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**DISTRIBUCIÓN Y ABUNDANCIA DE TRUCHAS EN RELACIÓN A LAS
COMUNIDADES DE MACROINVERTEBRADOS Y VARIABLES FISICO-
QUÍMICAS EN RÍOS ALTO ANDINOS**

Disertación previa a la obtención del título de Licenciado en Ciencias Biológicas

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Certifico que la disertación de la Licenciatura en Ciencias Biológicas del candidato Rubén Darío Abad Godoy ha sido concluida con conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación correspondiente.

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1. RESUMEN

Cada vez existe una mayor conciencia y necesidad de saber los efectos de las invasiones biológicas. La trucha arcoíris (*Oncorhynchus mykiss*) pertenece al grupo de peces Salmónidos, esta especie se la ha introducido a nivel global, lo que los convierte en un taxón ideal para estudios de invasión. En este estudio se realizó un muestreo a lo largo de un gradiente glaciar en diez estaciones (secciones de 25 m del río) de la Reserva Ecológica Antisana. En cada estación se determinó las características físico químicas e hidromorfológicas de cada río, posteriormente se recolectaron muestras de macroinvertebrados (Muestreo Surber) para así determinar su influencia en la abundancia y distribución de truchas, y finalmente mediante pesca eléctrica se realizó el muestreo de truchas. Los resultados mostraron que los taxones más predominantes en la dieta (análisis de contenidos estomacales) de las truchas fueron *Andesiops* sp. (Baetidae), *Hyallela* sp. (Hyallelidae) y Chironomidae (Diptera). En el análisis de Correspondencias Canónicas (CCA), se determinaron los factores ambientales más influyentes en el largo y peso promedio de las truchas, así como en su abundancia. A partir de nuestros resultados se podría plantear posibles guías base para gestionar estrategias de manejo de truchas en zonas de alto endemismo como los páramos, y así poder aplicar mecanismos apropiados de manejo de esta especie.

Palabras Clave: Abundancia de truchas, ríos alto-andinos, especies invasivas, dieta, densidad de macroinvertebrados.

2. ABSTRACT

Currently there is greater awareness about the necessity of understanding the effects of biological invasions. The rainbow trout (*Oncorhynchus mykiss*) is a Salmonid fish; that has been introduced globally, making it an ideal taxon for studies of invasion. In this study, we sampled throughout a glacier gradient in ten sites (25 m reaches) at the Antisana Ecological Reserve. At each site stream physicochemical and hydromorphological characteristics were determined. Subsequently macroinvertebrate samples (Surber sampling) were collected to determine their influence on the abundance and distribution of trout, and thus, eventually through electrofishing we realized the sampling trout. The results showed that the most dominant taxa in trout diet (analysis of gut contents) were *Andesiops* sp. (Baetidae), *Hyalella* sp. (Hyallelidae) and Chironomidae (Diptera). In the Canonical Correspondence Analysis (CCA), we determined the most influential environmental factors in the average weight and length of the trout, as well as in their abundance. From our results, it could pose functional guidelines for management strategies of trout in areas of high endemism as the paramos, and in that way be able to implement appropriate management mechanisms for this species.

Key Words: Trout abundance, high-Andean streams, invasive species, diet, macroinvertebrates density.

3. MANUSCRITO PARA PUBLICACION

REVISTA: FRESHWATER BIOLOGY John Wiley & Sons.

TITULO: Distribution and abundance of trout in relation to macroinvertebrate communities and physico-chemical variables in high-Andean streams

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INTRODUCTION

Invasive species are a major effect of global change and cause of biodiversity loss worldwide (Fausch *et al.*, 2001; Baxter *et al.*, 2004; Rahel & Olden, 2008; Labonne *et al.*, 2013). Primarily related to increased transport rates and human commerce, the introduction of non-native species has followed an exponential increase, profoundly altering terrestrial and marine ecosystems (Gurevitch & Padilla, 2004). The effects of invasive species can vary from undetectable to severe and their influence on freshwater systems can be at the individual, population, community or ecosystem level (Simon & Townsend, 2003). Among the alterations, changes in trophic networks are especially important, since their effect may spread across the network resulting in increased and/or decreased in biomass of a group of organisms at any of the trophic levels (Bell, Neill & Schluter, 2003). The effects of introducing non-native species has been studied mainly in temperate areas. There are considerably less published studies from tropical regions and very few from tropical mountains (Vimos, 2010).

The rainbow trout, *Oncorhynchus mykiss*, is one of the most widely introduced fish species worldwide (Fausch, 2007; Shelton, Samways & Day, 2015). It belongs to the order Salmoniformes, specifically, to the family Salmonidae. Adult and juvenile trouts are characterized by being opportunistic feeders, their diet consisting mainly of macroinvertebrates, fish, small amphibians, and occasionally, algae (Raleigh *et al.*, 1984; Toobaie & Grant, 2013). Numerous studies have reported their level of affection worldwide in at least 97 different countries and important regions including

Africa (Shelton, Samways & Day, 2015), Japan (Kitano, 2004), South America (Pascual *et al.*, 2001; Buria *et al.*, 2007; Vimos, 2010; Kadye *et al.*, 2013; Arismendi *et al.*, 2014), Holartic regions (Fausch *et al.*, 2001), New Zealand (Jowett, 1990), and to every continent except Antarctica (Crawford & Muir, 2008).

The Tropical Andes have been the subject of continuous introductions of aquatic animals for aquaculture (Saint-Paul, 1992; Ortega, Guerra & Ramírez, 2007). Specifically in the Ecuadorian Andes, rainbow trout introductions began in 1928, with embrionated eggs and from there, their fry populated the river systems of the interandean zones (Mora, Uyaguari & Osorio, 2004). Subsequent introductions of this and another species (*Salmo trutta*, commonly known as brown trout) have taken place ever since and are still going on, especially for sport fishing (Ron *et al.*, 2003), with little regard on their potential impact for native species and ecosystems. Both species of trout can be found in many rivers and reservoirs in the provinces of Azuay, Carchi, Imbabura, Pichincha and Napo (Mora, Uyaguari & Osorio, 2004). In spite of this, little is known about the impact they cause in these delicate ecosystems.

The paramos are exclusive Andean ecosystems with unique characteristics, such as: high pluviosity (1000-2000 mm annually), low mean temperatures (4-10° C), high humidity, deep organic soils, and high levels of endemism of both plants and animals (Pontón Cevallos, 2012). The paramos are very rich in water resources, which give birth to many headwater streams that are of vital importance as main sources of water, sediments, and biota for downstreams reaches (Sidle *et al.*, 2000; Clarke *et al.*, 2008).

Also, these streams are important sites for organic matter processing, nutrient cycling, and are therefore crucial for maintaining the “health” of the watersheds and to ensure the provision of ecosystem services (i.e. water purification and water quality) delivered by streams and their biota (Bernhardt *et al.*, 2005; Clarke *et al.*, 2008; Cardinale, 2011). A study by Dangles *et al.* (2011) found that even though paramo streams are poor in species, some of them may be considered crucial for maintaining ecosystem processes in these extreme ecosystems. In addition, these authors found that positive interspecific interactions may be increasing process rates in these streams. This implies that changes in species abundance or composition in these streams may have effects at the ecosystem functioning level. In spite of their importance, high-altitude streams in the tropics are, probably, one of the least studied ecosystems on earth (Jacobsen, 2008). More studies should be carried out to understand the functioning of these ecosystems and the potential impacts of global changes on the services they deliver.

We know very little about the distribution and abundance of invasive fish in paramo streams and even less about their impacts on native biota and ecosystem functioning (Vimos, 2010). Here we present the results of a study that aimed to investigate the distribution and abundance of trout in 10 high-Andean streams of different origin (glacier, rain or superficial drainage), in the Ecuadorian Andes. The selected streams vary dramatically in physicochemical and hydrological conditions (Jacobsen *et al.*, 2010; Andino, 2014; Espinosa, 2014), and we expect not to find any

trout in glacier streams, where conditions are very hostile and have limited availability of food. Also, through gut content analyses and surber sampling, we generated preliminary information about the possible effects of these fish on communities of stream benthic macroinvertebrates and on stream trophic chains.

METHODS

Study area

The current study was conducted in 10 streams located in the Antisana Ecological Reserve (REA in Spanish) in Ecuador, during the month of November of 2014. The Antisana Ecological Reserve (REA) is propitious for this study because it is rich in streams, whose biotic and abiotic characteristics have been studied for several years by the Stream Ecology team of PUCE in Quito, Ecuador. In addition, its wetlands and lagoons of its paramos supply water to a large part of Quito's metropolitan area (Rivera Rossi, 2007).

All sites are tributaries of the Antisana River, which flows into the Napo River, a main tributary of the upper Amazon River (Jacobsen *et al.*, 2010). The streams originate in the Antisana Volcano, located at the Eastern cordillera of the Ecuadorian Andes, 50 kilometers southeast from Quito, in the province of Napo (Rivera Rossi, 2007). All the streams included in this study present natural conditions with low anthropogenic alteration. There is a high inter stream variability with respect to physical and chemical conditions, related to the confluence of waters from different origins: glacier (G), spring (S), and superficial drainage (D) (Andino, 2014; Espinosa, 2014). The names of the streams used for this study correspond to those in Andino (2014) (Figure 1).

