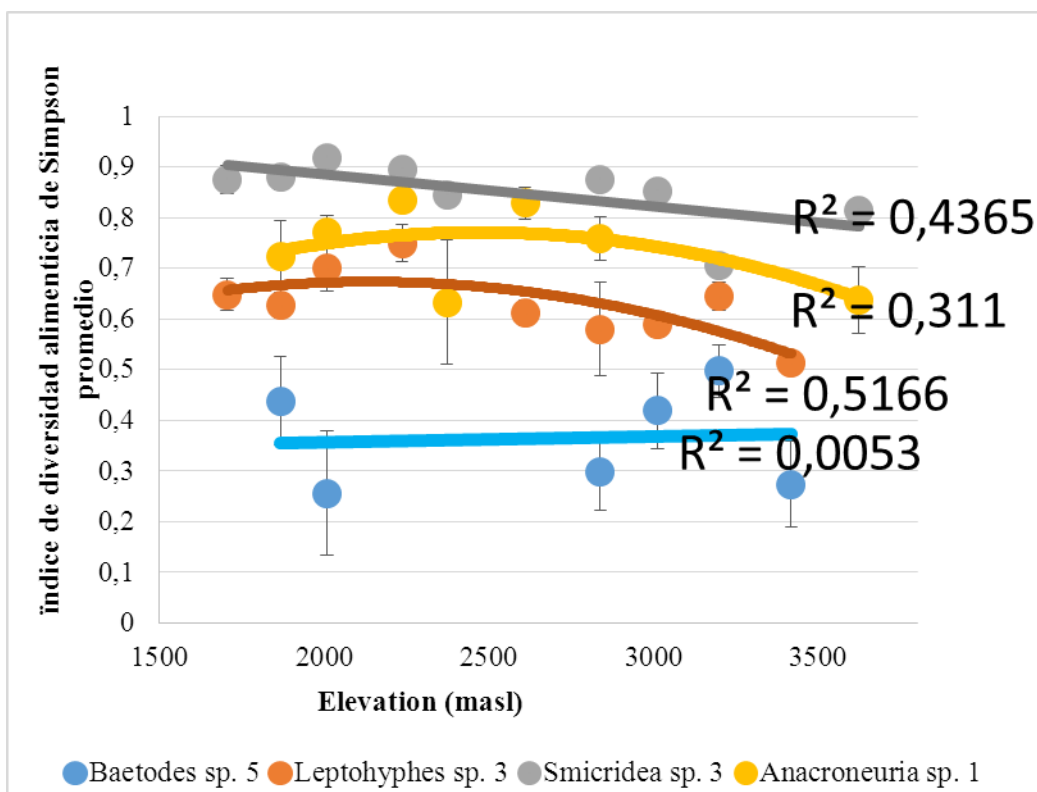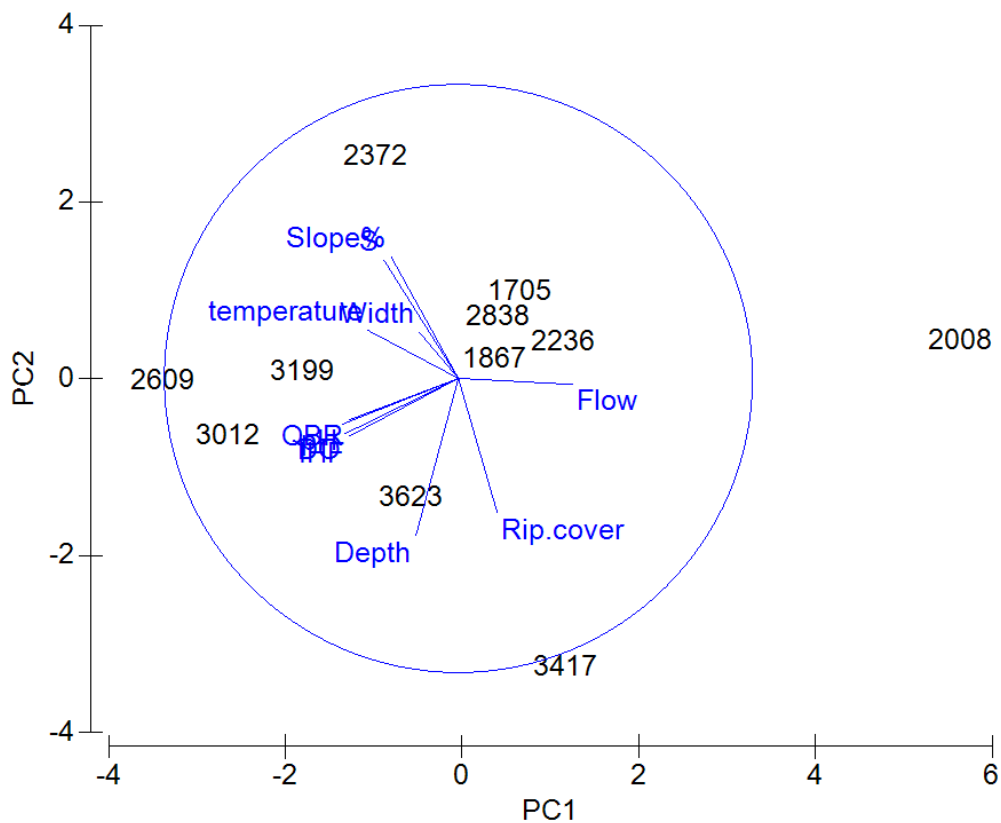
Figure 16. Mean Simpson's diet diversity index for *Anacroneuria* sp.1, *Baetodes* sp. 5, *Leptohyphes* sp. 3 and *Smicridea* sp. 3 along the elevation gradient (1705 – 3623 masl) from the Oyacachi watershed, Napo Province, Ecuador with linear or polynomial regression lines calculated over the mean and corresponding R squared values

Figure 17. Biplot graph of Principal Component Analysis (PCA) for environmental variables (pH,temperature, dissolved oxygen concentration (mg/L), standard conductivity, slope %, mean width, mean depth, flow, IHF, QBR and mean riparian cover % at 20 m) across eleven stream sites along the Oyacachi watershed, Napo Province, Ecuador