Ecological Stoichiometry of Neotropical Fishes Along Elevation Gradients of the Andes Mountains

A Thesis Presented in Partial Fulfillment

of the Requirements for the Degree of Master of Science

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ABSTRACT

Phosphorus and nitrogen are typically the most common limiting nutrients in freshwater ecosystems, where fish play important roles in the cycling of these nutrients. Variation among fish families indicates there is likely significant variation in the roles that different groups of fishes play in nutrient cycling. However, there is still much to be learned about nutrient cycling in tropical aquatic ecosystems. I aimed to determine whether carbon (C), nitrogen (N), and phosphorus (P) contents vary with elevation in Neotropical stream fishes inhabiting Andean Mountain streams of southwestern Ecuador. I also examined variation in C, N and P concentrations among taxonomic families, rivers, and among fish of different sizes. To do this, freshwater fishes were collected from ten sites located between approximately 72 and 935 meters above sea level in four rivers. Total carbon and nitrogen were measured from ground whole specimens using a CE Elantech Flash EA1112. Phosphorus was measured using a molybdovanadate with acid persulfate digestion method and analyzed colorimetrically. Elevation generally had a small or non-significant effect on variation in fish total C, N, and P content. Taxonomic family, species, river, and standard length accounted for more of the variation in these elements in fish tissues. There also seemed to be differences among species in what factors accounted for significant variation in fish total C, N, and P. Thus, the influence of different factors including elevation and variables correlated with elevation, on fish C, N, and P concentrations, is likely complex and varies among species.

CHAPTER 1. LITERATURE REVIEW

Introduction

All living things have a set of nutrients that are required to sustain life. Some of these essential nutrients are also limiting nutrients, which are nutrients that are scarce within ecosystems and limit the growth of organisms. In freshwater ecosystems, phosphorus and nitrogen are the most common limiting nutrients (Small, Pringle, Pyron, & Duff, 2011). Although there has been much research on nutrient cycling and limitation in freshwater ecosystems, there is still much to be learned, especially in tropical ecosystems exhibiting high rates of endemism, like those in the tropical Andes Mountains. Moreover, ecosystems in the Andes are highly threatened by many anthropogenic factors (Anderson & Maldonado-Ocampo, 2011).

Habitat loss and degradation related to agriculture, mining, dam construction, and global climate change are causing major alterations in stream communities inhabiting Neotropical mountain streams and even isolating high elevation aquatic communities in some cases (Anderson & Maldonado-Ocampo, 2011; Encalada et al., 2019). A case in point are the fish communities along the western slopes of the Andes in western Ecuador, which exhibit very high rates of endemism and are severely threatened (Anderson & Maldonado-Ocampo, 2011; Jiménez Prado et al., 2015; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000). The composition of the fish communities in Andean mountain streams varies substantially with elevation, as do many environmental factors that matter ecologically and physiologically for fishes, like temperature, conductivity, food resources, and nutrient composition (He et al.,

2016; Jacobsen, 2008; Körner, 2007). There have not been any studies on nutrient variation focusing on the freshwater fishes of western Ecuador or the specific roles of the endemic fish species that are common in this region on nutrient cycling in these ecosystems. There is also relatively little known about how nutrient content in fishes varies with elevation in Andean mountain streams, despite the large changes in biotic and abiotic conditions that are associated with changes in elevation. Thus, as these aquatic communities continue to degrade, there is little information available on the consequences of the loss of specific fish species on ecosystem functioning (Anderson & Maldonado-Ocampo, 2011; Encalada et al., 2019). In addition, it is unknown how nutrient cycling will be effected (Anderson et al., 2011).

The objective of my thesis is to determine how phosphorus and nitrogen contents vary in Neotropical stream fishes along elevation gradients of the Andes Mountains, as well as understand the contribution of species identity, river basin, and fish body size on this variation. This research will help to create a catalog of the phosphorus and nitrogen content in some common Neotropical stream fish species along the Andes Mountains of Ecuador and is the first research project on the ecological stoichiometry of freshwater fishes from western Ecuador.

In the rest of Chapter 1, I provide background information related to my research topic. Specifically, I provide an overview of stoichiometry (the balance of elements in an organism as well as through the ecosystem), elevation gradients, and Neotropical streams and fish. Chapter 2 presents the research component of my thesis in manuscript format. In it, I describe how nitrogen, phosphorus, and carbon are measured and vary in the stream water, sediment and fish sampled in southwestern Ecuador. In addition, I examine how these elements vary in fish from low to high elevations along the western slopes of the Andes Mountains in Ecuador, and

test for divergence in carbon, nitrogen and phosphorus among fish families, river drainages, and fish of different sizes.

Stoichiometry

Ecologists have long recognized that the processing of energy and matter are intricately linked (Vanni & McIntyre, 2016). Ecological stoichiometry is the balance of elements during foraging, maintenance, growth, reproduction, and waste production (Vanni & McIntyre, 2016). It provides a mechanistic justification for how animal species vary in mediating nutrient cycling (Elser & Urabe 1999, Vanni 2002).

All living organisms require essential nutrients to live and reproduce. Some common essential elements include carbon, hydrogen, oxygen, nitrogen, phosphorus, iron, and magnesium. Typically, animals are homeostatic and require a specific concentration of elements for their life cycle and reproduction (McIntyre & Flecker, 2010; Persson et al., 2010; Vanni, Flecker, Hood, & Headworth, 2002). These required elemental concentrations typically stay the same within species, but can differ between species, especially between more distantly related species, genera or taxonomic families. Vanni et al., (2002) showed that taxonomic identity may be an important factor in determining the ratios of nutrients recycling. For example, many studies, have shown that freshwater zooplankton exhibit stoichiometric variation. Genera like *Acanthodiaptomus* and *Heterocope* have a high C:P and N:P body content, however, *Ceriodaphnia*, *Daphnia*, and *Scapholeberis* have low N:P and C:P body content (Sterner & Elser 2002). Although these concentrations are often homogeneous within

families, this homeostatic stoichiometry can change during the organisms' life cycle, specifically as they grow and mature sexually (Sterner & Elser 2002).

Limiting nutrients are also an important aspect of stoichiometry. Limiting nutrients are those nutrients that are in low supply and thus will be exhausted first. These nutrients thus can limit the growth and development of organisms. Two important essential elements that are often limiting nutrients are phosphorus and nitrogen (Small et al., 2011).

Pure phosphorus is a waxy solid, colorless, translucent, and spontaneously combustible (Sterner & Elser 2002). Although required by organisms in small amounts, phosphorus availability in the environment is often extremely low, thus typically making it a limiting nutrient, especially in streams (McIntyre & Flecker, 2010). Phosphorus does not enter ecosystems through the atmosphere but through weathering of bedrock, so it is strongly influenced by climatic factors (Kitayama et al., 2000), in addition to runoff from fertilizers (Hart, Quin, & Long Nguyen, 2004; Steinman & Mulholland, 2007). At lower temperatures these weathering rates tend to be impaired and consequently the concentration of available phosphorus can be lower (Kitayama et al., 2000).

Pure nitrogen is a colorless, odorless gas that makes up a large component of the atmosphere (Sterner & Elser 2002). The major pathway by which nitrogen enters the ecosystem is through atmospheric nitrogen fixation. Nitrogen fixation is the process by which the atmospheric nitrogen, N_2, is transformed into organic useable compounds such as ammonia or nitrate ions. Due to temperature changes with elevation, these also tend to change with elevation (Lovett & Kinsman, 1990). In freshwater fish, ammonium is the primary nitrogenous

compound that is excreted and bioavailable (Mcintyre et al., 2008). Nitrogen is also a key nutrient required for proteins and structural materials thus, making up a substantial proportion of organism mass (Elser *et al.* 1996, Sterner & Elser 2002, McIntyre & Flecker 2010). Like phosphorus, nitrogen is often also a limiting nutrient.

The extent to which phosphorus and nitrogen are limiting nutrients varies among ecosystems. For example, since phosphorus is made available through the weathering of bedrock, which is affected by climatic factors such as temperature and precipitation rate, phosphorus availability can be lower at higher elevations (Kitayama et al., 2000). Unfortunately, there is surprisingly little known about how phosphorus and nitrogen vary with elevation in mountain streams, especially in Neotropical ecosystems, which are among the most biodiverse on earth.

Elevation Gradients

Viviroli et al. (2007) indicate that mountains cover approximately 39% of the land surface of the Earth. Elevation gradients, like mountains are great indicators of how climate change may affect ecosystems. This is because higher elevations are considered stressful environments for most species and thus, can demonstrate the ranges to which plants and animals are constrained by environmental conditions (Sundqvist, Sanders, & Wardle, 2013). With increased elevation there is decreased temperature and decreased land area (Sundqvist et al., 2013). These two factors along with other factors like precipitation play a large role in influencing the habitats of species along elevation gradients (Hodkinson, 2005; Körner, 2007).

The more stressful conditions found at higher elevations are typically thought to be the cause of the lower species richness (Anderson & Maldonado-Ocampo, 2011; El-Sabaawi et al., 2012; Winemiller, Agostinho, & Pellegrini Caramaschi, 2008). However, as elevation increases, the percentage of endemic species tends to increase (Anderson & Maldonado-Ocampo, 2011). Whether low or middle elevations exhibit the greatest species richness depends on the study and ecosystem, but the most common patterns seem to be that middle elevation ecosystems, between 500-2200 meters, exhibit the greatest species richness (Anderson & Maldonado-Ocampo, 2011). This may be because middle elevations have the optimal combination of environmental conditions including temperature and area to permit high species diversity (Sundqvist et al., 2013). Conditions at mid elevations can often also accommodate a combination of species from groups that are typically adapted to low and high elevations, increasing diversity (Sundqvist et al., 2013).

Many other abiotic and biotic factors vary with elevation. The structure and variety of biota at high elevation streams is drastically different than at lower elevations. Typically, high elevation streams have less distinct seasonality (Jacobsen, 2008), or fluctuations in the weather. In addition, high elevation streams generally have high slopes, fast currents with many ripples and rapids and lower water temperatures (Winemiller et al., 2008). Usually, human populations decrease with increasing altitudes, leading to better water quality than streams at lower elevations. However, high elevation streams can still be affected by pollution, mining, dams, and global warming (Jacobsen, 2008). At low elevations, stream currents are slow, sediments are rich with organic material, and temperatures are warm (Winemiller et al., 2008).

Covering over 5,000 miles along the western side of South America, the Andes Mountains are the longest terrestrial mountain range in the world. This mountain range is broken up into three sections, the Southern Andes, the Central Andes, and the Northern Andes. All three encompass varied terrain. With these differing terrains, the flora and fauna vary in addition to the species richness, making Andean streams among the most biodiverse and ecologically important streams in the Neotropics (Anderson & Maldonado-Ocampo, 2011). Unfortunately, they are also under severe pressure from anthropogenic sources including pollution, habitat loss, and severe threats to fish due to dams and overfishing (Anderson & Maldonado-Ocampo, 2011; Barletta et al., 2010; Encalada et al., 2019; Myers et al., 2000; Reis et al., 2016). Current scientific research and conservation in this area is focused on terrestrial flora and fauna, but extremely limited for fish. Tropical Andean fishes are among the least studied vertebrates on Earth (Anderson & Maldonado-Ocampo, 2011; Jacobsen, 2008).

Neotropical Fishes

Freshwater fish are an integral part of Earth's ecosystem, accounting for 25% of the vertebrate species on Earth (Stiassny, 1996; Winemiller et al., 2008). The Neotropics are considered to have the highest fish fauna richness, with an estimated 5,000 known species (Anderson et al., 2018; Anderson & Maldonado-Ocampo, 2011; Reis et al., 2016; Winemiller et al., 2008), accounting for approximately 50% of all freshwater fish (Anderson & Maldonado-Ocampo, 2011). They are also the most abundant vertebrates in Neotropical streams.

Fish are considered the most nutrient-rich organisms in streams and thus play an important role in nutrient cycling of aquatic ecosystems (Vanni *et al.* 2002, Mcintyre *et al.* 2008,

Small *et al.* 2011). It has been suggested that body size and temperature effect nutrient excretion rates of fish and thus are important in determining nutrient cycling rates (Vanni et al., 2002). Although body nutrient content can differ widely among species, fish typically constitute the dominant pool of phosphorus and nitrogen in stream ecosystems (Griffiths, 2006; Small et al., 2011). Therefore, when a species becomes extinct, it can affect the entire ecosystem. This is also true with pollution and the distribution of chemicals in an ecosystem. If a stream is contaminated with anthropogenic waste, these chemicals do not only affect the water and stream animals, but the entire ecosystem. Stream animals are eaten by land animals who take in these chemicals and transport them to their terrestrial habitats (Fausch *et al.* 2002, Sterner & Elser 2002). This makes fish extremely important to study.

In addition to fish being indicators of environmental quality, fish are very important in creating spatial hotspots of nutrient recycling. They may gather around certain physical structures or habitats, creating a high species richness area with high rates of nutrient cycling compared to surrounding areas (McClain et al., 2003; Mcintyre et al., 2008). Fish also can migrate long distances (i.e., for reproduction) or short distances (i.e., to feed) transporting nutrients from one area to another (Mcintyre et al., 2008). These hotspots can also change based on the species richness in an area. Each fish species requires essential nutrients at different concentrations for their life cycle, and thus release nutrients in different amounts into the environment (Small et al., 2011).

Taxonomic composition has a large impact on fish nutrient cycling. Nutrient concentrations are more strongly associated with family identity, than with species identity, and can help explain why nutrient levels and the severity of limiting nutrients (i.e. phosphorus

and nitrogen) vary among Neotropical streams (Vanni et al., 2002). Phosphorus is typically the most variable critical nutrient in fishes (El-Sabaawi, Warbanski, Rudman, Hovel, & Matthews, 2016; McIntyre & Flecker, 2010; Vanni et al., 2002). Most phosphorus in fishes is found in the bones making it an important element of body mass (Sterner & Elser 2002, Hendrixson *et al.* 2007). Because phosphorus is a large part of skeletal bone and the amount of bone varies considerably among fish families, phosphorus cycling is strongly influenced by taxonomic identity and body size (Hood, Vanni, & Flecker, 2005; Vanni et al., 2002). For example, suckermouth catfishes in the family Loricariidae can be encased in bone and exhibit correspondingly high concentrations of phosphorus, while other families like the Characidae (tetras) possess bones only in the internal skeleton and exhibit much lower phosphorus concentrations (Vanni et al., 2002).

Variation in phosphorus among fish groups matters both because of the differences in total phosphorus required and because of variation in excretion rates. Differences among fish families in the amount of phosphorus required means that some species may be more severely impacted by low amounts of phosphorus in the environment than others because of their evolutionary history. Thus, the species present and their abundance in an ecosystem with low phosphorus levels may be significantly impacted by their ability to deal with these low phosphorus concentrations. Fish species with high body phosphorus content also typically excrete phosphorus at lower rates than species with lower body phosphorus, impacting the amount of phosphorus available in the ecosystem for other species (Elser & Urabe 1999, Small *et al.* 2011). Body size and temperature may also affect nutrient excretion rates and thus nutrient cycling rates (Vanni et al., 2002). Phosphorus is also found in nucleotides involved in

the processing of genetic information (Sterner & Elser 2002). Thus, species with high growth rates need lots of phosphorus rich RNA and thus exhibit a high body phosphorus content (Elser *et al.* 2003, Vanni 2010). Young fish will also need more phosphorus because of their higher growth rates (Elser *et al.* 1996, McIntyre & Flecker 2010).

CHAPTER 2. Ecological Stoichiometry of Neotropical Fishes Along Elevation Gradients of the Andes Mountains

INTRODUCTION

All living things have a set of nutrients that are required to sustain life. Within these essential nutrients there are also limiting nutrients, which are nutrients that are scarce within ecosystems. Nitrogen and phosphorus are the most common limiting nutrients in freshwater ecosystems (Hecky and Kilham, 1988; Small et al., 2011). Nitrogen is essential for growth and development, including the production of proteins and nucleic acids (Elser *et al.* 1996, Sterner & Elser 2002, McIntyre & Flecker 2010). Phosphorus is required for cell development and is a key component of ATP, DNA, and bone and teeth (Elser *et al.* 1996, Sterner & Elser 2002).