Environmental variables

At each sampling site we characterized the physicochemical variables by measuring water temperature, conductivity (at 25°C), pH, and dissolved oxygen. These were obtained using WTW portable meter series (WTW GmbH, Xilem Inc, Munich, Germany). All measurements were performed before conducting the trout sampling (see below), in order to minimize changes in physico-chemical conditions related to electro fishing. In the streams with glacier influence we performed the measurements during morning hours to avoid possible changes caused by an increased flow related to glacier melt.

To estimate benthic algal biomass we measured Chlorophyll a (ChloA) concentration at each site. For this we collected 3 pebbles (avoiding those with filamentous algae) from 3 different segments along a 25m reach. The 3 pebbles were stored together in plastic containers, covered with 96% ethanol and left in the dark for 1-7 days until further processing in the laboratory. Later, a sample of ethanol was analyzed in a spectrophotometer and absorption (*Abs*) was measured at 665 and 750 nm. Stone surface area was estimated using the formula: $A = ((LW) + (LH) + (WH)) * 1,15$, where *L* is length, *W* is width, and *H* is height (all in cm), and 1,15 corresponds to a fixed factor to correct for the irregular shapes of the stones (Graham, McCaughan & McKee, 1988; Jacobsen *et al.*, 2010). We used equation 1 to calculate Chlorophyll α concentration:

$$ChloA = \frac{Abs_{(665-750)} \cdot V \cdot 10000}{83.4 \cdot A} \quad (1)$$

Where V is volume in ml, 83.4 corresponds to the absorption coefficient for chlorophyll in 96% of ethanol, and 10000 is the coefficient used to transform stone area (A) from cm^2 to m^2 (Københavns Universitet, 1977; Jacobsen *et al.*, 2010). Also, we complemented our environmental results with data obtained by Andino (2014).

Macrobenthos sampling

In the aforementioned reaches at each stream, three quantitative Surber samples (500 cm^2 ; mesh size $200 \mu\text{m}$) were collected from pebble-cobble substratum in riffle/run habitats. All samples were collected during daytime (before glacier melt at glacier streams) and preserved in 75% ethanol. In the laboratory, samples were rinsed through a 200μ sieve and sorted without the use of magnification. Invertebrates were identified to family or genus level according to North and South American macroinvertebrate keys (Roldán-Pérez, 1988; Domínguez & Fernández, 2009), and counted using a stereoscope (OLYMPUS SZ-6145).

Electrofishing

Electrofishing is the most common fish sampling technique used in rivers, streams and wadeable waters (Lobón-Cervía, 1991; Péfaur, 1995; Sostoa, García de Jalón & García-Berthou, 2005; Maldonado-Ocampo *et al.*, 2005). This technique is particularly useful in situations where other techniques, such as netting, may be

ineffective due to the nature of the species you wish to capture (Barony College, 2007). For the application of this method conductivity (in $\mu\text{S}/\text{cm}$) measured at the fishing site is used to grade the intensity of the power converter. Also, conductivity determines the ease with which electricity passes through a conductor (Barony College, 2007). In this step, the intensity of current necessary for fishing decreases as conductivity increases. In streams with low conductivity ($< 75 \mu\text{S}$) the voltage needed is about 1kW. In streams with higher conductivities stronger power units are needed because the batteries are too rapidly discharged (Bohlin *et al.*, 1989). The temperature also influences the conductivity (conductivity increases with temperature) thus, electrofishing is more effective in streams with higher temperatures. (Sostoa, García de Jalón & García-Berthou, 2005).

For this study, at each site, a 25 m-long stream reach was isolated, using two fine mesh (4 mm) stop nets at the upstream and downstream ends of the reach, in order to keep fish from escaping from the sampling reach and also from arriving from other reaches. A multipass electrofishing campaign was then conducted. We passed the number of times necessary until no more fish were collected (two or three passes were enough for all streams). For further information on electrofishing see Bohlin *et al.* (1989); Sostoa, García de Jalón & García-Berthou. (2005). Captured fish were placed in plastic buckets filled with water, and then transferred to containers installed on the shore. Each captured trout was weighed (in g) using a digital weighing scale, and measured in mm (total length TL) using a measuring board. For our records, each

specimen was photographed next to a ruler (Sostoa, García de Jalón & García-Berthou, 2005).

Finally, we induced the captured trout to vomit, and the regurgitated sample was stored in Ziploc bags in 75% alcohol with their respective tags (Vimos, 2010). Regurgitated samples were brought to the lab for later identification.

Data analyses

We performed a Canonical Correspondence Analysis (CCA) in order to understand the influence of environmental variables on trout metrics (abundance, average weight and average size). Initially, data for seventeen environmental variables were analyzed for correlations in order to avoid over fitting our model, of these seventeen variables, we only obtained four of them (pH, conductivity, temperature and oxygen), the others were obtained by Andino (2014). Eight of these variables were kept (Appendix 1) and transformed them into a logarithmic scale with $\log_{10}(x+1)$ to obtain homogeneity of variance and normality on the data. These procedures were performed using the PAleontological STatistics (Past) Software V.2.17. We also analyzed the relationship between GI and trout abundance through a regression analysis.

In order to understand the relationship between taxa found in Surber samples and that found in gut contents of trout at each site, we first computed Simpson's diversity index (Simpson 1-D) for each. Additionally, we obtained the Evenness (Evenness

e^H/S) and Fisher alpha (Fisher Alpha) indices (Appendix 5). All of these diversity indices were calculated using the (Past) Software V 2.17. To test diet composition vs. food availability in the benthos (taxa in Surber samples), we used a simple index of percent use minus percent availability (Carlisle *et al.*, 2012). For each site, we subtracted the percentage of taxa available (combining all the Surber samples for each site) from the percentage of each taxa in gut contents (combining all trout from each site). Then we used a sign test to determine which taxa is significantly preferred or avoided. The sign test was realized using Statistical Package for the Social Sciences (SPSS) software.

RESULTS

Sampling trout in streams

The only species of trout found in the study was *Oncorhynchus mykiss* (rainbow trout). We collected and analyzed a total of 230 individuals of *O. mykiss* with sizes between 23 and 383 mm in length, and weight between 0.1 and 209.5 g (Figure 2).

Abundance and distribution of trout and its association with biotic and hydromorphological variables.

Abundance of rainbow trout varied widely between streams in relation to the glacier index (GI), showing variable rates of trout abundance in streams. Also, we didn't found a tendency between both variables. Also, we found no significant relationship between them. ($R^2 = 0.004$; $P = 0.874$). (Figure 3).

Environmental and hydromorphological variables considered for this study varied greatly depending on the stream. Of the seventeen different variables considered for this study (Appendix 1), only eight were used for the Canonical Correspondence Analysis (CCA). These were: pH (l/logH⁺), conductivity ($\mu\text{S}/\text{cm}$), turbidity (NTU), channel slope (m), discharge (l/s), chlorophyll, temperature and depth CV (Figure 4). In the Canonical analysis we considered three response variables: (abundance, average weight and average length of trout). These factors contributed to explain the amount of variance between stream, environment and trout interaction. Our results show that conductivity, chlorophyll and pH are associated with the abundance of trout, and

conductivity is slightly associated with the average weight of trout. The streams most influenced by turbidity are G1 and G2, with significantly lower conductivities and temperature than the other streams, and at the same time the turbidity was the variable most highly related to the length of the trout, in addition to discharge, channel slope, temperature, and depth CV.

Macroinvertebrates and trout

A total of 15 102 specimens were collected, 8326 in stomach contents (Appendix 3) and 6776 in Surber samples (Appendix 4). Regarding the stomach contents, all the specimens were captured from nine out of ten sampled sites (one was excluded for absence of trout specimens). Most numerous taxa belonged to Diptera (36.01%), Amphipoda (31.76%), Ephemeroptera (12.04%), Trichoptera (9.23%) and Coleoptera (7.37%) (Table 1).

In the correlation between density of macroinvertebrates (MI) and trout abundance we found a direct relationship and a highly significance effects between both ($R^2=0.6458$; $P = 0.009$) (Figure 5a). Also, the correlation between Surber diversity (SD) and Diet diversity (DD) showed no correlation between both indices and no significant interaction among these two variables ($R^2= 0.029$; $P = 0.661$) (Figure 5b).

Taxa most commonly found in diets were *Andesiops* sp. (Baetidae), *Hyallela* sp. (Hyallelidae) and Chironomidae (Diptera) with percentages ranging from 28 to 69 % (Appendix 2).

