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Macroinvertebrate Community Composition, Food Web Structure, and Emergence Rate in Neotropical Cloud-Forest Streams in Mindo, Ecuador

Anna M. Harris
Grand Valley State University

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Macroinvertebrate community composition, food web structure, and emergence rate in

Neotropical cloud-forest streams in Mindo, Ecuador

Anna M. Harris

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Abstract

Tropical cloud forest streams are one of the most threatened and understudied ecosystems in the world. Understanding how these ecosystems function is essential for effective conservation. In this study, macroinvertebrate community composition, functional feeding group analysis, ecosystem attributes, and physicochemical parameters were used to evaluate biophysical stream conditions of 3 low-order Neotropical cloud forest streams at Reserva Las Gralarias in Mindo, Ecuador. Additionally, food web structure was analyzed via stable isotope analysis and aquatic insect emergence rate was also examined. As stream size increased from 1st to 3rd order, the macroinvertebrate communities shifted from being collector-gatherer dominated (65.2 to 29.8%, respectively) to being scraper dominated (17.9 to 56.3%, respectively). Shredders were poorly represented in all streams (2.7, 3.3, and 2.0% for 1st, 2nd, and 3rd order streams, respectively) similar to reports from other tropical systems. The analyses used in this and other tropical stream studies are based on temperate-based theories, which have been found to be inapplicable to tropical systems. Until tropical-based theoretical predictions are established, however, conservation efforts based on temperate theories should be implemented. Stable isotope analysis revealed a typical food web structure with basal resources having the lowest $\delta^{13}C$ and $\delta^{15}N$ signatures and these values increasing up the food web. Generally, $\delta^{15}N$ signatures in our systems were depleted when compared to other tropical studies. Lastly, aquatic insect emergence was not correlated with rain or the moon cycle. Results from this study provide base-line physical, chemical, and biological data on these streams that can be effectively used to track environmental changes in land-use via long-term monitoring. Furthermore, results from this study provide basic data on tropical stream ecosystem function that will be valuable as stream theories with specific predictions for the tropics are created, which will lead to better

monitoring efforts and more effective restoration and protection of these threatened and

disappearing systems.

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Abbreviations

BOM – benthic organic matter

C – carbon

CCA – canonical correspondence analysis

CPOM – coarse particulate organic matter

DO – dissolved oxygen

FFG – functional feeding group

FPOM – fine particulate organic matter

KC – Kathy's Creek

LC – Lucy's Creek

N – nitrogen

PAR – photosynthetically active radiation

RCC – river continuum concept

RLG – Reserva Las Gralarias

RSR – Rio Santa Rosa

VIF – variance inflation factors

Chapter 1: Introduction

1. Introduction

Tropical montane cloud forests ('cloud forests') are one of the most biodiverse ecosystems on the planet and contain an incredible number of endemic species (Bruijnzeel et al. 2010, Hamilton et al. 1995). Gentry (1992) suggests that local endemism in cloud forests is 10-24% for plant species. There are also high rates of endemism in animals. For example, nearly a third of Peru's endemic mammals, anurans, and birds are found in cloud forest habitats (Leo 1995). Cloud forests are also are one of the most threatened ecosystems and are considered a conservation priority. The largest threats to these ecosystems are climate change, air quality, and land conversion (Hamilton et al. 1995). Cloud forests provide numerous ecosystem services such as clean drinking water, nutrient cycling, and protection against erosion (Hamilton et al. 1995, Bruijnzeel et al. 2010, Martínez et al. 2009) and the loss of these ecosystems means the loss of their ecosystem services as well.

The river continuum concept (RCC) is a theory that examines longitudinal changes in macroinvertebrate community composition in streams and predicts the importance of basal resources as streams increase in size (Vannote 1980). Methods based on these predictions have been established to assess ecosystem health and function. For example, macroinvertebrates can be categorized into functional feeding groups (FFGs) based on how they acquire resources (Cummins and Klug 1979). As several species may be redundant in how they obtain resources, focusing on FFGs is a more stable and predictable method to study ecosystem function than species abundance or composition alone (Hawkins and MacMahon 1989). Exploring FFGs provides a way to examine food web structure and dynamics and can be used to assess how land-use changes impact resource availability (Hawkins and MacMahon 1989, Bondada et al. 2006).

This approach reflects longer-term stream conditions than chemical data alone and thus better represents the effects of land-use change on an ecosystem (Bücker et al. 2010).

The RCC is based-upon temperate streams and many studies have found specific predictions of this theory to be inapplicable to tropical systems (Dudgeon et al. 2010; Greathouse and Pringle 2006). For instance, according to the RCC, low-order forested streams are dependent on coarse particulate organic matter (CPOM) that comes from allochthonous inputs (Vannote et al. 1980). In low-order tropical streams, CPOM is the most dominant basal food source in terms of biomass but not in terms of consumption. Mantel et al. (2004) found that although CPOM was the dominant basal source in a stream in southern China, fine particulate organic matter (FPOM) and periphyton were more prominent in the gut content of macroinvertebrates. Other studies on tropical streams have determined CPOM to serve more as a substrate than a food source (Li et al. 2009, Uieda and Carvalho 2015).

In terms of the macroinvertebrate community, shredding specialists and collectors are predicted to be codominant FFG in low-order forested streams according to the RCC (Vannote et al. 1980). However, macroinvertebrate communities of low-order forested tropical streams are typically dominated by collectors while shredding specialists are a rarity (Dudgeon et al. 2010, Mantel et al. 2004, Ramírez and Pringle 1998). Although not all tropical streams follow this trend (Cheshire et al. 2005, Cummins et al. 2005), the vast majority do (e.g. Dobson et al. 2002; Mathuriau and Chauvet 2002; Lau et al. 2009). In most cases where shredding specialist macroinvertebrates are absent there is typically a larger macroconsumer, such as shrimp and fish, covering that niche (Moulton et al. 2010, Ocasio-Torres et al. 2015, Wright and Covich 2005). There is still more investigating to do in this area of tropical streams as basic questions about ecosystem function remain unanswered.

Furthermore, aquatic macroinvertebrates have been found to be more plastic in their feeding than their FFG implies (Dangles 2002). Lancaster et al. (2004), for example, used gut content and stable isotope analyses to examine if omnivory was occurring in aquatic macroinvertebrates that are traditionally considered predatory. Gut content results indicated that all 6 study taxa consumed large quantities of algae while stable isotope mixing models estimated 3 of the 6 taxa to be true omnivores with nearly half of their nitrogen being derived from algae. On top of this, some tropical streams have higher rates of omnivory by macroinvertebrates than their temperate counterparts (Frauendorf et al. 2013, Blanchette et al. 2014, but see Dudgeon et al. 2010) potentially making the findings of temperate-based methods questionable in this region.

An essential component of the RCC is the aquatic-terrestrial linkage. This is the exchange of materials between the terrestrial and the aquatic ecosystems, such as a stream and its riparian zone. Historically, it was thought that the aquatic ecosystem relied on terrestrial inputs more than riparian zones relied on aquatic inputs (Baxter et al. 2005). This is due to the assumption that more material exchange occurs from the riparian zone to the stream (Power et al. 2001). However, just because materials are being exchanged between ecosystems does not mean they are being assimilated by organisms. Stable isotopes analysis of carbon and nitrogen is a tool used by ecologists to quantitatively examine aquatic-terrestrial energy exchange and determine food web structure (Peterson and Fry 1987). The amount of $\delta^{13}C$ found in an organism gives insight into where the plant sources of carbon originate while the amount of $\delta^{15}N$ indicates its trophic position in the food web (Fry 1991). Bartels et al. (2012) conducted a meta-analysis to determine whether terrestrial or aquatic ecosystems receive more energy. In terms of quantity, aquatic ecosystems are receiving more inputs than terrestrial ecosystems. However, stable isotope analyses revealed that in terms of carbon being assimilated by organisms, the two

ecosystems are equal. Without conducting the stable isotope analyses, it is difficult to determine

that both systems are in fact energetically contributing equally (Bartels et al. 2012).

Before the meta-analysis from Bartels et al. (2012) quantified this energy exchange,

Nakano and Murakami (2001) described this aquatic-terrestrial exchange of materials as

"reciprocal subsidies," meaning terrestrial inputs, such as falling leaves and insects, fuel the

aquatic food web while emerging aquatic insects provide energy to the terrestrial food web. In

this groundbreaking study, they found the diets of terrestrial predators to follow the pattern of

aquatic prey abundances over time. When aquatic insects were emerging from their study

stream, they comprised a larger portion of terrestrial predator's diets than when they were not

emerging from the streams. The same was found for fish – as more terrestrial insects were

falling into the stream, there were more of these terrestrial invertebrates incorporated into their

diets (Nakano and Murakami 2001). Other studies have also found this relationship to exist (e.g.

Baxter et al. 2004, Nakano et al. 1999) and some found it to be so strong that the abundance of

terrestrial predators is significantly influenced by the abundance of their aquatic prey (Sabo and

Power 2002).

Bartels et al. (2012) developed a generalization about aquatic-terrestrial inputs based on

their meta-analysis and the results suggested aquatic ecosystems receive more subsidies than

terrestrial ecosystems. However, of the over 200 studies analyzed in this paper only a handful

were conducted outside of the temperate zone. This illustrates the lack of study on energy flow

between terrestrial and aquatic ecosystems in the tropics. Bartels et al. (2012) acknowledge that

the results from their study were biased towards temperate locations and that there is a need for

further investigations in non-temperate regions. Regardless, they believe their observed patterns

are present globally. As temperate and tropical streams seem to operate and function in different

ways (Dudgeon et al. 2010), this pattern cannot be considered a global generalization until the tropics are better represented in this area of research. For example, Frauendorf *et al.* (2013) conducted a study on a headwater stream in Panama that quantified energy flow via tadpole and macroinvetebrate gut content and secondary primary production estimations. After comparing their results with temperate streams of equal size, they found their Panamanian stream to have lower macroinvertebrate production. The authors predicted that this was primarily due to hydrological disturbances, such as floods during the rainy season, as they reduce invertebrate biomass and resource availability. Due to lack of flooding events in the dry season, greater amounts of food sources were ingested at higher rates during this time of year.