Fish are considered important reservoirs of these nutrients in freshwater streams (Kitchell *et al.* 1979, Sterner & George 2000, Griffiths 2006). They can play critical roles in nutrient recycling through excretion of nutrients and decomposition (Vanni, 2002). However, there is significant variation in the nutrient concentrations among fish families, which is often associated with structural variation, body size and growth rate (Sterner & George 2000, Hendrixson *et al.* 2007, McIntyre & Flecker 2010). This variation among fish families indicates there may be significant variation in the roles that different groups of fishes play in nutrient recycling. A major gap in knowledge is how elevation impacts nutrient recycling of fishes in Neotropical ecosystems and the extent to which different Neotropical fish families are affected. Many abiotic (temperature, conductivity, UV radiation, water currents, land area, etc.) and biotic factors vary with elevation including concentrations of nitrogen and phosphorus (Jacobsen, 2008; Körner, 2007; Sundqvist et al., 2013). The structure and variety of biota in high

elevation streams differs substantially from those at lower elevations. Typically, high elevation streams have less distinct seasonality (Jacobsen, 2008), or fluctuations in the weather. In addition, high elevation streams generally have higher slopes, fast currents with many ripples and rapids, and lower water temperatures (Winemiller et al., 2008). The reduced human populations at higher altitudes, also leads to better water quality compared to streams at lower elevations with greater human density.

It is important to study nutrient cycling in Neotropical regions given the high levels of biodiversity and increasing threats that these ecosystems face. Western Ecuador is a region with particularly high rates of endemism, with approximately 45% of freshwater fishes being endemic (Anderson & Maldonado-Ocampo, 2011; Encalada et al., 2019; Myers et al., 2000; Sierra, Campos, & Chamberlin, 2002). However, habitat loss related to agriculture, mining, and dam construction, pollution, the introduction of exotic species, overfishing, etc., are causing major alterations to the fish communities inhabiting Neotropical mountain streams in the region and even isolating high elevation fish communities in some cases (Anderson & Maldonado-Ocampo, 2011; Jiménez Prado et al., 2015; Mcintyre, Jones, Flecker, & Vanni, 2007; Taylor, Flecker, & Hall, 2006). Understanding the role that fish play in nutrient cycling is important so that we help protect this key ecosystem service in threatened areas. Western Ecuador is also a good place to study ecological processes in mountain streams because the Andes run close to the Pacific Ocean there, resulting in a series of high gradient rivers, in which environmental conditions change rapidly, that are in close geographic proximity to one another but run independently into the ocean.

In this study, I examine whether carbon (C), nitrogen (N), and phosphorus (P) contents vary with elevation in Neotropical stream fishes inhabiting Andean Mountain streams from close to sea level to approximately 1000 m in elevation in southwestern Ecuador. This elevation range was selected because it include the greatest species richness for mountain stream fishes in the region, and is broad enough to include substantial changes in environmental factors while being narrow enough to allow collection of individuals of some of the same fish species along the entire range of elevation. I will also examine whether fish total C, N and P differs among taxonomic families, fish inhabiting different rivers, and fish differing in body size. I hypothesize that fish nutrient content will change along elevation gradients of the Andes (higher concentrations at lower elevations) and differ significantly among fish families, river drainages, and with body size. Fish were collected from 10 sites located at different elevations in four rivers in southwestern Ecuador. C, N, and P concentrations were measured from whole ground fish specimens of all species collected. N and P concentrations were also measured from water and soil samples from each site, as were standard environmental variables like water temperature and conductivity. These are the first data focused on the ecological stoichiometry of freshwater fish communities from inhabiting Andean mountain streams in western Ecuador and will provide insight into the factors affecting stoichiometric variation among the imperiled fish species in a region of South America characterized by extremely high rates of endemism.

METHODS

Sampling Sites and Sample Collection

Fish were collected from ten different sites, four rivers at varying elevations from 72 meters to 935 meters along the Andes Mountains of western Ecuador during the dry season, July and August 2018 (Figure 1). The rivers sampled for this study were selected because they were relatively close to roads that allowed them to be accessed at different elevations. Two of the rivers, the Cristal and Chimbo rivers, form part of the Guayas drainage basin, which is the largest drainage basin in western Ecuador and includes the greatest diversity of freshwater fishes in the region. Both rivers are in the southeastern portion of the Guayas drainage. The third river sampled was the Chaucha River, which forms part of the Balao River drainage, and is much smaller than the Guayas basin, from which it is approximately 80 km south. The final river sampled was the Jubones River, which forms its own drainage basin close to the city of Machala, approximately 55 km from the border with Peru. The Jubones is also a relatively small drainage basin. Unfortunately, all rivers in western Ecuador, including those sampled for this study, are affected to different degrees by anthropogenic factors including agriculture, pollution from human populations, mining, dams, and overfishing (Jiménez Prado et al., 2015). Sampling sites were put into three elevation categories: low (<300 m), middle (301-600 m), and high (>601 m).

Fish were collected using a Smith-Root LR-24 electrofishing backpack, seines, and cast nets. Sampling was done in shallow areas of streams (about <60cm deep). Fish were immediately separated by species and put in bags on ice. They were then frozen until being processed, which typically occurred a few days later. Of the 433 fish collected, 171 were

measured for C, N, and P. One specimen was omitted from P measurement as there was not enough ground fish to measure. Not all species were found at each site (Table 1). Water and sediment samples from each site were collected, transported on ice and then refrigerated (water) or frozen (sediment) until N and P were measured. N and P were measured from water samples within 24 hours of collection. In addition to fish, water and sediment collections, elevation, water temperature, conductivity, pH, dissolved oxygen, approximate length of the stream sampled and average depth of the water in which fish were collected, were measured at each site (Table 2).

Specimen Preparation

Frozen fishes were thawed, identified to species (Jiménez Prado et al., 2015) (Figure 2), and the body length and wet weight recorded. Organs were removed from the visceral cavity of each specimen. The specimens were then oven-dried at 60°C for a minimum of 24 hours. Their wet and dry weights were measured, and then each individual specimen was ground to a fine powder using a mortar and pestle. Any pieces that did not grind up easily were cut into small pieces using scissors and reground with the mortar and pestle. The dried and ground specimens were brought back to DePaul University, in Chicago, Illinois. Specimens with a dry weight of 0.5 g or greater were ground in a SPEX SamplePrep 8000M Mixer Mill before nutrient testing. All specimens less than 0.5 g were re-ground by hand before nutrient testing.

Measurement of C, N, and P from Site Water and Sediment

Water and sediment samples were collected at each sampling site and put on ice in coolers for transport to the Ecotoxicology Laboratory in the *Facultad de Ciencias de la Vida, Escuela Superior Politécnica del Litoral*, in Guayaquil, Ecuador, where they were measured by lab personnel.

Two liters of undisturbed stream water were collected at each site in replicate one-liter glass bottles and put on ice in the field until transport to the lab, where they were refrigerated and processed within 24 hours. Total P was measured using the Molybdovanadate with Acid Persulfate Digestion Method, while total N was measured using the Persulfate Digestion Method. Both procedures were conducted using Test 'N Tube kits from Hach following the manufacturer's instructions. Total N and P were measured from both unfiltered and filtered water samples, with a 45-micron Teflon filter used for filtering.

Three sediment/soil samples were collected at each site and placed in separate gallon Ziploc bags in a cooler with ice until transport to the lab. The first sediment sample was collected from the stream bottom in shallow water a meter or more from the shore. The second sample was collected on land at the edge of the stream. The third sample was of soil collected on land approximately three to five meters from the stream's edge and was typically dug from under vegetation. In the lab, a calcium sulfate extraction for soil was conducted for measurement of soil N while a Mehlich 2 extraction for soil was conducted for measurement of soil P, both using Hach's Soil and Irrigation Water Test Kit (SIW-1) following the manufacturer's instructions. Total P and N were then measured using the same methods described above for

the water samples conducted with Test 'N Tube kits from Hach following the manufacturer's instructions.

Measuring Fish Total C, N and P

Each individual fish was analyzed for C and N using a CE Elantech Flash EA1112 Organic Elemental Analyzer (Appendix 1). The Flash EA1112 oxidizes the sample through combustion, which is then reduced to CO_2 and N_2. Before measurement, samples were re-dried at 60° C for approximately one week and then reweighed. Approximately 5.0 mg of each sample was weighed into a tin capsule. Aspartic acid was used to create a standard curve (at approximately 3.0mg, 6.0mg, 9.0mg, and 12.0mg) from which the percent N and percent C for each sample was calculated. Samples ground with the Mixer Mill were measured once, while samples ground by hand were measured twice and the average was taken. Any samples with a variation of 5% or more were measured a second or third time and the average taken.

Each individual fish was analyzed for P following a molybdovanadate with acid persulfate digestion method (Appendix 1). Approximately 2.0 mg of ground fish was weighed out and put into a tube from a HACH Test N' Tube kit for High Range Total Phosphorus. Five milliliters of deionized water was added to each tube. The HACH method 10127 was followed except for the DRB200 Reactor being set to 100°C instead of the 150°C. Immediately after the Molybdovanadate reagent was added, the vials were centrifuged for two to three minutes to ensure all particulates settled to the bottom of the tube. A matrix blank was used to standardize the method. Results were measured at 420nm and read in mg/L as P. All fish were analyzed in duplicates and averages taken. Any replicate samples with a difference of 1% or more were measured again and the average taken of the four measures. A few fish were

randomly selected for replicate measurement at the University of Georgia Stable Isotope Lab for testing to ensure accuracy.

Data Analysis

All analysis was done in RStudio version 1.2.5033 (R Core Team, 2019).

I examined environmental variation of total N and P at all sampling sites. Patterns of variation in N and P among drainages and with elevation were qualitatively described since not enough sites were sampled for statistical analysis. Correlation analysis was used to test whether N and P were associated in the river water and sediment samples using the Hmisc package (Harrell & Dupont, 2020).

Before conducting other analysis, I examined variation in body size and water content among specimens. ANOVA was used to test whether body size differed significantly among species and a Tukey test was used to test which species differed in size from each other. The percent water content of each specimen was calculated by subtracting the specimen's wet weight and from their dry weight, then dividing by their wet weight and multiplying by 100. Correlation analysis was used to examine if there was a relationship between specimen length (mm) and water content (%).

Since all specimens were measured multiple times for C, N, and P, averages per specimen were taken for each nutrient and used in the analysis. The variation in C, N, and P among taxonomic families was also analyzed using ANOVA, and a Tukey test was used to determine which families differed significantly from each other. Correlation analysis was used to determine if there was a relationship between N and P among families. Families were divided into two groups for the correlation analyses. The first group including all families from

the order Characiformes (tetras and their relatives), while the second group included all families in the order Siluriformes (catfishes). All specimens of the same species and site were averaged. To look at the taxonomic variation further, the variation in C, N, and P among taxonomic species was also analyzed using ANOVA, and a Tukey test was used to determine which species differed significantly from each other.

To examine how elevation and river affected fish total C, N, and P content, both individual and combined analyses of these variables were performed. For the individual analyses, ANOVA was used to test whether C, N, and P differed significantly by elevation level across all species, and a separate ANOVA was conducted to test whether C, N, and P differed significantly by rivers across all species. A Tukey test was used to test which rivers differed in C, N, and P content. For the combined analysis, variation in fish total C, N, and P were modeled as a function of river, elevation (categorical variable with three levels: high, mid and low elevations), species, and their interactions using factorial ANOVA.

Since Species and River accounted for a large amount of the variation in fish total C, N, and P (see results), a multiple regression was used to examine which variables accounted for variation in fish C, N, and P within each sampled river, and within species for the three most commonly collected fish species. For the analyses of variation of fish total C, N, and P within each river, variation in fish total for C, N, and P for each river to test the significant difference of body size, elevation level (as a categorical variable), and species. Another multiple regression was done for C, N, and P for three common species collected. These species were *Astroblepus cyclopus*, *Astroblepus cf. longifilis,* and *Brycon atrocaudatus.* They were each tested separately to test the significant difference of body size, elevation (as a continuous variable), and river.

RESULTS

Environmental variation of total N and P at sampling sites

The sites sampled were relatively typical for Andean mountain streams in the region (Figure 3). The river water temperature ranged from 18.0°C-24.2°C, pH from 7.6-8.4, and conductivity ranged from 95.1-268.1 uS (Table 2).

Total N in the river water ranged from undetectable to 1.4 mg/L with an average of 0.53 mg/L ±0.49, while the total P in the river water ranged from undetectable to 1.9 mg/L with an average of 0.95 mg/L ±0.65 (Table S1). There was little difference for N and P content for the filtered river water and unfiltered river water, so the unfiltered water measures were used in all analyses. Total N and P exhibited substantial variation among rivers and sites at different elevations (Figure 4). Total N and P were so low in the water of the Chaucha River that they were undetectable. Total N also seemed to be substantially lower in the Cristal River than the Chimbo and Jubones rivers. In all three rivers in which N was measurable, it was highest at the high elevation site, suggesting a tendency to increase with elevation. Total P varied less among the three rivers in which it was measurable, although values for the Cristal and Jubones rivers did not overlap, with the Cristal River exhibiting higher P concentrations. In all three rivers in which P was measurable in the water, it tended to decline with elevation which was the opposite of what was observed for N, such that in all three rivers, the lowest concentration of P was measured at the highest site.

There was generally less divergence among rivers for the sediment concentrations of N and P although the Chaucha River, for which water N and P were unmeasurable, also had substantially lower concentrations of N and P in the sediment samples than seen in the other

rivers (Figure 4). There was a general tendency of increasing N with elevation as seen for the water samples, while P also tended to increase with elevation in three of the four rivers, which was the opposite pattern to that observed in the water samples. Total N in the river water and sediment were not significantly correlated (correlation analysis, r=0.31, df=8,p=0.39), however, P in the river water and sediment were significantly correlated (correlation analysis, r=0.79, df=8,p=0.01) (Figure S1).

Taxonomic diversity, body size, and water content of fishes

Fish belonging to a total of 12 species in 8 families and three orders were collected. The Characiformes included representatives of five species in three families of which the Characidae were the most common, while the Siluriformes included representatives of six species in four families. The Cyprinodontiformes were represented by a single poeciliid species in the genus *Pseudopoecilia*. Although Cichliformes like *Andinoacara rivulatus* and *Mesoheros festae* and Gymnotiformes in the genus *Brachyhypopomus* are relatively common in southwestern Ecuador (Jiménez Prado et al., 2015), no representatives were collected. The species collected were not distributed uniformly by drainage basin or elevation.

In terms of drainages, Astroblepids were also the most common family collected in the Balao and Guayas drainages. However, in the Jubones drainages, no Astroblepids were found and Loricariids were most common. The Trichomycterid *I. laticeps* was also collected fairly broadly in three of the four rivers sampled and from high to low elevation sites, although typically it was found in low numbers. For the Characiformes, *B. atrocaudatus* was the most commonly collected species, collected in all rivers and across all elevation levels. The characid

E. ecuadorensis was also collected fairly widely in three of the four rivers sampled, although at fewer sites. The characids *B. bucayensis* and the parodontid *S. wagneri* were the least widely distributed, both being collected only in sites of the Guayas drainage.

The Astroblepidae was the most common family collected at high elevations (28 fish) and middle elevations. *A. cf. longifilis* and *A. cyclopus* were collected in similar numbers at high and middle elevation sites. At low elevations, Bryconids and Characids were the most common families collected. *B. atrocaudatus*, the only Bryconid collected, was the most common species found at the low elevation levels. All Characids, *R. altipinna*, *B. bucayensis*, and *E. ecuadorensis* were collected in similar numbers at low elevations, although *B. bucayensis* was only collected at one low elevation site, whereas the other characids were also collected at other sites and elevations. The Heptapterid catfish *P. elongata* was also collected primarily at low and mid elevation sites in two rivers. Surprisingly, the Loricariidae were collected in similar numbers at all elevation levels, however, the two Loricariid species seem to occur most commonly at different elevations. *C. bifurcum* was collected mostly at low or high elevations (7 fish and 5 fish, respectively), while *T. santarosensis* were found mostly at middle elevations (5 fish) and none found at low elevations.

The specimens collected were relatively small in size ranging from 20 to 131mm in length. There was significant variation in size among species (ANOVA, $F=6.93$, $df=11,159$, $p=1.68 \times 10^{-9}$) with the average largest species being *Pimelodella elongata* (89.44mm ±24.50), *Saccodon wagneri* (78.21mm ±24.25), and *Astroblepus cf. longifilis* (66.52mm ±12.49), and the average smallest species being *Pseudopoecilia* sp. (20.81 mm ±4.49) (Figure S2).

Fish water content ranged from 63.48% to 89.69% (Table S2), and there is a significant negative correlation between specimen length (mm) and water content (%) (correlation analysis, r= -0.39, df=169, p=1.43 x 10^{-7}) (Figure 5). Most families seemed relatively similar in typical water content with averages in the high 70's and low 80's. The most divergent families were the Loricariidae and Parodontidae, which had much lower water content than the other families, averaging 71.45% ± 3.75 and 70.13% ±4.45, respectively.

Variation in total C, N, and P among taxonomic families and species

There was a relatively strong taxonomic signal to the stoichiometric variation among the fishes collected, especially in N and P. Suckermouth catfishes in the family Loricariidae were the most divergent from the other families for all three elements.

