SEASONAL VARIABILITY IN MACROINVERTEBRATE COMMUNITIES IN PAIRED PERENNIAL AND INTERMITTENT STREAMS IN COSTA RICA

by

DARIXA D. HERNÁNDEZ ABRAMS

(Under the Direction of Scott Connelly)

ABSTRACT

Streamflow regimes influence the ecological integrity of freshwater ecosystems. The combined effects of global climate change and water abstraction may alter flow regimes causing disturbance in the form of flooding or drying. There is limited information on what these changes will cause to neotropical systems that are at risk of facing extremes in hydrological events. This thesis evaluated the monthly effects of flooding and drying on aquatic macroinvertebrate community compositions from adjacent intermittent and perennial streams in the Pacific North of Costa Rica over a one-year period. Macroinvertebrate communities were different between the two streams and changed seasonally as well. Richness was higher in the intermittent stream during both wet and dry seasons. Taxa that are considered sensitive to flow disturbance declined during both seasons. Our results identify patterns that may be useful to understanding survival strategies and life history traits in neotropical streams of varying flow regimes.

INDEX WORDS: Invertebrate assemblage, intermittent, perennial, seasonality, drought, flood, disturbance, tropical streams, flow, Costa Rica

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DEDICATION

To my parents for all of the sacrifices they made to give me the best education, enabling me to get this far. My dad’s love for science influenced me to pursue this degree and my mother’s hard work taught me that anything can be done if you put in the time and energy.

And to Puerto Rico. Such beautiful and resilient ecosystems will always be my inspiration.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Literature Review

Flow regimes are at the heart of sustaining the ecological integrity of stream ecosystems (Poff *et al*., 1997). High or low flows occur naturally in stream ecosystems; however, these lotic systems are at risk of additional hydrological flow alteration driven by increasing water demand and the effects of climate change. This hydrological alteration may cause extreme changes to flow regimes resulting in streambed drying or flooding. A decline in water flow may reduce lateral and longitudinal connectivity and decrease riffle habitats and increase isolated pools (Reich & Lake, 2015). Droughts interrupt stream continuums resulting in patchiness and habitat fragmentation (Lake, 2000). Contrary to droughts, flood events increase habitat connectivity and can produce streambed scouring. Both flooding and drought disturbances have the potential to significantly alter stream ecosystems by influencing both biotic (e.g. community composition alteration) and abiotic (e.g. chemistry and geomorphology) components.

Global climate change models suggest alterations in the amount and distribution of rainfall for most of the tropics (IPCC, 2014). The combined effects of climate change and human water extraction have been identified as important threats to tropical freshwater ecosystems. However, there is limited information on the effects of these extreme hydrological events on tropical stream ecosystems and potential impacts on tropical biodiversity. In Central America, the Continental Divide largely determines climatic patterns found on the Caribbean Sea and

Pacific Ocean sides of the landmasses. The Pacific slope of Costa Rica has a more defined wet and dry season, while the Caribbean slope has similar distribution of precipitation throughout the year. Adding to the long term patterns found, El Niño events in Costa Rica typically cause cyclical droughts over the Pacific North region of the country, while La Niña events cause an increase in precipitation and subsequent flooding (Alvarado *et al.*, 2012).

The district of Monteverde, located in the Pacific North of Costa Rica, is one of the most visited areas in the country during the high tourism season (Acuña, Villalobos & Ruíz, 2006). The number of permanent residents in Monteverde is approximately 4,000, yet the tourism industry has a much larger influence in the district's water consumption. Dallas (2005) found that daily water consumption per guest staying in high-end hotels was 3 to 13 times greater than general domestic consumption per person (178 ± 93 L/person/day). However, an estimated 85% or more of all household greywater is discharged directly into the environment (e.g. lawns, streets, rivers and streams) while big hotels usually dispose their greywater in septic wastewater tanks. Agriculture, a large end user of water resources, was mainly focused on cattle ranching in the mid-1990's when 210 families supplied milk to the local Monteverde Cheese Factory (Griffith, Peck & Stuckey, 2014). The economy and development in the area have since shifted to ecotourism as the main source of employment and income. Both population size and tourism are expected to increase in Montverde, leading to an increase in water demand. The combined effects of climate change and water abstraction for human use can lead to perennial streams becoming streams with intermittent flow regimes (Larned *et al.*, 2010), which has the likelihood to alter aquatic biodiversity (Dudgeon *et al.*, 2006), water availability, water quality (King *et al.*, 2015) and the restructuring of regional river basins.

Aquatic macroinvertebrates are commonly used as indicators of disturbance in stream ecosystems because of their sensitivity or tolerance to changes in their habitat. Community metrics such as taxon richness, abundance and biomass are used to quantify ecosystem changes. Aquatic macroinvertebrates possess functional attributes (e.g. feeding guilds, mobility, developmental speed, and reproduction) that may be associated with environmental disturbances (Vieira *et al*., 2006). Macroinvertebrates may survive perturbation through mechanisms related to adaptations in morphology (e.g. body armoring, size, and development time) and behavioral response (e.g. seeking refugia, drifting, and oviposition). Many times, life history adaptations are tied to seasonal timing and predictability as organisms synchronize life stages to patterns of long term flow dynamics (Lytle & Poff, 2004). The availability of refugia (defined by Robson *et al.* (2008) as physical spaces that provide shelter from one or more disturbance or sources of potential colonists after the occurrence of a disturbance) is also critical for macroinvertebrates to resist or recolonize freshwater hydrological disturbances.

Limited information exists on the effects of hydrological alteration caused by increased anthropogenic water demand and projections of climate change in the tropics. However, macroinvertebrates in Costa Rica are relatively well known, compared to other tropical countries (Springer, 2008), which brings an opportunity to better understand the effects of disturbances on these organisms. Understanding potential biotic responses to disturbances is important for predicting how ecological dynamics in aquatic organisms may respond to flow disturbances.

Project Objectives

We assessed the effects of both flooding and drought disturbances in paired perennial and intermittent neotropical headwater streams in Monteverde, Costa Rica. Seasonal changes in macroinvertebrate community composition were documented through monthly sampling over one year. We also attempted to quantify characteristics that enable some macroinvertebrate taxa to persist through hydrological alterations, while others decline in numbers. The goal of this work was to contribute to the limited information that exists on these vulnerable and rapidly changing tropical stream ecosystems.

In Chapter 2, we conducted a one-year study to: [1] quantify differences in aquatic macroinvertebrate community composition (i.e. taxon abundance, richness, biomass and FFGs) in neighboring perennial and intermittent streams; [2] document potential shifts in community composition within both streams across dry and wet seasons; and [3] characterize physical, chemical and biological parameters that may potentially affect changes in macroinvertebrate composition. In Chapter 3, we described the ecological reasoning, challenges and recommendations for the use of three sampling techniques that we ultimately found problematic in our study system.

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CHAPTER 2

SEASONAL VARIABILITY IN MACROINVERTEBRATE COMMUNITIES IN PAIRED PERENNIAL AND INTERMITTENT STREAMS IN COSTA RICA

[1] Hernández-Abrams, D.D., Connelly, S., Wenger, S.J. & Freeman, M.C. To be submitted to *Freshwater Biology*

Abstract

Rising global temperature is a driver altering intensity and duration of precipitation in tropical wet and dry seasons. Extremes in hydrological events combined with increasing population and tourism in Costa Rica are projected to affect local water availability. We quantified the effects of seasonal flow variation on aquatic macroinvertebrate communities within two headwater streams, one intermittent and the other perennial, in the Pacific North of Costa Rica. We sampled macroinvertebrates monthly over a year in riffle, pool and leaf pack habitats. A total of 72 invertebrate taxa were found. Non-metric multidimensional scaling (NMDS) analyses indicated dissimilarities in macroinvertebrate abundance and biomass composition between the two streams ($p<0.05$). We found lower macroinvertebrate abundance in leaf pack habitat during the wet season and higher richness in benthic habitat in the intermittent stream compared to the perennial stream ($p<0.05$). Our results may be explained by life history traits related to stream velocity preference or tolerance; short life cycles that limit exposure to disturbances; and dispersal capacities and feeding mechanisms that are dependent on water movement. Understanding the ecological response of these relatively under-studied systems will help us better assess the effects of projected changes in stream flows.

Introduction

Flow regime is critical to structuring the biotic and ecological integrity in stream ecosystems (Poff *et al*., 1997). Streams are characterized by high levels of spatiotemporal heterogeneity which are linked through four dimensions described by Ward (1989) as longitudinal (upstream-downstream path), lateral (stream channel and riparian zone), vertical (stream channel and groundwater), and temporal dimensions. A reduction in stream flow may reduce riffle habitats, lateral and longitudinal connectivity, and increase the number of isolated marginal pool habitats. And at the final stages of persistent drought, these microhabitats could disappear entirely (Reich & Lake, 2015). Flood events can alter stream ecosystem structure by mobilizing sediment and debris, altering the distribution and connectedness among riffles, runs and pools, and by reducing habitat diversity (Death, Fuller & Macklin, 2015). The frequency and intensity of flow as a physical disturbance can have important effects on benthic macroinvertebrate richness, abundance, and diversity (Townsend & Scarsbrook, 1997; Dudgeon *et al*., 2006) by exerting changes in geomorphology, (Death, Fuller & Macklin, 2015), organic matter transport and retention (Dewson, James & Death, 2007), stream metabolism (Acuña, 2004), refugia (Dole-Olivier, Marmonier & Beffy, 1997), and water chemistry (Gómez, Vidal-Abarca & Suárez, 2009).

Freshwater ecosystems are among the most vulnerable systems to effects of climate change (Omerod, 2009), and, although at risk of extremes in hydrological events, tropical streams remain relatively understudied (Wantzen, Ramírez & Winemiller, 2006). Unlike temperate regions, seasonality in the tropics is mainly characterized by temporal changes in precipitation rather than changes in temperature or duration of daylight. Projections of climate change suggest an alteration in the frequency and magnitude of precipitation during both wet and dry seasons in many tropical regions (IPCC, 2014; Trenberth & Fasullo, 2013; Seager, Naik & Vecchi, 2010). Specifically, there is a projected decrease in precipitation in many areas of the subtropics distributed through intense episodic rainfall events and longer dry periods (IPCC, 2014). The limited information we have on extreme hydrological events in the tropics comes primarily from studies in Puerto Rico (Covich, Crowl & Scatema, 2003; Benstead *et al*., 1999; Covich *et al*., 1991) and Andean streams (Ríos-Touma, Encalada & Prat Fornells, 2011; Jacobsen & Encalada, 1998; Dudgeon, 1993).

Additional stressors to tropical streams include consequences of increases in population, including urbanization (Montgomery, 2008), tourism, and agricultural expansion (DeFries & Rosenzweig, 2010), each resulting in increasing demand for limited water. The combined effects of climate change and increasing water demand may not only lead to anthropogenic water shortages but may have further-reaching ecological consequences (Larned *et al*., 2010). For example, in Costa Rica, El Niño events cause droughts over the Pacific North region of the country, and La Niña events cause an increase in precipitation and subsequent flooding (Alvarado *et al*., 2012). The Regional Committee for Hydrological Resources in Costa Rica (2008), part of the National Meteorological Institute, projected an overall reduction in annual precipitation over the Pacific North during the next century. However, the effects of reduced precipitation have already been felt, as during the past several decades, lower than normal stream discharge and precipitation have been reported during the dry season in the Tilarán Corrridor near the Monteverde region of the Pacific North of Costa Rica (Pounds, Fogden & Campbell, 1999).

The ability of an organism to modify and respond to its immediate environment may allow it to survive changes in natural flow regimes. Aquatic organisms may be resistant or sensitive to changes in their habitat caused by disturbances. The response of aquatic macroinvertebrates to disturbance may be reflected in changes in taxa abundance, richness, biomass, and functional feeding group (FFG) composition. Many life history adaptations are tied to seasonal timing and environmental predictability as organisms synchronize life stages to patterns of long-term flow dynamics (Resh *et al*., 1988; Lytle & Poff, 2004). Desiccation-resistant stages that allow for survival during drought (e.g. cysts, eggs, dormancy) may help favor dominance of benthic composition of fly larvae (Order: Diptera), some small crustaceans (e.g. Orders: Cyclopoida, Harpacticoida and Class: Ostracoda) and aquatic worms (Subclass: Oligochaeta) (Datry, 2012). It is known that macroinvertebrate families with high rheophily favor environments that are cooler and have a higher amount of dissolved oxygen (DO), while families with low rheophily are more tolerant of warm hypoxic environments, which are more common during droughts (Chessman, 2015). Because of this, macroinvertebrate community composition may shift from taxa adapted to low flow during drier years to those taxa that are adapted to high flows during periods of more precipitation (Bêche & Resh, 2007). However, the physical characteristics of a stream can mediate aquatic invertebrate survival during disturbances (Robson, Chester & Austin, 2011). Refugia from high flows can be provided by stream features such as large boulders that remain immobile during high flow events (Fingerut *et al*., 2014), areas of lower velocity (e.g. pools), and woody debris accumulations that can provide consistent habitat.