Although these RCC-based analyses may not be as accurate in tropical streams, it does provide a starting point for ecological evaluation. As more evidence piles up to support the inapplicability of the RCC predictions to tropical headwater streams, it is likely that they will require their own theories and predictions (Dudgeon et al. 2010, Mantel et al. 2004). While the world waits for these breakthroughs to come about, using temperate-based practices provide a solid starting point in the management, restoration, and conservation of these systems. Ultimately, however, more basic research on ecosystem function is needed to create effective conservation plans for tropical streams (Moulton and Watzen 2006).

2. Purpose

The purpose of this study was to examine and compare 3 low-order (1st-3rd) forested Neotropical streams located in Mindo, Ecuador. Macroinvertebrate community composition, FFG analysis, ecosystem attributes, and physicochemical parameters were used to evaluate biophysical stream conditions. Base-line stable isotope data was also examined in all 3 streams to determine how a typical Neotropical stream food web is structured. Furthermore, aquatic adult insect emergence was monitored weekly for 6 weeks in the 2nd order stream to examine emergence patterns.

3. Scope

This study was conducted in the cloud forest of Reserva Las Gralarias (S 0°00'33", W 078°44'15"; 1750-2400 m a.sl.) in Mindo, Ecuador during the summer of 2015. We had 3 sample sizes: Kathy's Creek (1st order), Lucy's Creek (2nd order), and Rio Santa Rosa (3rd order). Macroinvertebrate community composition, physicochemical parameters, and stable isotope analysis samples were collected from May 31st – June 6th, 2015 during the transition from the wet to dry season. Aquatic adult emergence rate was collected 6 times from June 8th to July 21st, 2015 at Lucy's Creek.

4. Assumptions

Our macroinvertebrate community composition sampling took place over a few days during the transition from the wet to dry season in 2015. This means that the community composition we found is really only a snapshot of the community. We assume that this snapshot is representative of what the community looks like, on average, for the entire wet season. The same lack of seasonal replication can be applied to all of our sampling.

5. Hypotheses

We hypothesized that 1) macroinvertebrate communities will change along a size (order) gradient and FFG analyses will reveal healthy systems, 2) stable isotope analyses will reveal typical aquatic food web patterns, and 3) aquatic emergence rates will be constant or correlate with the moon cycle.

6. Significance

Results from this study provide base-line physical, chemical, and biological data on these streams and mark the beginning of a long-term monitoring effort. The long-term monitoring data collected at these sites will be used effectively to track environmental changes in land-use in the area. Results from this study will also provide basic data on tropical stream ecosystem function. This is also the first study to conduct stable isotope analyses in Ecuador streams and cloud forest streams and will provide valuable information on aquatic food webs in this area. This information will be valuable as stream theories with specific predictions for the tropics are created and eventually lead to better monitoring efforts and more effective restoration and protection of these threatened systems

7. Definitions

<u>Bioindicator</u> – an organism used as an indication of an ecosystem's health

<u>Cloud forest</u> – a type of rainforest characterized by persistent low-level clouds

<u>Collector-gatherers/gathering collectors</u> – aquatic macroinvertebrates that feed by foraging the

      streambed

<u>Endemism</u> – organisms that are unique to a defined geographic location

<u>Collector-filterers/filtering collectors</u> – aquatic macroinvertebrates that feed by filtering the water

      column

<u>Functional feeding groups</u> – classifications based on how organisms gather food

<u>Macroconsumers</u> – larger omnivorous macroinvertebrates, such as shrimp and crabs, as well as

      small vertebrates, typically fish and tadpoles.

<u>Neotropical</u> – the tropics located in the Western hemisphere

<u>Omnivory</u> – consumption of many different types of food resources

<u>Predators</u> – organisms that eat other organisms

<u>Riparian zone</u> – the interface between a stream and the land/forest that surrounds the stream

<u>Scrapers</u> – aquatic macroinvertebrates that physically remove their food source (most commonly

      periphyton) from physical surfaces such as rocks

<u>Shredders</u> – aquatic macroinvertebrates that feed on coarse particulate organic matter

<u>Stable isotope analysis</u> – a tool used by ecologists that looks at naturally occurring isotopes in

      organisms to determine where they belong in a food web as well as to examine how

      energy is transferred between ecosystems

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Chapter 2: Macroinvertebrate community composition, food web structure, and emergence rate of Neotropical cloud-forest streams in Mindo, Ecuador

1. Abstract

Tropical cloud forest streams are one of the most threatened and understudied ecosystems in the world. Understanding how these ecosystems function is essential for effective conservation. In this study, macroinvertebrate community composition, functional feeding group analysis, ecosystem attributes, and physicochemical parameters were used to evaluate biophysical stream conditions of 3 low-order Neotropical cloud forest streams at Reserva Las Gralarias in Mindo, Ecuador. Additionally, food web structure was analyzed via stable isotope analysis and aquatic insect emergence rate was also examined. As stream size increased from 1st to 3rd order, the macroinvertebrate communities shifted from being collector-gatherer dominated (65.2 to 29.8%, respectively) to being scraper dominated (17.9 to 56.3%, respectively). Shredders were poorly represented in all streams (2.7, 3.3, and 2.0% for 1st, 2nd, and 3rd order streams, respectively) similar to reports from other tropical systems. The analyses used in this and other tropical stream studies are based on temperate-based theories, which have been found to be inapplicable to tropical systems. Until tropical-based theoretical predictions are established, however, conservation efforts based on temperate theories should be implemented. Stable isotope analysis revealed a typical food web structure with basal resources having the lowest $\delta^{13}C$ and $\delta^{15}N$ signatures and these values increasing up the food web. Generally, $\delta^{15}N$ signatures in our systems were depleted when compared to other tropical studies. Lastly, aquatic insect emergence was not correlated with rain or the moon cycle. Results from this study provide base-line physical, chemical, and biological data on these streams that can be effectively

used to track environmental changes in land-use via long-term monitoring. Furthermore, results

from this study provide basic data on tropical stream ecosystem function that will be valuable as

stream theories with specific predictions for the tropics are created, which will lead to better

monitoring efforts and more effective restoration and protection of these threatened and

disappearing systems.

2. Introduction

Tropical streams are extremely threatened ecosystems (Malmqvist and Rundle 2002) and those that are found in cloud forest are under even more pressure (Astudillo et al. 2016) as these forests are disappearing faster than any other ecosystem (Hamilton et al. 1995). Deforestation is the biggest threat to cloud forest streams with effects of this process including increased nutrients and deposited sediments (Buss et al. 2014). Although they only comprise 0.14% of the Earth's land, cloud forests are considered biodiversity hot spots (Bruijnzeel et al. 2011). Protection is essential in cloud forest stream conservation as these streams are extremely sensitive to change and even the smallest alteration in land use can have large ecological impacts (Astudillo et al. 2016) including the loss of endemic species and a decrease in water quality (Martínez et al. 2009). Pristine tropical streams that are located within large protected areas deserve immediate attention by conservationists and researchers as they can be set aside and preserved before being impacted. The preservation of undisturbed cloud forests will allow the collection of basic information that will aid in conservation of these imperiled systems (Dudgeon et al. 2006). In order to effectively restore disturbed cloud forests, an understanding of how they function is essential (Moulton and Wantzen 2006). However, tropical stream ecosystem function remains largely understudied (Jackson and Sweeney 1995).

Macroinvertebrate functional feeding groups (FFG) can be used as bioindicators to assess ecological function of aquatic systems and to examine how different land-use changes impact stream food web availability (Bonada et al. 2006). Bioindicators were originally used in streams to assess human impacts such discharge sewage. Original methods consisted of ranking organisms based on sensitivity levels and their abundances but has evolved to focus on function (Karr and Chu 1999). Using macroinvertebrates as bioindicators reflects the longer-term

perturbations of a stream more so than chemical data alone and thus better represents the effects of land-use changes on an ecosystem (Bücker et al. 2010). This approach is based on the river continuum concept (RCC), which examines longitudinal changes in macroinvertebrate community composition and predicts the relative importance of basal resources as a stream increases in size (Vannote et al. 1980). However, the RCC is based upon temperate forested streams and studies have determined certain predictions of this theory to be inapplicable to tropical systems (Dudgeon et al. 2010, Greathouse and Pringle 2006). Additionally, aquatic macroinvertebrates have been found to be more plastic in their feeding than their FFG implies (Dangles 2002) and tropical streams in particular have higher rates of macroinvertebrate omnivory than temperate streams (Frauendorf et al. 2013). However, while the applicability of FFG analyses is questionable in these systems, it does provide a starting point for ecological evaluations and in particular, functional aspects of the stream ecosystem such as food web structure and energy flow.

An essential element of a stream's ecological function is the terrestrial-aquatic linkage. Terrestrial inputs, such as falling leaves and insects, fuel the aquatic food web while emerging aquatic insects provide energy to the terrestrial food web. These inputs work with one another through a process described as a "reciprocal subsidy" by Nakano and Murakami (2001). Stable isotope analysis of C and N is a tool commonly used by ecologists to quantitatively examine this energy exchange and determine food web structure. The amount of $\delta^{13}C$ found in an organism gives insight into where the plant sources of carbon originate while the amount of $\delta^{15}N$ indicates its trophic position in the food web (Fry 1991). Both $\delta^{13}C$ and $\delta^{15}N$ increase with each trophic transfer with $\delta^{13}C$ increasing about 0.0-1.0 ‰ per trophic level and $\delta^{15}N$ increasing by 3-5 ‰ per trophic level (Peterson and Fry 1987). Because of this, when plotted on a figure, a typical food

web should create a positive slope with basal resources sitting in the bottom-left of the plot and the highest consumer positioned in the top-right.