Total C did not vary much among the Characiformes, which had an average C content of 44.83% ±3.62. The Cyprinodontiformes had a similar average total C percentage of 44.59% ±0.52. Taxonomic families in the order Siluriformes exhibited an average that was generally similar to that of the other orders although there was more variation among families. The difference in total C among families was statistically significant (ANOVA, F=13.72, df=7, 163, p=6.79x 10^{-14}), with the Loricariidae having the lowest C content out of all the families, averaging 37.68% ±2.53 (Figure 6a). To explore this more, separate analyses were conducted at the level of species within the orders Characiformes and Siluriformes. There was no significant difference in total C among the Characiformes species (ANOVA, F=1.50, df=5,64, p=0.20), but there was a significant difference among Siluriform species (ANOVA, F=19.71, df=5, p=2.0 x 10^{-13}). Post hoc comparisons using the Tukey HSD test indicated that the mean total C for the

Loricariids *C. bifurcum* and *T. santarosensis* were significantly different from *A. cf. longifilis*, *A. cyclopus*, *P. elongata*, and *I. laticeps* ($p < 0.05$). However, *C. bifurcum* and *T. santarosensis* did not differ significantly from each other. Post hoc comparisons also showed that *I. laticeps* significantly differed from *A. cf. longifilis* ($p<0.05$) (Table 3).

Variation in total N among families was more apparent. The Siluriformes had a lower mean average N (10.35% ±1.39), with the Loricariidae having a substantially lower N content than the other families in this order. The Characiformes had a slightly higher average of 10.70% ±1.02, with a declining trend from the Bryconidae, to the Characidae, to the Parodontidae. The Cyprinodontiformes had the highest average N content with an average of 11.19% ±0.36. There was a significant difference in total N among all families (ANOVA, $F=34.67$, $df=7,163$, $p=2 \times 10^{-16}$) (Figure 6b). Exploring variation in total N within orders, there were significant differences among species in both the Characiformes and Siluriformes (ANOVA, $F=17.21$, $df=4,61$, $p=1.69 \times 10^{-9}$; $F=70.46$, $df=5,95$, $p< 2.0 \times 10^{-16}$, respectively). Post hoc comparisons using the Tukey HSD test indicated that mean N for *R. altipinna* and *B. atrocaudatus* were significantly different than *E. ecuadorensis* ($p<0.001$ for both) and *S. wagneri* ($p<0.001$, $p<0.0000001$, respectively). In addition, *S. wagneri* and *E. ecuadorensis* were significantly different from each other ($p=0.02$) (Table 4). For Siluriformes, the total N content for the loricariid *C. bifurcum* was significantly different from all other Siluriformes collected except the other loricariid *T. santarosensis*. Total N content for *A. cf. longifilis* was also significantly different from all other Siluriformes collected except for *P. elongata* and *I. laticeps*. *A. cyclopus* and *T. santarosensis* total N content were significantly different from all Siluriformes collected (Table 4).

There was also notable variation in total P content among families. The Characiformes had an average of 5.48% ±1.24 P content, with the Parodontidae exhibiting higher total P than the Bryconidae and Characidae. The Cyprinodontiformes had an average P of 4.71% ±1.05. The Siluriformes had the highest P content with an average of 6.55% ±2.09, however, this higher average is mostly due to the Loricariidae having the highest P content of any family in the study with an average of 10.30% ±1.06 (Figure 6c). There was significant variation in total P among families (ANOVA, $F=43.11$, $df=7,163$, $p=2 \times 10^{-16}$). In terms of differences among species within orders, Characiform species differed significantly (ANOVA, $F=6.21$, $df=4,61$, $p<0.001$), with *S. wagneri* differing from all other Characiform species collected (Table 4). Total P content also differed significantly among Siluriform species (ANOVA, $F=52.84$, $df=5,95$, $p< 2.0 \times 10^{-16}$), with the Loricariids *C. bifurcum* and *T. santarosensis* differing from all other Siluriformes collected, except from each other (Table 4).

There was no significant correlation between average N and P for the Characiformes (correlation analysis, $r=-0.27$, $df=15$, $p=0.29$) (Figure 7a), however, there was a strong negative correlation in the Siluriformes that was highly significant (correlation analysis, $r=-0.83$, $df=28$, $p=1.27 \times 10^{-8}$) (Figure 7b).

Influence of river, elevation, and species identity on total C, N, and P in fishes

To put the differences in total C, N, and P among species in the context of variation among rivers and site elevation, I conducted separate and combined analysis of variation of these elements by river and elevation.

For the analyses conducted separately, average fish C, N, and P content did not differ significantly between low, middle, and high elevation sites when pooled across all species (ANOVA, $F=1.62$, $df=2, 168$, $p=0.20$; $F= 0.17$, $df=2,168$, $p=0.84$; $F=0.47$, $df=2,168$, $p=0.63$,respectively) (Figures 8 and 9). The fish C content differed significantly among rivers (ANOVA, $F=7.98$, $df=3,167$, $p= 5.28 \times 10^{-5}$), as did fish N (ANOVA, $F=3.27$, $df=3, 167$, $p= 0.02$), and P (ANOVA, $F=6.53$, $df=3, 167$, $p<0.001$), suggesting that drainage basin had a stronger influence on fish stoichiometric variation than elevation.

For the combined analyses, variation in total C, N, and P were modeled as a function of River, Elevation (categorical variable with three levels: high, mid and low elevations), species, and their interactions. For total C in fishes, river and species accounted for a highly significant portion of the variation, while elevation and the interactions among variables were not significant (Table 5). Consistent with the analyses reported above, river and species accounted for highly significant portions of the variation in both total N and P in fishes (Table 5). Elevation also accounted for significant variation in total N and P in fishes, although it was only marginally significant for N. The interaction between river and elevation was also only marginally significant for total N while the other interactions were not significant. None of the interactions were significant for total P in fishes. Thus, species identity and river again seemed more important than elevation in accounting for variation in total C, N, and P, although elevation accounted for a statistically significant component of the variation of N and P in these combined analyses.

Variation in fish total C, N, and P within rivers

Given the strong influence of river on variation in fish total C, N, and P, I further analyzed variation for each river separately. First, the relationship between total C, N, and P in fish species within each river was examined. For these analyses, fish total C, N, and P were averaged for each species by collection site, and the pairwise correlations between these elements in each river were calculated. Average total N and P were moderately to strongly negatively correlated in fishes in the Cristal ($r=-0.72$, $df=15$, $p<0.001$), Chimbo ($r=-0.66$, $df=17$, $p=0.002$), and Jubones ($r=-0.99$, $df=3$, $p<0.001$) rivers, while the correlation was also negative in the Chaucha River, but was not statistically significant ($r=-0.42$, $df=6$, $p=0.30$). Fish average total C and P was also negatively correlated in the Cristal ($r = -0.49$, $df = 15$, $p = 0.04$), Chimbo ($r = -0.72$, $df = 17$, $p<0.001$), and Jubones ($r = -0.98$, $df = 3$, $p<0.001$) rivers, and was again similar in the Chaucha River, but not statistically significant ($r = -0.59$, $df = 6$, $p = 0.13$). Average total C and N were significantly positively correlated in the Chimbo ($r = 0.57$, $df = 17$, $p= 0.01$) and Jubones ($r = 0.95$, $df = 3$, $p= 0.01$) rivers, but the relationship was not significant in the Cristal ($r = 0.04$, $df = 15$, $p = 0.87$) or Chaucha rivers ($r = 0.00$, $df = 6$, $p = 0.99$). Therefore, there was a general tendency for fish total N and P and fish total C and P to be negatively correlated within rivers, while fish in two of the four rivers also exhibited a significant positive correlation between total C and N.

Analyses for fish total C, N, and P were conducted separately for each river with C, N, or P as individual response variables and standard length, elevation level (as a categorical variable), and species as predictor variables (Table 6). Species was statistically significant for all four rivers for total N, however it was only significant for C and P in two rivers, the Chimbo and

Jubones rivers (Figure 10), and was only marginally non-significant for total P in the Cristal River (F=2.06, df=9,35, p=0.06). In addition to Species, Standard Length was significant for total N in two rivers, the Cristal and Jubones rivers (F=7.09, df=1,34, p=0.01, F=7.90, df=1,158, p=0.01, respectively) (Figure 10). Elevation did not account for a significant portion of the variation in fish total C, N, and P in any of the rivers although it was marginally non-significant for total C in the Cristal River (F=3.19, df=2,42, p=0.051).

Variation in fish total C, N, and P for the most common species

Since taxonomic identity strongly influenced variation in fish total C, N, and P, analyses of the influence of environmental variables were conducted separately for the species with the largest sample sizes collected at the most sites: *Brycon atrocaudatus, Astoblepus cyclopus*, and *A.* cf. *longifilis*. Fish total C, N, or P were the individual response variables and fish standard length, elevation (in meters as a continuous variable), and river (numbered 1-4 from north to south) were the predictor variables (Table 7).

The results varied by species, but all three predictor variables accounted for significant variation of at least one element in one species. Elevation and standard length were significant for total N in *B. atrocaudatus* (F=11.23, df=1,22, p=0.003; F=5.67, df=1,22, p=0.03, respectively) (Figure 11). Total C for *A. cyclopus* varied as a function of Standard Length and River (F=4.59, df=1,29, p=0.04; F=9.28, df=1,29, p=0.01, respectively) (Figure 11). None of the predictor variables accounted for a significant portion of the variation in fish total C, N, or P in *A. cf. longifilis*, although total N and P were only marginally non-significant for body length (F=3.95, df=1,26, p=0.06; F=3.53, df=1,26, p=0.07, respectively). Thus, different factors seemed to be

acting on different elements in these three species, suggesting species specific patterns to the stoichiometric variation within species.

DISSCUSSION

The goal of our study was to determine whether C, N, and P contents vary with elevation in Neotropical stream fishes inhabiting Andean Mountain streams of southwestern Ecuador. In addition, the study aimed to examine variation in C, N and P concentrations among taxonomic families, between river systems, and among fish of different sizes. Elevation generally accounted for a relatively small or non-significant portion of the variation in fish total C, N, and P content. Taxonomic family, species, river, and standard length accounted for more of the variation in these elements. There also seemed to be differences among species in what factors accounted for significant variation in fish total C, N, and P. Thus, the influence of different factors, including elevation and variables correlated with elevation, on fish C, N, and P concentrations, seems to vary by species.

Environmental variation in N and P

N and P were negatively correlated in the river water in our sampling sites. N tended to increase as elevation increased, while P decreased as elevation increased. More P at lower elevations could be because the phosphorus cycle tends to move P downstream as the current carries decomposing plant and animal tissue and dissolved P eroded from rocks (US EPA, n.d.). More N at higher elevations could be because there is often less canopy cover at higher elevations. Less canopy cover impacts the light availability which impacts photosynthesis. Photosynthesis then affects carbon fixation and elemental ratios. At higher elevations, this results in more light and thus in more phytoplankton and epilithon. Phytoplankton and epilithon have high chlorophyll count and chlorophyll is high in N (Kohler et al., 2012; Martyniuk, Modenutti, & Balseiro, 2016).

There did not appear to be a strong relationship between water and sediment N and P. In sediment, N increased with elevation as seen for the water samples, while sediment P also tended to increase with elevation in three of the four rivers, which was the opposite pattern to that observed in the water samples. Total N could be increasing in sediment with altitude because it is believed that low temperatures seen in high elevations limit cycling or organic matter (He et al., 2016). Decreasing temperatures associated with increasing elevation can directly influence the community and nutrients by limiting metabolism, process rates, and nutrient mineralization rates for plants and soil microbes (Sundqvist et al.,2014). However, it is unknown as to exactly why C, N, and P vary as they do for this study system, as studies have been inconsistent on changes in the plant and soil nutrients along elevation gradients (He et al., 2016). Sundqvist et al. (2014) had similar results in their study conducted on plant and soil microbial communities in the Swedish subarctic tundra, and Fisher et al. (2013) found declining foliar N:P ratios and increasing plant growth as elevation increased in Peruvian ecosystems.

Variation in C, N, and P among taxonomic families and species

Total C, N, and P content varies significantly among taxonomic families and species. This was predicted as several studies have shown that taxonomic identity is important in explaining the variation in nutrient content and recycling rates in Neotropical streams (Hendrixson et al., 2007; McIntyre & Flecker, 2010; Vanni et al., 2002). It is generally thought that animals are homeostatic regulators such that, their bodies elemental composition is consistent within species (El-Sabaawi et al., 2016; Hood et al., 2005; McIntyre & Flecker, 2010; Persson et al., 2010; Vanni et al., 2002). In animals and specifically fishes, this is due to skeletal investment

and fundamental needs, while their growth and reproduction may become limited by nutrients (McIntyre & Flecker, 2010). Although individual fish may be homeostatic, fish are not homogenous across families and species, as was seen in this study. I found substantial variation in total C, N, and P among families and species.

Other factors that may play a role in variation among families and species include body size, phylogenetic affinities, body morphology, and growth rates (McIntyre & Flecker, 2010). Body size is an important factor explaining variation in nutrient excretion rates among species in many other studies, however the effect of differences in body size has not been consistent in magnitude or direction across studies (Hendrixson et al., 2007). In this study, body size did not seem to have a large effect on the variation of total body C, N, and P in fishes. It was significantly negatively correlated with fish water content. It was also significant in explaining variation in fish N in the Cristal and Jubones rivers when looking at the variation for each river separately, for N in *B. atrocaudatus* and for C in *A. cyclopus* when looking at the variation for the most common species, but was not a significant factor in most of the other analyses.

Loricariids had the highest body P content out of all the families collected, and generally had a total P content that was 3.5-5.6% higher than other families. This is a very large difference as some families, such as the Poeciliiade and Trichomycteridae had a total P content of only 4.7% and 5.2%. This is likely because of structural demands arising from skeletal investment since much of the P in fishes is in their bones (Hendrixson et al., 2007; McIntyre & Flecker, 2010; Sterner & George, 2000) and Loricariids are encased in bony armor to ward off predators (Sterner & Elser 2002, McIntyre & Flecker 2010). Other families tend to have a greater proportion of muscle and less bone than Loricariids and are consequently faster

swimmers, suggesting a trade-off between investment in bone and muscle that can impact stoichiometric ratios in fishes (Elser *et al.* 1996, McIntyre & Flecker 2010).

Interestingly, the families Loricariidae and Parodontidae, which are in different taxonomic orders (Siluriformes and Characiformes, respectively) and are very different morphologically, varied in similar directions for total C, N, and P from other families within their respective orders. These two families had the least total N and the most total P. This is interesting because species within both of these taxonomic families perform similar ecological roles in Andean streams, they are bottom fish that occur in fast flowing streams with hard substrates from which they scrape algae and detritus (Jiménez Prado et al., 2015; Restrepo-Gómez & Mancera-Rodríguez, 2014). The two loricariid species collected were much more extreme in their elemental composition, having very high P and low N content relative to all other species measured (Fig. 7). This is consistent with their extreme ecological and morphological specialization. Loricariids, commonly known as suckermouth catfishes, are flattened dorso-ventrally, have large mouths highly adapted for scraping substrate for food, and are encased in bone (Hood et al., 2005). Although Paradontids are also specialized for a similar ecological niche, they are not as specialized morphologically as Loricariids are and exhibited less dramatic divergence in P and N than Loricariids. Parodontid species vary in their morphology and especially in their mouths depending on their habitat and the available resources. Some species have developed highly specialized teeth to scrape algae while others have teeth to consume aquatic insects (Restrepo-Gómez & Mancera-Rodríguez, 2014). The Parodontid collected in this study, *Saccodon wagneri*, is a species that is endemic to western Ecuador and

northwestern Peru, and has a ventral mouth adapted to scraping algae and organic material from rocks (Jiménez Prado et al., 2015).

Conversely, *Bryconamericus bucayensis* and *Eretmobrycon ecuadorensis* are very similar morphologically, both are small tetras in the family Characidae that are active swimmers and likely omnivorous (Jiménez Prado et al., 2015; Román P. & Román-Valencia, 2017). However, they appeared to exhibit some divergence in their nutrient contents, especially in their total P content (Fig. 7). *E. ecuadorensis* was collected at several sites in different drainages and was generally homogeneous in N and P. *B. bucayensis* was only collected at one site but it did not overlap with the samples of *E. ecuadorensis* measured. Characids are the largest family of Neotropical fishes and the phylogenetic relationships of many genera, including *Bryconamericus* and *Eretmobrycon,* have been controversial due to their great diversity, lack of synapomorphies, and high intraspecific variation (García-Melo et al., 2019). There is also virtually nothing known about the ecological differences between these two species in western Ecuador because of the lack of studies on them. However, *B. bucayensis* appears to generally be limited to low elevation river sites in southwestern Ecuador from the Guayas drainage southward, while *E. ecuadorensis* appears to have a broader distribution and has been recorded from a broader elevational range as well (Windsor Aguirre, personal observation). Differences in their ecology or physiological requirements may account for differences in their stoichiometry, but more data are needed to understand the magnitude and causes of the difference between them.