Studying tropical headwater streams within Costa Rica at locations that are relatively free from human disturbance provides an opportunity to quantify the effects of hydrological disturbance, such as from flooding or drying, on macroinvertebrate composition without confounding environmental stressors such as altered temperatures or anthropogenic contamination. We conducted a one-year study to: [1] quantify differences in aquatic macroinvertebrate community composition (i.e. taxon abundance, richness, biomass and FFGs) in neighboring perennial and intermittent streams; [2] document potential shifts in community composition within both streams across dry and wet seasons; and [3] characterize physical, chemical and biological parameters that may potentially affect changes in macroinvertebrate composition. We hypothesized that because a perennial stream would have a more stable flow regime, higher taxa richness and abundance would be found in this stream. Second, we hypothesized that streambed drying would result in lower taxa richness during the dry season in the intermittent stream. We also hypothesized that high rainfall events would increase mortality or downstream movement of macroinvertebrates resulting in lower taxa richness and abundance following high flows.

Methods

Location

We studied two pre-montane cloud forest headwater streams at the University of Georgia's satellite campus (UGA-CR) located in the Pacific North of Costa Rica in the community Altos de San Luis, Monteverde (10.28°'N, 84.79°'W). The San Luis Valley lies within the Bellbird Biological Corridor, a regional conservation zone, and is located in the Puntarenas Province near the continental divide on the Pacific slope of the Cordillera de Tilarán, where climate patterns are driven by the Intertropical Convergence Zone, orographic effects and cold fronts, tropical storms, and (uncommonly) hurricanes (Clark, Lawton & Butler, 2014). The district of Monteverde is characterized by having marked dry (~December - ~March) and wet seasons (~May - ~October), with November and April serving as transitional months between each season. During July and August, trade winds intensify over the region and typically cause a reduction in precipitation which is locally called *veranillo*. Using data from the onsite weather station, we defined *a priori* the wet season in San Luis as May through October and the dry season as November through April.

Agriculture in the Monteverde district was mainly focused on cattle ranching in the mid-1990s but the economy and development have since shifted to ecotourism as the main source of employment and income (Griffith, Peck, & Stuckey, 2014). Monteverde is one of the most visited areas in Costa Rica during the high tourism season (Acuña, Villalobos & Ruíz, 2006), and tourism is recognized as the largest driver of water consumption in the area (Dallas, 2005). Both population size and tourism are expected to increase in Monteverde, leading to a rise in water demand while effects of climate change may magnify the need for freshwater water availability.

The UGA-CR campus (~4000m^2) is a former small-scale coffee and dairy farm, that was converted to an education and research facility in 2002. Sixty percent of the land is protected forest, 30% is used for sustainable agriculture practices, and 10% is developed. The campus is in close proximity to the Monteverde Cloud Forest Reserve and the San Luis Biological Reserve. The population of permanent residents in San Luis is approximately 350. The larger town of Santa Elena, the regional tourism center, sits above San Luis at a distance of ~7.8km.

Study sites

Within the UGA-CR campus are two parallel headwater streams, Alondra (1.74 km^2 drainage area, perennial stream) and Bruja (0.828 km^2 drainage area, intermittent stream), which are ~125m apart (Fig. 2.1) and at an elevation of ~1200m. The perennial stream flows continuously during both wet and dry seasons and the intermittent stream typically dries from

February - March. The streams converge ~400m below our study site, and subsequently flow into the San Luis River. Average annual precipitation at the location is ~3000mm. The vegetation surrounding the streams is secondary forest dominated by *Cecropia obtusifolia*, *Heliocarpus americanus*, *Piper auritum*, *Trema micrantha*, *Inga mortoriana*, *Lonchocarpus oliganthus*, *Inga sierra*, *Conostegia xalopensis*, and *Saurauia montana* (less common).

Macroinvertebrates

We sampled 100m reaches in each stream on a monthly basis over the course of one year (May 2016 - April 2017). Macroinvertebrates were collected in five riffles and five pools each month using a $0.093m^2$ Surber sampler (243µm mesh). A $0.023m^2$ Mini-Surber was used during the months of February-April to sample the intermittent stream's riffles due to difficulties with fitting the larger-sized Surber in the available wetted areas as a consequence of low flow. We combined riffle and pool samples prior to our data analyses to describe benthic macroinvertebrate communities as a whole rather than separating them into habitats. We will refer to these as benthic samples. We also collected four leaf litter grab samples (mostly submerged underwater in riffles) monthly in each stream from June 2016-April 2017 at haphazardly chosen locations from riffle accumulations using 16.8cm x 14.9cm plastic sandwich bags filled to capacity. In order to avoid causing a decline in macroinvertebrate abundance by oversampling, no samples were collected during consecutive months from the exact same location. Macroinvertebrates were preserved in 70% ethanol, transported to the laboratory, and identified to lowest possible taxonomic level, generally genus. The family Chironomidae was classified to subfamily (Tanypodinae or Non-Tanypodinae) and the subclass Oligochaeta was left at this level. Local (Springer, Ramírez & Hanson, 2010) and regional guides (González-

Córdoba, Zúñiga & Manzo, 2015; Manzo & Archangelsky, 2008; Roldán, 1988) were used to identify macroinvertebrates, supplemented by a North American guide (Merritt, Cummins & Berg, 2008). We estimated macroinvertebrate biomass by measuring individuals to the nearest 0.25mm and using published length-mass relationships (Benke *et al*., 1999; Sabo, Bastow & Power, 2002; Méthot *et al*., 2012). Where genus level length-mass values were unavailable, the next highest available taxonomic level was used (i.e. family, sub-family, subclass, or order). We assigned each taxon to a functional feeding group according to Merritt, Cummins & Berg (2008) and Domínguez & Fernández (2009).

Physical and chemical parameters

Average daily precipitation and temperature data were obtained from the on-site UGA Costa Rica weather station. Water temperature and depth were estimated using a HOBO® data logger (HOBO U20L-01, Onset Computer Corp., Bourne, Massachusetts). Two data loggers were placed in pools in each stream and a third was tied to a nearby tree in order to record atmospheric barometric pressure.

We collected and froze one water sample from each stream monthly in acid-washed 125mL bottles to analyze nutrient concentrations. The twenty-four samples were frozen and transported to the US on dry ice to analyze for TP, TN, NO_3^--N, NH_4^+-N, and SRP-P. A persulfate digest for water total nitrogen and total phosphorus was performed following Koroleff (1983) protocol as modified by Qualls (1989). All samples were processed at the University of Georgia Center for Applied Isotope Studies.

Chlorophyll-a

We sampled periphyton from September to March using a syringe/brush sampler modified after Loeb (1981). We collected five subsamples of periphyton in Whirl-packs® monthly from September 2016 - March 2017 in five areas with similar canopy cover along our two 100m reaches. Samples were transported to the laboratory where they were kept frozen until processing, at which time they were thawed and filtered through Whatman glass fiber filters (0.7μm). Filters were placed in aluminum foil packets, frozen, and transported to the University of Georgia, Athens. Chlorophyll-a was extracted from filters using a hot ethanol method (Sartory & Globbelaar 1984) and acidification with 0.1 M HCL. Samples were read using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan).

Characterization of habitat composition

Habitat composition was described by following the Montgomery & Buffington (1997) protocol modified by Hauer & Lamberti (2007). We measured width, depth, percent canopy cover (using a spherical densitometer), percent sediment composition (boulder, cobble, pebble, gravel, sand and silt; estimated visually), and the proportion of pools, riffles and runs in each stream at every 10m within the 100m reaches. Stream width and depth and the percentage of pools and riffles was measured once during the wet season (September 2016) under the assumption that these would remain similar throughout the season. However, these measurements were taken every month from February - April 2017 during the dry season in order to record changes in stream width and depth and proportion of pools, riffles, and runs as drying progressed. We defined riffles as fast flowing and shallow areas in the streams, runs as fast flowing but deeper areas, and pools as areas of little or no movement of water, greater depth, and deposited sediment. Runs were not sampled for macroinvertebrates due to difficulty of sampling in deep areas of fast moving water.

Data analyses

For our analyses, we examined the total numbers, biomasses and richness of invertebrates collected in either benthic samples or leaf packs, in each month and stream, as independent response variables. We examined macroinvertebrate community composition in the two streams using non-metric multidimensional scaling (NMDS) with Bray-Curtis distances to obtain similarity matrices of [1] abundance and [2] biomass of taxa. Rare macroinvertebrates occurring in only one sampling month and site were excluded from these analyses. For biomass, we excluded the pseudothelphusid crab, *Ptychophallus*, due to its much larger mass compared to other taxa. Elmid larvae and adults were treated as separate taxa. We conducted separate ordinations for benthic samples and for leaf litter samples. For each ordination we calculated a stress value, which indicates the degree to which the distance between samples in the reduced dimensional space matches the original multivariate distance between samples. A stress lower than 0.2 is considered to be a good fit (Clarke, 1993). For all of our ordinations we determined that a three dimensional solution was optimal based on stress values. We used analysis of similarities (ANOSIM) with 999 permutations to test for differences in macroinvertebrate composition between the perennial and intermittent stream with $p<0.05$. We then used similarity percentages tests (SIMPER) to identify taxa with the greatest contribution to dissimilarities. All ordination analyses were done using the “vegan” package in R version 3.2.4 (R Core Team, 2016).

Stage height was a key variable for explaining seasonal macroinvertebrate community dynamics. Unfortunately, we had a substantial gap (109 days) in the stage height time series for the intermittent stream due to the loss of a data logger during a high flow event. We filled this gap by constructing a Random Forest model (package “randomForest” in R) using daily stage height in the perennial stream, daily precipitation, and precipitation from the prior day as predictor variables to predict daily stage height in the intermittent stream. The resulting model explained 68% of the variability in the observed time series, which we judged to be sufficient to use as the basis for predicting the missing days of stage height.

We used multiple linear regression models to test total monthly abundance, biomass, and richness of benthic samples as a function of stream (perennial vs intermittent) and season in order to test for seasonal differences in communities. If we found a significant result, we tested for interactions between stream and season. We repeated this process with the leaf pack samples. We also used multiple linear regressions to test for relationships between total abundances, richness, biomass, and taxa from abundance SIMPER analyses and rainfall measurements from 31 days prior to sampling in each stream (Table 2.1). We considered results to be significant at $p<0.05$.

Results

Precipitation and stage height

Highest precipitation (~60-120mm/day) occurred during the months of May, June, September, and December (2016 rainy season) and early May (2017 rainy season). Lowest continuous daily precipitation (near 0mm/day) occurred from late February to the end of April (2017 dry season). The amount of daily precipitation was reflected in each stream’s stage height

(Fig. 2.2); however, the two streams responded differently to rainfall. The perennial stream maintained stage heights between 0.15 and 0.35m during the dry season, while the intermittent stream exhibited flashier behavior (0.05-0.43m).

Average canopy cover was 79% in the perennial stream and 70% in the intermittent stream (Table 2.2). Habitat types differed between the two streams and they also changed seasonally (Table 2.2). Riffle habitats composed 53% of the perennial stream during the wet season and 56% of the intermittent stream (Table 2.2). Pool habitats made up 28% of the perennial stream while they formed 32% of the intermittent stream. Habitat availability gradually decreased in both streams during the dry season (Table 2.2). Deep and fast runs formed the remaining habitat. Stream width was similar between streams during the wet season (Table 2.2) and decreased in the intermittent until partial or complete drying (60m of 100m sampling reach) of the streambed during from February to April. Sediment in both streams was mainly composed by large boulders, cobble, and finer sediment such as sand, silt, and clay (Table 2.2).

General macroinvertebrate composition

We identified and measured 18,632 aquatic macroinvertebrate individuals belonging to 72 genera, 49 families, and 14 orders. Refer to Appendix A for a complete list of taxa. A total of 74 taxa were found in benthic habitats, and 54 taxa were found in leaf pack samples. Total monthly abundance ranged from 86-671 individuals in benthic habitats and from 98-1,855 individuals in leaf litter (Table 2.3). The most abundant taxa belonged to Diptera (66%), followed by Coleoptera (12%), Ephemeroptera (9%), and Trichoptera (7%). Only one family and two genera of Plecoptera are known in Costa Rica, but macroinvertebrates from this order contributed 4% of the total taxa abundance. Non-insects belonging to Decapoda, Amphipoda, Gastropoda, and Oligochaeta had a minor contribution (1%) of the total invertebrate abundance. Total biomass in benthic habitat was dominated by hydropsychids *Leptonema* (58%) and *Calosopsyche* (8%) in the perennial stream and by hydropsychid *Leptonema* (51%) and ptilodactylid *Anchytarus* (11%) in the intermittent stream. In leaf litter habitat, biomass was dominated by *Anchytarsus* (38%) and *Leptonema* (19%) in the perennial stream and by *Anchytarsus* (25%) and perlid *Anacroneuria* (14%) in the intermittent stream. Macroinvertebrates from 7 functional feeding groups were identified. Collector-gatherer (47% of total abundance) was the most abundant group with an abundance of 2,401 individuals found in benthic habitats and 6,584 individuals in leaf litter. The second most abundant group was predator (25%), with 831 individuals found in benthic habitats and 2,293 individuals in leaf litter. Filterer (18%) and shredder-detritivore (8%) were the third and fourth most abundant groups.