Sign test for diet (consumption vs. availability) showed preference and significant avoidance of food in two taxa. The two significantly avoided taxa were *Alluaudomyia* sp (P = 0.004) and *Stilobezzia* sp (P = 0.004). Only a few taxa showed positive values, for they were highly consumed. The highly consumed species were *Andesiops* sp. (Baetidae), and *Hyallega* sp. (Hyallegidae), followed by *Prionocyphon* sp. (Scirtidae). In contrast, the most avoided species were Chironomidae (Diptera), followed by *Neoelmis* sp. (Elmidae) and *Stilobezzia* sp. (Diptera). Additionally, at the stomach contents we found other taxa that were not present at the Surber samples. These taxa represented only a small percentage, and most of them were flying adults (Table 2).

DISCUSSION

Sampling trout in streams

As previously mentioned, two species of trout were introduced in the rivers and streams of Ecuador (Mora, Uyaguari & Osorio, 2004). Rainbow trout, *O. mykiss*, has been previously found in high altitude streams in Ecuador (Ron *et al.*, 2003) although, to our knowledge, no data exists from extremely high altitude streams like the ones sampled in this study. Our data shows a high number of these exotic fish along the sampled streams. Also, their sizes and weights were very variable (Figure 2), and this could be due to the availability of resources like food from the stream (highly related with the abundance of macroinvertebrates in the stream). In general terms, as glacier influence declines, the size of the trout increases, and its ranges of sizes are more variable (pers. obs). According to a study demonstrated that in temperate zones the food availability varies widely, due to the seasons, and this affects the size and weight of the trout. While in tropical zones the length-mass relationship did not vary too much because there are no seasons. (Allan, 1982).

Abundance and distribution of trout and its association with hydromorphological variables.

The correlation between the GI and abundance of trout was better adjusted to a lineal model (Figure 3). Streams with medium glacier influence show relatively high channel stability and warmer temperatures that probably allow other zoobenthic taxa

to colonize these reaches, or even reaches close to the glacier when glacial runoff is low (Milner *et al.*, 2001). Therefore GI represents a stress or severity gradient and constitutes a natural disturbance to communities. However new results indicate that species richness is maximized under intermediate glacial contribution which could be in accordance to the intermediate disturbance hypothesis (McGregor *et al.*, 1995; Brittain & Milner, 2001; Catford *et al.*, 2012; Andino, 2014).

Stream conditions are influenced greatly by the confluence of their waters. Streams can be thus classified by their origin. In the Antisana catchment streams originate from either glaciers (G), springs (S), or superficial drainage (D) (Jacobsen, 2008; Andino, 2014; Espinosa, 2014). Glacier streams (G1 and G2) have the most variable conditions. At the same time these glaciers are particularly sensitive to climate change because they are constantly close to the daily melting conditions (Vuille *et al.*, 2008), a dynamic typical for tropical glacier streams (Espinosa *et al.*, 2010; Jacobsen *et al.*, 2010). Also, we found that G1 and G2 streams had the lowest levels in both temperature and conductivity. In temperate zones, temperature is much lower in glacier, compared to spring and superficial drainage fed streams, having a maximum water temperatures below 10⁰C (Milner *et al.*, 2001). While in the tropics, temperature of glacier-fed streams did not follow that model, because it is highly variable throughout the day (Jacobsen *et al.*, 2010). Water turbidity tended to increase with altitude as one gets closer to the glacier snout (Espinosa, 2014). Glacier streams indicates a higher rate of dissolved solids (Cadbury, Milner & Hannah, 2011). CCA

shows that turbidity is one of the variables that most influenced the length of the trout probably due to the low penetration of light into the water and the presence of glacier sediment (Jacobsen & Bojsen, 2002; Espinosa *et al.*, 2010), which might affect directly the acquisition of food. According to the results of our CCA, depth CV influences negatively trout abundance and weight, this could be due to the fact that the glacier streams have a depth CV highly variable throughout the day, making it difficult to obtain food for trout and to seek more stable streams (Jowett, 1990). Also, chlorophyll showed a clear relationship with abundance. A study about the relationship between brown trout to chlorophyll showed that this is clearly related with the macroinvertebrates. These fish are strong visual predators that feed primarily during the day, they feed macroinvertebrates highly related with herbivory, which are capable of removing a large fraction of phytoplankton (measured in chlorophyll a concentration) (Simon & Townsend, 2003; Baxter *et al.*, 2004). Therefore, the abundance of trout will be higher in streams with higher concentrations of chlorophyll (Figure 4).

Macroinvertebrates and trout

Contrary to the study of Vimos (2010) and Jowett (1990) streams with high density of macroinvertebrates also reported high trout abundance (Figure 5a); except for one spring stream (S1), which, despite having a medium density of MI (462), presented no trout. We suspect this may be due to the fact that the stream bed was mainly composed of sand and trout probably need a different substrate for spawning

and as shelters (Vimos, 2010). According to the Simpson's diversity indexes (Figure 5b), Surber diversity and Diet diversity showed no correlation (S1 stream was not considered for the analysis and had a value of 0,6291). But the diversity at the Surber samples were bit higher in our samples. According to other studies, rainbow trout forage selectively on invertebrates (Nakano *et al.*, 1999), and trout can quickly shift to foraging on benthos when drifting prey are reduced (Fausch, Nakano & Kitano, 1997). In agreement to Baxter *et al.* (2004), this selective behavior has important food web implications by reducing the Diet diversity.

Three groups of MI were the most predated: *Andesiops* sp. (Baetidae), Chironomidae (Diptera) and *Hyallela* sp. (Hyallelidae) (Appendix 2). This is consistent with what was reported by Buria *et al.* (2007) and Vimos *et al.* (2010). The preference of these taxa must be due to the fact that they are the dominant taxa in the drift, and this is consistent with our study because in all the streams the most predated taxa were also the most frequent into drift (Cueva, 2013). The distribution of Chironomids and other important groups like *Andesiops* sp. and *Hyallela* sp. and changes in the distribution of these taxa is of particular interest in glacier-fed rivers as the degree of glacial influence changes (Milner *et al.*, 2001). According to Dangles *et al.* (2011), they explain that *Hyallela* sp. and *Andesiops* sp. are species of high interest to grass decomposition rates. Therefore, trout could be affecting ecosystem processes on the streams. Also these groups may display considerable species richness and notably, glacial floodplains can represent hot-spots of environmental and species

diversity in view of their considerable temporal and spatial heterogeneity (Brittain & Milner, 2001). So, effects of trout may be difficult to anticipate without in-depth understanding of food web relationships.

In the analysis of consumption vs. availability, the most preferred species were *Andesiops* sp, *Hyallela* sp and *Prionocyphon* sp, and the most avoided species were Chironomidae, *Neoelmis* sp and *Stilobezzia* sp (Table 2). Other taxa were consumed frequently but in proportions relatively equal to or less than their availability. In spite of Chironomidae being one of the most consumed species, it's not one of the most preferred. The values in the Surber sample overcast the consumed value meaning that the percentage of Chironomidae in the environment is higher in contrast to the diet sample. We suggest Chironomidae is being avoided by trout, in relation to the percentage eaten. Also, this test didn't show consistent values between the consumptions vs. availability, so the values were only determined by positive and negative signs of preference (Carlisle *et al.*, 2012).

Although there are extensive studies on the impacts of trout in many scales. This study provides preliminary results about their impact on native communities in areas of high endemism such as paramos and how the distribution of this aggressive species adapts easily in the ecosystem. Moreover, at the Ecuadorian Andes there are limited and almost null studies about the effects of trout. So, this study could also serve as a base document to generate appropriate conservation decisions.

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4. NORMAS PARA PUBLICACION

Instrucciones para autores

A single file should be prepared containing the title page, Summary, Text, Acknowledgments, References and tables (see guidelines below).

Additional files may be created for each figure. Microsoft Office 2007/2010 file formats (i.e. .docx, .xlsx etc.) are acceptable on SIM.

- Please leave the right-hand margin unjustified
- Turn the hyphenation option off
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(a) *Title page.* This should include the title. List of author's names, Institute or laboratory of origin, Name, Postal address and email address of the author to whom proofs should be sent. an abbreviated title for use as a running head line and five keywords, which should be relevant for literature searching and each normally comprising not more than two words.

(b) *Summary.* All papers should include a summary. In short numbered paragraphs, limited to about 3% of the length of the text, and in any case to not more than 500 words. This should provide a concise statement of the scope of the work and its principal findings and be fully intelligible without reference to the main text.

(c) **Introduction.** This should contain a clear statement of the reason for doing the work. Outlining essential background information but should not include either the results or conclusions.

(d) **Methods.** This should be concise but provide sufficient details to allow the work to be repeated.

Product and manufacturer names: Where specific named materials/products are mentioned or named equipment used (including software packages). These should be identified by their manufacturer. Followed by the manufacturer's location (e.g. town. state. country), or a source reference should be given if a standard or replicated procedure is being followed.

(e) **Results.** This should not include material appropriate to the Discussion.

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There are no formal limits to the length of papers, but page space in the journal is tight, and most papers (except review articles) should be no longer than 9,000 words in total (text plus references, excepting Figs and Tables).