The purpose of this study was to examine and compare 3 low-order (1st-3rd) forested Neotropical streams located in Mindo, Ecuador. Macroinvertebrate community composition, FFG analysis, ecosystem attributes, and physicochemical parameters were used to evaluate biophysical stream conditions. Base-line stable isotope data was also examined in all 3 streams to determine how a typical Neotropical stream food web is structured. Furthermore, aquatic adult insect emergence was monitored weekly for 6 weeks in the 2nd order stream to examine emergence patterns. We hypothesized that 1) macroinvertebrate communities will change along a size (order) gradient and FFG analyses will reveal healthy systems, 2) stable isotope analyses will reveal typical food web patterns, and 3) aquatic emergence rates will be constant or correlate with the moon cycle. Results from this study provide base-line physical, chemical, and biological data on these streams and mark the beginning of a long-term monitoring effort. The long-term monitoring data collected at these sites will be used effectively to track environmental changes in land-use in the area. Results from this study will also provide basic data on tropical stream ecosystem function. This information will be valuable as stream theories with specific predictions for the tropics are created and eventually lead to better monitoring efforts and more effective restoration and protection of these threatened systems.

3. Methods

3.1 Study area

Reserva Las Gralarias (RLG) is a 405-ha Neotropical cloud forest reserve in the Mindo Parish, Pichincha province, Ecuador (S 0°00'33", W 078°44'15"; 1750-2400 m a.sl.). It contains primary and secondary forests, regenerating pasture, several permanent and seasonal streams (Hutter and Guayasamin 2012) and is located within the Chocó/Western Ecuador biodiversity hotspot (Myers et al. 2000). The wet season is from October to May with an annual total rainfall of 2400 mm. Our sampling took place from May 31st - July 21st, 2015 during the transition from wet to dry season. The 3 RLG streams sampled in this study included Kathy's Creek (KC; 1st order), Lucy's Creek (LC; 2nd order), and Río Santa Rosa (RSR; 3rd order). These systems were selected based on accessibility. The streams are independent tributaries that never connect and eventually drain into Esmeraldas River. KC is the smallest of the 3 and has a streambed entirely composed of clay. LC is larger than KC and is characterized by its large boulders. RSR is the largest of the 3 systems in terms of depth and width and is the only one of the study streams to support fish. These 3 systems are not anthropogenically impacted. RSR has experienced some light grazing about 1 km in distance and 300 m in elevation upstream of the study location but no negative effects of grazing can be detected. The land surrounding these systems includes primary and secondary cloud forest and old pasture land that is in a re-growth phase where active efforts have been put forth successfully for the last decade to plant trees and re-grow the cloud forest (personal communication, J. Lyons). At the locations where sampling occurred there were no signs of impact to the riparian zones within 100 m or more of the stream.

3.2 Data collection

3.2a Benthic macroinvertebrate community

Benthic macroinvertebrate abundance (individuals/m^2) was measured using a modified Surber net sampler (area = 0.18 m^2). Three samples were taken within a 100-m reach at each stream during the morning (8:00 to 10:00 am). Contents of the samples were stored in 95% ethanol until the sorting and identification processes. Specimens were enumerated and identified to family and FFG primarily using tropical-based references (e.g. Cummins et al. 2005, Encalada et al. 2011) and North American-based references when necessary (e.g. Merritt and Cummins 1984). Diversity was calculated for each Surber sample using Shannon's diversity index. Richness (number of families present) was also determined for each sample. Ecosystem attributes were determined using FFG ratios as described in Hauer and Lamberti (1996). This method allows for the monitoring of land-use practices as FFG ratios will respond to changes in food resource availability.

3.2b Physicochemical parameters

Physicochemical parameters were measured on one occasion at each site at the time of benthic macroinvertebrate sampling (Table 1). A YSI hand-held probe (Yellow Springs, Ohio, USA) was used to measure water temperature (°C), dissolved oxygen (percent and mg/L), conductivity (S/m), and total dissolved solids (ppm). Elevation was determined using a GPS or topographic maps when necessary. Median substrate data were obtained from unpublished data collected in May 2014 (personal communication, E.B. Snyder) and was collected using the modified Wolman Pebble Count method (Wolman 1954). Parameters obtained from the YSI were measured 3 times within each 100-m stream reach while elevation and substrate were only measured once. Discharge was determined by measuring cross sectional area and estimating

velocity of a floating object 3 times. Light irradiance as photosynthetically active radiation

(PAR) was measured 10 times at each site in the morning using a LiCor underwater quantum

senor (Li 192 UWQ; Lincoln, Nebraska) and a LiCor hand-held meter (Li 250A). Periphyton

was scraped from rocks in LC and RSR. At KC periphyton was collected off of large pieces of

clay as rocks were not present. Chlorophyll-α level was also measured at each stream.

Periphyton was sampled from a 4.9 cm^2 area of the rock (or clay) surface onto a 0.45 μm fiber

glass filter and was kept in a freezer until transported to the Aquatic Ecology lab at the

Universidad San Francisco de Quito for further analysis. Pigment extraction methods were

conducted with 100% ethanol and follow spectrophotometric analysis of Chlorophyll-α methods

of Hauer and Lamberti (2006). An Agilent Cary 60 spectrophotometer (Agilent Technologies)

was used to measure chlorophyll-α levels.

3.2 c Stable isotope analyses

Coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM)

were sampled using sets of nested nets (>1 mm for CPOM, 0.63 μm-1 mm for FPOM). Nets

were secured upstream of sampling sites for a minimum of 2 hours. There was not enough

biomass to create samples for FPOM at KC and LC (Table 5). Periphyton was scraped off of

rocks and collected on fiber glass filters (0.45 μm) using a hand-held vacuum pump. In lab,

periphyton was scraped off of the filters before the drying process. Riparian plants and moss

were randomly sampled from each stream. Riparian plants were later identified to family and

bulked to produce one composite sample per stream. The δ^{13}C for LC's riparian plants was

unable to be detected. To ensure mixing of isotopic signatures, moss was sampled from several

different rocks at each stream and bulked to create one composite sample. Spiders near the

stream and within its riparian zone were collected at each stream and bulked to create a composite sample per stream.

Aquatic benthic macroinvertebrates were sampled from random reaches of each stream using d-frame kick nets. One composite sample per FFG (predators, shredders, collector-gatherers, collector-filterers, and scrapers) was created using individuals large enough to identify without the use of a microscope to ensure there would be enough biomass to produce stable isotope samples after further processing. Benthic organic matter (BOM) samples were collected from these same d-frame kick net samples. RSR was the only stream in which enough biomass of all FFG was collected for analyses. There was not enough biomass for shredders and collector-filterers in LC. In KC, there was not enough biomass for collector-filterers, collector-gatherers, or scrapers.

Emerged aquatic insects and terrestrial insects were collected using a light trap. The trap was set up for an hour after sunset (6:00-7:00 pm) at the edge of each stream. Individuals were collected in plastic vials and transferred to the lab for identification. Aquatic adults were bulked into one group regardless of functional feeding group. While sampling for terrestrial insects at RSR and LC several large beetles were collected and also included in the analysis as a separate sample. Cicadas (Cicadoidae) collected at RSR were also included in the analysis.

Pristimantis appendiculatus was also sampled to examine how a common terrestrial amphibian in the area fits into the aquatic-terrestrial food web. Three *P.appendiculatus* (total length = 21.0, 31.0. and 37mm) were sampled at least 100 m away from the streams and were included in the analysis. After being captured, individuals were submerged in a clove oil solution for at least an hour before their stomachs were removed before the drying process. When sampling for aquatic benthic insects in RSR a fish (*Astroblepus spp.*) was captured (length

= 20.0 mm). The stomach was removed and the fish was dried, ground up, and included in the stable isotope analysis.

All samples were dried for at least 8 hours using a home-made oven composed of a large cooking pot and a 70 w light bulb. Following drying, a mortar and pestle were used to grind up the samples into a fine powder. Samples were stored in a freezer until August 2015 when they were returned to GVSU and were furthered processed for analysis. Samples were weighed into tin capsules with 1.0 mg (+/- 0.05 mg) of each composite sample sent to USGS in Denver, CO for further analyses. C and N signatures from composite samples were averaged with each stream acting as a replicate.

3.2 d Aquatic adult emergence

Lucy's Creek served as a long-term emergence site where trapping events occurred weekly from June 8th to July 21st, 2015 skipping the week of June 15 (6 trapping events total). LC was chosen as the long-term emergence site as it easier to access than RSR and has greater emergence than KC. Just as for the light trapping events used for the stable isotope sampling, the trap was set up at the edge of the stream for an hour after sunset (6:00-7:00 pm). Emergence was calculated for the overall amount of individuals and for each aquatic insect order (Ephemeroptera, Tricoptera, and Plecoptera) as the number of individuals captured per light trapping event. Aquatic Diptera were not included in the analyses as it was not possible to distinguish them from terrestrial Diptera while sampling. "Capture" was defined as being placed in a plastic vial during the trapping event or still being attached to the light trap when being packed up at the end of the trapping event. "Effort" was consistent as two people participated in capture efforts during all trapping events. Individuals collected were stored in 95% ethanol and sorted into order.

3.3 Statistical Analysis

All analyses were performed on R version 3.1.2 (R Core Team, Vienna, Italy). One-way ANOVAs were used to test for differences in total abundance, FFG densities, taxon richness, and Shannon's diversity index among sites. If significant differences were found among streams ($P < 0.05$) a Tukey HSD was used for mean separation. If data failed a normality test, a Kruskal-Wallis test was used. A canonical correspondence analysis (CCA) was used to examine differences in macroinvertebrate community composition among sites and which environmental variables influenced these differences. Since several of the measured variables were highly intercorrelated, we used stepwise selection based on variance inflation factors (VIF) to determine which variables to include in the analysis. Ultimately, the variables that were chosen were width, substrate, and DO (mg/L) as they were not highly correlated with one another, differed at least slightly between streams, and had VIF values less than 3. Unique macroinvertebrate species (not present in all 3 streams) were eliminated from the analysis in order to increase plot clarity. A permutation test with 1000 steps was performed on the final CCA plot to determine if the model, axes, and variables were significance. C and N signatures were averaged for each composite sample with the streams serving as replicates. Simple linear regression was used to examine the slope of the food web using the average C and N signatures. Lastly, a multiple linear regression was used to determine if emergence rates were caused by the moon cycle and the presence of rain. Trapping events where constant heavy rain occurred were coded as "1", light or on-and-off rain during trapping events were coded "0.5", and no rain was coded "0". Trapping events were assigned a moon cycle number from $0.0 - 1.0$ based on what the moon cycle was the night of the trapping event. Shapiro-Wilk normality tests were run to ensure that the emergence data was normal before performing the regressions.