Differences in macroinvertebrate assemblage between streams

There was a pattern of higher macroinvertebrate abundance and higher taxon richness ($p<0.05$; Table 2.4) in the intermittent stream in benthic habitats (2796 vs 2349 individuals and 33 vs 26 taxa) and in leaf litter habitat (7208 vs 6279 individuals and 27 vs 24 taxa; Fig. 2.3). A total of 16 taxa (15 genera, 1 family) were unique to the intermittent stream and 6 genera were unique to the perennial stream (Table 2.5). There was also a trend towards more biomass in the perennial stream benthic habitats (5108mg perennial vs 4623mg intermittent) and leaf litter packs (3252mg perennial vs 2312mg intermittent) (Fig. 2.3).

The NMDS analysis of abundance showed significant differences between the communities in the two streams for both benthic data ($R=0.21$, $p=0.01$; Fig. 2.4) and leaf data

(R=0.24, p=0.007; Fig. 2.5). Clustered groups of contiguous months within each stream show seasonal similarities in community abundance. Perennial stream points were more clustered than those of the intermittent stream, indicating less temporal dissimilarity in composition abundance in the perennial stream. SIMPER analysis indicated that hydropsychids *Leptonema* and *Calosopsyche*; coleopterans *Anchytarsus* and *Heterelmis;* ephemeropterans *Leptohyphes*, *Farrodes*, and *Baetodes*; and the perlid *Anacroneuria* were the highest cumulative contributors (>76%) to these differences in benthic habitat (Table 2.6). Taxa that contributed to >76% to differences in community abundance in leaf litter habitat were dipterans *Non-Tanypodinae*, *Tanypodinae* and *Simulium* (Table 2.6). The NMDS analysis based on biomass showed a distinction between the two streams in leaf pack communities (R= 0.12, p=0.03; Fig 2.6) and a less clear distinction in benthic communities (R= 0.07, p=0.08; Fig. 2.7). SIMPER analysis identified *Calosopsyche* (Trichoptera), *Anchytarsus* (Coleoptera), *Hetaerina* (Odonata), *Anacroneuria* (Plecoptera), and *Argia* and *Brechmorhoga* (Odonata) as most influential cumulative contributors of the differences in biomass in benthic communities (Table 2.7).

Seasonal differences in macroinvertebrate assemblage

We found that benthic richness varied as a function of both stream and season ($p<0.05$; Table 2.4). Richness in benthic habitat was higher in the intermittent stream and during the dry season (Table 2.4). We found lower abundance in leaf pack samples during the wet season ($p<0.05$) and an overall pattern towards lower abundance and richness at the beginning of the wet season and near the end of the dry season (Fig. 2.3). Abundance peaked during January in the intermittent stream and biomass followed the same pattern (Fig. 2.3). Biomass was lower in leaf pack samples during the wet season ($p<0.05$; Table 2.4). We found six significant responses from 199 tests evaluating effects of precipitation on macroinvertebrate metrics ($p<0.05$; Table 2.8). There was a negative relationship between number of days with precipitation above 15, 20 and 30mm and total monthly *Anchytarsus* abundance, and a positive relationship between number of days with precipitation of less than 15mm and total monthly *Anchytarsus* abundance ($p<0.05$; Table 2.8). We also found a negative response of *Baetodes* abundance to number of days with precipitation above 20mm and a positive response of *Baetodes* abundance to number of days with precipitation of 0mm ($p<0.05$; Table 2.8). Non-Tanypodinae (Diptera) and *Anchytarsus* (Coleoptera) occurred at higher numbers in benthic habitat in the intermittent stream during the dry season (Fig. 2.8). The trichopterans *Calosopsyche*, *Leptonema* and ephemeropterans *Leptohyphes* and *Farrodes* declined to abundances near zero during the dry season in the intermittent stream (Fig. 2.8). *Farrodes*, *Anchytarsus* and *Calosopsyche* numbers were highest during the wet season (Fig. 2.8). In leaf packs, dipertans from subfamilies Non-Tanypodinae and Tanypodinae occurred in higher numbers in the beginning of the dry season and then declined to numbers near zero from February to April (Fig. 2.9). There was a trend towards more *Simulium* larvae in the perennial stream during the dry season; these declined to numbers near zero from February to April (Fig. 2.9).

The trends in total abundances are reflected in functional feeding groups. Filter feeders, which are dominated by *Simulium, Calosopsyche* and *Leptonema* dropped to near zero from February - March (dry season) in the intermittent stream (Fig. 2.10). Predators, influenced mainly by *Tanypodinae and Anacroneuria*, were consistently higher in the intermittent stream during the months of December to March (dry season) and peaked in December in all sampling types (Fig. 2.10). The collector-gatherer group, dominated by Non-Tanypodinae dipterans, was more abundant during the months of January-April (dry season) in the intermittent stream in both sample types (Fig. 2.10).

Nutrients and chlorophyll a

Total-P and SRP-P were consistently higher in the perennial stream, with mean Total-P concentrations of 29µg/L in the perennial stream and 9µg/L in the intermittent stream and mean SRP-P concentrations of 43µg/L in the perennial stream and 22µg/L in the intermittent stream (Fig. 2.11). Total-N concentrations varied among the two streams; however, NO_3-N and NH_4-N concentrations were generally higher in the intermittent stream, with mean NO_3-N concentrations of 167µg/L in the intermittent stream and 155µg/L in the perennial stream and mean NH_4-N concentrations of 5µg/L in the intermittent stream and 2.4µg/L in the perennial stream (Fig. 2.11).

Chlorophyll-a concentrations were similar between streams. The perennial stream had average concentrations ranging from 0.54mg/m^2 to 5.03mg/m^2 while average concentrations ranged from 0.92mg/m^2 to 1.59mg/m^2 in the intermittent stream (Fig. 2.12). We were unable to obtain chlorophyll-a data in February in the intermittent stream due to streambed drying.

Discussion

Summary of findings

Our year-long study of two tropical headwater streams showed substantial differences in macroinvertebrate communities between seasons and between streams, although our findings did not always support our original hypotheses. We expected to find higher macroinvertebrate richness and abundance in the perennial stream, but rather found higher richness and trends of higher abundance in the intermittent stream. Our hypothesis that seasonality, and related streambed drying, would be associated with low taxon richness in the intermittent stream was also not supported. Instead, we found higher richness during the dry season in the intermittent stream. Our third hypothesis, that we would find lower abundance and taxon richness related to high rainfall events, was partially supported; we found lower abundance in leaf pack habitat during the wet season, lower richness in benthic intermittent stream samples during the wet season and a trend towards lower abundance in benthic samples. We also found a decrease in total monthly abundance of *Anchytarsus* following the number of days with precipitation above 15, 20, and 30mm, and a decrease in total monthly abundance of *Baetodes* following the number of days with precipitation above 20mm. Overall, our results show that macroinvertebrate community composition was different between streams with differing flow regimes, between wet and dry seasons, and may also suggest declines associated with high rainfall events.

Invertebrate composition between stream types

Although the perennial and intermittent streams are adjacent to each other, and have similar riparian vegetation and surrounding land use, our NMDS analyses showed that the macroinvertebrate community abundance composition differed significantly between streams in benthic and leaf pack samples. Our finding that taxon richness was significantly higher in the intermittent stream differs from our initial hypothesis. An intermediate level of disturbance in terms of intensity and frequency is thought to favor a community composition with higher diversity (Connell, 1978). We anticipated that the combination of drying and high flow events would constitute a major ecological disruption, and ultimately result in lower species richness in the intermittent stream. Although we did not quantify this, we observed a steeper elevational gradient with more cascades over large boulders in the perennial stream compared to the intermittent stream. This higher elevational gradient may result in higher velocities and scouring in the perennial stream, and we suggest that this difference in abiotic conditions within the perennial stream, compared with conditions found within the intermittent stream, creates less favorable conditions for many macroinvertebrates. On the other hand, higher velocities in the perennial stream may be preferred by some species in the family Leptophlebiidae (Rankin & Yoder, 2009) that have gills to obtain oxygen. We found more than double the number of individuals from the leptophlebiid *Farrodes* in the perennial stream (Table 2.3). Another possible explanation for higher richness of some taxonomic groups in the intermittent stream may be that the occurrence of both flooding and drying disturbances in the same stream may be selecting for different macroinvertebrate communities with characteristics that allow for coexistence in the same habitat. For example, within the intermittent stream we found 16 unique taxa that mostly have rapid life cycles and adult flying stages (Table 2.5). Rapid life cycles may allow for different cohorts to coexist in different life stages. The combination of short life cycles and adult flying stages may also minimize the time of exposure to disturbance events inside the stream. Our findings of higher richness in the intermittent streams differ from Szoeke, Crisman & Thurman (2016), who found higher abundance and richness in perennial versus intermittent dry forest streams in Costa Rica. However, the latter streams have been altered hydrologically and structurally for irrigation; their study area receives a different rainfall frequency and intensity from ours and therefore other mechanisms may be structuring their macroinvertebrate communities. It is possible that macroinvertebrates in these human-altered streams have not adapted to recent hydrological changes and therefore, lack life history traits that allow for survival. Additionally, Datry *et al*. (2016) found no differences between perennial and intermittent invertebrate communities in headwaters of the Bolivian Amazon, but attributed these results to the possibility of high humidity and frequent rainfall increasing the probability of invertebrate survival during dry conditions. The variation in overall findings from these studies suggests that it is difficult to generalize or predict patterns of macroinvertebrate community change in neotropical streams in response to differing hydrological alteration.

Environmental measurements

Groundwater contribution to the perennial stream's baseflow throughout the year may be playing a key role in this stream's more consistent flow, while the flashiness in the intermittent stream during the dry season may indicate lack of groundwater contribution. Tropical island streams have been found to have naturally flashy hydrographs in spite of surrounding urban or forested land use or seasonality, which has been attributed to high elevational gradients, soil saturation and small watersheds (Ramírez *et al*., 2009). The small drainage area and high elevation of our sites, combined with soil saturation from the wet season may also be contributing to flashiness in our streams.

Groundwater contribution may also influence nutrient levels in streams. We believe that the higher levels of Total-P and SRP-P in the perennial stream indicate a higher groundwater influence in this stream. Our finding of consistently higher levels of Total-P and SRP-P in the perennial stream may be driven by phosphatized rock weathering in groundwater influencing the perennial stream. Although few soil studies have been conducted in the area of Monteverde (Clark, Lawton & Butler, 2014), Costa Rica is a volcanic landscape composed of relatively young, igneous basaltic rocks with younger silicic rock formations in the northwest region (Deering *et al*., 2012). Pringle, Triska & Browder (1990) found high variability in chemical properties (e.g. Total-P, SRP-P, SO_4 and conductivity) of northeastern Costa Rican streams within the same watershed and attributed these differences to patchiness of volcanic geology within the system. Although SRP-P and Total-P levels in our sites weren't as high as the ones found at La Selva Biological Station, which are associated with direct influence of spring-fed geothermal water, levels of SRP and TP within streams at our study site lie between what Pringle, Triska & Browder (1990) considered phosphorus-rich and phosphorus-poor sites. Our findings suggest possible indirect influence of geothermal phosphorus enrichment in the perennial stream.

Although we did not find a significant response of total monthly macroinvertebrate abundance, richness or biomass to number of days with low or high amounts rainfall, we did find a significant response in the abundance of some specific taxa, when analyzed at a higher level of resolution than at the community level, and evaluating taxa from the abundance SIMPER analysis. We found that abundance of *Anchytarsus* decreased with number of days with precipitation above 15, 20 and 30mm, but decreased with number of days with precipitation below 15mm. Taxa from the genus *Anchytarus* have been classified as sprawlers and have been described as preferring slow moving water (Viera *et al.*, 2006; Poff *et al.*, 2006), meaning that they may more likely drift downstream during high rainfall events, and perhaps may favor slower moving water to avoid drift. Similar to *Anchytarsus*, *Baetodes* monthly abundance decreased with number of days with precipitation above 15mm and increased with number of days with no precipitation. It is possible that declines in *Baetodes* abundance are also a response to drifting or dislodgement after high rainfall events. We also found a significant response of lower macroinvertebrate community abundance in leaf packs during the wet season and lower richness in benthic samples of the intermittent stream during the wet season. A 15-yr study in

Costa Rican lowland streams found that temporal changes in macroinvertebrate assemblages responded strongly to rainfall and discharge associated with El Niño Southern Oscillation (ENSO) events (Gutiérrez-Fonseca, Ramírez & Pringle, 2018). Our one-year study was under an El Niño neutral phase and was too short in duration to attribute community shifts to specific flow patterns, but we consider that the resolution of our monthly sampling was rigorous enough to document community response shortly after events of high rainfall and within two distinct seasons that would not have been perceived in sampling of lower frequency.