5. FIGURES

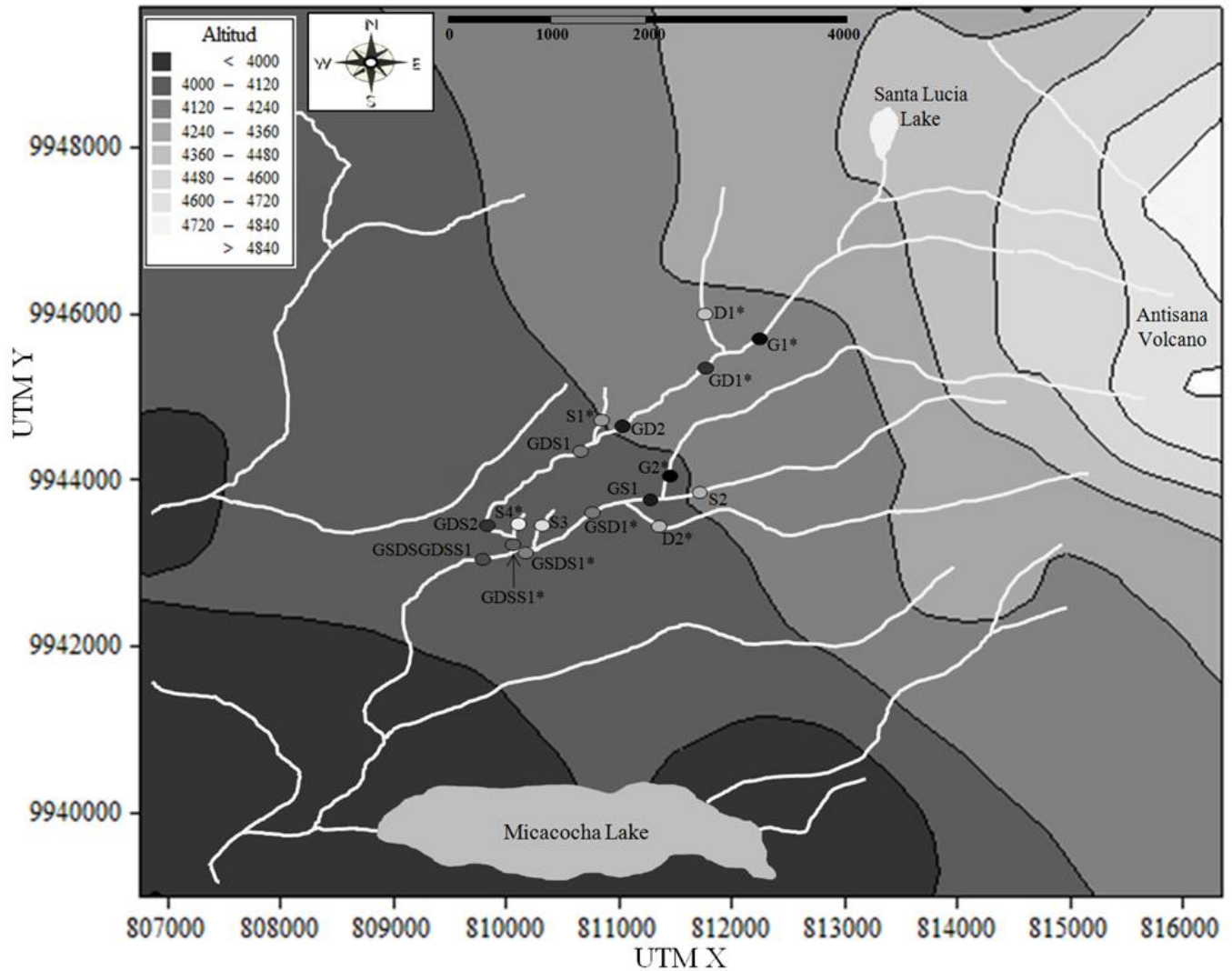


Figure 1. Topographic map of the study area with the 17 study sites at the foothills of the snowcapped Antisana volcano studied by Andino (2014). The streams were named according to their source: glacier (G), spring (S), and superficial drainage (D). The study sites were G2, D2, GSD1, GD1, G1, D1 GDS2, S1, S4, GDSD1. Taken from Andino (2014).

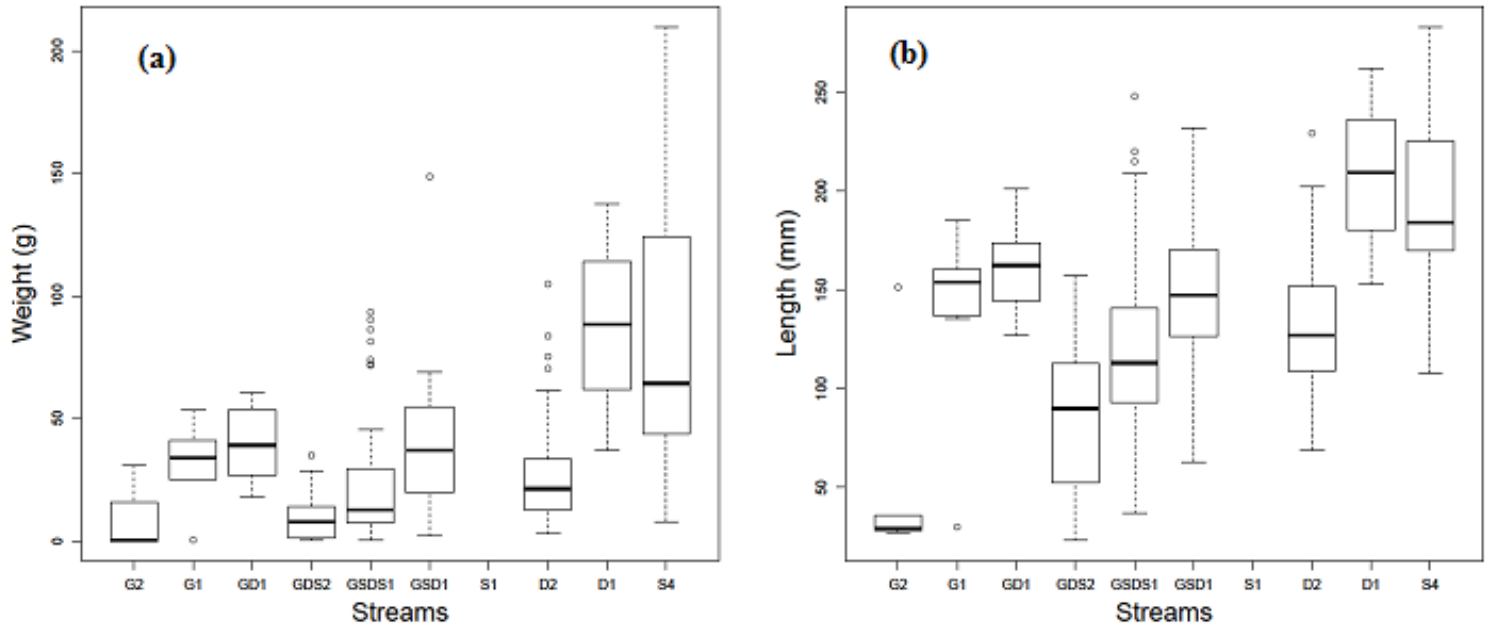


Figure 2. (a) Box plots of the variation of weight (g) of trout found in the 9 study sites (S1 was excluded for absence of trout); (b) Box plots of the variation of length (mm) of trout found in the 9 study sites (S1 was excluded for absence of trout).