4. Results

Total macroinvertebrate abundance ranged from 900-3983 individuals/m^2 (Table 2) and was not significantly different among streams (Table 3). Shredder and filtering-collector densities did not differ among streams while collector-gatherer, scraper, and predator densities did differ among streams (Fig. 1). As stream size increased from 1st to 3rd order, the most abundant FFG shifted from collector-gatherers to scrapers with LC (2nd order) having an intermediate density for both groups. Taxon richness and Shannon's Diversity Index were not significantly different among sites.

FFG ratio methods suggested that all 3 streams were low in CPOM (0.04-0.06) and low in transport fine particulate organic matter (TFPOM, 0.15-0.29; Table 4). KC and LC were heterotrophic while RSR was autotrophic. KC was the only stream to have an unstable channel (0.40). Both KC and RSR had a lower than normal top-down predator control (0.05 and 0.08, respectively) while LC had normal top-down predator control (0.13).

The CCA plot indicated some separation among study sites (Fig. 2). A permutation test (steps=1000) determined the overall model to be significant (P=0.01). The first two axes explained 93.5% of the variation (CCA 1 = 77.8%, CCA 2 = 15.7%). Axis 1 was significant (P = 0.008) and positively correlated with stream width (r = 0.31), substrate (r = 0.83), and DO (r = 0.35). Axis 2 was not significant (0.09) and was positively correlated with stream width (r = 0.86) and negatively correlated with substrate (r = -0.53) and DO (r = -0.84). Stream width was the only significant environmental variable (P = 0.001) in the model. RSR was separated from the other streams along axis 1, while KC and LC were separated from one another along axis 2.

Linear regression showed that the relationship between δ^{15}N and δ^{13}C was positive (slope = 1.06) and significant (F = 37.74, P = 1.88 x10^{-5}; Figure 3). All basal resources (periphyton,

moss, FPOM, CPOM, BOM, and riparian plants) were similar in δ^{13}C and δ^{15}N signatures (overlapping standard deviations) and were the least enriched in both δ^{13}C and δ^{15}N (Table 6, Figure 4). Aquatic primary consumers (scrapers, shredders, collector-gatherers, and collector-filterers) were within the same range of δ^{15}N and δ^{13}C signatures and were more enriched in both than the basal resources. Predators and aquatic adults were similar in δ^{13}C to the basal resources and the primary consumers but were higher in δ^{15}N. Terrestrial insects were also similar in δ^{13}C signature to the basal resources but were more enriched in δ^{15}N. The cicadas and beetles that were sampled in RSR had similar δ^{13}C and δ^{15}N signatures to the aquatic primary consumers. Spiders also had similar δ^{13}C signatures as the aquatic primary consumers but were much more δ^{15}N -enriched. Spiders had the second highest δ^{15}N signatures with the fish sampled in RSR having the highest δ^{15}N signatures. The fish was the most depleted in δ^{13}N but was close to the range of the spiders' δ^{13}N signatures. The δ^{13}C and δ^{15}N signatures of *P.appendiculatus* were within the range of the spiders.

A total of 371 aquatic adults were captured during the 6 trapping events (Figure 5). Tricoptera composed 95.4% (354) of the insects collected. Only 16 Ephemeroptera and 1 Plecoptera were sampled. Shapiro-Wilk determined emergence data to be normal (P = 0.055). There was no correlation between emergence and moon cycle (R^2_{Adj} = 0.37, P = 0.20), rain (R^2_{Adj} = 0.37, P = 0.20), or moon cycle and rain (R^2_{Adj} = 0.68, P = 0.085).

5. Discussion

As our stream size increased from 1st to 3rd order, the macroinvertebrate communities shifted from being collector-gatherer dominated to being scraper dominated, which follows predictions of the RCC. As order increases and stream width widens, more light is available to support the periphyton community and thus scraping macroinvertebrates increase in abundance (Vannote et al. 1980). The collector-gatherer dominance found in KC and LC is similar to what Ramírez and Pringle (1998) found in their Costa Rican streams. They found gathering collectors to comprise 59.5% and 56.4% in pool and riffle habitats, respectively. However, the streams examined in Ramírez and Pringle (1998) are larger (4th order) than all of our study systems. Regardless, we argue that this comparison is still valid, as order increases, more light is available to support the periphyton community and thus scraping macroinvertebrates increase in abundance (Vannote et al. 1980) which appears to occur sooner in cloud forest streams as their canopies are of shorter stature than other types of tropical forests (Hamilton 1995). PAR and chlorophyll-α measurements generally support these results with KC (1st order) and RSR (3rd order) having average PAR readings of 10.0 and 30.5, respectively, and average chlorophyll-α measures of 2.23 μg/cm^2 and 6.13 μg/cm^2, respectively.

The rarity of shredders in these low-order streams contradicts the predictions of the temperate systems of the RCC (Vannote et al. 1980) but matches patterns found in other tropical streams of similar sizes in Central America (Ramírez and Pringle 1998), Asia (Dudgeon et al. 2010, Lau et al. 2009), Africa (Dobson et al. 2002), and South America (Mathuriau and Chauvet 2002). The role of shredding macroinvertebrates in low-order streams is to aid in the breakdown of leaf litter and while shredding macroinvertebrates are scarce in tropical streams, leaf litter is not (Dobson et al. 2002 and Mantel et al. 2004). Although leaf litter was not measured in this

study, it was observed to be abundant at all 3 sites, all of which had riparian zones with closed canopy covers.

There is evidence to suggest that leaf litter serves as physical substrate for macroinvertebrates rather than a food source in lowland tropical streams (Aggie et al. 2008, Li et al. 2009, Uieda and Carvalho 2015). If this is the case and shredding specialist macroinvertebrates are not filling the role of leaf litter processing, then how is this material being incorporated into the food web in tropical streams? Several studies have shown macroconsumers, such as shrimps, crabs, and fish, to fill the shredding niche in tropical streams. An exclusion leaf pack experiment by Moulton et al. (2010), for example, found macroconsumers in Brazilian streams to be more important in leaf litter processing than shredding specialist macroinvertebrates. Using 3 different mesh sizes they found the leaf packs with the largest mesh sizes that did not exclude larger macroconsumers to have faster leaf litter processing rates than those that excluded larger macroconsumers. In the 2 smaller mesh sizes, there was no difference in leaf litter processing rates between those that included macroinvertebrates and those that excluded them indicating that shredding macroinvertebrates are not significantly contributing to this process. Similar results have been found in other leaf pack and exclusions studies throughout the tropics (Ocasio-Torres et al. 2015, Uieda and Carvalho 2015, Wright and Covich 2005). Crabs are present at all 3 RLG streams studied and RSR supports several types of fish. Future research on these systems should examine the abundances and potential importance of these macroconsumers in leaf litter processing in these small tropical streams.

According to the ecosystem attributes as determined by FFG ratios, it was found that as stream order increased the streams became more autotrophic. This is to be expected and follows along with the predictions of the RCC that as streams increase in width they become reliant on

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autochthonous materials (Vannote et al. 1980). Stability also increased with order with KC (1st order) being categorized as unstable. KC has the smallest median substrate size of the 3 systems and the streambed is entirely composed of clay whereas LC and RSR are characterized by large rocks and boulders. Furthermore, 1st order streams are more dramatically impacted by events such as flooding which only adds to their instability (Junk et al. 1989). All 3 systems were characterized as having low TFPOM meaning the FPOM was either of poor quality or there simply was not much being made available for filtering collectors (Cummins et al. 2005). The latter is likely the reasoning as low amounts of FPOM were observed in nested nets used to sample organic matter for the stable isotope analysis conducted at these sites at the same time macroinvertebrate collection occurred.

Although CPOM was observed in high abundance, the FFG ratios predicted all 3 sites to be low in CPOM. However, this attribute is determined by comparing the number of shredding specialist macroinvertebrates to the total number of collectors (filtering and gathering) and is therefore largely biased by the absence of shredders. The analysis of this ratio according to Hauer and Lamberti (1996) assumes that if there are few shredders that their food source (CPOM) must also be in low abundance. This would typically be the case in larger systems, not in 1st and 2nd order heavily forested streams such as KC and LC (Vannote et al. 1980). Dobson et al. (2002) also found high levels of CPOM but few shredders in their African streams. In addition, using a combination of stable isotope and gut content analyses, Mantel et al. (2004) determined that although CPOM was the dominant basal resource in their shredder-scarce study streams, FPOM and periphyton were more important in aquatic insect diets.

The multivariate analysis showed some separation among the streams primarily due to median substrate size. RSR was distinctly separated from KC and LC. As previously

mentioned, KC is characterized by clay while LC is made up of large boulders. RSR has an intermediate sized substrate which drives its macroinvertebrate community to differ from KC and LC. KC and LC differed along axis 2 which was found to be insignificant. Due to logistical constraints in the field, our sample size (n=9) was relatively low for a multivariate analysis and the data interpretation is not necessarily robust. Regardless, we are confident that if sample size were increased a greater separation between KC and LC would exist. Substrate type and stability has been known to influence macroinvertebrate communities (Beisel et al. 1998, Cobb et al. 1992) even in the Neotropics where Buss et al. (2004) found substrate type to be more important for community composition than water quality or other environmental factors. (Buss et al. 2004).

While this present study and several others use FFG analyses in tropical systems, this method was inspired by the RCC, which was derived in a temperate climate. The majority of tropical studies, including this one, have found some predictions of the RCC to be inapplicable to tropical systems. The RCC serves as a model to interpret longitudinal patterns in stream food webs (Vannote et al. 1980). To best apply this concept to tropical systems, a study that examines longitudinal food web patterns in several tropical streams from throughout the world is necessary to generate predictions that are specific to the tropics (Greathouse and Pringle 2006). Additionally, omnivory is more common in tropical systems than in temperate systems (Frauendorf et al. 2013, Blanchette et al. 2014) so it is not clear if FFGs are even relevant in tropical streams. The development of tropical-based stream theories that focus on function will be essential in the successful conservation of these systems as management plans that focus on function are considered the most efficient way to preserve aquatic ecosystems (Moss 2000, Dudgeon et al. 2006). When it comes to management, restoration, or conservation of tropical streams, temperate-based practices may provide a good starting point but more research on

ecosystem function is needed to create effective conservation plans (Moulton and Watzen 2006) and further advances our ecological knowledge, which is where the results from this study will be useful.