Macroinvertebrate community differences between seasons

The higher abundance and richness in the intermittent stream that we documented during the dry season could be the direct response of animals concentrating in the remaining wetted areas (Smith & Pearson, 1987). However, our finding that richness was higher in the intermittent stream's benthic habitats during the wet season, compared to the perennial stream, indicates that higher richness was not simply a concentration effect. However, our regression results showed significantly higher richness, but not abundance, in the intermittent stream compared to the perennial stream. If concentration was the factor driving this response, we would expect macroinvertebrate abundance to be higher as well. It is possible that isolated pools in the intermittent stream, combined with transects of remaining flowing water, are sufficient for macroinvertebrates to persist in the intermittent stream. Our results are consistent with an Andean stream study, in which Ríos-Touma, Encalada & Prat Fornells (2011) found seasonal changes in macroinvertebrate community composition, with higher richness and densities during the dry season, which they argued were driven by invertebrate habitat preference in the absence of floods. Similar to our study, Ramírez, Pringle & Douglas, (2006) also found higher invertebrate densities in La Selva, Costa Rica during the dry season when flooding disturbance was minimal.

Life history traits and FFGs

Functional Feeding Groups (FFGs) and life history traits can provide additional information for understanding macroinvertebrate community response to disturbance events. Filter feeder taxa, dominated by the genera *Simulium*, *Calosospsyche* and *Leptonema,* were generally less abundant throughout the year in the intermittent stream and declined to numbers near zero during the dry season. This result is expected since this group relies heavily on water movement in order to feed. Additionally, these genera are characterized by having low larval mobility (Poff *et al*., 2006), thus making them vulnerable to low flow conditions because of the lack of food delivery. Conversely, macroinvertebrates classified as collector-gatherers typically show a preference for slow moving water in order to feed on fine benthic organic matter (FBOM) suspended in the water column (Ramírez & Gutiérrez- Fonseca, 2014a). We found higher numbers of this FFG group, dominated by Non-Tanypodinae chironomids, during the months from January to March in the intermittent streams, when water was likely to be at its lowest velocity. We also found predators dominated by Tanypodinae and *Anacroneuria* at higher abundances during the dry season in the intermittent stream. This result may suggest that predation was not a mechanism decreasing richness in the reduced wetted area of the intermittent stream during drying.

The process of recolonization from nearby population sources following disturbance is important for invertebrate community recovery. The neighboring perennial waters of the San Luis River and Quebrada Alondra (our perennial study site) may be playing an important role in the recolonization process of aquatic macroinvertebrates in the intermittent stream following disturbances. Perennial surface waters can be important sources of recolonizers in intermittent stream macroinvertebrate communities (see review in Stubbington & Datry, 2013). It is known that tropical streams do not have large fluctuations in temperatures between seasons; therefore, macroinvertebrates in the tropics are adapted to narrow temperature ranges and may respond to relatively small temperature changes. However, temperatures in the tropics may vary greatly along an elevational gradient, limiting dispersal ability (Shah *et al*., 2017). Dispersal in our study sites should not be limited by large ranges of elevational temperature variation. Strong adult fliers such as the hydropsychids *Leptonema* and *Calosopsyche* occurred at higher numbers in the perennial stream throughout the year, which could be an indication of the perennial stream acting as a source for these macroinvertebrates. Drift from upstream reaches or dispersal from areas downstream can also play an important part in recolonization or seasonal changes in macroinvertebrate composition. For instance, declines in total leaf pack macroinvertebrate abundance during the wet season could be the result of movement in taxa with high drift dispersal. Results of a study by Ríos-Touma, Prat & Encalada (2012) indicated that larvae of *Simulium* blackflies are known to have higher drift dispersal during the wet season in tropical streams.

Broader significance

Our findings add to the limited number of studies in the tropics on macroinvertebrates responding to changes in hydrology. This study provides information on tropical macroinvertebrate communities and how they may change on a monthly and seasonal basis. Few studies have looked at monthly changes in aquatic organisms, especially in the tropics. Important community changes could be missed in less frequent sampling. It is also important to note that long term studies linking hydrological changes to changes in macroinvertebrate assemblages in the tropics are rare and only two have been published (Covich, Crowl & Heartsill-Scalley, 2006; Gutiérrez-Fonseca, Ramírez & Pringle, 2018) to our knowledge. The heterogeneity of precipitation intensity and distribution across the tropics makes it difficult to generalize macroinvertebrate response to changes in flow regime. Therefore, more studies should be conducted in different tropical regions for a better understanding of the effects of hydrological alteration on macroinvertebrate communities.

Freshwater ecosystems, some of the most vulnerable to the effects of climate change, are changing rapidly. Predictions of global change suggest that in the future tropical streams will experience less uniformity in the amount of daily precipitation during the rainy season and longer dry periods in the subtropics. Tropical cyclones rarely affect the Pacific North of Costa Rica; however, four months after collections for this study were completed, tropical storm Nate brought torrential rains that caused debris flow down the perennial stream valley which destroyed the existing channel and essentially created a new stream. The lack of previous exposure to such events may affect the level of resistance and resilience of these systems to this extreme hydrological disturbance. For instance, findings from Smith *et al*. (2005) suggest that basins in Puerto Rico, commonly affected by tropical cyclones responsible for some of the largest discharge flood peaks in U.S. Geological Survey stream gage records, have efficient drainage networks with large channel capacities. Systems in Puerto Rico may be adapted to such extreme and episodic rainfall events, while streams in the Pacific North of Costa Rica may be less adapted and therefore more vulnerable.

Tropical stream ecosystems are exposed to many ecological threats, one of which is disturbance of the natural flow regime, not only impacted by multiple anthropogenic stressors, but also intense atmospheric events (e.g. tropical cyclones) and cyclical ENSO events. Although monthly sampling is valuable for understanding effects of disturbance events, it is recognized that characterizing the effects of cyclical disturbances (i.e. El Niño and La Niña events) should also be incorporated, when feasible, during long term ecological studies. Although studies of aquatic invertebrates in tropical streams have increased in recent decades, many important knowledge gaps remain (Ramírez & Gutíerrez-Fonseca, 2014b). Studies of macroinvertebrate community responses to hydrological alterations across different tropical regions are important for understanding variations in ecosystem structure and function in these diverse systems. Results from our study suggest that tropical streams may vary greatly in terms of community response to disturbances, even when located in close proximity. Interestingly, we found higher benthic richness in the intermittent stream compared with the perennial stream, suggesting that streams that appear more disturbed could actually serve as refuges for some taxa. This study also provides information on how macroinvertebrate communities may recover from drought or flood events in streams with varying flow regimes through possible explanations from functional feeding group characteristics and survival strategies, contributing to limited knowledge about the natural history of these taxa in Neotropical ecosystems. The monthly resolution and yearlong scale of changes to macroinvertebrate communities from this study provide nuanced insights into immediate biotic responses to disturbance in both perennial and intermittent reference streams, information that is critical for identifying the ways in which hydrological alterations may impact tropical freshwater streams.

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Table 2.1: List of the multiple linear regression model macroinvertebrate response variables for benthic and leaf pack samples and different precipitation predictor variables tested. Each of the response variables was tested as per month totals and each of the predictor variables was calculated 31 days prior to each sampling month for each stream.

Response variables tested	**Predictor variables tested**
Total benthic abundance	Average monthly precipitation
Total leaf pack abundance	# of days with precipitation >15, 20, 30 and 40mm
Total biomass (mg)	# of days with precipitation < 15, 10 and 5mm
Total benthic richness	# of days with precipitation 0mm
Total leaf pack richness	
Abundance from genera in SIMPER abundance analyses for benthic and leaf pack macroinvertebrate samples	

Table 2.2: Perennial and intermittent stream habitat characterization. All values are shown as means of measurements taken at very 10m in 100m reaches in each stream. Wet season values were measured in September 2016 and dry season values were measured in March 2017.

	Wet season				**Dry season**			
	PER	**SD**	**INT**	**SD**	**PER**	**SD**	**INT**	**SD**
% pool	28		32		42		54	
% riffle	53		56		40		33	
% run	19		12		17		13	
wetted width (m)	3.6	1.10	3.42	1.40	3	0.904	1.75	1.15
mean depth (cm)	12.4	3.7	9.08	3.90	9.78	10.3	3.8	2.9
% sand, silt, mud	17		21		10		19	
% gravel	8		9		8		13	
% pebble	12		14		12.5		12	
% cobble	23		21		23		18	
% boulder	40		35		46.5		38	
% canopy cover	79.64	3.98	70.18	5.74				

PER, perennial stream; SD, standard deviation; INT, intermittent stream

Table 2.3: Summary of total monthly taxon abundance, biomass and richness in each stream and sample type. Benthic macroinvertebrate samples were taken from May 2016-April 2017 and leaf pack macroinvertebrate samples were taken from June 2016-April 2017.

	Abundance		**Richness**		**Biomass (mg)**	
	Perennial	**Intermittent**	**Perennial**	**Intermittent**	**Perennial**	**Intermittent**
Benthic						
May	85	73	20	24	194	132
June	99	90	20	17	307	215
July	301	217	16	22	424	545
August	347	88	19	24	432	203
September	176	268	19	26	636	568
October	117	196	24	27	496	432
November	145	136	19	26	442	245
December	173	249	20	27	491	542
January	285	660	26	26	310	873
February	146	346	24	33	458	522
March	256	296	19	29	232	299
April	186	171	20	21	685	81
Leaf pack						
May	NA	NA	NA	NA	NA	NA
June	146	98	14	13	262	66
July	332	341	13	11	167	100
August	211	913	17	21	254	167
September	565	410	19	15	216	72
October	247	811	14	21	219	212
November	1855	820	19	13	483	86
December	1052	1313	23	17	392	280
January	1023	867	22	25	634	490
February	365	592	15	23	252	473
March	360	689	18	18	293	297
April	123	354	15	13	80	69

Table 2.4: Multiple linear regression results for benthic taxon richness and leaf pack total monthly abundance as an effect of site type and season.

	Coefficient	**Standard Error**	**t-Statistic**	***P***
Benthic richness				
Intercept	21.958	1.182	18.571	1.64e-14
Intermittent stream	4.917	1.365	3.601	0.0001**
Wet season	-2.917	1.365	-2.136	0.0444*
Benthic abundance				
Intercept	234.33	43.70	5.362	2.56e-05
Intermittent stream	39.50	50.46	0.783	0.442
Wet season	-82.67	50.46	-1.638	0.116
Benthic biomass				
Intercept	450.42	69.61	6.470	2.06e-06
Intermittent stream	-37.50	80.38	-0.467	0.646
Wet season	-49.67	80.38	-0.618	0.543
Leaf pack richness				
Intercept	18.371	1.412	13.003	6.6e-11
Intermittent stream	0.0909	1.679	0.054	0.957
Wet season	-2.616	1.685	-1.552	0.137
Leaf pack abundance				
Intercept	742.19	147.96	5.016	7.67e-05
Intermittent stream	84.45	175.80	0.480	0.636
Wet season	-377.02	176.53	-2.136	0.0459*
Leaf pack biomass				
Intercept	361.81	50.24	7.202	7.69e-07
Intermittent stream	-85.45	59.69	-1.432	0.169
Wet season	-145.58	59.94	-2.429	0.0252*

Significant p-values ‘**’ ,0.01, ‘*’ 0.05

Table 2.5: List of unique taxa found in each stream, sample type, season, and total abundance.

Stream type taxon	**Taxon order**	**Sample type**	**Season**	**Abundance**
Intermittent				
Allenhyphes	Ephemeroptera	benthic	dry	2
Austrodinodes	Trichoptera	benthic	wet	1
Camelobaetidius	Ephemeroptera	benthic	wet/dry	5
Centromacronema	Trichoptera	benthic	wet/dry	3
Cora	Odonata	benthic	dry	1
Corydalus	Megaloptera	benthic/leaf	dry	5
Culoptila	Trichoptera	benthic	dry	1
Cyrnellus	Trichoptera	benthic	wet	4
Euthyplocia	Ephemeroptera	benthic	wet	1
Haplohyphes	Ephemeroptera	benthic	wet	6
Heteragrion	Odonata	benthic	dry	19
Limnophora	Diptera	benthic	dry	1
Marilia	Trichoptera	benthic/leaf	wet/dry	14
Planorbidae	Gastropoda	benthic	wet	1
Polycentropus	Trichoptera	benthic	wet/dry	7
Tricorythodes	Ephemeroptera	benthic	wet	1
Perennial				
Culicidae	Diptera	benthic	wet	1
Limonia	Diptera	benthic	wet	1
Lutrochus	Coleoptera	benthic	wet	1
Microvelia	Hemiptera	benthic	wet/dry	2
Petrophila	Lepidoptera	benthic/leaf	wet	3
Tabanidae	Diptera	benthic/leaf	dry	5

Table 2.6: Summary of total abundance Similarities Percentages analysis of benthic and leaf samples. Taxa having a cumulative contribution of 76% to the Bray-Curtis dissimilarity between the intermittent and perennial stream, as estimated by SIMPER analysis, are shown.