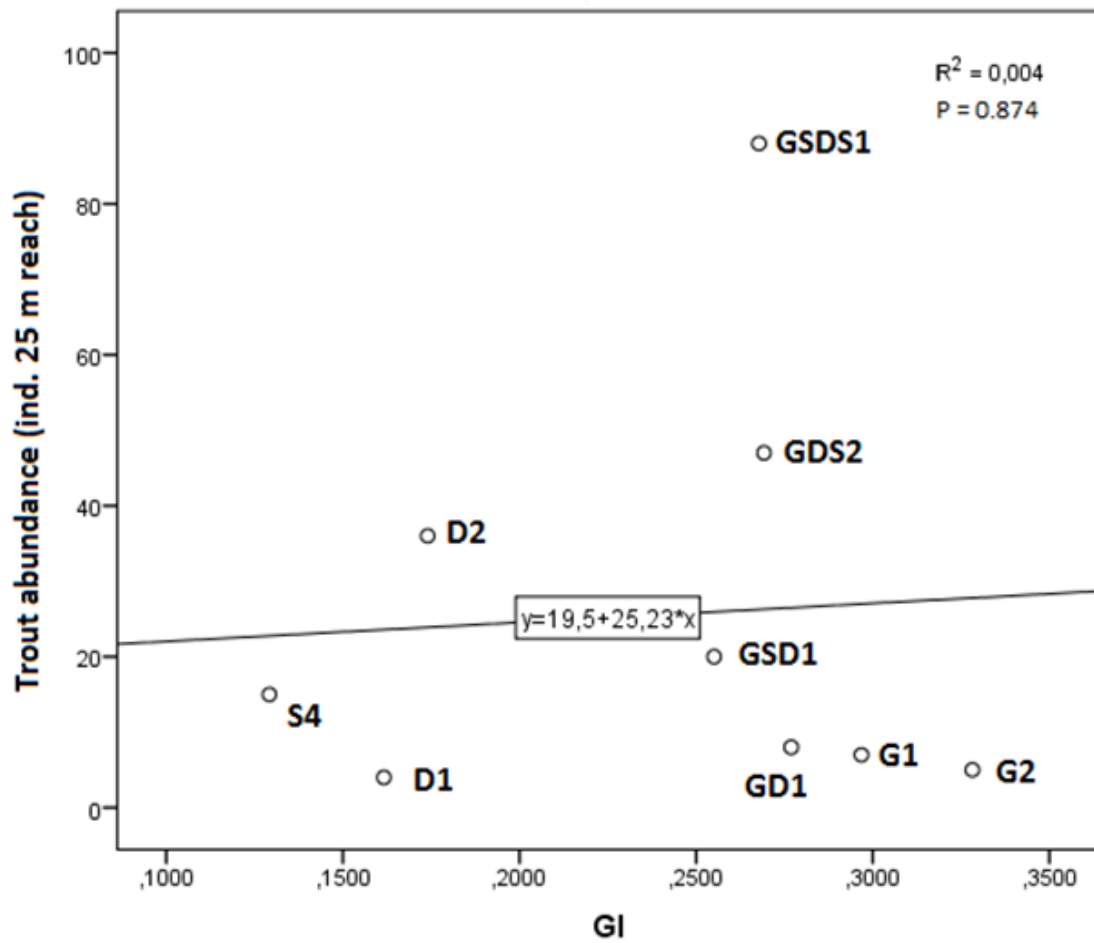


Figure 3. Plot of the relationship between glacier index and trout abundance for the 10 study sites (S1 was excluded for absence of trout). R^2 = coefficient of determination. P = Statistical significance. The line represents the lineal model adjusted to the data.

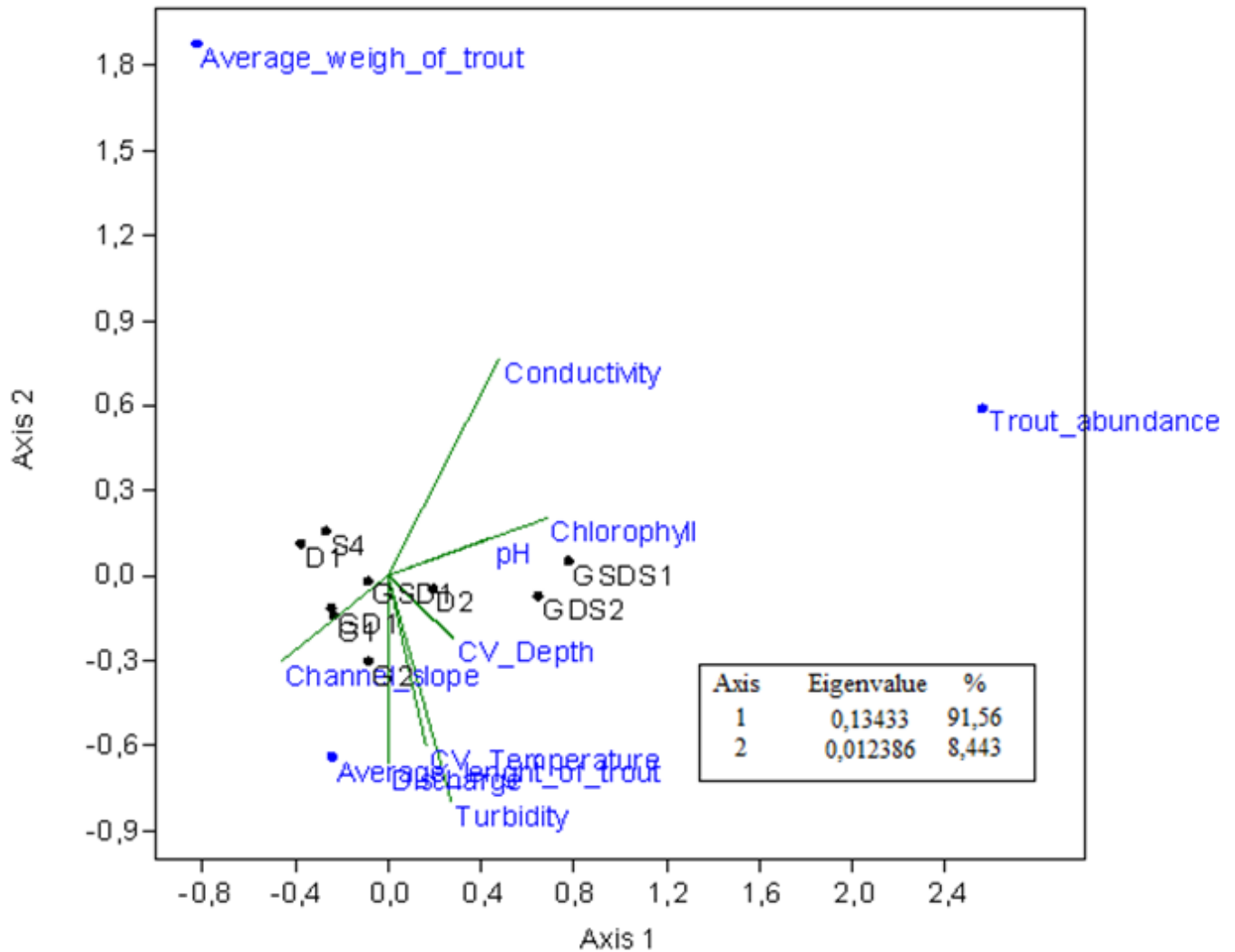


Figure 4. Canonical correspondence analysis (CCA) of the biotic and abiotic variables and the trout variables sampled in 9 study sites (S1 was excluded for absence of trout). Environmental variables plotted as a correlation with site scores; including respective axis Eigenvalues. Environmental variables were Log (x + 1) transformed.

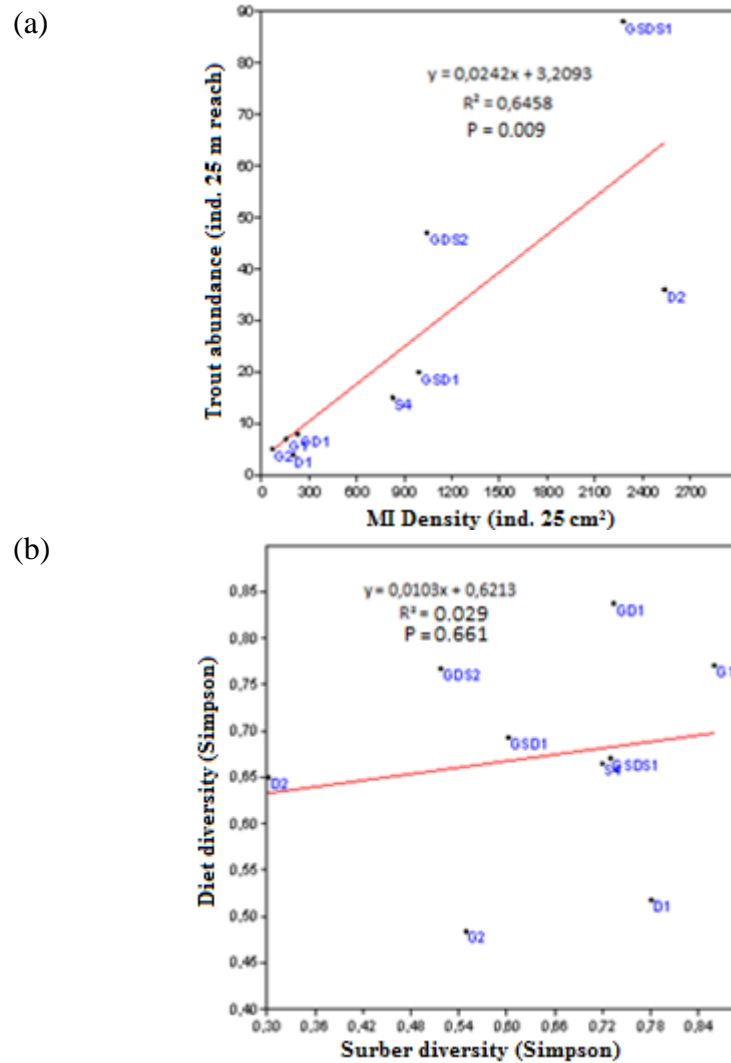


Figure 5. (a) Relationship between macroinvertebrates density and trout abundance (S1 was excluded for absence of trout). (b) Relationship between Surber samples diversity and Diet samples diversity through data obtained in the Simpson's diversity index (S1 was excluded for absence of trout). R^2 = Coefficient of determination. P = Statistical significance. The red lines represent the linear models adjusted to the data.