The linear regression from the δ^{13}C and δ^{15}N signatures produced a positive slope, as expected (Figure 3), indicating that the food webs in these streams are typical. Basal resources had the lowest δ^{13}C and δ^{15}N signatures and these values increased up the food web indicating that the FFGs are operating properly. When comparing our δ^{13}C and δ^{15}N values to other stable isotope studies conducted in tropical streams, there were mixed results. Our periphyton signatures were similar to what Verburg et al. (2007) found in Panamanian streams but were depleted in both δ^{15}N and δ^{13}C when compared to periphyton from studies conducted in Puerto Rico (March and Pringle 2003) and Mexico (Coat et al. 2009). Our streams have very dense, tall canopies which limits the sunlight that reaches the water surface. This likely causes a decrease of in-stream primary production (Lau et al. 2009, March and Pringle 2003). Another explanation is low N content levels in the water of our streams which would lead to low N signatures in periphyton (Coat et al. 2009). This was not directly measured in this study but the conductance of these streams ranged from 18.3-59.8 S/m (Table 1) potentially suggesting low levels of dissolved nitrogen fractions.

FPOM isotopic signature was another basal resource that differed from the literature values. The two Panamanian streams from Verburg et al. (2007) had δ^{15}N values of 5.35 ‰ and 4.23 ± 0.77, while our FPOM δ^{15}N signature was 0.3. Because of limited capture efficiency, we only have a single composite sample for FPOM, however, the difference between the δ^{15}N values is large. Our FPOM δ^{15}N signature was also depleted when compared to streams from Hong Kong where one stream had a δ^{15}N value of 5.93 ± 0.84 for FPOM (Lau et al. 2009).

Higher $\delta^{15}N$ values in FPOM can be due to high denitrification rates in stream sediments (Fry 2006). As our basal resources were depleted in $\delta^{15}N$ when compared to the literature, low rates of denitrification could be occurring in these systems. CPOM isotopic signature, on the other hand, was much more similar to the literature than FPOM. All streams studied in March and Pringle (2003) and Burress et al. (2013) had signatures that matched our results. Coat et al. (2009) had CPOM $\delta^{13}C$ signatures that matched our results but was much more N-enriched (4.3 $\pm$ 1.4) when compared to our streams.

As $\delta^{13}C$ and $\delta^{15}N$ signatures of basal resources differed from the literature, it was no surprise that the rest of the food web also differed from values found in the literature. Our predator signatures matched the literature, while scrapers from literature were N-enriched when compared to our data (Verburg et al. 2007, Lau et al. 2009). When compared to Panamanian streams, our collector-filterers had similar $\delta^{13}C$ signatures but were N-depleted. While we only had one sample for collector-filterers, again due to sampling constraints, the difference between $\delta^{15}N$ signatures was large (1.4‰ versus 4.25 $\pm$ 0.61‰; Verburg et al. 2007). Our collector-filterers and collector-gatherers were also N-depleted when compared to data from streams in China and Uruguay (Lancaster et al. 2008, Burress et al. 2013).

In conclusion, we found more support for the scarcity of shredding specialist macroinvertebrates in tropical streams and a dominance of collectors. These findings match patterns found in other tropical streams and further question the applicability of specific RCC predictions in tropical streams. Despite the differences in macroinvertebrate community composition, stable isotope analyses revealed a typical aquatic food web. However, $\delta^{13}C$ and $\delta^{15}N$ signatures often differed from values observed in other tropical systems. Currently, the best conservation plan for tropical streams is to maintain natural habitat that is minimally impacted,

such as the systems of RLG, and with the pressures faced by tropical cloud forest streams especially, this conservation strategy should be implemented immediately (Rawi et al. 2013). For example, our results suggest that shredding specialist macroinvertebrates are irrelevant in tropical stream ecosystem function and thus should not be a specific concern of conservationists. Instead, macroconsumers should be a focus of conservationists as they play a greater role in the shredding niche. As pristine tropical streams are becoming a rarity, these and other protected sites can serve as reference sites for future research and restoration efforts. It is especially important to protect low-order streams as they are the most easily affected by landscape changes and their impacts are accumulated downstream. The long-term monitoring efforts that are now established at the RLG streams will allow for early detection of any land-use changes that may occur in the future. As these ecosystems are slowly disappearing, theories with specific predictions for tropical streams are needed immediately so that tropical stream function can be properly monitored and more effectively restored and protected.

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Table 1. Summary of mean physicochemical data for Kathy's Creek (KC), Lucy's Creek (LC) and Rio Santa Rosa (RSR), Mindo, Ecuador. Physicochemical parameters were taken on the same day as macroinvertebrate sampling. PAR = photosynthetically active radiation. *PAR measurements for LC were taken on a day with heavy overcast.

Physicochemical Parameters	KC	LC	RSR
Order	1st	2nd	3rd
Elevation (m a.s.l.)	2011	1850	1771
Temperature (°C)	15.4	15.8	15.6
Width (m)	0.9	1.6	4.5
Depth (m)	0.09	0.15	0.25
Discharge (m^3/s)	0.01	0.03	0.82
PAR ($\mu mol/m^2$/s)	10.0	1.57*	30.5
Chlorophyll-α ($\mu g/cm^2$)	2.23	2.47	6.13
Median substrate (mm)	< 2	256-512	45-64
Specific conductance (μS)	18.3	59.2	59.8
Total dissolved solids (ppm)	0.01	0.04	0.04
Dissolved oxygen (%)	84.6	89.9	89.6
Dissolved oxygen (mg/L)	8.47	8.93	8.77

Table 2. Macroinvertebrate taxa at Kathy's Creek (KC), Lucy's Creek (LC), and Rio Santa Rosa (RSR), Mindo, Ecuador. Values are for mean abundance (individuals/m^2) and the percent that each taxon contributes to the total macroinvertebrate abundance for each stream. Values in parentheses represent the standard error (n=3 replicates per stream). Aquatic taxon are categorized into the following functional feeding groups (FFG): shredders (SH), filtering-collectors (FC), gathering-collectors (GC), scrapers (SC) and predators (PR). Abundance and percent are also given for each FFG and order at each stream as well as the richness and Shannon's diversity index found there. Bolded values are those that significantly differed among streams (one-way ANOVA $p<0.05$) with Tukey HSD ($p<0.05$) groupings signified by superscript letters.

Order	Family	FFG	KC $\bar{x}$ (±SE)	%	LC $\bar{x}$ (±SE)	%	RSR $\bar{x}$ (±SE)	%
Ephemeroptera	Baetidae	GC	128 (14)	5.01	61 (29)	4.02	68 (13)	2.54
	Euthyplociidae	GC			2 (2)	0.12		
	Leptohyphidae	GC	28 (15)	1.09	9 (2)	0.61	91 (41)	3.36
	Leptophlebiidae	SC	209 (11)	8.20	111 (23)	7.32	30 (24)	1.10
	Oligoneuriidae	FC			4 (2)	0.24	15 (4)	0.55
Odonata	Calopterygidae	PR	20 (10)	0.80			2 (2)	0.07
	Coenagrionidae	PR	2 (2)	0.07				
	Gomphidae	PR			2 (2)	0.12	4 (2)	0.14
	Libellulidae	PR					11 (8)	0.41
	Polythoriidae	PR			6 (3)	0.37		
Plecoptera	Perlidae	PR	33 (6)	1.31	37 (11)	2.44	43 (2)	1.58
Trichoptera	Calamoceratidae	SH	20 (10)	0.80	13 (5)	0.85	4 (4)	0.14
	Ecnomidae	PR	2 (2)	0.07				
	Glossosomatidae	SC	2 (2)	0.07	2 (2)	0.12		
	Heliopsychidae	SC	161 (60)	6.31	130 (26)	8.54	1296 (867)	47.98
	Hydrobiosidae	PR	11 (3)	0.44	37 (15)	2.44	4 (4)	0.14
	Hydropsychidae	FC	111 (75)	4.35	54 (34)	3.54	39 (16)	1.44
	Hydroptilidae	SC	37 (7)	1.45	43 (37)	2.80	43 (40)	1.58
	Leptoceridae	PR			46 (13)	3.05	82 (43)	3.02
	Philopotamidae	FC	4 (4)	0.15	11 (6)	0.73		