Habitat type taxon	**Taxon order**	**Cumulative % contribution**
Benthic		
Non.Tanypodinae	Diptera	0.2610
Calosopsyche	Trichoptera	0.3786
Anchytarsus	Coleoptera	0.4643
Leptonema	Trichoptera	0.5374
Leptohyphes	Ephemeroptera	0.6047
Farrodes	Ephemeroptera	0.6582
Heterelmis	Coleoptera	0.6989
Anacroneuria	Plecoptera	0.7366
Baetodes	Ephemeroptera	0.7647
Leaf pack		
Non.Tanypodinae	Diptera	0.3442
Tanypodinae	Diptera	0.6040
Simulium	Diptera	0.7623

Table 2.7: Summary of taxon biomass Similarities Percentages results of benthic and leaf samples. Taxa having a cumulative contribution of 76% to the Bray-Curtis dissimilarity between the intermittent and perennial stream, as estimated by SIMPER analysis, are shown.

Habitat type taxon	**Taxon order**	**Cumulative % contribution**
Benthic		
Leptonema	Trichoptera	0.4336
Calosopsyche	Trichoptera	0.5175
Hetaerina	Odonata	0.5904
Anchytarsus	Coleoptera	0.6588
Anacroneuria	Plecoptera	0.7139
Argia	Odonata	0.7675
Leaf pack		
Anchytarsus	Coleoptera	0.2497
Leptonema	Trichoptera	0.4033
Anacroneuria	Pleacoptera	0.5128
Phylloicus	Trichoptera	0.5688
Hyallelidae	Amphipoda	0.6160
Non-Tanypodinae	Diptera	0.6615
Simulium	Diptera	0.7405

Table 2.8: Multiple linear regression results for total monthly abundance of *Anychytarsus* in benthic samples as an effect of site type and precipitation predictor variables. Each of the predictor variables was calculated 31 days prior to each sampling month for each stream.

	Coefficient	**Standard Error**	**t-Statistic**	***P***
***Anchytarsus* abundance**				
Intercept	31.553	7.070	4.463	0.0002
p15	-2.955	1.260	-2.346	0.0288*
Intermittent stream	4.098	6.182	0.663	0.5146
Intercept	30.300	6.961	4.353	0.0002
p20	-3.45	1.574	-2.194	0.0396*
Intermittent stream	4.220	6.262	0.674	0.5077
Intercept	27.343	6.108	4.476	0.0002
p30	-4.614	2.184	-2.113	0.0467*
Intermittent stream	5.083	6.293	0.808	0.4282
Intercept	-61.560	34.824	-1.768	0.0916
pb15	3.012	1.300	2.317	0.0307*
Intermittent stream	4.079	6.199	0.658	0.5176
***Baetodes* abundance**				
Intercept	7.0905	1.9233	3.687	0.00137
p20	-0.9533	0.4349	-2.192	0.0398*
Intermittent stream	0.9283	1.7302	0.537	0.5962
Intercept	0.5719	1.8586	0.308	0.7615
p0	0.3591	0.1556	2.308	0.0313*
Intermittent stream	1.1367	1.7096	0.665	0.5133

p15, number of days with precipitation above 15mm; p20, number of days with precipitation above 20mm; p30, number of days with precipitation above 30mm; pb15, number of days with precipitation below 15mm; 0mm, number of days with precipitation at 0mm; significant p-value '*' 0.05

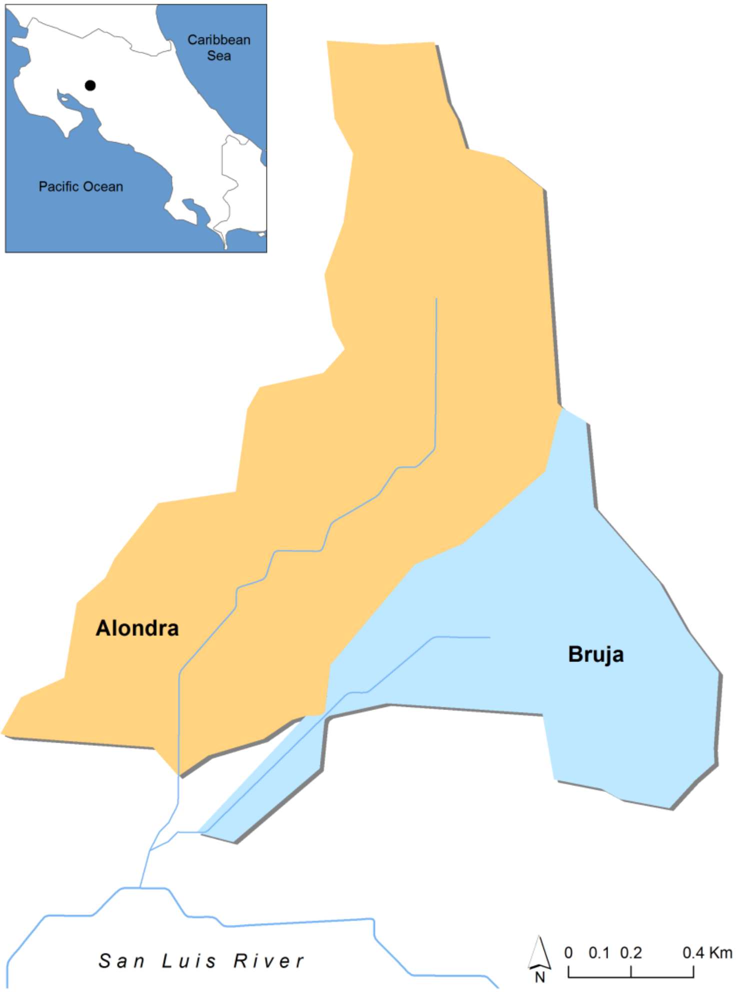

Figure 2.1: Map of drainage areas above our sampling reaches in Alondra (perennial stream) and Bruja (intermittent stream) in San Luis, Costa Rica. These streams converge in the San Luis River

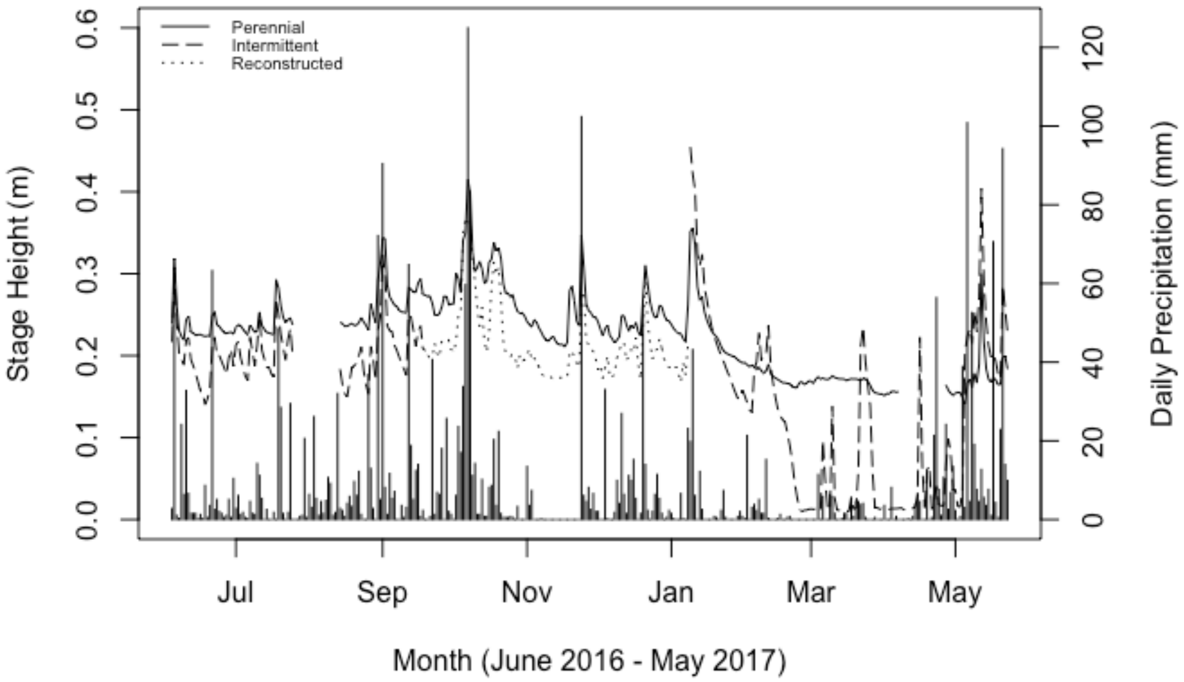

Figure 2.2: Mean daily stage height of perennial and intermittent stream and precipitation. Vertical lines indicate cumulative daily (June 2016 to Apr 2017) precipitation over study sites. The dotted line from mid-Sept to January indicates daily stage height in intermittent stream reconstructed using precipitation data from previous day and perennial stream stage data.

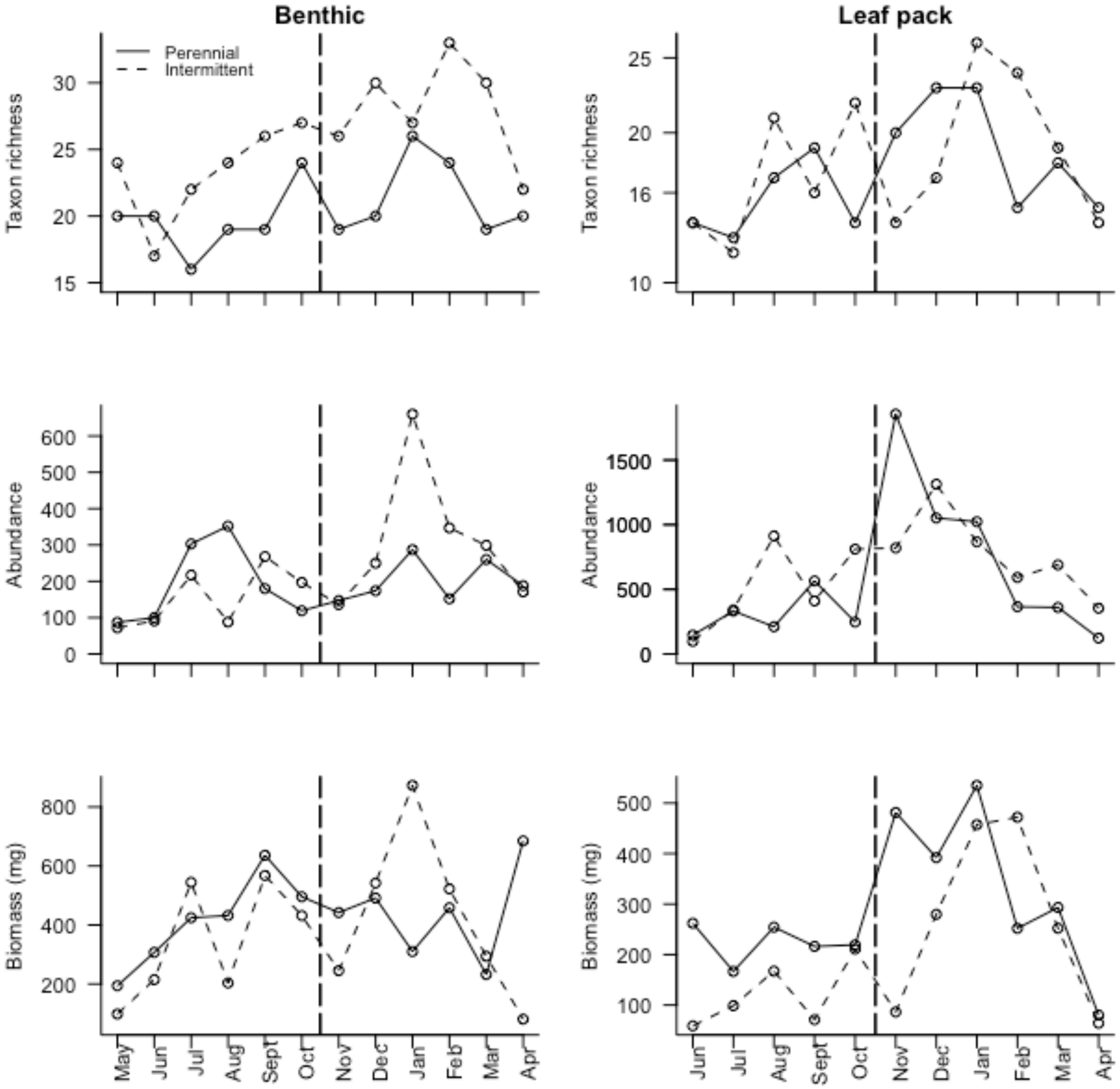

Figure 2.3: Macroinvertebrate community general metrics over time (May 2016 to April 2017). Plotted values are monthly totals for benthic and leaf pack samples in each stream. The dashed vertical line separates the wet season (May to October) from the dry season (November to April).