6. TABLES

Table 1. Percentages of abundance of orders from stomach contents of trout.

Stomach contents (%)	Taxa
36.01	Diptera
31.76	Amphipoda
12.04	Ephemeroptera
9.23	Trichoptera
7.37	Coleoptera
3.25	Haplotaxida
1.05	Plecoptera
0.93	Nematoda
0.14	Lumbriculida
0.10	Seriata
0.09	Acari
0.03	Hemiptera
0.02	Lepidoptera
0.01	Scolopendromorpha
0.01	Hymenoptera
0.01	Dermaptera
0.01	Rhynchobdellida
0.01	Bassomatophora

Table 2. Sign test comparing Diet vs. availability of food in 9 study sites (S1 was excluded for absence of trout). Negative values indicate avoidance (or lack of use) of a category of prey, whereas positive values indicate preference (use greater than availability).

** = taxa with P values minor than 0.01.

Stream	<i>n</i>	<i>Alaudomyia</i> sp. **	<i>Andesiops</i> sp.	<i>Anomalocosmoecus</i> sp.	<i>Caitloma</i> sp.	<i>Chelifera</i> sp.	Chironomidae indet.	Chironomidae indet. Pupa	<i>Claudioperta</i> sp.	<i>Contulma</i> sp.	Diptera pupa	Hirudinea	Eggs of rainbow trout	<i>Hyalella</i> sp.	HYDRACARINA
G2	11	-0,732	71,454	-	-	-	0,141	-	-2,196	-	-0,146	-	-	-	-0,146
D2	14	-0,026	-	-0,167	-0,057	-0,070	-52,415	-3,294	-	-	-	-	-	53,893	-
GSD1	21	-1,159	1,600	-0,325	-0,526	-0,003	-17,638	3,184	-	-	-	-	-	11,228	-
GD1	14	-6,472	-6,932	-	-	-	-19,345	4,350	-	-	-	-	-	3,594	-1,769
G1	19	-6,448	6,646	-	-	-0,198	33,234	-	-0,198	-	-0,198	-4,166	-1,984	-0,198	-0,396
D1	14	-6,185	-1,93	-	-	-	-26,000	0,880	-	-	-	-	-	54,681	-2,680
GDS2	21	-2,983	30,243	0,558	-	0,708	-53,481	0,266	0,787	-0,459	-	-	-	9,783	-0,344
S4	21	-0,790	34,884	4,662	2,419	-0,790	-30,100	-0,964	1,700	-	-0,296	-	-	-5,634	-0,098
GSDS1	21	-0,691	-5,432	-1,728	-0,148	-0,444	29,377	0,840	-1,136	-	-0,296	-	-	-22,817	0,049

Table 1. Continued.

Stream	<i>Limmophora</i> sp.	Lumbriculiidae	<i>Molophilus</i> sp.	<i>Moroniella</i> sp.	Muscidae	Naididae	<i>Nectopsyche</i> sp.	Nematoda	<i>Neelmis</i> sp.	Nepticulidae	<i>Neotrichia</i> sp.	<i>Ochrotrichia</i> sp.	Planariidae	<i>Prionocyphon</i> sp.	<i>Simulium</i> sp.	Sphaeriidae	<i>Stiobezzia</i> sp. **
G2	-	-	-	-	-	-	-0,439	-	-64,57	-	-	-	-2,049	-	-0,292	-	-1,024
D2	-	0,180	0,206	-	-	-1,689	1,956	1,174	0,395	-	-	-	-	-	-	-	-0,083
GSD1	-	-0,105	-0,108	0,309	-0,105	-0,315	1,855	-	-0,958	-	-	0,100	-0,105	4,557	0,309	-0,105	-1,689
GD1	-	-	-	-	-	-2,949	2,506	-	1,585	-	1,953	15,630	-	16,921	-1,474	-	-7,956
G1	-	-0,793	-	-	-	-5,952	-6,448	-	-1,091	-	7,043	-4,464	-	-	-0,198	-0,793	-13,392
D1	-	-	-0,824	-	-	-5,979	-	-	-1,930	-	-	-0,206	2,511	8,077	-9,278	-	-11,13
GDS2	-0,144	-	-0,229	-	-	20,82	-0,918	1,017	-0,873	-	1,424	-0,087	-	-	-0,379	-0,574	-5,166
S4	-	-	-	-0,098	-	-0,098	-0,743	-	-1,952	-0,098	-0,790	-0,691	-1,707	-	1,586	-	-0,395
GSDS1	-	-	-	1,235	-	4,884	2,620	-	2,225	-0,098	-0,395	-0,345	-2,075	-	-0,395	-	-0,345

7. APPENDIXES

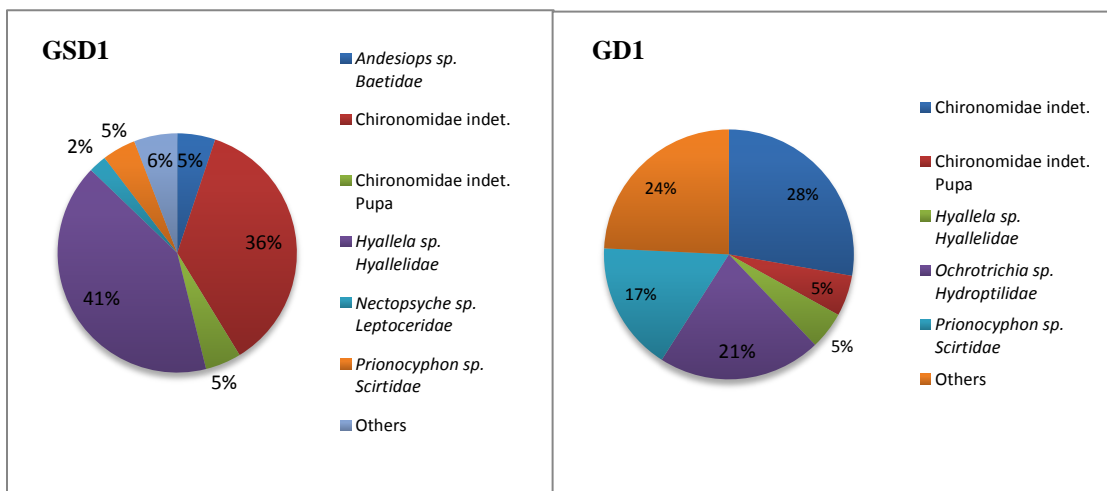
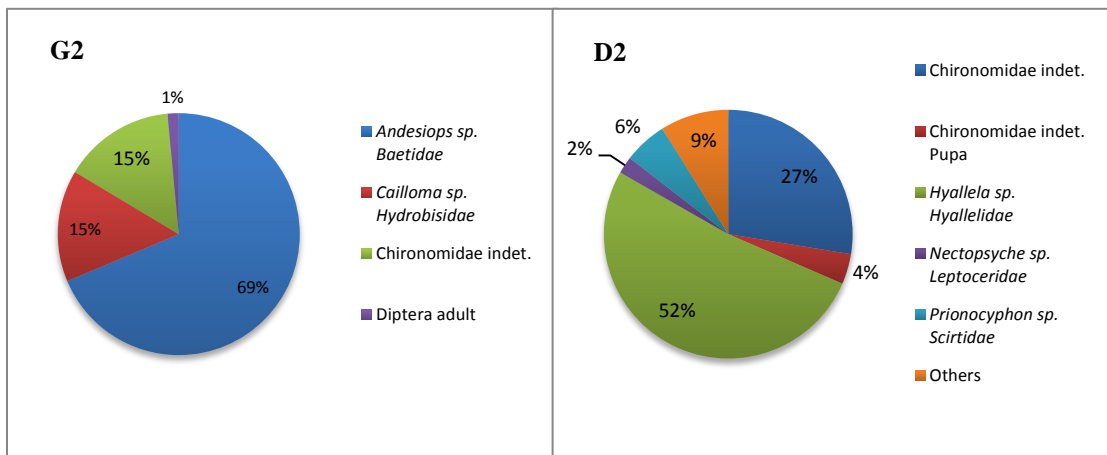
Appendix 1. Physico-chemical and hydromorphological variables of the 10 study sites. * = Variables taken in this study.