			Count (SD)	%	Count (SD)	%	Count (SD)	%
	Polycentropodidae	FC	37 (24)	1.45	6 (6)	0.37		
Coleoptera	Dytiscidae	PR					2 (2)	0.07
	Elmidae (adults)	SC	48 (8)	1.89	111 (70)	7.32	135 (35)	5.00
	Elmidae (larvae)	GC	24 (5)	0.94	43 (15)	2.80	217 (80)	8.02
	Gyrinidae	PR	2 (2)	0.07			9 (9)	0.34
	Psephenidae	SC			9 (5)	0.61	7 (5)	0.27
	Ptilodactylidae	FC	15 (2)	0.58	33 (22)	2.20	44 (17)	1.64
	Scirtidae	FC	11 (3)	0.44	4 (2)	0.24		
Diptera	Ceratopogonidae	GC	19 (7)	0.73	11 (6)	0.73	31 (8)	1.17
	Chironomidae	GC	1465 (621)	57.40	526 (153)	34.63	393 (143)	14.53
	Dolichiopididae	PR	22 (15)	0.87	4 (4)	0.24	2 (2)	0.07
	Dixidae	FC	17 (10)	0.65				
	Empididae	PR	2 (2)	0.07	15 (15)	0.98		
	Limoniidae	SH					2 (2)	0.07
	Simuliidae	FC	63 (30)	2.47	113 (56)	7.44	72 (21)	2.67
	Tipulidae	PR	24 (8)	0.94	20 (15)	1.34	26 (10)	0.96
Other	Crambidae	SH			2 (2)	0.12	4 (2)	0.14
	Hyalellidae	SH			2 (20)	0.12		
	Naucoridae	PR			4 (4)	0.24	7 (7)	0.27
	Planariidae	SH	33 (6)	1.31	50 (18)	3.29	11 (6)	0.41
	Tetrigidae	GC	2 (2)	0.07			7 (7)	0.27
	Veliidae	PR					2 (2)	0.07
	Total		2552 (721)		1518 (491)		2702 (1373)	
	Richness		23 (1)		22 (2)		21 (3)	
	Shannon's Diversity Index		1.82 (0.18)		2.29 (0.08)		1.96 (0.11)	
FFG	Shredders		69 (10)	2.7	50 (25)	3.3	54 (23)	2.0
	Filtering collectors		243 (94)	9.5	191 (99)	12.6	126 (34)	4.7
	Gathering collectors		1665 (629)	65.2[b]	652 (198)	42.9[a,b]	807 (288)	29.9[a]
	Scrapers		457 (65)	17.9[a]	456 (124)	30[a,b]	1522 (959)	56.3[b]
	Predators		118 (35)	4.6[a]	170 (54)	11.2[b]	193 (71)	7.1[a,b]
Order	Ephemeroptera		365 (14)	14.30	187 (16)	12.32	204 (76)	7.54
	Odonata		22 (12)	0.87	7 (5)	0.49	17 (6)	0.62
	Plecoptera		33 (6)	1.31	37 (11)	2.44	43 (2)	1.58
	Trichoptera		385 (99)	15.09	341 (121)	22.44	1467 (971)	54.28
	Coleoptera		100 (3)	3.92	200 (111)	13.17	415 (139)	15.35
	Diptera		1611 (645)	63.13	689 (238)	45.37	526 (184)	19.47
	Other		35 (7)	1.38	57 (18)	3.78	31 (18)	1.17

Table 3. Results from one-way ANOVA measuring the differences in total abundance, richness, Shannon's diversity index, predator densities, collector-gatherer densities, and scraper densities among 3 tropical streams in Mindo, Ecuador. As shredder and filtering-collector densities failed normality tests, a Kruskal-Wallis was used to measure the differences in these values among sites. All degrees of freedom are 2.

One-way ANOVA	P	F
Total abundance	0.42	0.51
Family Richness	0.89	0.11
Shannon's Diversity index	0.098	3.52
Predators	0.012	10.2
Scrapers	0.017	8.75
Kruskal-Wallis	P	χ^2
Shredders	0.96	0.089
Filtering-collectors	0.73	0.62

Table 4. Ecosystem attributes for Kathy's Creek (KC), Lucy's Creek (LC), and Rio Santa Rosa (RSR) Mindo, Ecuador determined by using averaged functional feeding group ratios (n = 3 per stream) as described in Hauer & Lamberti (1984). Abbreviations: P/R = Production/Respiration, CPOM = Coarse particulate organic matter, FPOM = Fine particulate organic matter, TFPOM = transport FPOM, and BFPOM = FPOM deposited in benthic macroinvertebrates. General criteria: P/R > 0.75 = autotrophic, CPOM/FPOM > 0.5 = normal, substrate stability > 0.5 = stable, top-down predator control 0.10-0.20 = normal predator to prey abundance, TFPOM/BFPOM > 0.5 = FPOM available in suspension.

	KC		LC		RSR	
Ecosystem attribute	Ratio	Evaluation	Ratio	Evaluation	Ratio	Evaluation
P/R	0.23	heterotrophic	0.51	heterotrophic	1.54	autotrophic
CPOM/FPOM	0.04	low CPOM	0.06	low CPOM	0.06	low CPOM
Substrate stability	0.40	unstable	0.92	stable	1.91	stable
Top-down predator control	0.05	low ratio	0.13	normal ratio	0.08	low ratio
TFPOM/BFPOM	0.15	low TFPOM	0.29	low TFPOM	0.16	low TFPOM

Table 5. Contents of the composite macroinvertebrate stable isotope samples collected at each stream. Describes the family (or order) and the number of individuals present in the composite samples. Plant families represented in the riparian plant composite samples are also described.

	KC		LC		RSR	
	Family	#	Family	#	Family	#
Predators	Calopterygidae	1	Aeshnidae	1	Corydalidae	1
	Gerridae	6	Gerridae	10	Gomphidae	5
	Perlidae	5	Naucoridae	4	Gyrinidae	1
	Veliidae	59	Perlidae	4	Naucoridae	2
			Polythoridae	9	Perlidae	8
			Veliidae	14		
Shredders	Tipulidae	6			Leptoceridae	18
					Tipulidae	2
Collector-gatherers			Ameletidae	1	Baetidae	8
			Hydropsychidae	23	Elmidae (larvae)	7
			Leptoceridae	1	Leptophlebiidae	2
Collector-filterers					Hydrosychidae	18
					Oligoneuriidae	3
					Psephenidae	1
Scrapers					Elmidae (adults)	40
					Helicopsychidae	18
Aquatic Adults	Unknown Diptera	33	Calamoceratidae	8	Calamoceratidae	18
	Calamoceratidae	3	Perlidae (Anacroneuria sp.)	0	Unknown Diptera	15
	Philopotamidae	3	Unknown Ephemeroptera	8	Leptoceridae	10
	Unknown Ephemeroptera	1	Philopotamidae	2	Hydropsychidae	1
	Unknown Tricoptera	4	Unknown Tricoptera	2	Philopotamidae	2
			Tipulidae	1	Perlidae (Anacroneuria sp.)	1
Terrestrial Insects	Lepidoptera	17	Lepidoptera	12	Lepidoptera	13
	Hemiptera: Cicadellidae	1	Hemiptera: Cicadellidae	1	Unknown Diptera	2
	Coleoptera	1	Blattodea	1	Coleoptera	2

			Unknown Diptera	1	Cicadoidea*	3
			Unknown	2		
			Coleoptera*	4		
Spiders	Uknown	21	Unknown	38	Unknown	25
Riparian Plants	Araceae		Araceae		Araceae	
	Arecaceae		Arecaceae		Arecaceae	
	Blechnaceae		Blechnaceae		Cyatheaceae	
	Poaceae		Campanulaceae		Davalliaceae	
	Cyatheaceae		Melastomataceae		Euphorbiaceae	
	Euphorbiaceae		Cyatheaceae		Melastomataceae	
	Fabaceae		Moraceae		Musaceae	
	Melastomataceae		Bromeliaceae		Pteridaceae	
	Heliconiaceae		Marantaceae		Smilacaceae	
	Bromeliaceae		Smilacaceae		Solanaceae	
	Polypodiaceae					
	Pteridaceae					
	Solanaceae					
	Borginaceae					

*denotes group that formed their own composite sample

Table 6. $\delta^{15}N$ and $\delta^{13}C$ signatures for composite samples from Kathy's Creek (KC), Lucy's Creek (LC), and Rio Santa Rosa (RSR).

	KC		LC		RSR	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Periphyton	0.1	-29.6	1.8	-30.6	-0.1	-29.7
Moss	0.1	-31.2	-1.7	-30.7	-2.4	-30.2
FPOM					0.3	-27.1
CPOM	2.8	-28.5	0.9	-29.8	-0.8	-29.4
BOM	0.5	-29.0	0.7	-29.9	-2.2	-31.3
Riparian Plants	1.1	-33.8	0.8		-1.4	-27.7
Shredders	1.4	-28.6			1.8	-27.3
Collector-filterers					1.4	-29.0
Collector-gatherers			2.4	-27.9	1.2	-29.0
Scrapers					1.3	-26.9
Predators	4.8	-27.6	4.4	-28.0	2.7	-26.8
Aquatic Adults	5.1	-27.4	3.8	-27.4	2.7	-28.3
Terrestrial Insects	4.7	-30.8	1.0	-27.1	1.5	-29.7
Cicadas					1.9	-27.4
Beetles			1.9	-27.5		
Fish					6.0	-25.3
Spiders	4.6	-27.6	5.9	-26.9	4.1	-25.8

Figure captions

Figure 1. The average density (in percent) of each functional feeding group for Kathy's Creek (KC), Lucy's Creek (LC), and Rio Santa Rosa (RSR) Mindo, Ecuador as determined by Surber net samples (n=3 per site). Letters represent significant groupings (One-way ANOVA, TukeyHSD $p<0.05$) per functional feeding group.

Figure 2. Canonical correspondence analysis triplot displaying how macroinvertebrate community structure differs among 3 streams in Mindo, Ecuador sites due to environmental variables. The first two axes explain 93.5% of the variation with axis 1 explaining 77.8% of the variation and axis 2 explaining 15.7%. Total inertia for the plot was 0.5751. Site abbreviations: kc = Kathy's Creek, lc = Lucy's Creek, and rsr = Rio Santa Rosa. Species abbreviations: Bae = Baetidae, Cala = Calamoceratidae, Cera = Ceratopogonidae, Chir = Chironomidae, Doli = Dolichopodidae, Elm = Elmidae, Heli = Helicopsychidae, Hyb = Hydrobiosidae, Hyp = Hydropsychidae, Hypt = Hydroptilidae, Lepp = Leptophlebiidae, Leph = Leptohyphidae, Perl = Perlidae, Plan = Planariidae, Ptil = Ptilodactylidae, Sim = Simulidae, and Tip = Tipulidae.

Figure 3. Average $\delta^{15}N$ and $\delta^{13}C$ signatures for all composite samples from RLG streams sampled in this study. Error bars represent standard error (n=1, 2, or3, depending on the composite sample). All FFG refer to aquatic macroinvertebrates.

Figure 4. Average $\delta^{15}N$ and $\delta^{13}C$ signatures bulked into main groupings (basal resources, aquatic insects, and terrestrial insects) as well as the top consumers (fish, spiders, and frog). Error bars represent standard error.