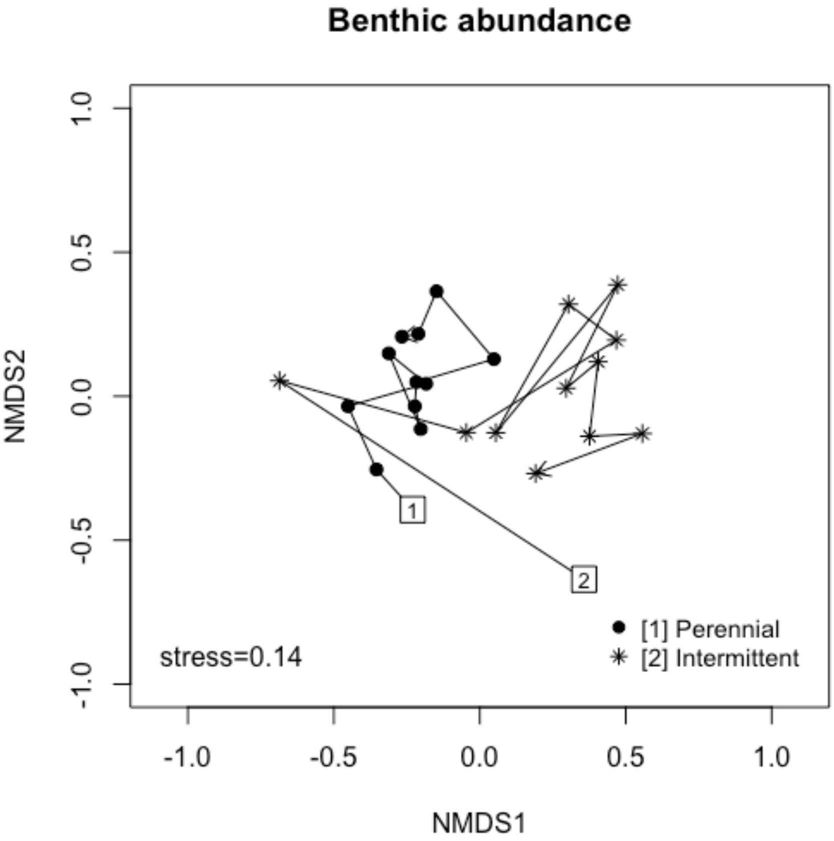

Figure 2.4: Non-metric multidimensional scaling (NMDS) plots of stream taxa abundance found in benthic habitats. Each point displays Bray-Curtis dissimilarities in total monthly taxon abundance between the intermittent and the perennial stream.

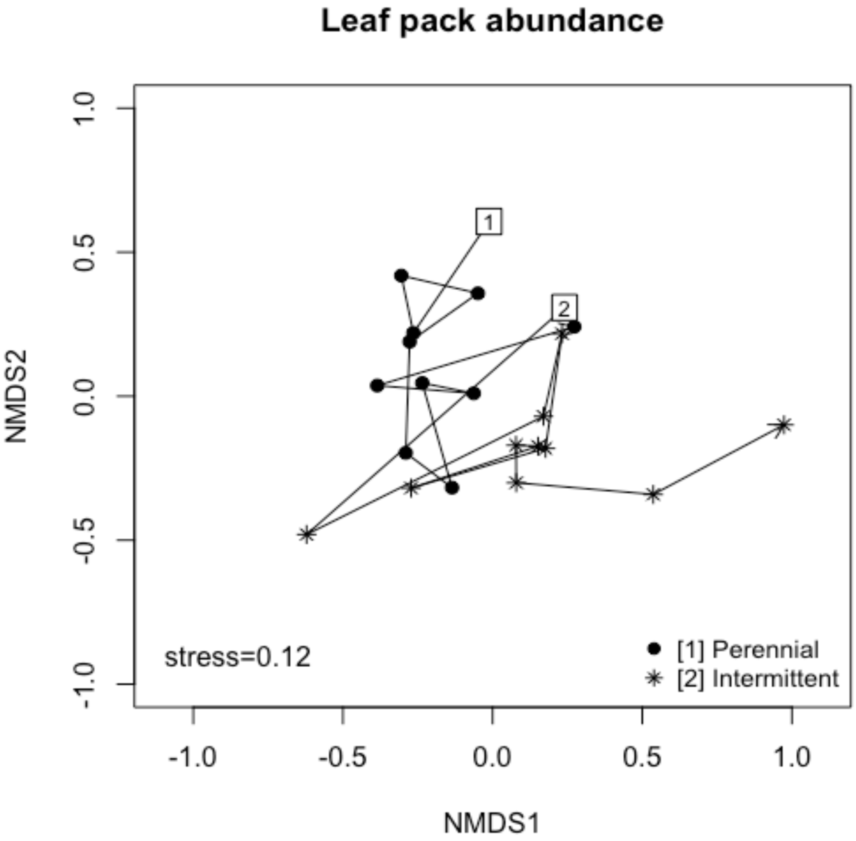

Figure 2.5: Non-metric multidimensional scaling (NMDS) plots of stream taxa abundance found in leaf litter habitat. Each point displays Bray-Curtis dissimilarities in total monthly taxon abundance between the intermittent and the perennial stream.

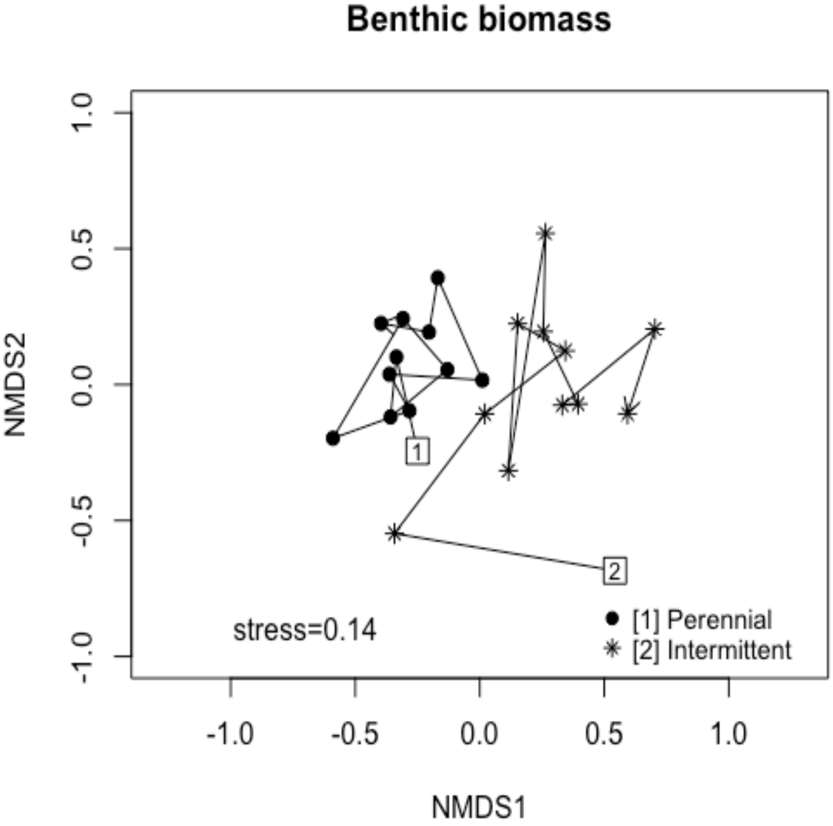

Figure 2.6: Non-metric multidimensional scaling (NMDS) plots of stream taxa biomass (mg) found in benthic habitats. Each point displays Bray-Curtis dissimilarities in total monthly taxon abundance between the intermittent and the perennial stream.

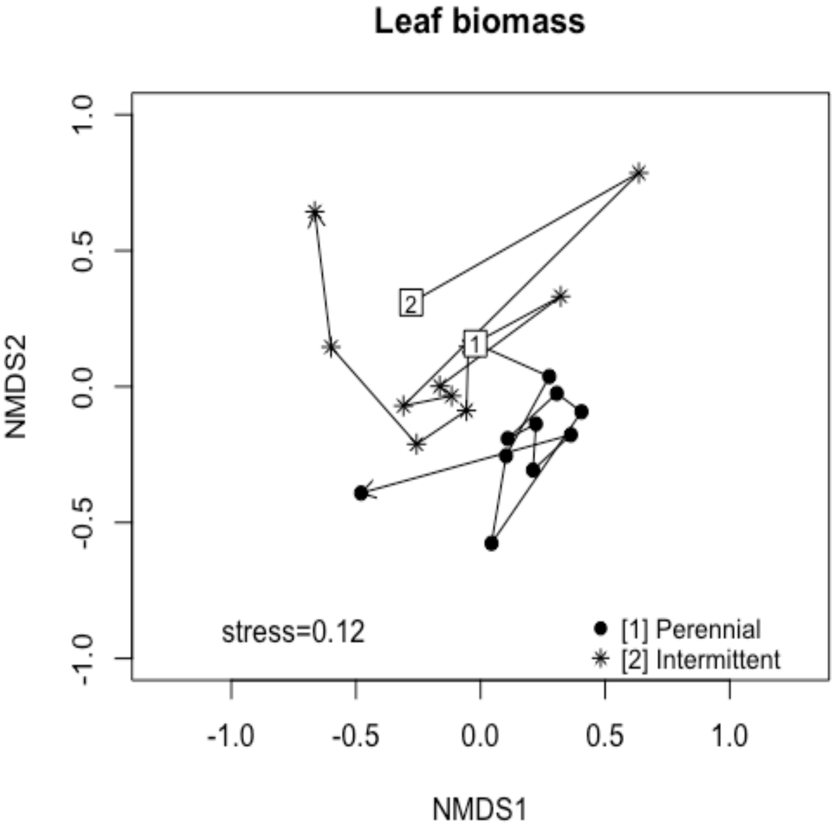

Figure 2.7: Non-metric multidimensional scaling (NMDS) plots of stream taxa biomass (mg) found in leaf litter habitat. Each point displays Bray-Curtis dissimilarities in total monthly taxon biomass from the intermittent and the perennial stream.

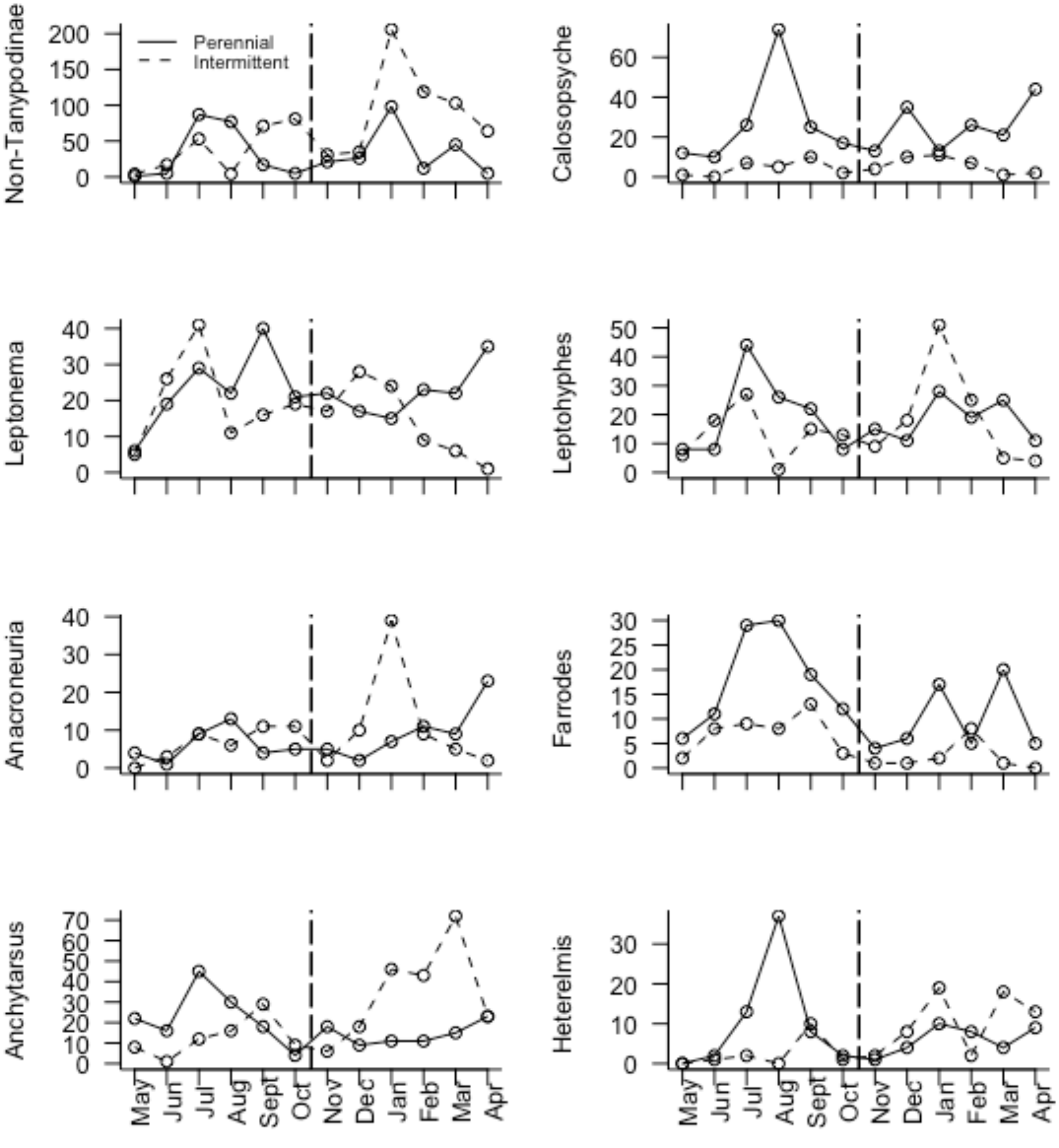

Figure 2.8: Macroinvertebrates at lowest taxonomic resolution with highest contribution to benthic abundance NMDS dissimilarities over time. Plotted values are monthly totals for benthic and leaf pack samples in each stream. The dashed vertical line separates the wet season (May to October) from the dry season (November to April).