The other characid collected, *Rhoadsia altipinna*, is much more divergent morphologically and probably phylogenetically. It occurs form the Guayas drainage basin south

to Peru and a congener, *R. minor*, occurs north in the Esmeraldas drainage basin (Malato et al., 2017). *Rhoadsia* is larger than the other two characids, growing to 17 cm in length, and occurs over broad elevational ranges from sea level to approximately 1300 m (Jiménez Prado et al., 2015). Although very little is known about its ecology, it appears to feed primarily on detritus (Aguirre, personal observation). *R. altipinna* seemed to exhibit substantial variation in total P, overlapping with *E. ecuadorensis* and *B. bucayensis*, but exhibited a higher total N content (Fig. 7). It is not clear why this is because fish with greater total N content tend to be higher on the food chain (McIntyre & Flecker, 2010), which does not appear to be the case with *R. altipinna*. More detailed studies of its ecology and life history are needed. Nonetheless, the three characid species collected show evidence of differing in their total N or P content, suggesting that interesting patterns of stoichiometric divergence may occur even among species of the same taxonomic family.

It is also interesting to look at the differences between the two loricarioid catfish families, the Astroblepidae and Loricariidae. Astroblepids are naked suckermouth catfishes and feed primarily on aquatic invertebrates and occur at high elevations in Andean mountain streams, while Loricariids are armored suckermouth catfishes and are primarily obligate grazers of algae and detritus (Hood et al., 2005; Moody, Lujan, Roach, & Winemiller, 2019). Astroblepids have much more N and much less P than Loricariids. Because Astroblepids are typically found at higher elevations, which typically lack larger fish, they do not need armor to protect themselves from predacious fishes. This results in a need for less bone and consequently less P. Loricariids, however, are found at lower elevations with many predators. The armor serves as a defense against predacious fishes and results in higher bon content and

consequently higher P content. (Moody et al., 2019). In addition, diet could play a role explaining the difference seen in N and P since most insects, which Astroblepids primarily feed on, have high N content and some are poor in P (McIntyre & Flecker, 2010). In addition, the water content at high elevations had a high N and low P, while at low elevations the pattern was reversed. This is consistent with the difference in these elements between Loricariids, which are more common at lower and mid elevations generally and have low N and high P, and Astroblepids, which are most common at high elevations and are high in N and low in P.

Finally, there was an interesting pattern of divergence in total N content between the two species of *Astroblepus* collected, with *A. cf. longifilis* tending to have a higher total N content than *A. cyclopus*. Unfortunately, there is virtually nothing known about the ecology of these two species in western Ecuador, although they were collected sympatrically, suggesting that they likely exhibit significant ecological divergence to be able to coexist at the same sites (Macarthur & Levins, 1967). Differences in total N often reflect differences in trophic level (McIntyre & Flecker, 2010) suggesting that these two sympatric *Astroblepus* may be diverging in feeding habits, which would allow them to coexist and perhaps explain the differences in total N content seen. Given that there are a large number of *Astroblepus* species over a very broad elevational range in Andean mountain streams (Jiménez Prado et al., 2015), including a the highest elevations at which native Andean fishes occur, this may be a particularly good group to study the influence of different ecological factors and adaptation to different elevations on variation in stoichiometric ratios.

Factors influencing variation in C, N, and P in fish species

The rivers in which samples were collected had a significant influence on the total C, N, and P variation seen in the fishes. There are many factors that make rivers differ from one another such as sediment type, plant and animal species part of the ecosystem, canopy cover, oxygen levels, channels and flows, and land use by humans (Encalada et al., 2019; Jacobsen, 2008; Kohler et al., 2012; Winemiller et al., 2008). Although these factors and others could vary based on elevation as well, my data indicate that sites within a river were relatively homogenous compared to the differences seen among sites in different rivers.

Although river seems to be more important in accounting for variation in fish total C, N, and P, there was some differences in elemental composition that were associated with elevation. In the stream water, elevation played a factor with N and P content- N increased as elevation increased, while P decreased as elevation increased. Elevation also significantly influenced total N and P in fishes, though only marginally for N, and the interaction between river and elevation was also only marginally significant for total N. When looking at the variation in total C, N, and P for the most common species, elevation was significant for N in *B. atrocaudatus*. Therefore, elevation was not a large factor in the variation of total C, N, and P in fishes. This could be because fishes are more homeostatic and need to keep a consistent nutrient content in their body (El-Sabaawi et al., 2016; Hood et al., 2005; McIntyre & Flecker, 2010; Persson et al., 2010; Vanni, 2002), while plants, water and sediment are able to easily hold nutrients at differing concentrations (McIntyre & Flecker, 2010).

Another factor that influenced variation in C, N, and P in fishes was standard length. This is especially true in the Cristal and Jubones rivers as standard length was found to be significant in both rivers for N. Body size has been found to influence C, N, and P in other studies as well,

however, in other studies had a larger effect (Elser et al., 1996; Hall et al., 2007; Hendrixson et al., 2007; Mcintyre et al., 2008; Vanni, 2010; Vanni et al., 2002; Vanni & McIntyre, 2016), but most of these studies measured excretion and not total body C, N, and P. For example, Vanni et al. (2002), conducted a study in the Andean piedmont of Venezuela and their results showed that body size was important in determining nutrient recycling rates and ratios. Another study by McIntyre et al. (2008) conducted at the same location as Vanni et al. (2002) found that the patterns of fish body size can influence aggregate excretion rates as much as the taxonomic structure of the community.

Conclusions and Future Directions

I conclude that the effect of elevation on fish total C, N, and P content was typically relatively small or not significant. Taxonomic family, species, river, and standard length accounted for more of the variation in these elements. The influence of different factors, including elevation and variables correlated with elevation, on fish C, N, and P concentrations, seems to be complex and likely varies among species.

More research needs to be done to better understand the role that these fish species play in nutrient cycling in Andean ecosystems, especially since many of the rivers in the study area are under strong pressure from anthropogenic factors. Future studies should sample at more sites along broader elevational gradients and aim to include more species from more taxonomic families, as well as larger sample sizes. This study was limited as to how many fishes could be collected, which could affect the analysis of the influence of different factors on total C, N, and P. A key improvement would be to measure C, N, and P from larger samples of fish of

the same species per site. By having larger sample sizes, some of the marginally significant and non-significant results documented in this study could become significant. In addition, future studies can collect more fish at more sites in the same rivers sampled in this study and combine those data with the data from this study to better understand what is happening. It would also be interesting to get a better idea of nutrient cycling by measuring total C, N, and P excretion in the fishes in this region. This would allow us to better understand the nutrient content that these fishes require and if rivers in the region are N-limited or P-limited.

LITERATURE CITED

Anderson, E. P., Jenkins, C. N., Heilpern, S., Maldonado-Ocampo, J. A., Carvajal-Vallejos, F. M., Encalada, A. C., … Tedesco, P. A. (2018). Fragmentation of Andes-to-Amazon connectivity by hydropower dams. *Science Advances*, *4*. Retrieved from http://advances.sciencemag.org/

Anderson, E. P., & Maldonado-Ocampo, J. A. (2011). A Regional Perspective on the diversity and conservation of tropical Andean fishes. *Conservation Biology*, *25*(1), 30–39. https://doi.org/10.1111/j.1523-1739.2010.01568.x

Anderson, E. P., Marengo, J. A., Villalba, R., Roland Pierre Halloy, S., Young, B., Cordero, D., … Carrascal, D. R. (2011). Consequences of climate change for ecosystems and ecosystem services in the tropical Andes. Retrieved from https://www.researchgate.net/publication/265030120_Consequences_of_Climate_Change_for_Ecosystems_and_Ecosystem_Services_in_the_Tropical_Andes

Barletta, M., Jaureguizar, A. J., Baigun, C., Fontoura, N. F., Agostinho, A. A., Almeida-Val, V. M. F., … Corrêa, M. F. M. (2010). Fish and aquatic habitat conservation in South America: a continental overview with emphasis on neotropical systems. *Journal of Fish Biology*, *76*, 2118–2176. https://doi.org/10.1111/j.1095-8649.2010.02684.x

El-Sabaawi, R. W., Kohler, T. J., Zandoná, E., Travis, J., Marshall, M. C., Thomas, S. A., … Flecker, A. S. (2012). Environmental and organismal predictors of intraspecific variation in the stoichiometry of a neotropical freshwater fish. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0032713

El-Sabaawi, R. W., Warbanski, M. L., Rudman, S. M., Hovel, R., & Matthews, B. (2016). Investment in boney defensive traits alters organismal stoichiometry and excretion in fish. *Oecologia*, *181*(4), 1209–1220. https://doi.org/10.1007/s00442-016-3599-0

Elser, J. J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., … Sterner, R. W. (2003). Growth rate-stoichiometry couplings in diverse biota. *Ecology Letters*, *6*, 936–943. https://doi.org/10.1046/j.1461-0248.2003.00518.x

Elser, James J., Dobberfuhl, D. R., MacKay, N. A., & Schampel, J. H. (1996). Organism size, life history, and N:P stoichiometry. *BioScience*, *46*(9), 674–684.

https://doi.org/10.2307/1312897

Elser, James J., & Urabe, J. (1999). The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Available from: J. Urabe Retrieved On*, *80*(3), 735–751. https://doi.org/10.2307/177013

Encalada, A. C., Flecker, A. S., Poff, N. L. R., Suárez, E., Herrera, G. A., Ríos-Touma, B., … Anderson, E. P. (2019). A global perspective on tropical montane rivers. *Science*, *365*, 1124–1129. https://doi.org/10.1126/science.aax1682

Fausch, K. D., Torgersen, C. E., Baxter, C. V., & Li, H. W. (2002). Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. *BioScience*, *52*(6). Retrieved from https://watermark.silverchair.com/52-6-483.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAngwggJ0BgkqhkiG9w0BBwagggJlMIICYQIBADCCAloGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMwvNkenlm8tIhDUgLAgEQgIICK8PMwa_fJx7t7d6jMrxkzpLJ0FZVT61zVUCDLChPO9DDEq

García-Melo, J. E., Oliveira, C., Da Costa Silva, G. J., Ochoa-Orrego, L. E., Garcia Pereira, L. H., & Maldonado-Ocampo, J. A. (2019). Species delimitation of neotropical Characins (Stevardiinae): Implications for taxonomy of complex groups. *PLoS ONE*, *14*(6), 1–22. https://doi.org/10.1371/journal.pone.0216786

Griffiths, D. (2006). The direct contribution of fish to lake phosphorus cycles. *Ecology of Freshwater Fish*, *15*(1), 86–95. https://doi.org/10.1111/j.1600-0633.2006.00125.x

Hall, R. O., Koch, B. J., Marshall, M. C., Taylor, B. W., & Tronstad, L. M. (2007). How body size mediates the role of animals in nutrient cycling in aquatic ecosystems. In A. G. Hildrew, D. G. Raffaelli, & R. Edmonds-Brown (Eds.), *Body Size: The Sturcture and Function of Aquatic Ecosystems* (pp. 286–305). Cambridge University Press. Retrieved from file:///C:/Users/Jessica/Downloads/BodySizeTheStructureandFunctionofAquaticEcosystems (1).pdf

Harrell, F. E. J., & Dupont, C. (2020). Hmisc: Harrell Miscellaneous. Retrieved from https://cran.r-project.org/package=Hmisc

Hart, M. R., Quin, B. F., & Long Nguyen, M. (2004). Phosphorus runoff from agricultural land and direct fertilizer effects: A review. *Journal of Environmental Quality*, (33), 1954–1972.

https://doi.org/10.2134/jeq2004.1954

He, X., Hou, E., Liu, Y., & Wen, D. (2016). Altitudinal patterns and controls of plant and soil nutrient concentrations and stoichiometry in subtropical China. *Scientific Reports*, *6*(January), 1–9. https://doi.org/10.1038/srep24261

Hendrixson, H. A., Sterner, R. W., & Kay, A. D. (2007). Elemental stoichiometry of freshwater fishes in relation to phylogeny, allometry and ecology. *Journal of Fish Biology*, *70*(1), 121–140. https://doi.org/10.1111/j.1095-8649.2006.01280.x

Hodkinson, I. D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, *80*, 489–513. https://doi.org/10.1017/S1464793105006767

Hood, J. M., Vanni, Æ. M. J., & Flecker, A. S. (2005). Nutrient recycling by two phosphorus-rich grazing catfish : the potential for phosphorus-limitation of fish growth. *Ecosystem Ecology*, *146*, 247–257. https://doi.org/10.1007/s00442-005-0202-5

Jacobsen, D. (2008). Chapter 8: Tropical High-Altitude Streams. In D. Dudgeon (Ed.), *Tropical Stream Ecology* (1st ed., pp. 219–256). London: Academic Press.

Jiménez Prado, P., Aguirre, W., Laaz Moncayo, E., Navarrete Amaya, R., Nugra Salazar, F., Rebolledo Monsalve, E., … Valdiviezo Rivera, J. (2015). *Guía de peces para aguas continentales en la vertiente occidental del Ecuador*. Esmeraldas: PUCESE, MECN, UDA. Retrieved from https://www.academia.edu/12936223/Guia_de_peces_para_aguas_continentales_en_la_vertiente_occidental_del_Ecuador

Kitayama, K., Majalap-lee, N., & Aiba, S. (2000). Soil Phosphorus fractionation and Phosphorus-use efficiencies of tropical rainforests along altitudinal gradients of Mount Kinabalu , Borneo. *Oecologia*, *123*(3), 342–349.

Kitchell, J., O'Neill, R., Webb, D., Gallepp, G., Bartell, S., Koonce, J., & Ausmus, B. (1979). Consumer Regulation of Nutrient Cycling. *American Institute of Biological Sciences*, *29*(1), 28–34. Retrieved from https://www.jstor.org/stable/pdf/1307570.pdf

Kohler, T. J., Heatherly, T. N., El-Sabaawi, R. W., Zandonà, E., Marshall, M. C., Flecker, A. S., … Thomas, S. A. (2012). Flow, nutrients, and light availability influence Neotropical epilithon

biomass and stoichiometry. *Freshwater Science*, *31*(4), 1019–1034. https://doi.org/10.1899/11-141.1

Körner, C. (2007). The use of “altitude” in ecological research. *Trends in Ecology and Evolution*, *22*(11), 569–574. https://doi.org/10.1016/j.tree.2007.09.006

Lovett, G. M., & Kinsman, J. D. (1990). Atmospheric pollutant deposition to high-elevation ecosystems. *Atmospheric Environment. Part A. General Topics*, *24*(11), 2767–2786. https://doi.org/10.1016/0960-1686(90)90164-I

Macarthur, R., & Levins, R. (1967). The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The American Naturalist*, *101*(921), 377–385. https://doi.org/10.1086/282505

Malato, G., Shervette, V. R., Navarrete Amaya, R., Valdiviezo Rivera, J., Nugra Salazar, F., Calle Delgado, P., … Aguirre, W. E. (2017). Parallel body shape divergence in the Neotropical fish genus Rhoadsia (Teleostei: Characidae) along elevational gradients of the western slopes of the Ecuadorian Andes. *PLOS ONE*, *12*(6), e0179432. https://doi.org/10.1371/journal.pone.0179432

Martyniuk, N., Modenutti, B., & Balseiro, E. (2016). Forest structure affects the stoichiometry of periphyton primary producers in mountain streams of Northern Patagonia. *Ecosystems*. https://doi.org/10.1007/s10021-016-9996-8

McClain, M. E., Boyer, E. W., Dent, C. L., Gergel, S. E., Grimm, N. B., Groffman, P. M., … Pinay, G. (2003). Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, *6*(4), 301–312. https://doi.org/10.1007/s10021-003-0161-9

McIntyre, P. B., & Flecker, A. S. (2010). Ecological stoichiometry as an integrative framework in stream fish ecology. *American Fisheries Society Symposium*, *73*, 539–558.

Mcintyre, P. B., Flecker, A. S., Vanni, M. J., Hood, J. M., Taylor, B. W., & Thomas, S. A. (2008). Fish distributions and nutrient cycling in streams : Can fish create biogeochemical hotspots? *Ecology*, *89*(8), 2335–2346.

Mcintyre, P. B., Jones, L. E., Flecker, A. S., & Vanni, M. J. (2007). Fish extinctions alter nutrient recycling in tropical freshwaters. *PNAS March*, *104*(11), 4461–4466. Retrieved from

https://www.pnas.org/content/pnas/104/11/4461.full.pdf

Moody, E. K., Lujan, N. K., Roach, K. A., & Winemiller, K. O. (2019). Threshold elemental ratios and the temperature dependence of herbivory in fishes. *Functional Ecology*, 1–11. https://doi.org/10.1111/1365-2435.13301

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*(6772), 853–858. https://doi.org/10.1038/35002501

Persson, J., Fink, P., Goto, A., Hood, J. M., Jonas, J., Kato, S., & Persson, J. (2010). To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *IAP World Services*, *119*, 741–751. https://doi.org/10.1111/j.1600-0706.2009.18545.x

R Core Team. (2019). R: A language and environment for statistical computing. Vienna, Austria. Retrieved from https://www.r-project.org/.