Figure 5. The number of aquatic adults (Emphemeroptera, Tricoptera, and Plecoptera) captured during each emergence sampling event at Lucy's Creek. Catch per unit effort = 1 hour of net time from 6:00 to 7:00 pm, which corresponded to the onset of dusk into complete darkness.
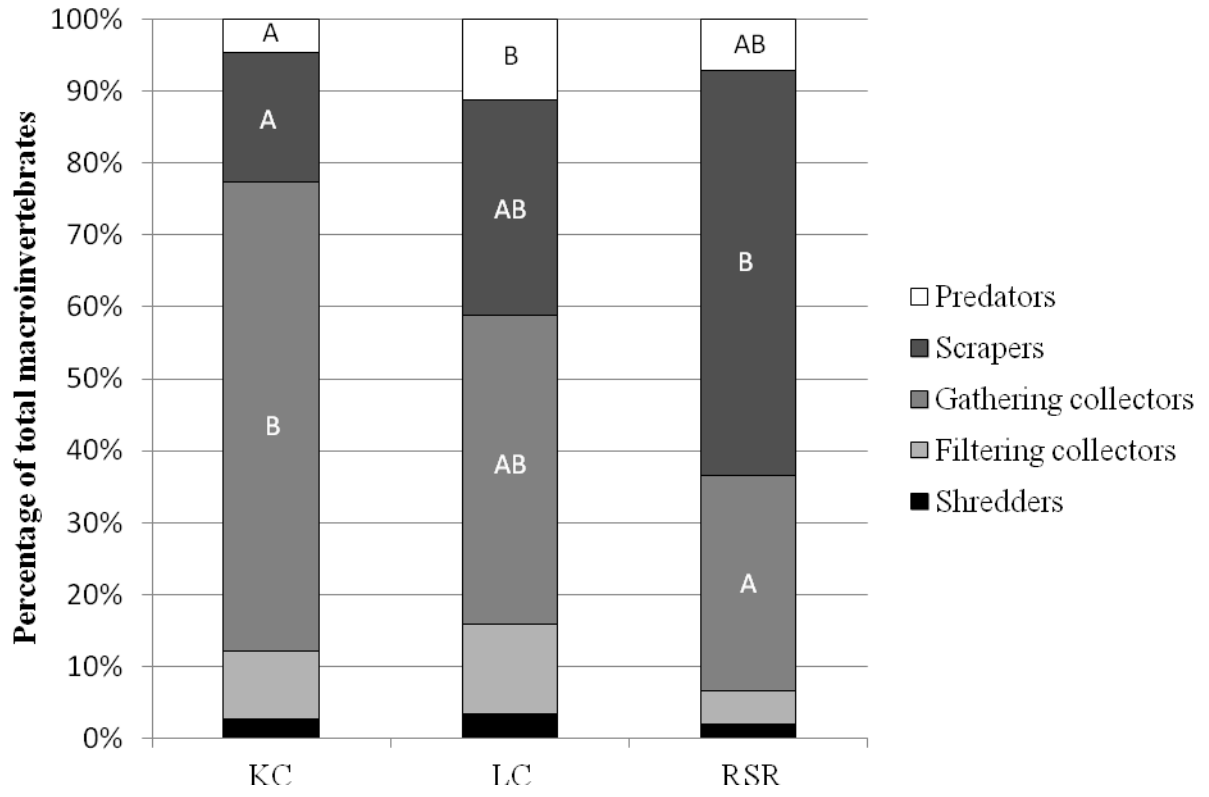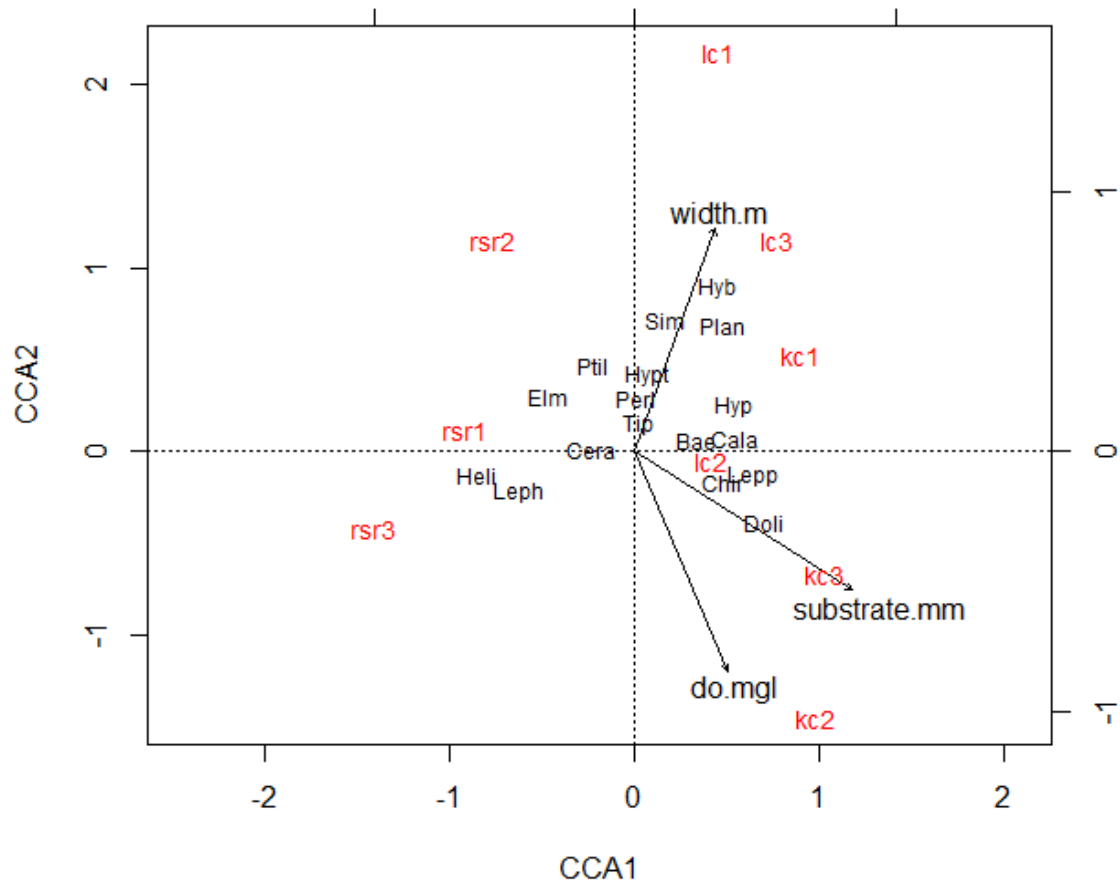
Fig. 1

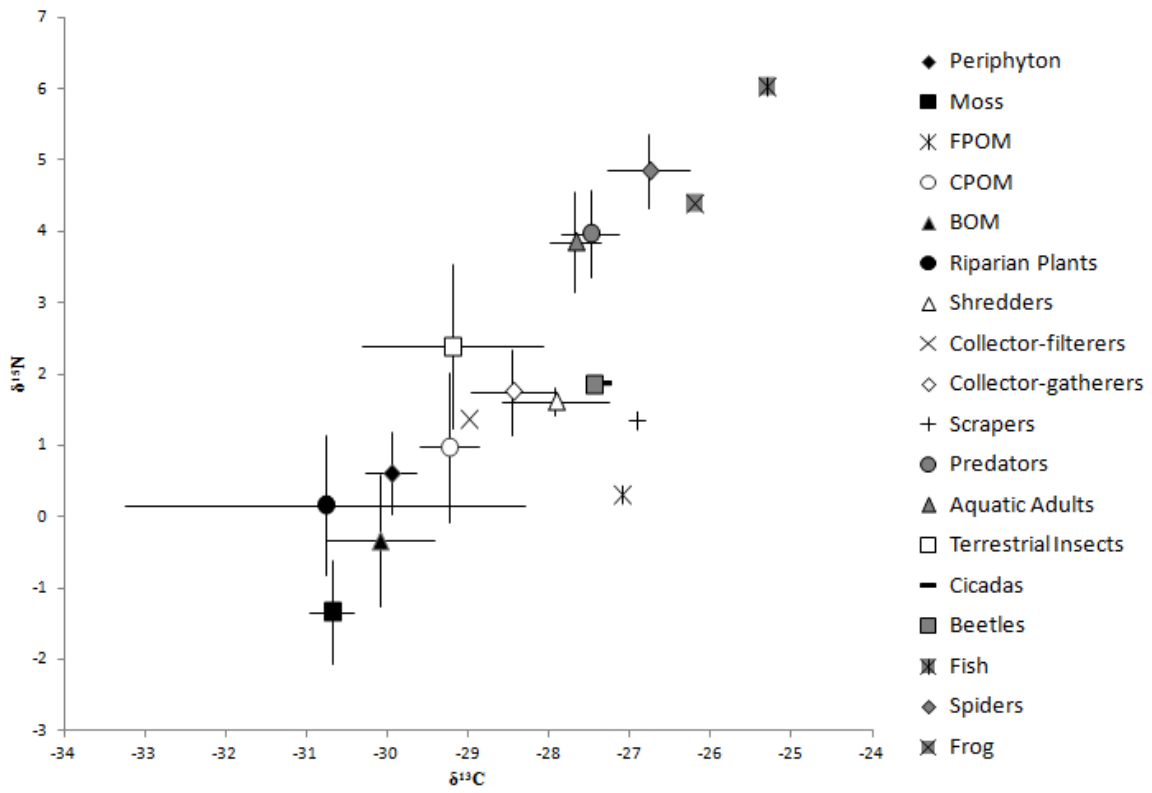**Fig 2.**
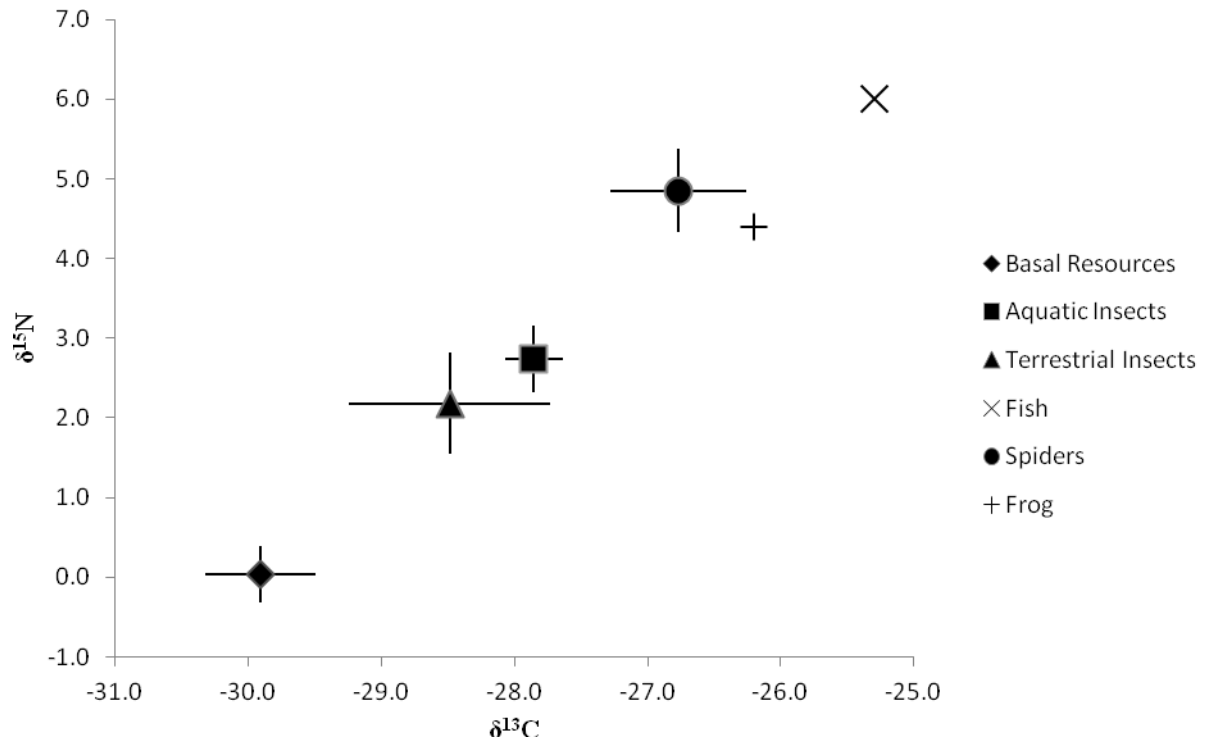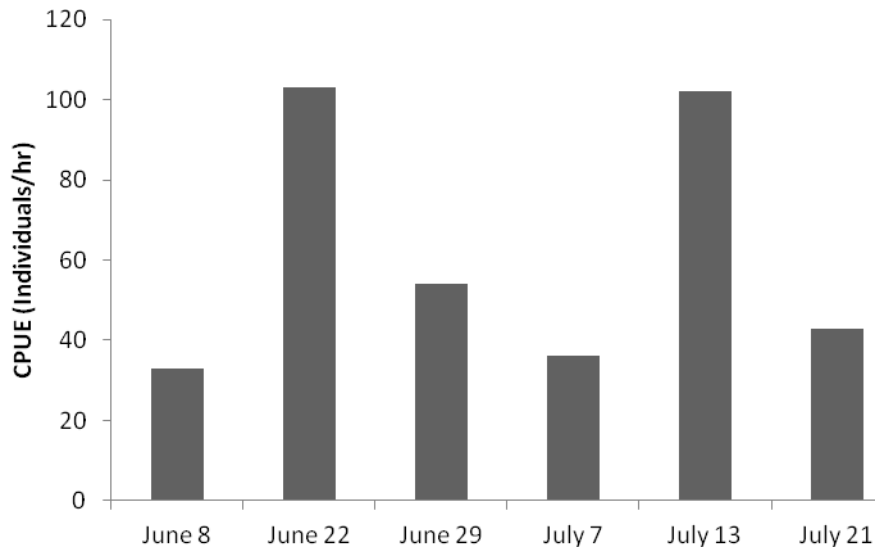
Fig 3.