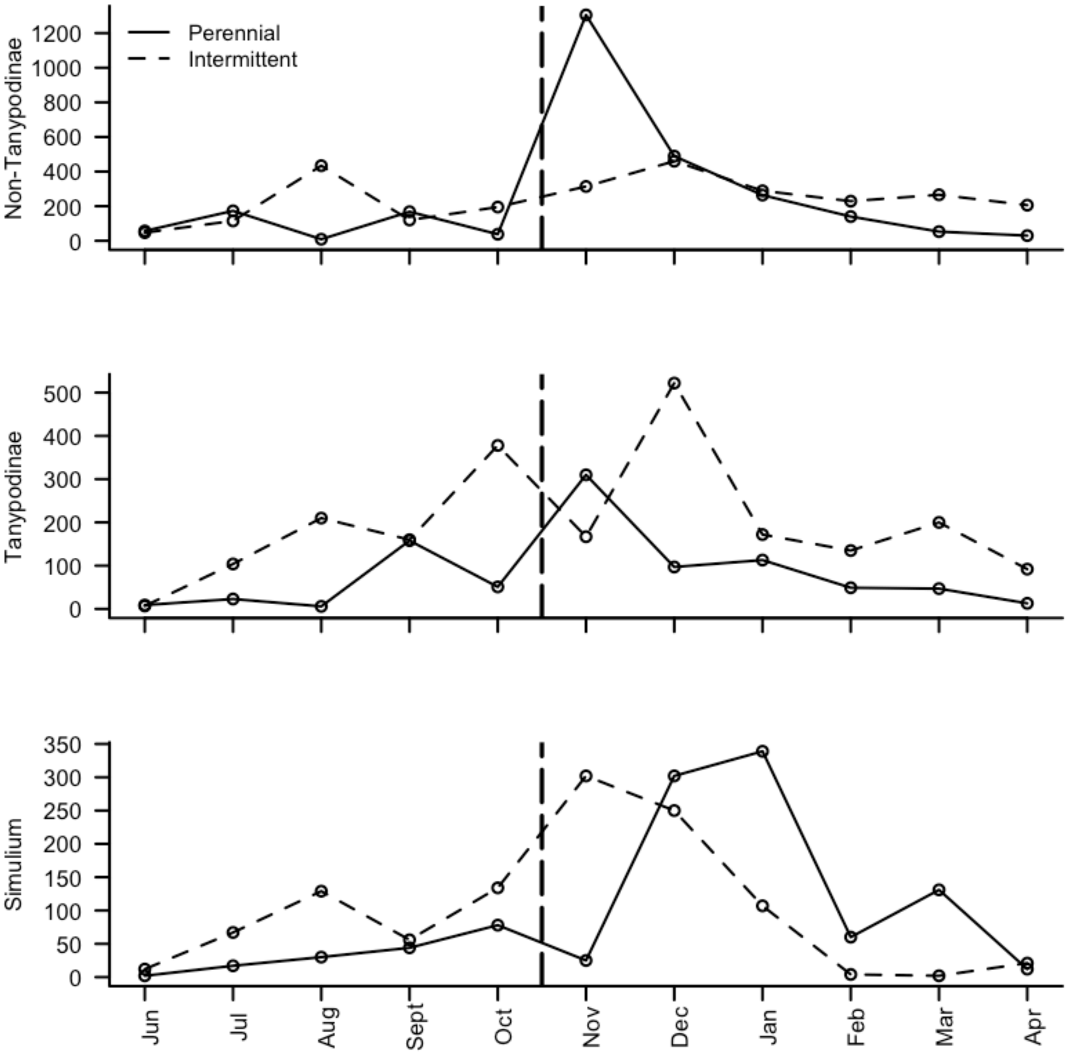

Figure 2.9: Macroinvertebrates at lowest taxonomic resolution with highest contribution to leaf pack abundance NMDS dissimilarities over time. Plotted values are monthly totals for benthic and leaf pack samples in each stream. The dashed vertical line separates the wet season (May to October) from the dry season (November to April).

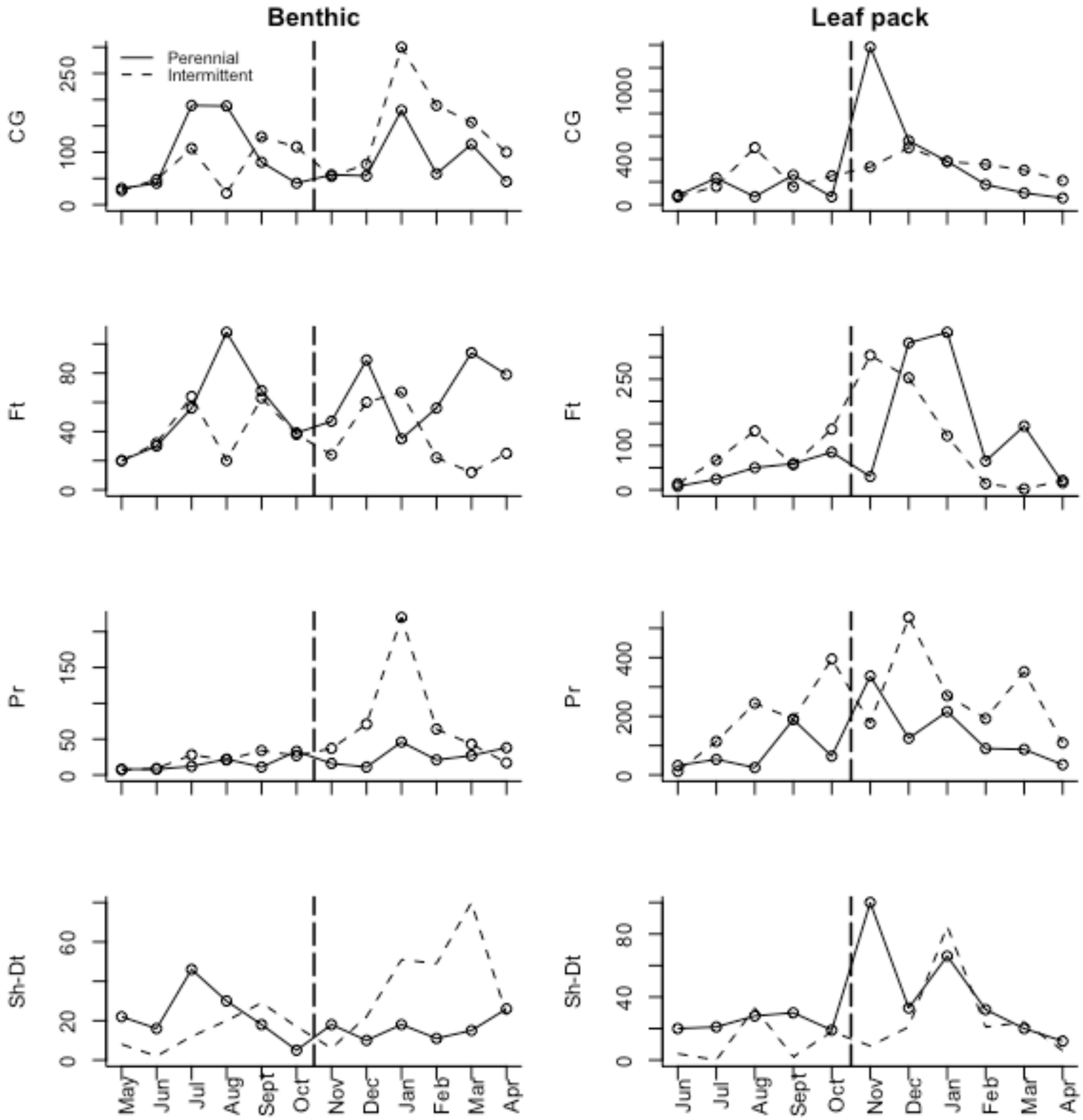

Figure 2.10: Most abundant function feeding groups over time (May 2016 to April 2017). Plotted values are monthly totals for benthic and leaf pack samples in each stream. The dashed vertical line separates the wet season (May to October) from the dry season (November to April).

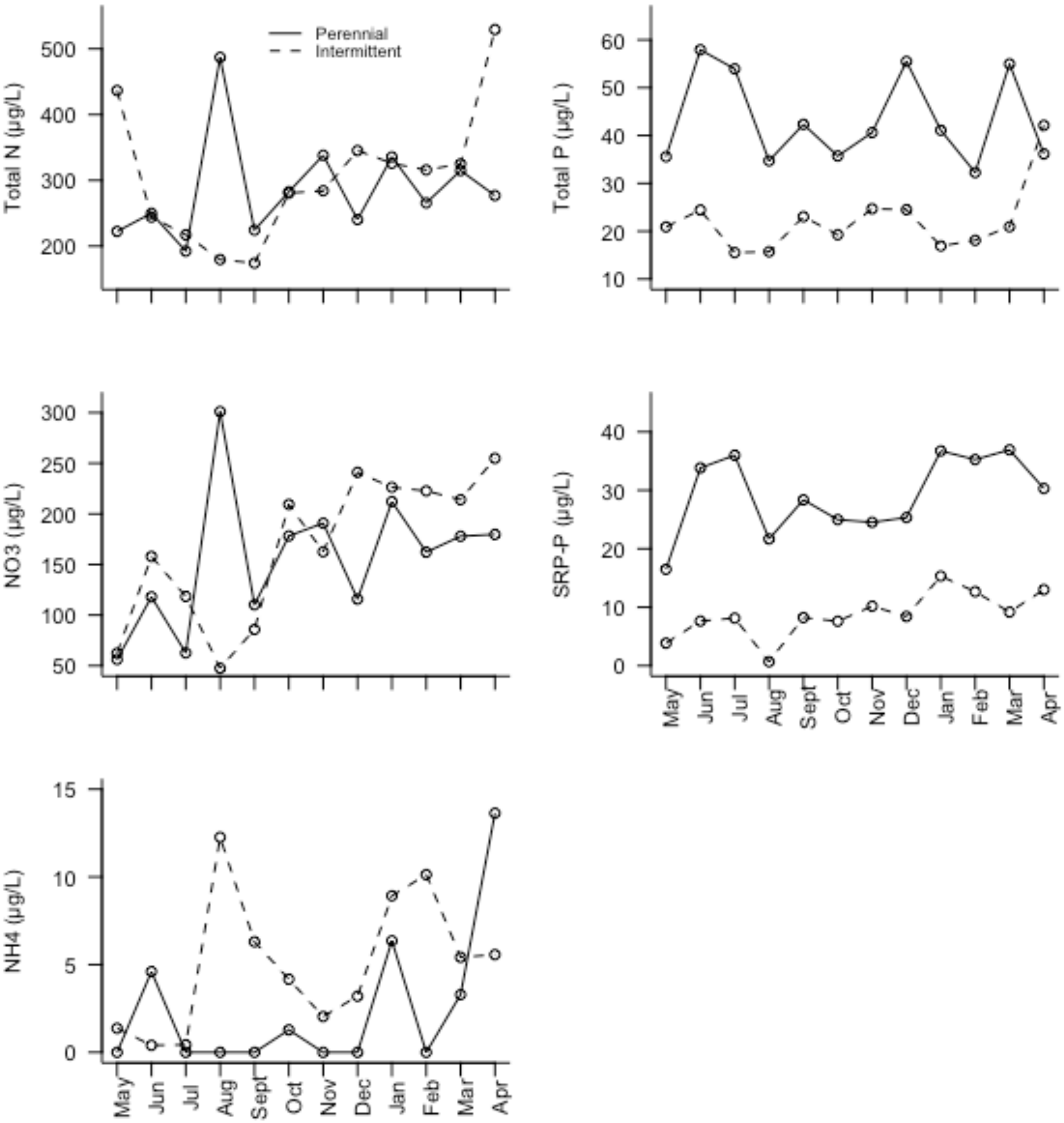

Figure 2.11: Nutrient concentrations over time (May 2016 to April 2017). Plotted values are monthly totals in each stream. The dashed vertical line separates the wet season (May to October) from the dry season (November to April).