Reis, R. E., Albert, J. S., Dario, F. Di, Mincarone, M. M., Petry, P., & Rocha, L. A. (2016). Fish biodiversity and conservation in South America. *Journal of Fish Biology*, *89*, 12–47. https://doi.org/10.1111/jfb.13016

Restrepo-Gómez, A., & Mancera-Rodríguez, N. (2014). Trophic ecology of Saccodon dariensis (Pisces: Parodontidae) in Guatapé River tributaries, Magdalena River Basin, Colombia. *Revista MVZ Cordoba*, *19*(1), 3930–3943.

Román P., C., & Román-Valencia, C. (2017). Diet and reproduction of Bryconamericus caucanus (Characiformes: Characidae) in the Venada Creek, Quindío River, Colombia. *Revista MVZ Córdoba*, *22*(3), 6296–6309. https://doi.org/10.21897/rmvz.1134

Sierra, R., Campos, F., & Chamberlin, J. (2002). Assessing biodiversity conservation priorities: ecosystem risk and representativeness in continental Ecuador. *Landscape and Urban Planning*, *59*(2), 95–110. https://doi.org/10.1016/S0169-2046(02)00006-3

Small, G. E., Pringle, C. M., Pyron, M., & Duff, J. H. (2011). Role of the fish astyanax aeneus (Characidae) as a keystone nutrient recycler in low-nutrient neotropical streams. *Ecology*, *92*(2), 386–397. https://doi.org/10.1890/10-0081.1

Steinman, A. D., & Mulholland, P. J. (2007). Phosphorus limitation, uptake, and turnover in

benthic stream algae. In *Methods in Stream Ecology* (2nd ed., pp. 187–212). Elsevier.

Sterner, Robert W., & George, N. B. (2000). Carbon, Nitrogen, and Phosphorus stoichiometry of Cyprinid fishes. *Ecology*, *81*(1), 127–140. Retrieved from https://esajournals.onlinelibrary.wiley.com/doi/pdf/10.1890/0012-9658%282000%29081%5B0127%3ACNAPSO%5D2.0.CO%3B2

Sterner, Robert Warner., & Elser, J. J. (2002). *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press. Retrieved from https://press.princeton.edu/titles/7434.html

Stiassny, M. L. J. (1996). An overview of freshwater biodiversity: With some lessons from African fishes. *Fisheries*, *21*(9), 7–13. https://doi.org/10.1577/1548-8446(1996)021<0007:AOOFB>2.0.CO;2

Sundqvist, M. K., Liu, Z., Giesler, R., & Wardle, D. A. (2014). Plant and microbial responses to nitrogen and phosphorus addition across an elevational gradient in subarctic tundra. *Ecology*, *95*(7), 1819–1835. https://doi.org/10.1890/13-0869.1

Sundqvist, M. K., Sanders, N. J., & Wardle, D. A. (2013). Community and ecosystem responses to elevational gradients: Processes, mechanisms, and insights for global change. *Annual Review of Ecology, Evolution, and Systematics*, *44*(1), 261–280. https://doi.org/10.1146/annurev-ecolsys-110512-135750

Taylor, B. W., Flecker, A. S., & Hall, R. O. (2006). Loss of a harvested fish species disrupts Carbon flow in a diverse tropical river. *SCIENCE*, *313*(5788), 833–836. https://doi.org/10.1126/science.1125485

US EPA, O. of W. (n.d.). 5.6 Phosphorus. Retrieved February 21, 2019, from https://archive.epa.gov/water/archive/web/html/vms56.html

Vanni, M. J. (2002). Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, *33*(1), 341–370. https://doi.org/10.1146/annurev.ecolsys.33.010802.150519

Vanni, M. J. (2010). Preface: When and where do fish have strong effects on stream ecosystem processes. *American Fisheries Society Symposium*, *73*, 531–538.

Vanni, M. J., Flecker, a S., Hood, J. M., & Headworth, J. L. (2002). Stoichiometry of nutrient

recycling by vertebrates in a tropical stream: Linking biodiversity and ecosystem function. *Ecology Letters*, *5*, 285–293. https://doi.org/10.1046/j.1461-0248.2002.00314.x

Vanni, M. J., & McIntyre, P. B. (2016). Predicting nutrient excretion of aquatic animals with metabolic ecology and ecological stoichiometry: A global synthesis. *Ecology*, *97*(12), 3460–3471. https://doi.org/10.1002/ecy.1582

Viviroli, D., Dürr, H. H., Messerli, B., Meybeck, M., & Weingartner, R. (2007). Mountains of the world, water towers for humanity: Typology, mapping, and global significance. *Water Resources Research*, *43*(7). https://doi.org/10.1029/2006WR005653

Winemiller, K., Agostinho, A., & Pellegrini Caramaschi, E. (2008). Chapter 5: Fish Ecology in Tropical Streams. In David. Dudgeon (Ed.), *Tropical Stream Ecology* (1st ed., pp. 107–146). London: Academic Press.

DATA TABLES AND FIGURES

	Site Code	G1	G2	G3	G4	G5	G6	J1	J2	B1	B2	
	Elevation Level (meters)	**815 (High)**	**450 (Middle)**	**171 (Low)**	**935 (High)**	**406 (Middle)**	**72 (Low)**	**900 (High)**	**169 (Low)**	**874 (High)**	**349 (Middle)**	**Total**
	Total	13 (41)	14 (25)	18 (31)	11 (32)	39 (93)	20 (89)	10 (20)	12 (18)	7 (17)	27 (67)	**171 (431)**
	Characiformes											
Characidae	***R. altipinna***	0	0	0	0	5 (6)	3 (4)	5 (9)	0	0	0	**13 (19)**
	B. bucayensis	0	0	3 (4)	0	0	0	0	0	0	0	**3 (4)**
	E. ecuadorensis	0	1 (1)	0	0	6 (13)	5 (35)	0	0	0	5 (14)	**17 (63)**
Bryconidae	***B. atrocaudatus***	0	3 (4)	3 (4)	1 (2)	5 (17)	4 (5)	0	4 (5)	0	5 (10)	**25 (47)**
Paradontidae	***S. wagneri***	0	0	3 (4)	0	5 (20)	0	0	0	0	0	**8 (24)**
	Cyprinodontiformes											
Poeciliidae	***Pseudopoecilia sp.***	0	1 (1)	0	0	0	0	0	0	0	3 (6)	**4 (7)**
	Siluriformes											
Astroblepidae	***A. cf. longifilis***	5 (9)	2 (3)	1 (1)	5 (15)	5 (6)	0	0	0	5 (14)	5 (12)	**28 (60)**
	A. cyclopus	6 (29)	5 (13)	5 (13)	5 (15)	5 (14)	0	0	0	2 (3)	4 (5)	**32 (92)**
Heptapteridae	***P. elongata***	0	0	0	0	1 (1)	5 (39)	0	5 (6)	0	0	**11 (46)**
Loricariidae	***C. bifurcum***	0	0	2 (4)	0	1 (2)	2 (5)	5 (11)	3 (6)	0	0	**13 (28)**
	T. santarosensis	1 (1)	0	0	0	5 (12)	0	0	0	0	0	**6 (13)**
Trichomycteridae	***I. laticeps***	1 (2)	2 (3)	1 (1)	0	1 (2)	1 (1)	0	0	0	5 (19)	**11 (28)**

Table 1: Specimens collected by site. The number listed is the number of specimens measured by site. Total number of specimens collected by site is shown in parenthesis.

Site Code	River	Drainage	Latitude (South)	Longitude (West)	Elevation (m)	Elevation Level	Water Temp. (°C)	Cond. (uS)	pH	Dissolved Oxygen (%)	Sample Length (m)	Average Depth (m)
G1	Cristal	Guayas	01.76854	79.17366	815	High	18.9	101.3	7.9	100	50	34.0
G2	Cristal	Guayas	01.77390	79.21638	450	Middle	21.7	112.5	8.1	122	50	30.0
G3	Cristal	Guayas	01.76727	79.26642	171	Low	22.6	113.4	8.1	100	40	23.3
G4	Chimbo	Guayas	02.11999	79.11493	935	High	18.5	97.8	7.6	129	65	40.0
G5	Chimbo	Guayas	02.17725	79.134	406	Middle	21.1	95.1	7.8	121.1	30	26.7
G6	Chimbo	Guayas	02.18333	79.33056	72	Low	24.2	268.1	8.4	135	40	26.7
B1	Chaucha	Balao	02.90265	79.48233	874	High	18.0	137.0	NA	99.2	30	26.7
B2	Chaucha	Balao	02.89849	79.56583	349	Middle	20.5	105.0	NA	100	30	30.0
J1	Jubones	Jubones	03.34651	79.38155	900	High	20.6	127.0	8.1	93.8	30	26.7
J2	Jubones	Jubones	03.32299	79.65786	169	Low	22.8	131.6	8.2	89.5	30	15.0

Table 2: Site Environmental Data. Temp. = Temperature and Cond. = Conductivity.

Siluriformes	*A. cf. longifilis*	*A. cyclopus*	*C. bifurcum*	*I. laticeps*	*P. elongata*	*T. santarosensis*
A. cf. longifilis	-					
A. cyclopus	1.6681	-				
C. bifurcum	**5.1629*** **	**6.8310*** **	-			
I. laticeps	**2.9763***	1.3082	**8.1392*** **	-		
P. elongata	-0.3671	-2.0352	**4.7957***	3.3435	-	
T. santarosensis	**-6.7701*** **	**-8.4382*** **	-1.6073	**-9.7465*** **	**-6.4030*** **	-

Table 3: Posthoc comparisons of pairwise differences in total C between Siluriform species based on Tukey's HSD test. * and bold indicates statistical significance at the 0.05 level, ** if p<0.01, and *** if p<0.001. There were no significant differences in C among Characiformes, so those results are not shown.

Characiformes	*B. atrocaudatus*	*B. bucayensis*	*E. ecuadorensis*	*R. altipinna*	*S. wagneri*
B. atrocaudatus	-	-0.7270	-0.4852	0.8032	**1.2346***
B. bucayensis	-0.9985	-	0.2418	0.0763	**1.9616***
E. ecuadorensis	**-1.1072*****	-0.1087	-	0.3181	**1.720***
R. altipinna	-0.0061	-1.0045	**-1.1132*****	-	**2.0378*****
S. wagneri	**-2.0655*****	-1.0670	**-0.9583***	**-2.0715*****	-

Siluriformes	*A. cf. longifilis*	*A. cyclopus*	*C. bifurcum*	*I. laticeps*	*P.elongata*	*T. santarosensis*
A. cf. longifilis	-	0.3350	**-4.4462*****	-0.5918	0.1855	**5.0115*****
A. cyclopus	**-1.2913*****	-	**-4.1112*****	-0.9268	-0.1495	**4.6765*****
C. bifurcum	**3.3325*****	**2.0412*****	-	**-5.0381*****	**-4.2607*****	0.5652
I. laticeps	-0.3989	**0.8924***	**2.9336*****	-	-0.7774	**5.6033*****
P. elongata	-0.0289	**1.2624*****	**3.3036*****	-0.3700	-	**4.8260*****
T. santarosensis	**-3.4862*****	**-2.1948*****	-0.1536	**-3.0873*****	**-3.4572*****	-

Table 4: Posthoc comparisons of pairwise differences in total N and P between Characiform (top) and Siluriform (bottom) species based on Tukey's HSD test. Mean differences in total nitrogen between species pairs are below the diagonal and mean differences in total phosphorus between species pairs are above the diagonal. Bold indicates statistical significance. * indicates $p<0.05$ level, ** indicates $p<0.01$, and *** indicates $p<0.001$.

Carbon	Sum of Squares	df	Mean Square	F	P
River	319.72	3	106.57	13.415	1.266×10^{-7}
Elevation Level	26.57	2	13.28	1.672	0.192
Species	840.56	11	76.14	9.619	2.484×10^{-12}
River: Elevation Level	77.49	4	19.37	2.439	0.051
River: Species	118.77	15	7.92	0.997	0.463
Elevation Level: Species	140.42	10	14.04	1.768	0.074
River: Elevation Level: Species	57.13	3	19.04	2.397	0.071
Residuals	969.16	122	7.94		

Nitrogen	Sum of Squares	df	Mean Square	F	P
River	14.855	3	4.952	11.339	1.299×10^{-6}
Elevation Level	2.692	2	1.346	3.082	0.049
Species	180.272	11	16.388	37.528	$<2.2 \times 10^{-16}$
River: Elevation Level	4.363	4	1.091	2.498	0.046
River: Species	6.516	15	0.434	0.995	0.465
Elevation Level: Species	5.017	10	0.502	1.149	0.332
River: Elevation Level: Species	0.752	3	0.251	0.574	0.633
Residuals	53.277	122	0.437		

Phosphorus	Sum of Squares	df	Mean Square	F	P
River	61.86	3	20.620	17.110	1.91×10^{-9}
Elevation Level	17.61	2	8.804	7.306	0.001
Species	323.99	11	29.454	24.440	$<2.2 \times 10^{-16}$
River: Elevation Level	6.30	6	1.049	0.871	0.518
River: Species	12.91	12	1.076	0.893	0.556
Elevation Level: Species	7.80	4	1.949	1.617	0.174
Residuals	159.08	132	1.205		

Table 5: ANOVA tables for the combined analysis to determine the influence of river, elevation level, and species identity on total C, N, and P in fishes.

	Carbon	Nitrogen	Phosphorus
Cristal			
Species	NS	**7.445×10^{-7}**	0.061
Elevation Level	0.0512	NS	NS
Body Length	NS	**0.0117**	NS
Chimbo			
Species	**2.512×10^{-8}**	**1.141×10^{-13}**	**9.52×10^{-8}**
Elevation Level	NS	NS	NS
Body Length	NS	NS	NS
Jubones			
Species	**0.001**	**$<2.2 \times 10^{-16}$**	**$<2.2 \times 10^{-16}$**
Elevation Level	NS	NS	NS
Body Length	NS	**0.006**	NS
Chaucha			
Species	0.204	**0.002**	0.177
Elevation Level	NS	NS	NS
Body Length	NS	NS	NS

Table 6: Model response variable p-values for Carbon, Nitrogen, and Phosphorus for each River. This analysis was conducted to determine the influence of species, elevation level, and body length on total C, N, and P of all species by river. Bold indicates statistical significance at the 0.05 level. NS indicates no statistical significance.

	Carbon	Nitrogen	Phosphorus
Brycon atrocaudatus			
Elevation	NS	**0.026**	NS
Body Length	NS	**0.003**	NS
River	NS	NS	NS
Astroblepus cyclopus			
Elevation	NS	NS	NS
Body Length	**0.041**	NS	NS
River	**0.005**	NS	NS
A. cf. longifilis			
Elevation	NS	NS	NS
Body Length	NS	0.058	0.071
River	NS	NS	NS

Table 7: Model response variable p-values for Carbon, Nitrogen, and Phosphorus for common species. This analysis was conducted to determine the influence of elevation, body length, and river on total C, N, and P of species with the largest sample sizes collected at the most sites: *Brycon atrocaudatus*, *Astroblepus cyclopus*, and *A. cf. longifilis*. Bold indicates statistical significance at the 0.05 level. NS indicates no statistical significance.