Fig. 4

Fig. 5

Chapter 3: Discussion

1. **Extended Review of Literature**

When searching for answers to differences between temperate and tropical streams ecosystems it is useful to keep the habitat template concept (Southwood 1977) in mind. This concept describes the relationship between a habitat and the species that inhabit it. Simply, if a habitat is suitable for a particular species or group of organisms, they should be present. Conversely, if the organisms are no longer present, the habitat may no longer be suitable for them to survive. This concept can also be applied to conservation efforts as alterations to ecosystems can create unsuitable conditions for certain species.

There is evidence to suggest that CPOM is more often used as a physical substrate rather than a food source in tropical streams (Aggie et al. 2008, Li et al. 2009, Uieda and Carvalho 2015). In fact, Mantel et al. (2004) determined that although CPOM was the dominant basal resource, FPOM and periphyton were more prominent in gut content analyses. In the present study, shredders were scarce although the dense closed canopies of the study sites provided an abundance of CPOM. Patterns of low shredders and high amounts of CPOM have been found in other studies conducted in tropical stream as well (Mantel et al. 2004, Dobson et al. 2002). If their food source is readily available, why are shredders a rarity in tropical streams?

Some suggest that the microbial portion of the aquatic food web is playing a role in leaf litter processing (Marthuriau and Chauvet 2002, Dodson et al. 2002, Wright and Covich 2005). As the tropics are warmer than the temperate zone, microbial rates should be higher (Suberkropp and Chauvet 1995, Boyero et al. 2011). Marthuriau and Chauvet (2002) found leaf litter breakdown rates to be fast, when compared to temperate rates, in their Colombian streams that were high in fungal biomass and low in shredders. Wright and Covich (2005) examined the

influence of fungi and bacteria on tropical leaf decomposition and found that the fastest

decomposition rates occurred in treatments with both bacteria and fungi. Through the use of

microcosms and different types of leaves, they determined fungi and bacteria have different roles

in leaf litter decomposition. Further information on the aquatic microbial community in RLG

streams is needed to fully understand their potential role in leaf litter decomposition.

Another explanation is that tropical leaf quality may not be suitable for shredding

macroinvertebrates. Leaf litter quality tends to be lower in the tropics than in the temperate zone

(Bruder et al. 2014, Graça and Cressa 2010). Aggie et al. (2009) tested the effects of toughness

and nitrogen content of leaves to litter breakdown rates in Hong Kong and found a negative

correlation between breakdown rates and leaf strength (tougher leaves decomposed more

slowly). There was also no link between litter quality and macroinvertebrate assemblage

indicating that litter has a substrate role as opposed to a food source role, as previously

discussed, and may be responsible for the lack of shredders in these ecosystems. In addition,

Boyero et al. (2011) surveyed 129 sites globally to examine diversity patterns for shredding

macroinvertebrates. Leaf toughness was not related to shredder abundance or richness.

Although leaf toughness and quality does not seem to be related to shredder distribution, there is

evidence to suggest chemical properties of leaf litter play an important role (Mathuriau and

Chauvet 2002).

Lastly, macroconsumers, such as crabs, shrimp, and fish, could be more important in leaf

litter processing than shredding macroinvertebrates. Several studies conducted in tropical

streams found this to be true (Moulton et al. 2010, Uieda and Carvalho 2015, Wright and Covich

2005). In some cases, that not only are macroconsumers important in the breakdown of leaf

litter, but shredding macroinvertebrates have little to no effect of leaf litter processing. Moulton

et al. (2010) conducted a leaf pack experiment in Brazilian streams and packs that excluded macroconsumers but allowed shredding macroinvertebrates to forage were not significantly different than packs that excluded all fauna. The few shredders that were present at those sites were not significantly contributing to leaf litter breakdown, the macroconsumers were. Crabs are present at all three RLG streams which could potentially contribute to the shredding niche in these systems.

To our knowledge, this is the first stable isotope study conducted in Ecuador and perhaps in cloud forest streams as well. We had mixed results when comparing our findings to the literature. Overall, the components of our stream food webs seemed to be N-depleted when compared to the literature. One explanation for this is low levels of in-stream primary production likely caused by the tall, dense canopies that surround these streams (Lau et al. 2009, March and Pringle 2003). A second explanation is low N contents in the water which would create low N signatures in periphyton and continue up the food web (Coat et al. 2009). N content in water was not measure in this study, however, conductance of our streams ranged from 18.3-59.8 uS/m which likely indicate low levels of N. Regardless, the patterns found in our stable isotope analysis were typical of aquatic food webs and reveal a linkage between the streams and their riparian zones.

2. Study limitations and recommendations for future research

This present study used numerical abundances to analyze macroinvertebrate community composition. Some have suggested that biomass is more important than numerical abundance in determining the relative importance of shredding macroinvertebrates in processing leaf litter (Chesire et al. 2005, Tonin et al. 2014). Using both methods to analyze FFGs in these systems may have altered our ecosystem attribute conclusions as numerical and biomass abundances can produce varied results. For example, Ramírez and Pringle (1998) used both methods to examine community composition in their Costa Rican stream. In terms of numerical abundances, collector-gatherers were the most dominant FFG in riffle habitats (56.4%) but in terms of biomass they composed only 18.77% of the community. Biomass abundances determined predators to be the most dominant FFG in this habitat (61.03%) while numerical abundances determined predators to only constitute 8.91% of the community. These discrepancies between methods highlights the importance of using various methods to examine community composition and future studies should aim to utilize both methods. Future studies at these sites should also focus on potential role of macroconsumers in leaf litter processing. For example, crabs are present at all 3 sites however their abundance and foraging strategies are currently unknown.

Stable isotope analyses revealed typical aquatic food webs that are linked to terrestrial ecosystems. As this analysis aimed to provide base-line stable isotope data, future studies should expand upon these efforts to produce more thorough results. For example, separating each macroinvertebrate into samples based on identification rather than FFG would provide more detailed results. This approach has been used successfully in other tropical streams (e.g. Mantel et al. 2004, Lau et al. 2009, March and Pringle 2003).

This study was also limited in seasonal and temporal repetitions. Sampling took place during the transition from the wet to dry season of 2015 which ultimately was an el Niño year. As season has an impact on macroinvertebrate community composition and emergence rates in tropical streams (Lau et al. 2009, Ramirez and Pringle 1998), future studies should aim to sample during both seasons. For effective monitoring of these sites, sampling should be conducted over several years so that changes in biophysical stream conditions can be properly reported and causes of such changes can be identified.

3. Literature Cited

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