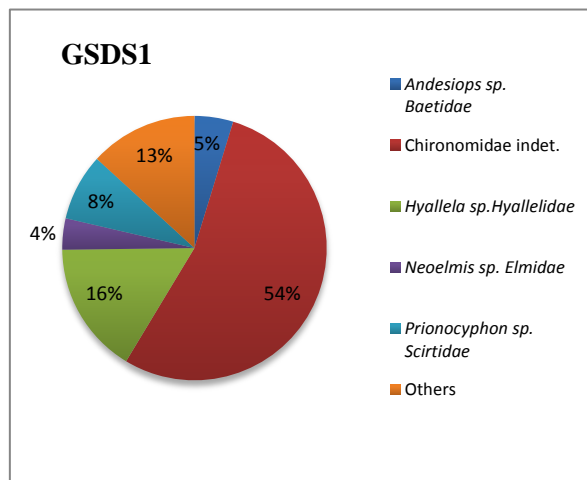
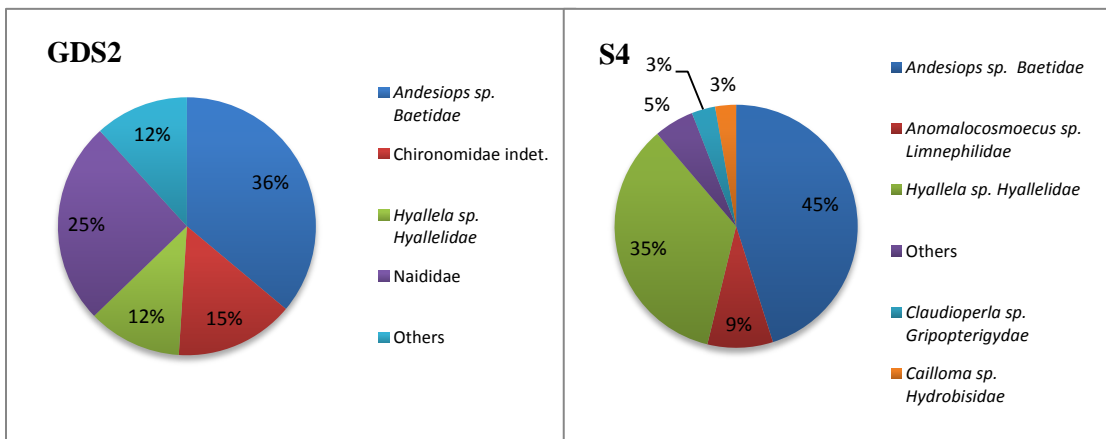
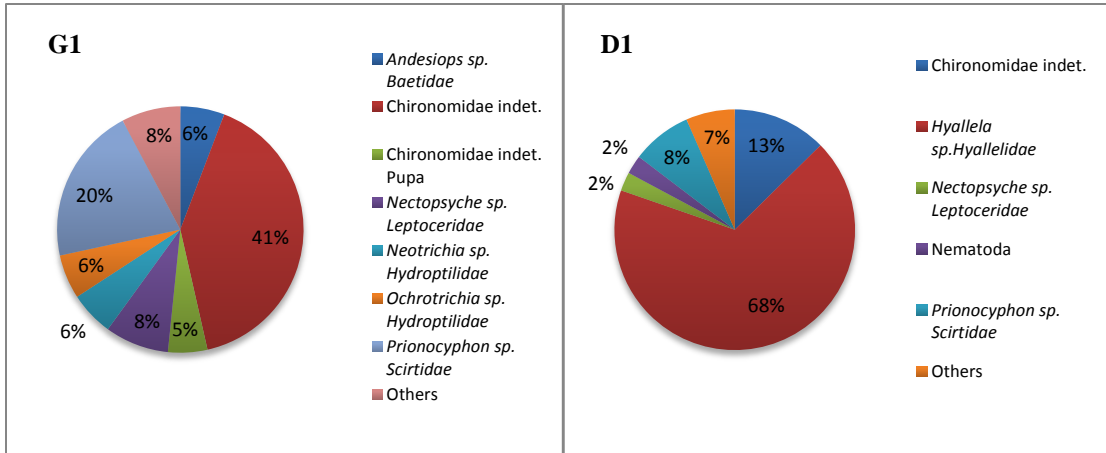
Stream	Stream order	Altitude (m.a.s.l.)	UTM X	UTM Y	pH (l/logH+)*	Conductivity (μ S/cm)*	Temperature ($^{\circ}$ C)*	O2 Concentration (mg/l)*	Turbidity (NTU)
G2	1	4109	811078	9943872	6.86	15.9	7.9	13	284
G1	1	4195	811725	9945452	7.67	22.8	5.4	13.3	144
GD1	2	4193	811710	9945398	7.4	39.2	6.5	12.8	131
GDS2	2	4056	809793	9943234	8.22	161.4	10.2	23.9	62
GSDS1	2	4042	809888	9943190	7.88	165.2	11.1	18.4	95
GSD1	2	4093	810941	9943760	7.53	155.5	8.8	24.8	40
S1	1	4090	809890	9944154	6.25	144.9	9.6	15.5	10
D2	1	4108	811088	9943738	7.37	145.4	8.9	19	6
D1	1	4202	811707	9945446	8.08	89.2	8.7	22.7	4
S4	1	4050	809919	9943238	6.62	149.2	7.2	22.2	1

Appendix 1. Continued.

Depth (cm)	Channel width (m)	Channel slope (‰)	Average Vel. (m/s)	Maximum Vel. (m/s)	Minimum Vel. (m/s)	Discharge (l/s)	Glaciality index	Temperature CV (°C)	Depth CV	Chlo 'a' (mg/m ²)
16.89	0.95	3.76	0.196	0.323	0.032	15.3	0.3282	0.3259	0.00114	19.07
23.76	0.66	5.60	1000	1000	0.086	149.5	0.2968	0.4178	0.00129	37.24
23.77	0.91	5.20	0.148	0.308	0.038	31.1	0.2769	0.2947	0.00103	31.28
19.47	1.00	6.38	0.400	0.733	0.093	18.9	0.2692	0.1593	0.00106	114.35
18.29	1.46	0.09	0.505	1250	0.165	5.9	0.2678	0.1483	0.00127	54.04
22.32	1.15	2.00	0.436	0.774	0.130	27.3	0.2551	0.2648	0.00098	43.79
25.26	3.98	6.00	0.205	0.422	0.045	10	0.2023	0.0479	0.00074	66.09
21.48	0.8	3.08	0.371	0.625	0.091	17.5	0.1740	0.1876	0.00102	27.34
30.28	0.56	38.75	0.062	0.153	0.007	1.3	0.1616	0.2167	0.00105	31.59
38.38	3.56	0.27	0.313	0.714	0.063	3.7	0.1292	0.0002	0.00105	28.77

Appendix 2. Percent (%) abundance of taxa in the diet of trout collected in 9 study sites (S1 was excluded for absence of trout).





Appendix 3. Taxon abundance from stomach contents samples.

Specimen	G2	D2	GSD1	GD1	G1	D1	GDS2	S4	GSDS1	TOTAL
<i>Alluaudomyia sp.</i> Ceratopogonidae	0	4	1	9	5	0	13	0	2	34
<i>Andesiops sp.</i> Baetidae	46	30	51	8	9	1	377	373	108	1003
<i>Anomalocosmoecus sp.</i> Limnephilidae	0	10	6	0	0	0	14	72	49	151
<i>Cailloma sp.</i> Hydrobisiidae	10	1	0	0	0	0	1	23	5	40
<i>Chelifera sp.</i> Empididae	0	3	2	0	0	0	12	0	7	24
Chironomidae indet.	10	700	358	63	63	25	156	10	1228	2613
Chironomidae indet. Pupa	0	100	48	12	8	2	11	1	39	221
<i>Claudioperla sp.</i> Gripopterygidae	0	26	8	4	2	1	14	26	7	88
<i>Contulma sp.</i> Anomalopsychidae	0	3	0	0	0	0	0	0	0	3
Dermaptera	0	1	0	0	0	0	0	0	0	1
Diptera adult	1	21	2	3	1	2	6	2	14	52
Diptera pupa	0	0	0	0	0	0	1	0	0	1
Dystiscidae	0	0	0	2	0	0	0	0	0	2

Appendix 3. Continued.

Eggs of rainbow trout	0	1	0	0	0	0	0	0	0	1
Formicidae	0	0	0	1	0	0	0	0	0	1
Glossiphoniidae	0	0	0	0	1	0	0	0	0	1
<i>Hyallela sp.</i> Hyallelidae	0	1313	407	11	0	134	123	289	368	2645
HYDRACARINA	0	4	1	0	0	0	0	0	3	8
<i>Lispe sp.</i> Muscidae	0	1	1	0	0	0	0	0	0	2
Lumbriculidae	0	11	0	0	0	0	1	0	0	12
<i>Molophilus sp.</i> Limoniidae	0	7	2	0	0	0	0	0	2	11
<i>Mortoniella sp.</i> Glossosomatidae	0	7	4	0	0	0	0	0	27	38
Muscidae	0	1	0	0	0	0	0	0	0	1
Naididae	0	3	0	0	0	0	266	0	2	271
<i>Nectopsyche sp.</i> Leptoceridae	0	56	24	8	13	5	0	2	73	181
Nematoda	0	29	10	0	0	5	14	1	19	78
<i>Neelmis sp.</i> Elmidae	0	32	7	6	1	1	4	1	87	139
<i>Nonagria sp.</i> Noctuidae	0	0	0	0	0	0	1	0	1	2

Appendix 3. Continued.