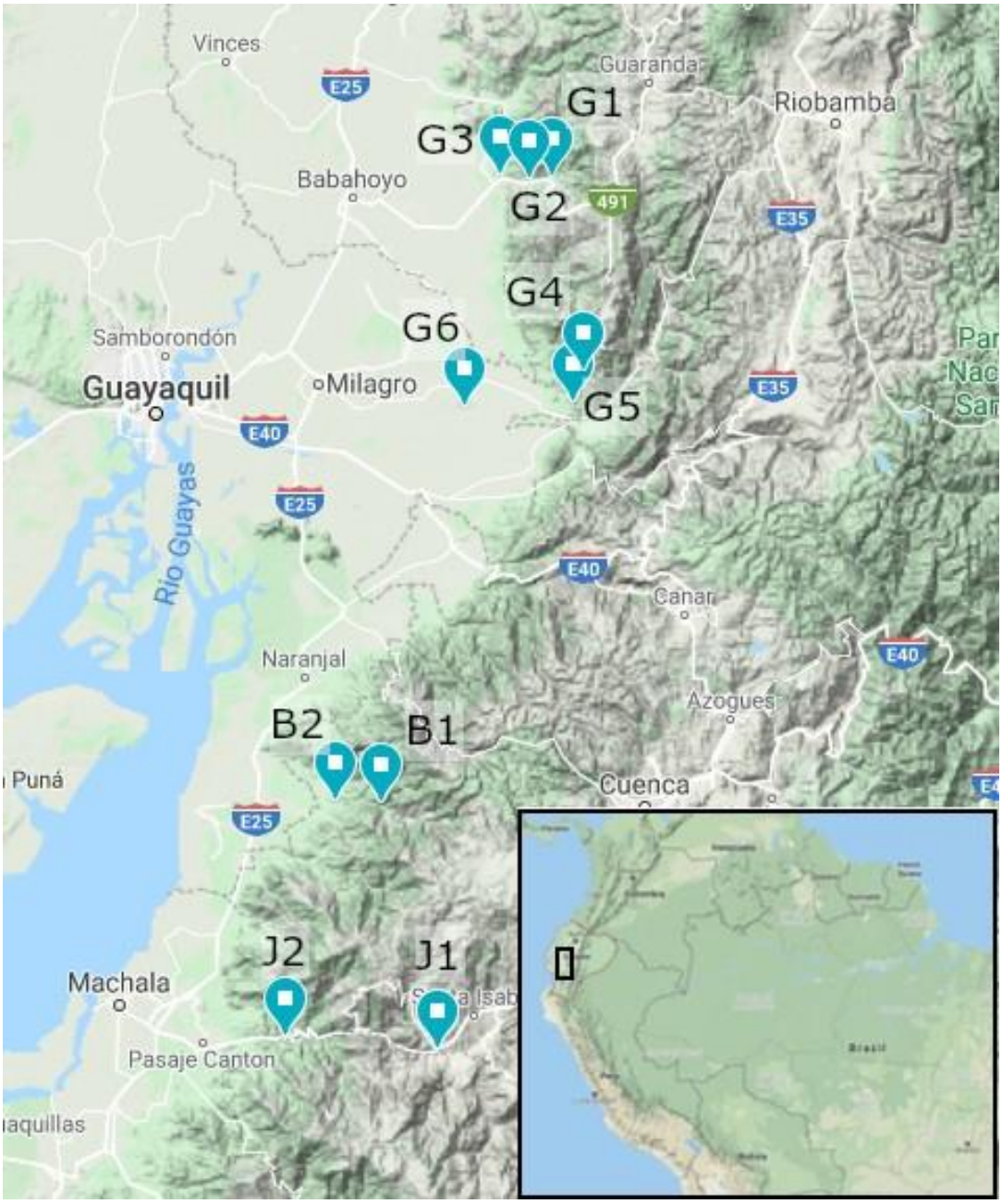

Figure 1: Collection site map. Sites G1-G3: Cristal river. Sites G4-G6: Chimbo river. Sites B1-B2: Chaucha river. Sites J1-J2: Jubones river. Images modified from Google Maps.

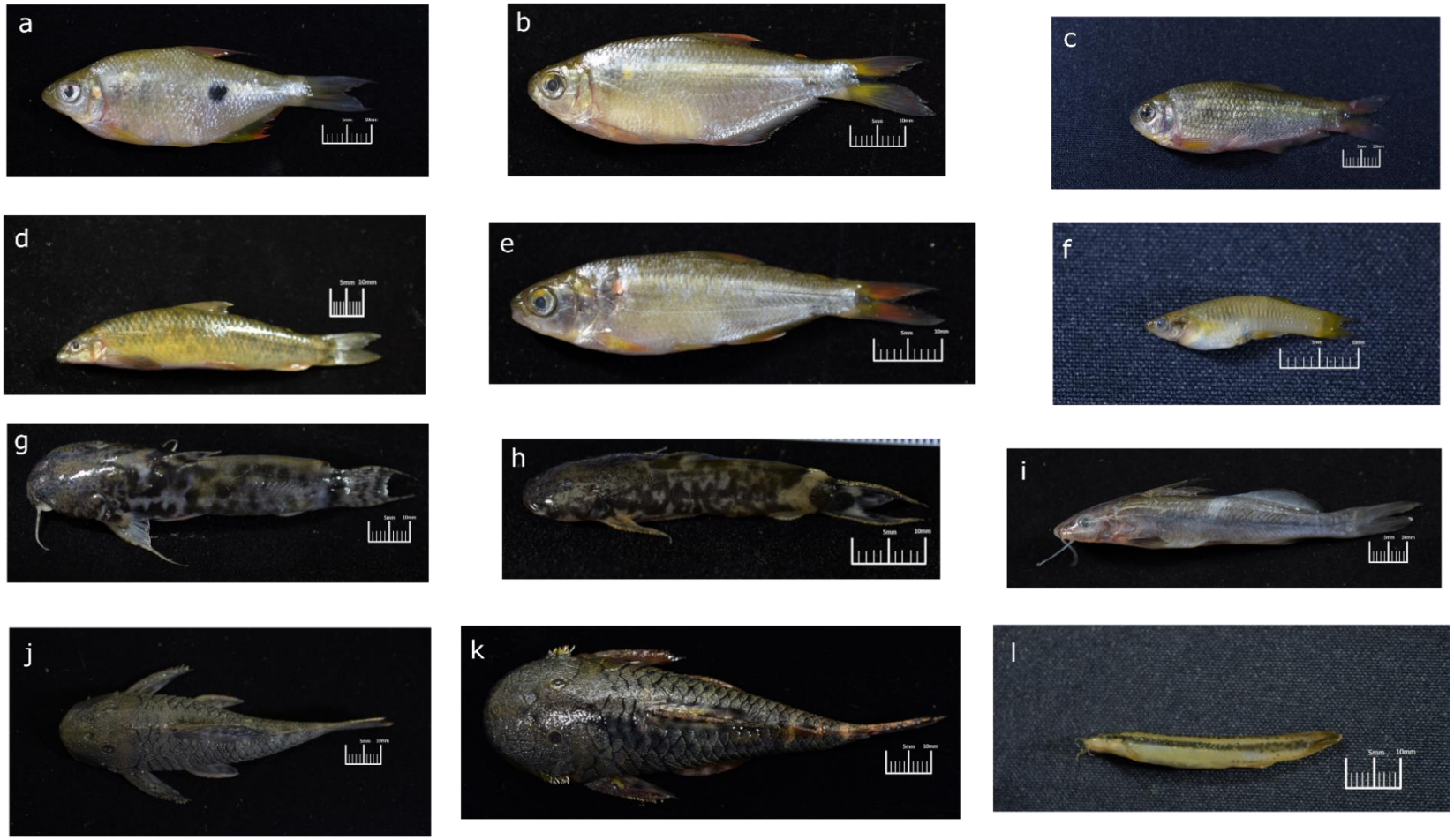

Figure 2: Species representative of fish measured. (a) *Rhoadsia altipinna* (b) *Bryconamericus bucayensis* (c) *Eretmobrycon ecuadorensis* (d*) Saccodon wagneri* (e) *Brycon atrocaudatus* (f) *Pseudopoecilia* sp. (g*) Astroblepus cf. longifilis* (h) *Astroblepus cyclopus* (i) *Pimelodella elongata* (j*) Chaetostoma bifurcum* (k) *Transancistrus santarosensis* (l) *Ituglanis laticeps.*

Figure 3: Collection sites. (a) Site G3, Cristal river at 171 meters. (b) Site G2, Cristal river at 450 meters. (c) Site G1, Cristal river at 815 meters. (d) Site G6, Chimbo river at 72 meters. (e) Site G5, Chimbo river at 406 meters. (f) Site G4, Chimbo river at 935 meters. (g) Site B2, Chaucha river at 349 meters. (h) Site B1, Chaucha river at 874 meters. (i) Site J2, Jubones river at 169 meters. (j) Site J1, Jubones river at 900 meters.

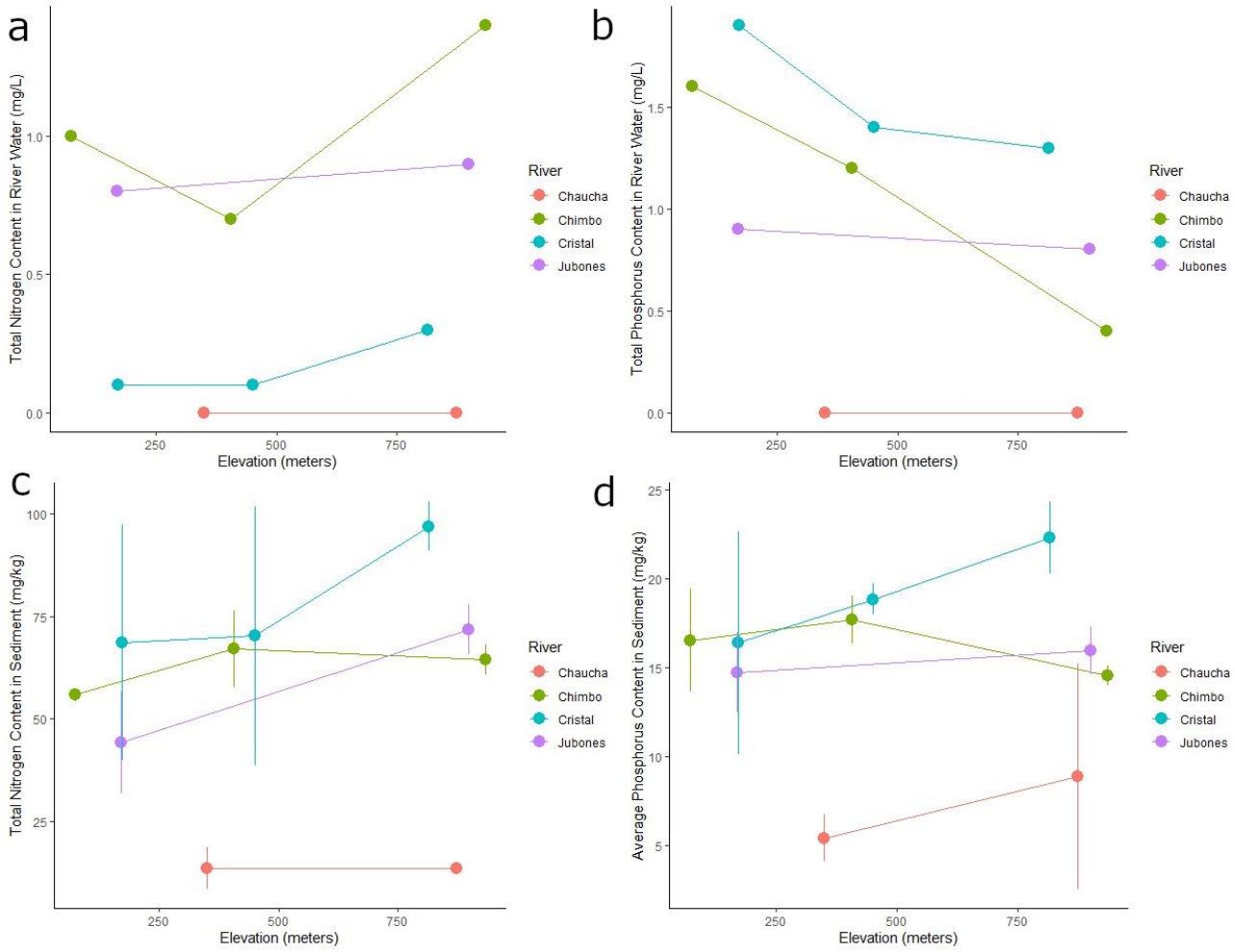

Figure 4 : Average total nitrogen and phosphorus in river water and sediment from sampling sites. (a) Total Nitrogen content in river water plotted against site elevation. (b) Total Phosphorus content in river water. (c) Average Nitrogen content in Soil. (d) Average Phosphorus content in Soil.

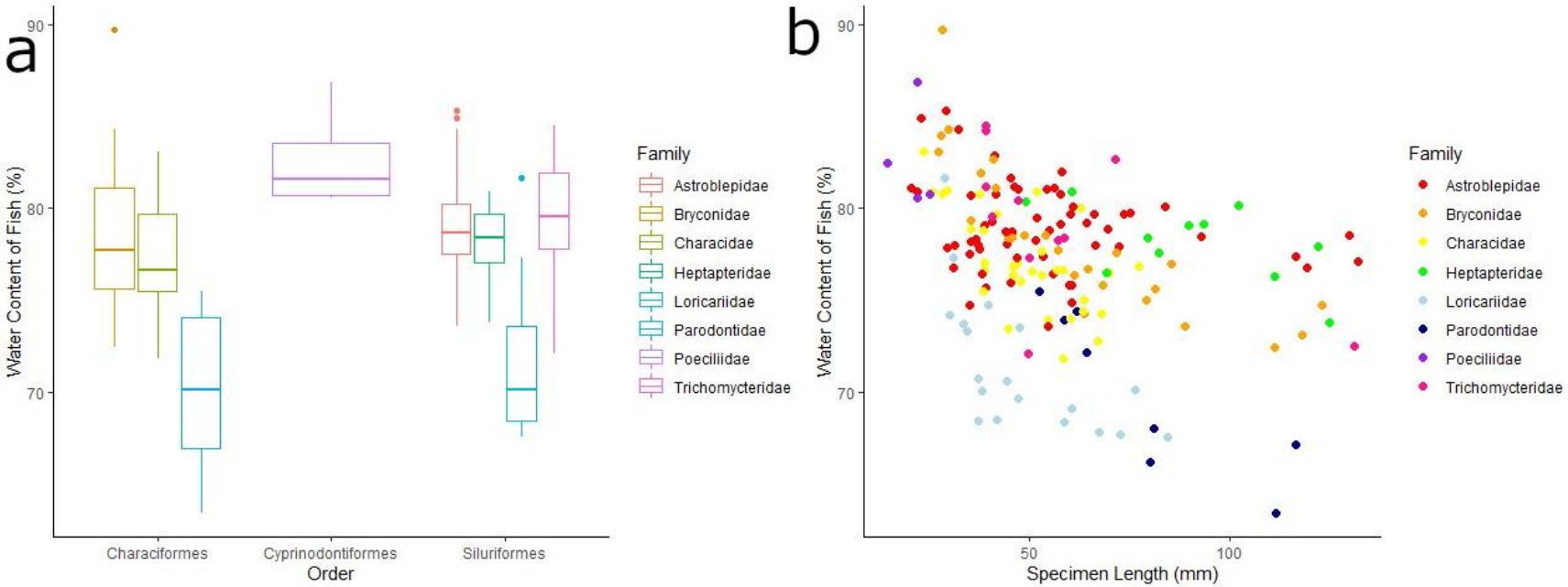

Figure 5: Water content in fishes. (a) Total water content (%) in different fish families listed by taxonomic order. (b) Total water content (%) in different fish families plotted against specimen length (mm).

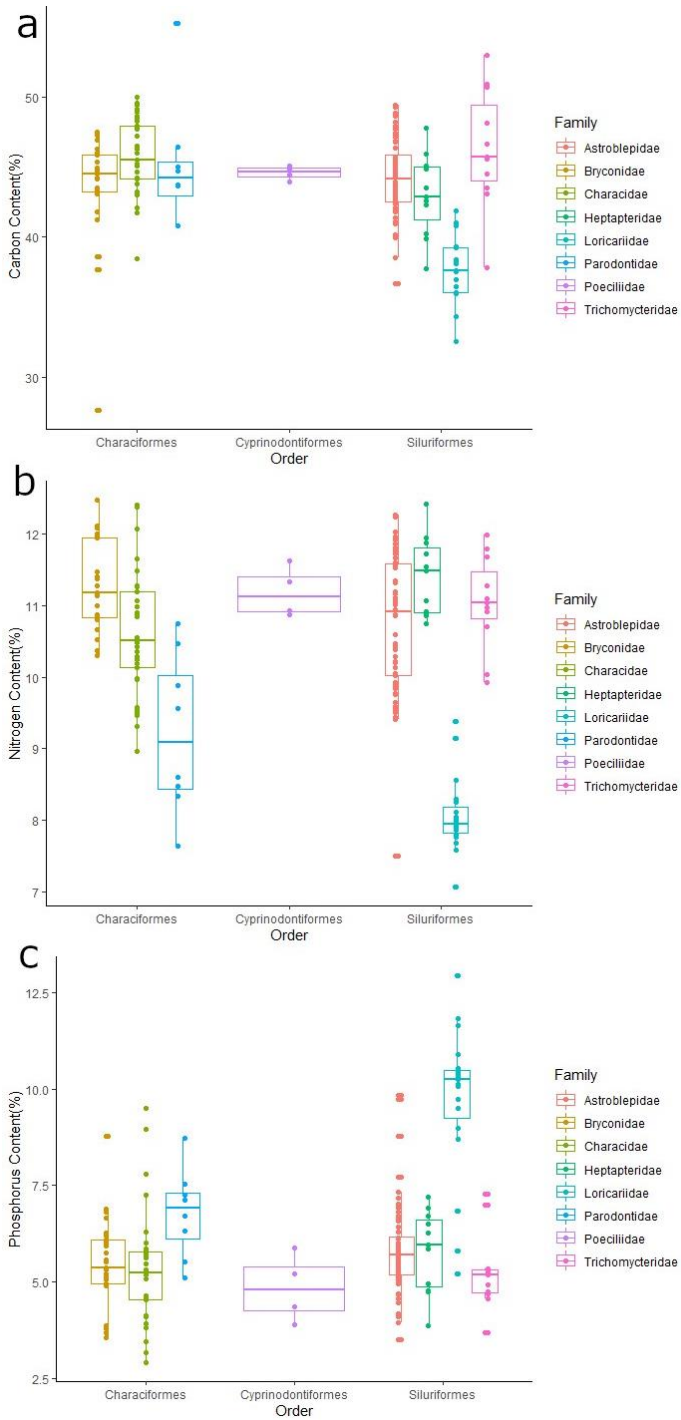

Figure 6: Total (a) Carbon, (b) Nitrogen, and (c) Phosphorus content (%) in different fish families listed by taxonomic order.