<i>Neotrichia</i> sp. Hydroptilidae	0	5	2	10	9	0	17	0	8	51
<i>Ochrotrichia</i> sp. Hydroptilidae	0	9	3	48	9	0	5	0	7	81
Planariidae	0	0	0	0	0	5	1	3	0	9
Planorbidae	0	0	0	0	1	0	0	0	0	1
<i>Prionocyphon</i> sp. Scirtidae	0	143	45	38	32	16	6	6	186	472
<i>Simulium</i> sp. Simuliidae	0	0	4	0	0	0	2	17	2	25
Scatopsidae	0	1	0	0	0	0	0	0	0	1
Scolopendridae	0	0	1	0	0	0	0	0	0	1
Staphylinidae	0	0	1	0	0	0	0	0	0	1
<i>Stridulivelia</i> sp. Veliidae	0	3	0	0	0	0	0	0	0	3
<i>Stilobezzia</i> sp. Ceratopogonidae	0	5	3	4	1	0	0	0	1	14
Tricoptera adult	0	8	0	0	0	1	0	1	33	43
TOTAL	67	2538	991	227	155	198	1045	827	2278	8326

Appendix 4. Taxon abundance from benthic samples. Data were obtained from three Surber samples for each stream.

Specimen	G2	D2	GSD1	S1	GD1	G1	D1	GDS2	S4	GSDS1	Total
<i>Alluaudomyia sp.</i> Ceratopogonidae	5	2	12	0	36	55	30	37	8	1	186
<i>Andesiops sp.</i> Baetidae	73	0	35	66	36	7	12	56	109	37	431
<i>Anomalocosmoecus sp.</i> Limnephilidae	0	6	9	37	0	0	0	7	42	40	141
<i>Atopsyche sp.</i> Hydrobidae	1	0	0	0	0	0	0	0	0	0	1
<i>Cailloma sp.</i> Hydrobidae	4	1	5	0	0	0	0	0	4	1	15
<i>Chelifera sp.</i> Empididae	0	2	2	0	0	1	0	4	8	1	18
Chironomidae indet.	121	822	520	67	164	116	192	598	317	225	3142
Chironomidae indet. Pupa	0	76	17	0	4	0	1	7	11	5	121
<i>Claudioperla sp.</i> Gripopterygidae	15	0	0	0	0	10	0	5	15	2	47
<i>Contulma sp.</i> Anomalopsychidae	0	0	0	0	0	0	0	4	0	0	4
Diptera adult	2	1	0	0	4	0	1	1	1	0	10
Diptera pupa	1	0	0	0	0	1	0	0	3	1	6
Hirudinea	0	0	0	3	0	21	0	0	0	2	26
Eggs of rainbow trout	0	0	0	0	0	10	0	0	0	0	10
<i>Hyallela sp.</i> Hyallelidae	0	38	294	262	5	1	88	19	415	57	1179
HYDRACARINA	1	0	0	1	6	2	13	3	1	1	28

Appendix 4. Continued.

<i>Limnophora sp.</i> Muscidae	0	0	0	0	0	0	0	1	0	0	1
Lumbriculidae	0	3	1	0	0	4	0	0	0	0	8
<i>Molophilus sp.</i> Limoniidae	0	1	3	2	0	0	4	2	0	4	16
<i>Mortoniella sp.</i> Glossosomatidae	0	0	1	0	0	0	0	0	1	8	10
Muscidae	0	0	1	0	0	0	0	0	0	0	1
Naididae	0	18	3	8	10	30	29	44	1	10	153
<i>Nectopsyche sp.</i> Leptoceridae	3	5	6	0	4	91	0	8	10	41	168
Nematoda	0	1	0	0	0	0	0	3	0	1	5
Nepticulidae	0	0	0	0	0	0	0	0	1	0	1
<i>Neoelmis sp.</i> Elmidae	441	10	16	0	4	10	12	11	21	15	540
<i>Neotrichia sp.</i> Hydroptilidae	0	0	0	0	9	5	0	2	8	3	27
<i>Ochrotrichia sp.</i> Hydroptilidae	0	0	2	0	22	63	1	5	7	0	100
Planariidae	14	0	1	0	0	0	1	0	21	5	42

Appendix 4. Continued.

<i>Prionocyphon sp.</i> Scirtidae	0	0	1	0	2	0	3	0	0	0	6
<i>Simulium sp.</i> Simuliidae	2	0	1	0	5	1	45	5	5	1	65
Sphaeriidae Bivalvia	0	0	1	15	0	4	0	5	0	1	26
<i>Stilobezzia sp.</i> Ceratopogonidae	7	3	19	1	32	72	54	45	4	5	242
TOTAL	690	989	950	462	343	504	486	872	1013	467	6776

Appendix 5. Univariate fauna metrics calculated on proportions of Surber and Diet benthic diversity data.

Site	Benthic			
	Evenness (e ^{H/S}) Surber	Fisher Alpha Surber	Evenness (e ^{H/S}) Diet	Fisher Alpha Diet
G2	0,2347	2,487	0,608	0,9328
G1	0,4816	3,902	0,4671	3,733
GD1	0,4256	3,203	0,5712	3,608
GDS2	0,181	4,101	0,2873	3,723
GSDS1	0,2812	5,074	0,2226	3,742
GSD1	0,1757	3,801	0,2108	4,208
S1	0,3843	1,802	-	-
D2	0,1399	2,509	0,1534	4,78
D1	0,4379	2,932	0,2787	2,811
S4	0,2461	3,966	0,2699	2,602

Appendix 6. Coorelation matrix between physico-chemical and hydromorphological variables. Correlation values are given in the lower triangle of the matrix, and the two-tailed probabilities are given in the upper. * = Variable chosen for the Canonical correspondence analysis (CCA).

0	pH*	Conductivity*	Temperature	Oxygen	Turbidity*	Depth	Channel width	Channel slope*	Average speed	Discharge*
pH	0	0,9021	0,57012	0,28417	0,94399	0,38652	0,0080455	0,27422	0,47767	0,63459
Conductivity	0,044845	0	0,012914	0,01532	0,013296	0,71055	0,22335	0,60012	0,81327	0,11618
Temperature	0,20491	0,74765	0	0,16642	0,36687	0,31761	0,70204	0,97672	0,42876	0,039139
Oxygen	0,37606	0,73558	0,47394	0	0,035796	0,39163	0,92679	0,5221	0,71217	0,22299
Turbidity	-0,02562	-0,74563	-0,32033	-0,66526	0	0,084262	0,26512	0,49439	0,68306	0,37397
Depth	-0,30805	0,13474	-0,35262	0,30491	-0,57166	0	0,12769	0,42099	0,59999	0,75386
Channel width	-0,77803	0,42291	0,13886	0,033504	-0,39008	0,515	0	0,40527	0,57773	0,37034
Channel slope	0,38332	-0,18946	0,010644	0,2303	-0,24539	0,28724	-0,29663	0	0,26849	0,7126
Average speed	0,25466	-0,085995	-0,28266	-0,13396	0,14809	-0,18952	-0,20096	-0,38756	0	0,0022761
Discharge	0,17205	-0,52864	-0,6567	-0,4232	0,31585	-0,11399	-0,31813	-0,13375	0,84139	0
Glacier index	0,22643	-0,51689	-0,11718	-0,50619	0,85145	-0,7893	-0,47407	-0,32442	0,35075	0,4346
CV Temperature	0,39088	-0,76733	-0,52444	-0,4472	0,66485	-0,48949	-0,79247	0,096332	0,4072	0,68061
CV Depth	0,53994	-0,35566	-0,22268	-0,17287	0,50249	-0,24883	-0,57364	-0,085903	0,59315	0,47551
Chlorophyll	0,32889	0,51132	0,56073	0,37103	-0,2395	-0,26457	0,12386	-0,087308	0,11285	-0,084444
Altitude	0,21759	-0,75051	-0,65406	-0,41353	0,18989	0,092222	-0,50367	0,58274	0,013676	0,49303

Appendix 6. Continued.

Glacier index	CV Temperature*	CV Depth*	Chlorophyll*	Altitude
0,52931	0,26405	0,10717	0,35345	0,54593
0,12605	0,0095771	0,31316	0,13091	0,012385
0,74717	0,11965	0,53633	0,091757	0,040212
0,13547	0,19503	0,63294	0,29118	0,23487
0,0017739	0,035953	0,13882	0,50513	0,59928
0,0066277	0,15102	0,48815	0,46009	0,79997
0,16629	0,0062641	0,082942	0,73318	0,13775
0,36042	0,79122	0,81347	0,81046	0,077073
0,32037	0,24283	0,070695	0,75627	0,97009
0,20944	0,030293	0,16482	0,81659	0,14764
0	0,02048	0,1974	0,70421	0,72959
0,71359	0	0,12014	0,39513	0,033172
0,44509	0,52386	0	0,60736	0,74588
0,13781	-0,30277	-0,18577	0	0,20677
0,12558	0,67236	0,11779	-0,4369	0

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PONTIFICIA UNIVERSIDAD CATOLICA DEL ECUADOR

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