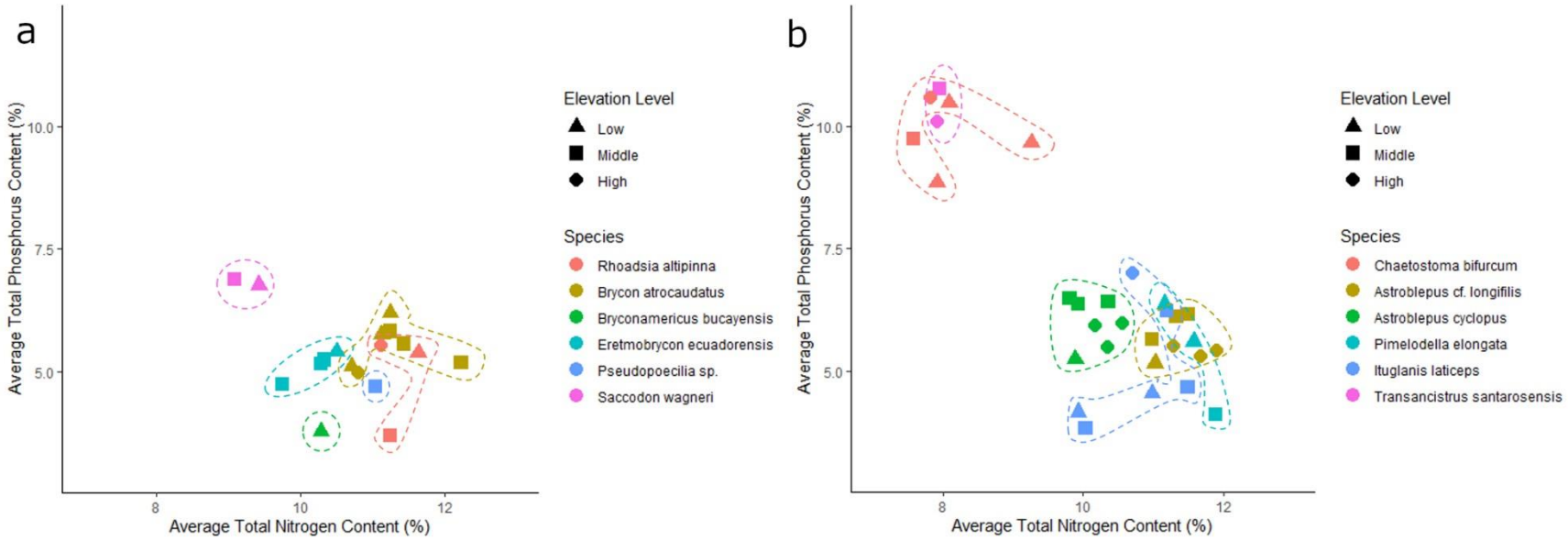

Figure 7: Average total nitrogen and phosphorus content (%) in different fish species separated by elevation. (a) Characiformes and Cyprinodontiformes. (b) Siluriformes. Each point represents the average for a species at sampling site. Hatched lines envelope site averages for each species.

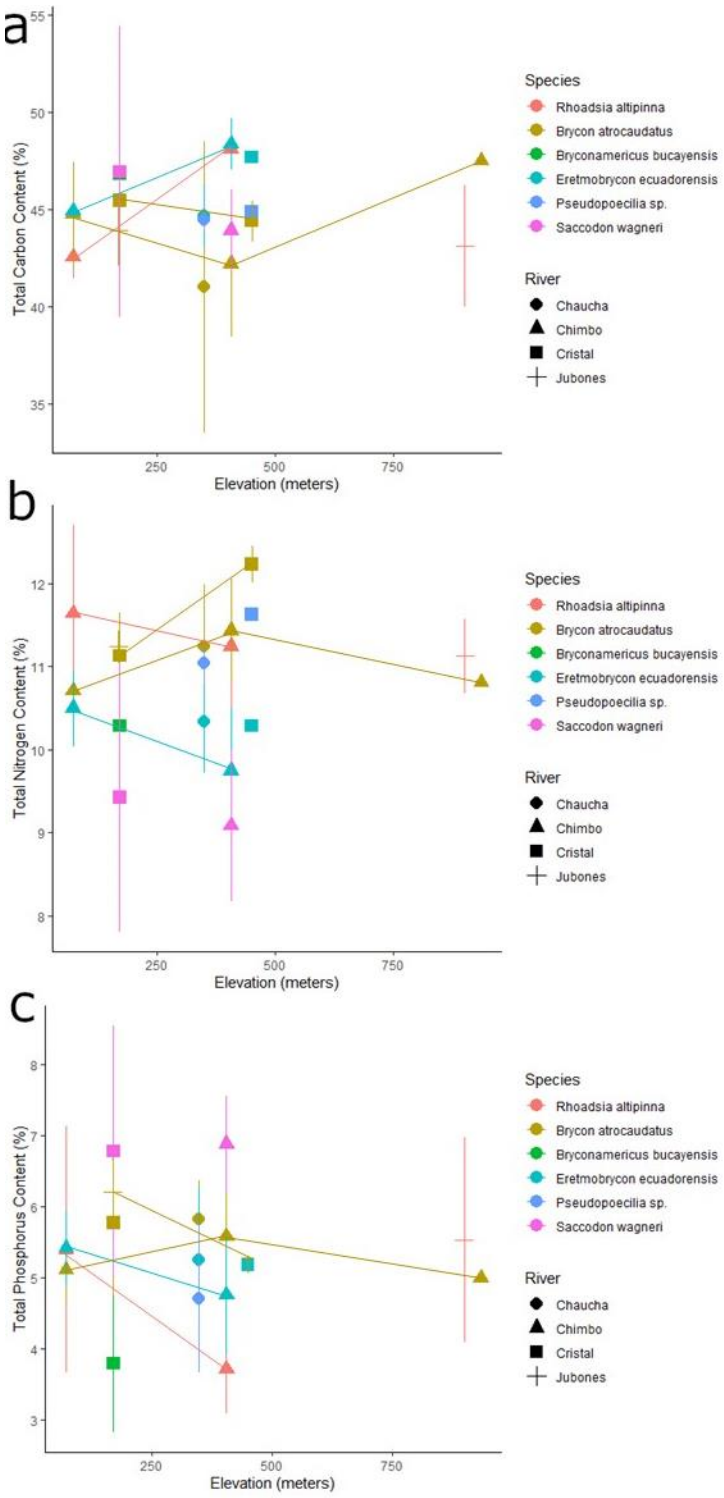

Figure 8: Total (a) Carbon, (b) Nitrogen, and (c) Phosphorus content (%) in Characiform and Cyprinodontiform species. Lines unite species site mean within same river.

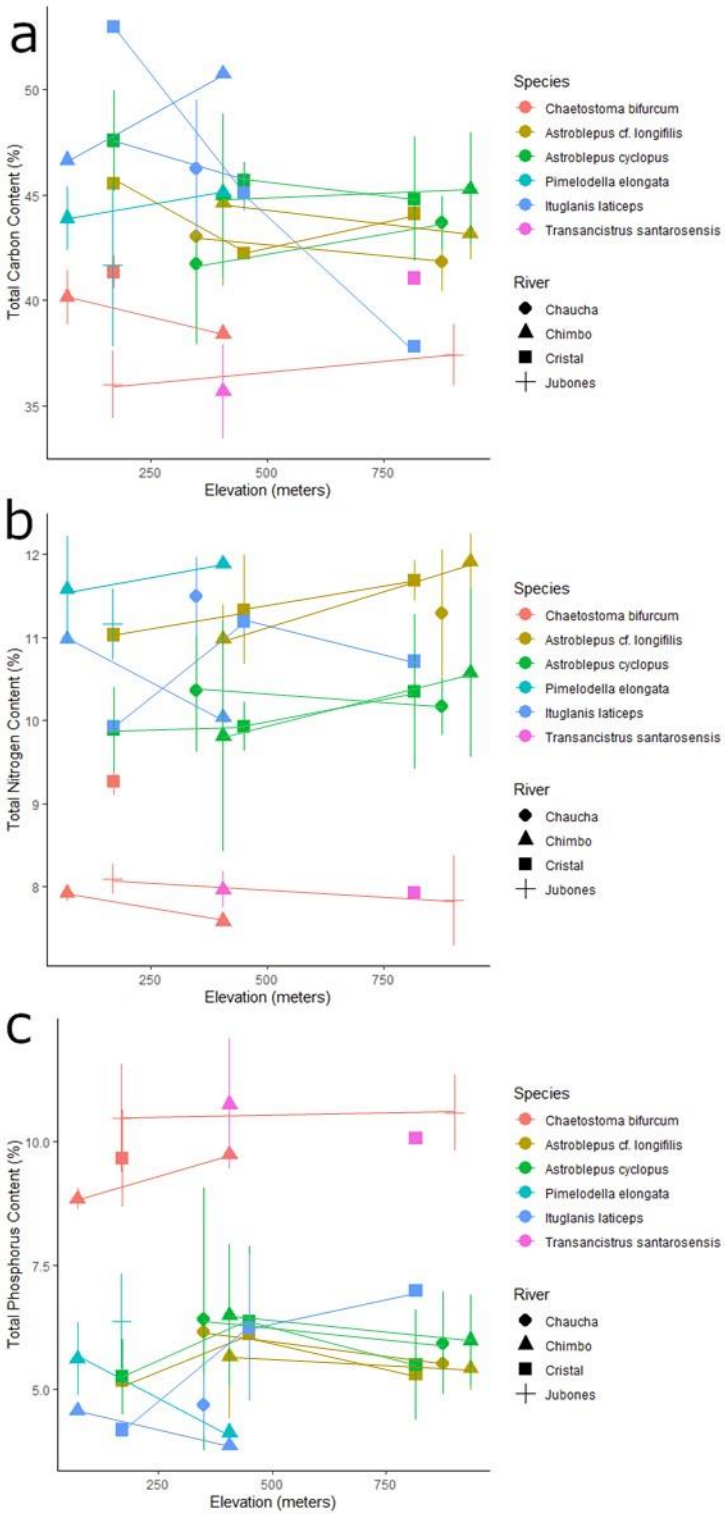

Figure 9: Total (a) Carbon, (b) Nitrogen, and (c) Phosphorus content (%) in Siluriform species. Lines unite species site mean within same river.

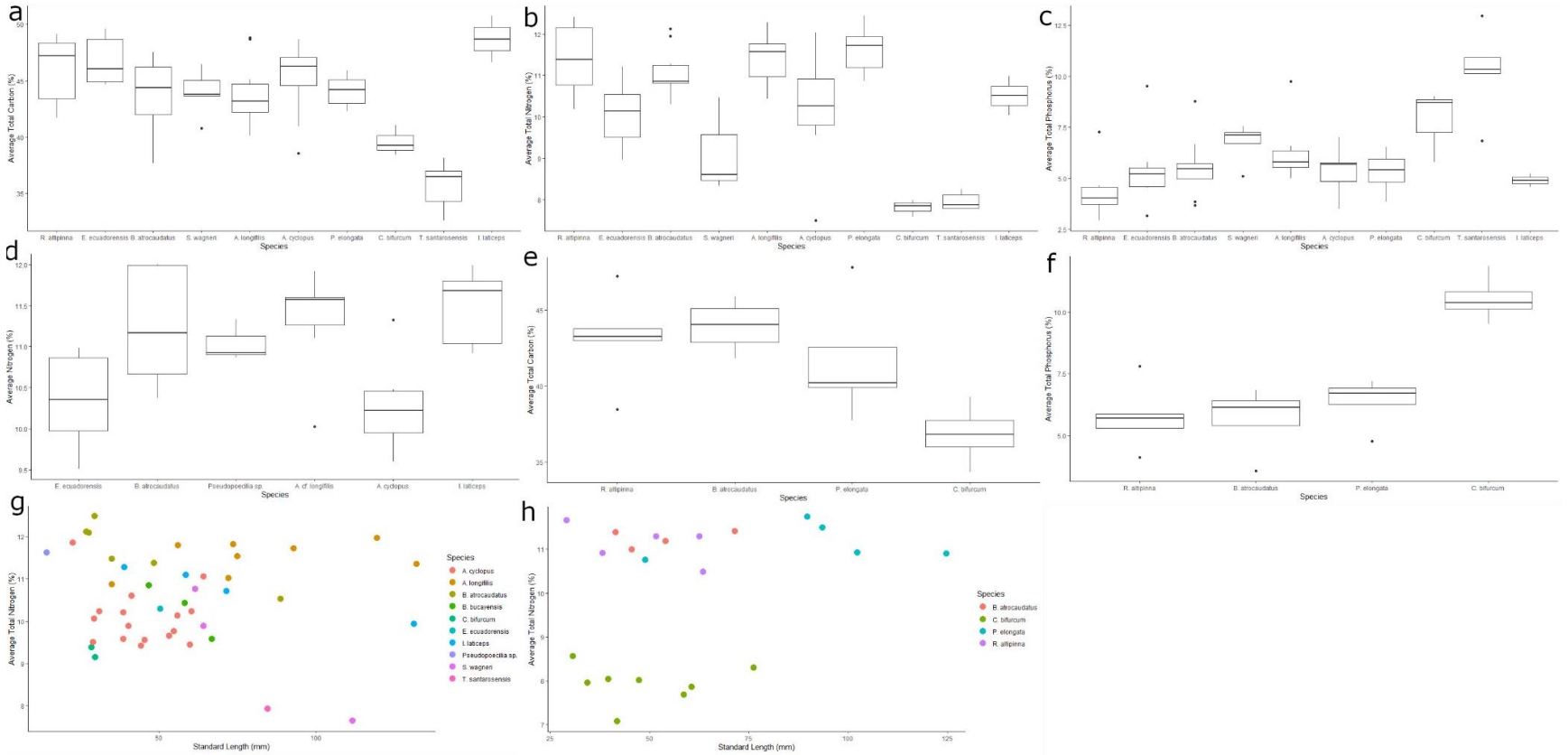

Figure 10: Effects of species and standard length on C, N, and P in fishes from each of the rivers sampled. Analyses for fish total C, N, and P were conducted separately for each river with C, N, or P as individual response variables and standard length, elevation level (as a categorical variable), and species as predictor variables. These are the effects found to be significant. (a) Average total Carbon for all species in the Chimbo River. (b) Average total Nitrogen for all species in the Chimbo River. (c) Average total Phosphorus for all species in the Chimbo River. (d) Average total Nitrogen for all species in the Chaucha River. (e) Average total Carbon for all species in the Jubones River. (f) Average total Phosphorus for all species in the Jubones River. (g) Average total Nitrogen by standard length for all species in Cristal River. (h) Average Total Nitrogen by body length for all species in the Jubones River.

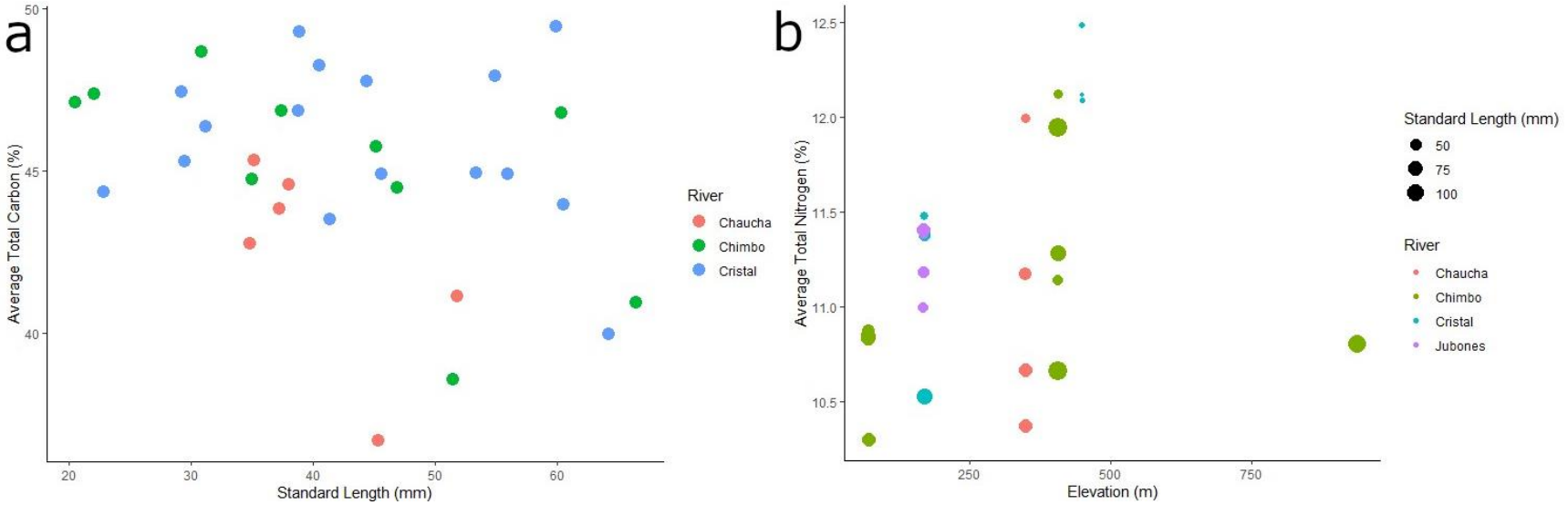

Figure 11: Significant effects on C, N, and P for species with the largest sample sizes collected at the most sites. (a) Variation in total Carbon in *A. cyclopus* plotted against standard length for specimens collected in different rivers. (b) Variation in total Nitrogen in *B. atrocaudatus* plotted against elevation for specimens of different standard length.

SUPPLEMENTAL INFORMATION

	River Water Total Phosphorus (mg/L)	River Water Filtered Phosphorus (mg/L)	River Water Total Nitrogen (mg/L)	River Water Filtered Nitrogen (mg/L)	River Sediment Phosphorus (mg/kg)	Shoreline Sediment Phosphorus (mg/kg)	Plant Sediment Phosphorus (mg/kg)	River Sediment Nitrogen (mg/kg)	Shoreline Sediment Nitrogen (mg/kg)	Plant Sediment Nitrogen (mg/kg)
Cristal										
171 m	1.9	1.8	0.1	0.1	22.1	17.4	9.7	96.6	70.2	39.2
450 m	1.4	1.4	0.1	0.1	18.6	19.8	18.1	74.9	99.3	36.5
815 m	1.3	1.2	0.3	0.1	24.1	20.1	22.7	98.3	90.5	102.2
Chimbo										
72 m	1.6	1.5	1.0	0.9	13.2	18.1	18.3	57.7	54.6	55.1
406 m	1.2	1.1	0.7	0.6	18.8	16.2	18.1	56.2	72.9	72.1
935 m	0.4	0.3	1.4	1.1	15.2	14.2	14.3	68.4	63.9	61.0
Chaucha										
349 m	NA	NA	NA	NA	6.9	4.4	4.9	19.4	11.5	9.8
874 m	NA	NA	NA	NA	7.6	3.3	15.8	13.4	14.1	12.7
Jubones										
169 m	0.9	0.7	0.8	0.7	12.8	17.2	14.2	32.6	57.3	42.6
900 m	0.8	0.8	0.9	0.8	14.5	17.2	16.2	65.3	77.4	72.9

Table S1: Nitrogen and phosphorus content in river water (filtered and non-filtered) and sediment separated by elevation and river.

Species	Average Length (millimeters)	Average Wet Weight (grams)	Average Dry Weight (grams)	Average Water Content (%)
Rhoadsia altipinna	45.71 ±17.92 (23.43-77.71)	3.74 ±4.20 (0.31-13.10)	0.83 ±0.99 (0.05-3.03)	79.69 ±2.30 (74.99-83.07)
Bryconamericus bucayensis	57.41 ±10.07 (46.91-66.98)	4.26 ±2.22 (2.11-6.54)	1.08 ±0.65 (0.49-1.78)	75.48 ±2.32 (72.81-77.00)
Eretmobrycon ecuadorensis	50.14 ±9.48 (35.17-68.00)	3.42 ±1.89 (0.98-7.96)	0.85 ±0.51 (0.21-2.05)	75.67 ±1.77 (71.86-78.90)
Brycon atrocaudatus	61.35 ±28.07 (27.15-122.97)	7.59 ±10.29 (0.36-38.02)	1.90 ±2.75 (0.04-10.23)	78.40 ±4.11 (72.47-89.69)
Saccodon wagneri	78.21 ±24.25 (52.32-116.57)	11.19 ±10.59 (2.61-30.38)	3.67 ±3.74 (0.64-9.97)	70.13 ±4.45 (63.48-75.52)
Pseudopoecilia sp.	20.81 ±4.49 (14.44-24.99)	0.20 ±0.09 (0.06-0.25)	0.03 ±0.02 (0.01-0.05)	82.65 ±2.91 (80.54-86.84)
Astroblepus cf. longifilis	66.52 ±28.12 (32.20-131.91)	10.12 ±13.47 (0.68-50.98)	2.19 ±3.05 (0.11-11.66)	79.52 ±2.07 (73.59-84.33)
Astroblepus cyclopus	42.82 ±12.49 (20.49-66.51)	2.25 ±1.68 (0.17-5.96)	0.50 ±0.39 (0.03-1.31)	78.39 ±2.47 (74.78-85.29)
Pimelodella elongata	89.44 ±24.50 (49.04-124.76)	10.33 ±7.51 (1.53-24.46)	2.34 ±1.89 (0.30-6.41)	78.20 ±2.10 (73.80-80.91)
Chaetostoma bifurcum	43.32 ±14.19 (28.75-76.22)	3.10 ±3.19 (0.75-11.70)	0.91 ±0.98 (0.14-3.49)	72.61 ±3.96 (68.42-81.67)
Transancistrus santarosensis	57.82 ±19.74 (37.22-84.56)	7.49 ±6.31 (1.42-16.05)	2.40 ±2.07 (0.42-5.20)	68.95 ±1.40 (67.57-70.77)
Ituglanis laticeps	56.59 ±26.71(39.02-131.01)	3.84 ±8.37 (0.60-29.02)	0.97 ±2.32 (0.09-7.97)	79.20 ±4.13 (72.12-84.52)

Table S2: Standard length + standard deviation for all specimens measured. Minimum and maximum are given in parenthesis.

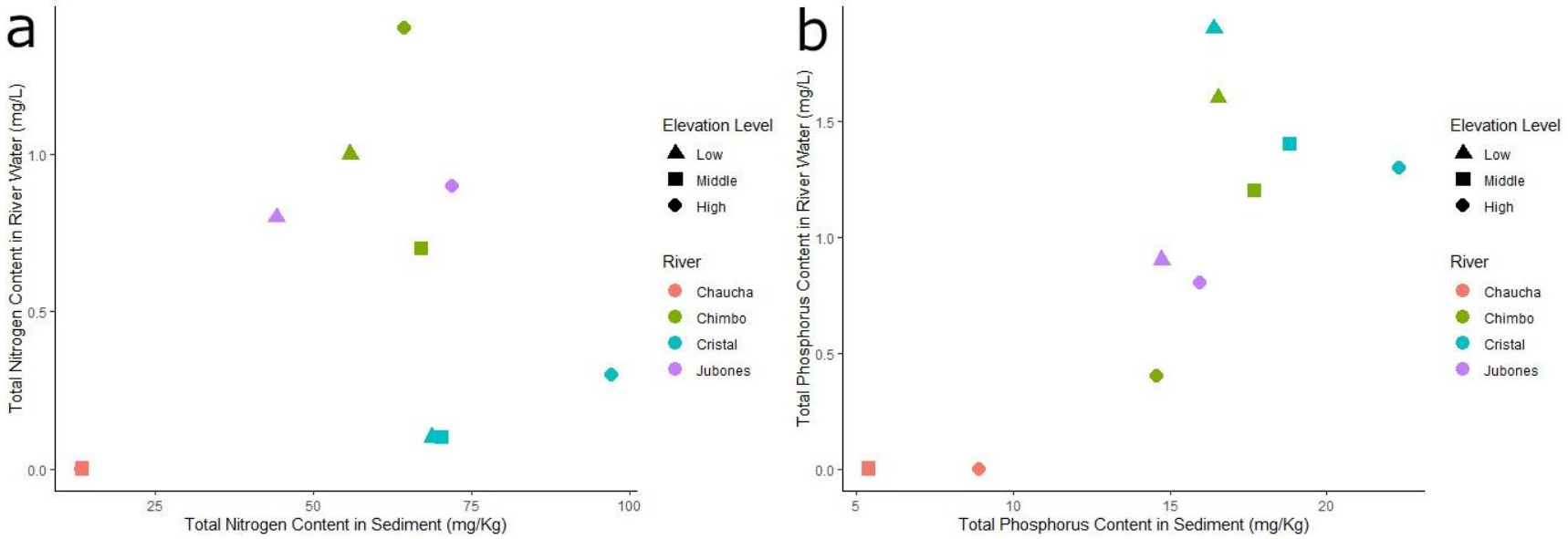

Figure S1: Total (a) Nitrogen and (b) Phosphorus content in river water plotted against sediment for each sampling site. Symbols separate samples by elevation level and colors by river.

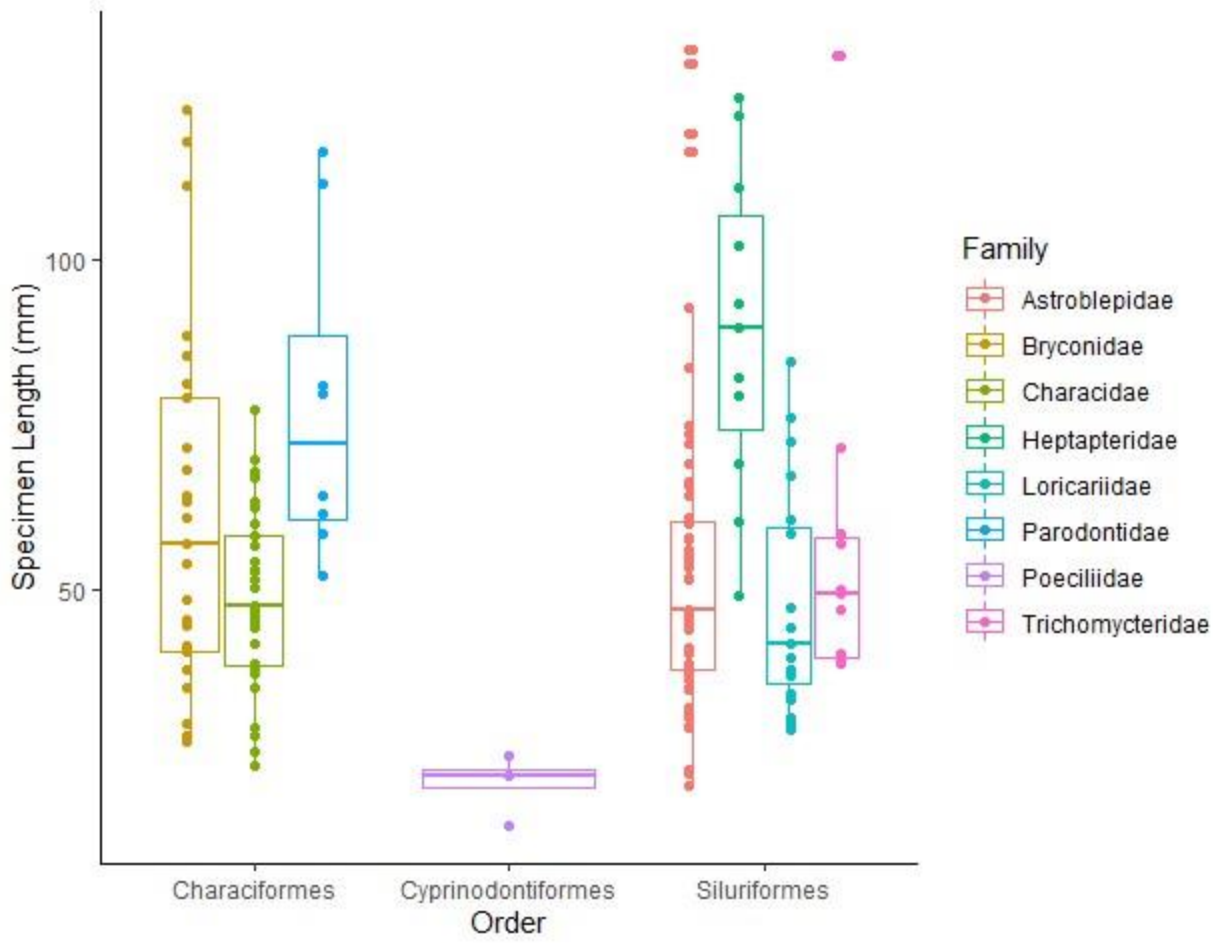

Figure S2: Specimen length (mm) in different fish families listed by taxonomic order.

Appendix 1: Lab Protocols Used to Measure C, N, and P from Fish Samples.

Analyzing Carbon and Nitrogen in Whole Fish

1. Dry whole fish in drying oven for approximately one week. Grind with mortar and pestle until powder is formed. Samples 0.5 grams and larger can be pulverized in the Mixer Mill.
    a. Scissors may be used to cut fish scales if not grinding up.
    b. Paint brushes can be used to help collect all fish powder after being pulverized in Mixer Mill.

2. Power on CE Elantech Flash EA1112 (CN analyzer) in McGowan South room 212. Sign- in to computer. Open the "Eager Xperience for Flash" program. Warm-up the analyzer.
    a. The analyzer will take about 45 minutes to warm-up.

3. While the analyzer is warming, prepare samples and fill out the sample table.
    a. In the sample table, enter the sample name, filename, type (e.g., blank, standard, unknown), and weight (mg).
    b. Shape a tin disk, 35mm into a tin cup. Tare on a scale and add approximately 5.00mg of sample into the tin cup. Pinch the top closed and insert into the appropriate location in the autosampler tray.
    c. Four standards of Aspartic acid should be used with the approximate following weights: 3.00mg, 6.00mg, 9.00mg, and 12.00mg. This will create your standard curve.

4. Once the analyzer is warmed up and your samples are prepared, run the CN analyzer.

5. To see the results once the run is done, click on the calculator and excel sheet icon (summarize results) at the top of the window.
    a. To open the results later, click on File→ Load Method and select the file to open. Then, follow the step above to open the results.

Analyzing Phosphorus in Whole Fish

1. Plug in the DRB200 Reactor and preheat to 100°C.

2. Prepare a blank tube with no ground fish, adding only 5mL of deionized water to a tube.

3. Weigh out approximately 2.0mg of ground fish into a weigh boat.

4. Carefully dump weighed out fish into a HACH TEST N' TUBE for High Range Total Phosphorus. Pipette 5mL of deionized water into the tube, rinsing the weigh boat as you pipette.

5. Add the contents of one Potassium Persulfate Powder Pillow to each vial and shake to dissolve the powder.

6. Insert all vials into the reactor and close the lid.

7. Start the instrument timer for 30 minutes.

8. While the vials are heating, turn on the DR5000 instrument. Start the program 542 P Total HR TNT. On the bottom right of the screen click on Options, then More… and select Chemical Form. Change the chemical form to P.

9. When the timer goes off, carefully remove each vial from the reactor and put into a test tube rack to cool to room temperature (approximately 30 minutes).

10. Once tubes are cooled to room temperature, pipette 2mL of 1.54 N Sodium Hydroxide Standard Solution into each vial. Invert the vial to mix.

11. Pipette 0.5mL of Molybdovanadate Reagent to each vial and invert to mix. Start a timer for a 7-minute reaction. During this reaction, centrifuge tubes for 2-3 minutes to settle any precipitate to the bottom of the tube (if needed).

12. After the 7-minute reaction time, clean the blank vial and insert into the cell holder. Zero the machine.

13. Clean each sample vial and insert into the cell holder to read the results. The results are in mg/L of P. All results must be read between 7-9 minutes after reaction begins.

Phosphorus Calculation

1. Take the reading from the machine in mg/L of P and multiply it by 0.01L (10mL in tube).

   P= 10.8mg/L weight of sample= 2.091mg

   10.8mg/L x 0.01L= 0.108mg

2. Take the answer from above and divide it by the weight of the sample in mg.

   0.108mg/2.091mg=0.0516

3. Take the answer from above and multiply by 100 to get the total phosphorus percent.

   0.0516 x 100= 5.16%