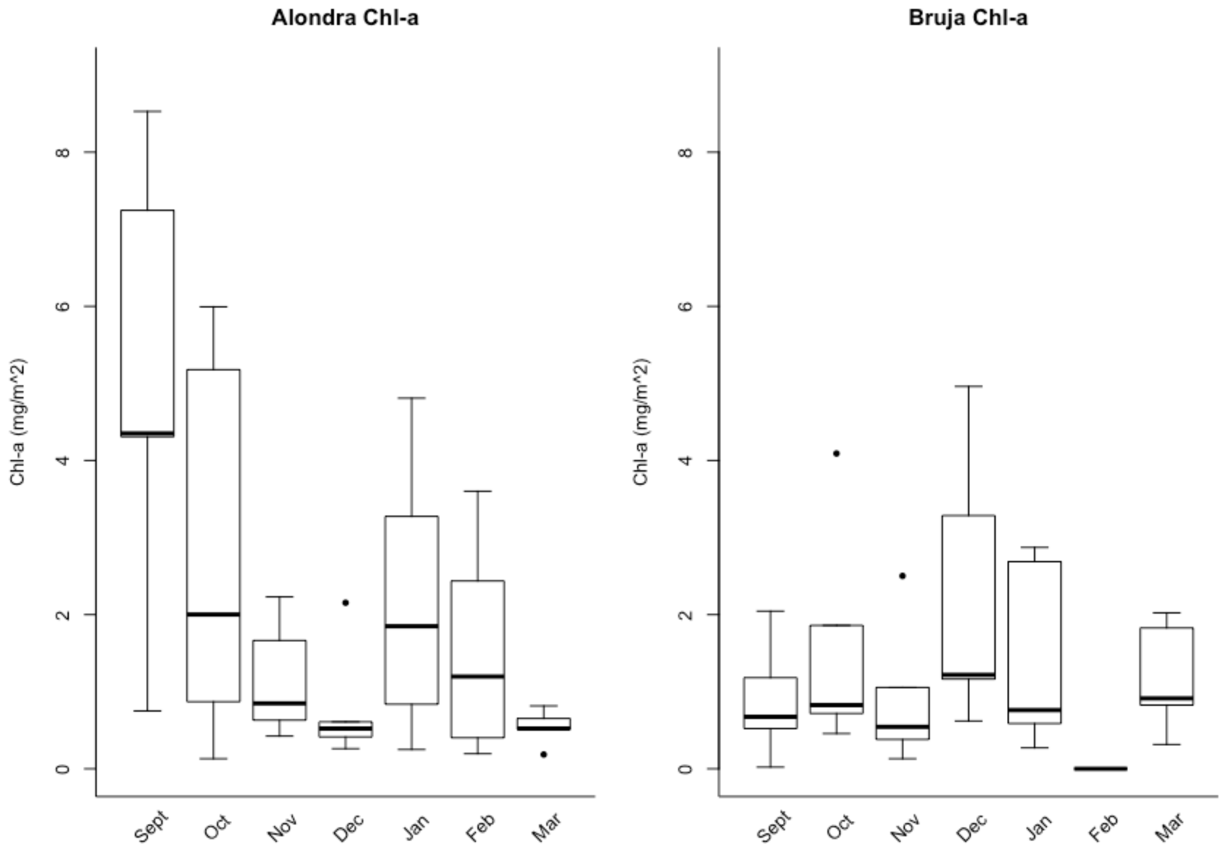

Figure 2.12: Chlorophyll-a concentrations over 7 sampling months a) perennial stream and b) intermittent stream. February was not sampled in the intermittent stream due to stream drying

CHAPTER 3

LIMITATIONS AND RECOMMENDATIONS ON MACROINVERTEBRATE SAMPLING TECHNIQUES

Introduction

Streams are complex systems with dynamic hydrological and geomorphological properties that are important drivers of ecological processes. The physical characteristics of a stream can influence the level of resistance or resilience of aquatic communities in response to a hydrological disturbance. Habitats that shelter aquatic invertebrates from disturbance have been defined as refugia and their function may differ depending on their location within a freshwater ecosystem, an organism's ability to access them, and the magnitude and timing of the disturbance (Sedell *et al*., 1990). Refugia from high flows can be provided by stream features such as patches of low flow (Lancaster & Hildrew, 1993), the hyporheic zone (Poole & Stewart, 1976), periphyton on large boulders, and woody debris accumulations that can provide more stable habitat. Lancaster & Belyea (1997) use the term refugia to describe physical spaces or times (life cycles or behavior) where organisms avoid negative impacts of a disturbance and therefore have a high probability of persisting after an adverse event. Robson, Chester, & Austin (2011) emphasize the importance of studying both behavior and physical space utilization as invertebrate refugia. Examples include flying to perennial waters, rheotaxis, use of a stable structure as shelter, aestivation in sediment, and life stages resistant to desiccation.

Because streams are such complex systems, ecologists have the need to utilize a variety of sampling methods, if the intention is getting the most complete understanding of the biotic communities within these systems. It can often be somewhat challenging to assess which methods are most appropriate to use in such physically heterogeneous systems without undergoing a process of trial and error. However, oftentimes unsuccessful sampling methods do not get published, and others repeat the same mistakes, inhibiting the advance of scientific understanding.

In this chapter, we briefly describe the application and limitations of multiple aquatic macroinvertebrate sampling techniques that we attempted in two headwater streams of San Luis de Monteverde, Costa Rica. Our initial objectives with trying these techniques were to [1] assess macroinvertebrate community structures in different refugia in a perennial and intermittent stream after flood and drying disturbances and [2] to quantify the recolonization patterns after wet and dry seasons resumed. Our intent was to better understand patterns of resistance and resilience of invertebrate communities through their use of refugia, and behavioral changes that allow for survival. The goals of this chapter are to describe the ecological reasoning, challenges and recommendations for the use of three sampling techniques that we ultimately found problematic in our study system.

Study area

Location

We studied two pre-montane cloud forest headwater streams at the University of Georgia's satellite campus (UGA-CR), located in the Pacific North of Costa Rica in the community Altos de San Luis, Monteverde (10.28°'N, 84.79°'W). The district of Monteverde is characterized by having marked dry (~December - ~March) and wet seasons (~May - ~October), with November and April serving as transitional months between each season. During July and

August, trade winds intensify over the region and typically cause a reduction in precipitation which is locally called *veranillo*. We were interested in how stream invertebrates respond to flow because both human population size and tourism are projected to increase in Monteverde, leading to a rise in water demand, with additional effects of climate change magnifying the need for freshwater water availability.

Study sites

Within the UGA-CR campus are two parallel headwater streams, Alondra (1.74 km^2 drainage area, perennial stream) and Bruja (0.828 km^2 drainage area, intermittent stream), which are ~125m apart and at an elevation of ~1200m. The perennial stream flows continuously during both wet and dry seasons and the intermittent stream typically dries from February-March. The streams converge ~400m below our study site, and subsequently flow into the San Luis River. Average annual precipitation at the location is ~3000mm. Sediment in both streams is mainly composed by large boulders, cobble and finer sediment such as sand, silt, and clay. More details on the study area are found in the "Study Sites" section in the second chapter of this thesis.

Method descriptions, challenges, results and possible explanations for results

Emergence sampling

We attempted to assess adult aquatic insect emergence as a measure of behavioral response to disturbance or demographic, reflecting survival to last instar. It is reasonable to assume that movement by macroinvertebrates with flying adult stages may be more pronounced during their adult stage. However, very little is known about some basic life history traits of these insects (such as degree of voltinism), and the degree to which reproductive episodes are synchronized with respect to seasonal disturbance, such as flooding or drying. We installed floating 47 x 47cm emergence traps over riffle and pool habitats. The traps were constructed using 2.55cm PVC tubing, mesh fabric and foam tubing material for floatation. To account for spatial heterogeneity and seasonal variation, we followed methods from Small *et al*. (2013) and placed traps within riffle and pool habitats for 3-4 days each month. We placed five traps in riffles and five in pools in each stream, monthly from May 2016- August 2016 (wet season) and from January- February 2017 (dry season). We also tested placing the traps over stable objects (e.g. boulders and logs) and near the banks with the idea that some larvae and nymphs in preparation to become adults would need a stable structure to emerge (e.g. some stoneflies and caddisflies). In order to protect the traps from flooding, we used rebar and zip ties to anchor the traps to the streambed and also tied them with a thin rope to sturdy riparian trees. We collected all insects on a daily basis using a handheld insect aspirator and transported these to the laboratory for identification under a stereoscope. The traps were inspected on a daily basis to remove any intruding terrestrial insects (e.g. spiders and grasshoppers) and patch any ruptures in the mesh fabric with duct tape or aquarium silicon glue.

We encountered multiple challenges with this method. During the wet season, events of high flow occurring overnight often overturned our traps or caused excessive debris accumulation forced against the mesh fabric. Spates also dismantled at least three traps on two separate occasions. Although the buoyant tubing material sealed the bottom of the traps with the water surface, and traps were inspected daily for ruptures, it was common to find spiders and other terrestrial insects inside the traps. Additional difficulties included transportation and storage of the traps which were not constructed with an easily collapsible design. We were unable to capture aquatic insect adult emergence.

The lack of emergence trap captures could be related to stream discharge flashiness, due to the high elevational gradient and the occasional high intensity of rainfall at our sites. Torrential rains during the wet season might have caused downstream movement of larvae or nymphs, resulting in insects emerging farther downstream from the headwaters of the San Luis River in areas with more stable flow. Small *et al*. (2013) were able to detect adult macroinvertebrate emergence using similar traps in Costa Rican lowland streams at La Selva Biological Station. Their success may be related to a more even distribution of rainfall in the Caribbean slope that potentially provides a more stable habitat for insects to develop, as opposed to the marked seasonality that our sites receive in the Pacific North, with the added advantage of less elevational gradient of their streambeds. Floating traps for insect emergence have also worked well in streams in Idaho with an elevation similar to ours but with different seasonal fluctuations in terms of temperature (Malison, Benjamin & Baxter, 2010). Temperature plays an important role in stream insect emergence as lifecycles may be synchronized with seasonality and is associated warmer temperatures. Emergence takes place in short pulses in the temperate streams, while it can occur continuously throughout the year in tropical regions (see review by Baxter, Fausch & Saunders, 2005). The probability of capturing adult insects may be higher in temperate areas if the study is conducted during a seasonal emergence peak.

Hyporheic zone

We intended to quantify the use of the hyporheic zone as refugia by burrowing macroinvertebrates. We constructed a stovepipe corer with a rubber seal in the bottom to sample areas where the sediment and streambed would allow for the corer to enter. We took five samples in each stream twice during the wet season and once during the dry season. The samples were placed in plastic bags and inspected in the lab under a stereomicroscope. However, we did not find burrowing macroinvertebrates in the samples.

This method may not be appropriate for our stream sites due to the particle size composition in both streams, as large boulders and bedrock make up a large part of the particle composition in both streams. Due to this, we were generally unable to press the corer much deeper than ~7 cm into the sediment. Areas of finer sediment in the stream are mostly composed of compacted clay which may be creating an anoxic environment. It is possible that macroinvertebrates in our study sites are adapted to high levels of oxygen from moving water. High water velocities after rainfall events may also scour the stream sediment providing an unstable habitat. Ramírez, Pringle & Douglas (2006) were able to apply this method in lowland streams of La Selva Biological Station; however, these sites had large accumulations of depositional leaf litter and sediments, where the technique seems more suitable.

Reactivation experiment

We conducted a laboratory experiment in order to address dormancy as a survival strategy in dry sediment with a modified methodology from Chester & Robson (2011). Five samples were taken from locations in the intermittent stream where wetted stream width and depth had decreased from February 2017 - March 2017 (dry season). We collected dry sediment using a soil corer in areas of accumulated fine particles after two weeks of complete streambed drying. The samples were taken to the lab and immediately placed in separate plastic cups, which were filled with deionized water and aerated with aquarium bubblers. We tested a total of 15 of these mini aquaria (3 replicas each of 5 core samples). We covered each cup with mesh fabric to prevent any emerging insects from escaping. The samples were inspected on a daily basis for a total of one week to observe possible hatching from potentially desiccation-resistant eggs. We waited an additional two weeks, refilling the cups with DI water when needed, and inspected the samples again for any emerging macroinvertebrates, although none were observed.

It is possible that dessication-resistant eggs are uncommon in dry sediment of the intermittent stream because our sites still receive small amounts of precipitation, and humidity remains relatively high, compared with some temperate locations, during our dry season. Similar methods have worked well in semi-arid Mediterranean streams where average annual precipitation ranges from 400-600mm (Chester & Robson, 2011) and environmental conditions may be intolerable for other life stages of aquatic organisms. A survival strategy such as dormancy may not be critical to macroinvertebrate persistence in our sites. Moreover, the limited depth of the hyporheic zone described earlier may also restrict the use of dry sediment as a sheltering habitat. Other factors that we did not account for in our experiment were replicating ambient stream temperatures, and diel light intensity and duration. The narrow ranges in temperature and diel variation in Costa Rica streams (Jackson & Sweeney, 1995) have been recognized to influence macroinvertebrate development time. It is possible that macroinvertebrates at our site have low thresholds to fluctuations in temperatures and light exposure and we recommend to account for control of these factors. Another way of potentially improving our design would be to use stream water instead of DI water in efforts to maintain chemical properties as similar as possible to natural conditions.

Conclusion

The aquatic macroinvertebrate sampling techniques discussed in this chapter were implemented in an effort to obtain information on the potential use of refugia and behavioral survival strategies in response to flood and drying disturbances. We adapted methods from other stream studies; however, differing physical characteristics such as precipitation, temperature and streambed particle composition may have contributed to the ineffectiveness of these methods in our stream sites. We have reported specific challenges that may serve as guidelines for understanding possible limitations in future studies. Apart of physical characteristics, there may be other chemical (e.g. nutrient availability) and ecological interactions (e.g. predation and competition) driving aquatic community assemblages which may not be related to failure in experimental design. In general, it is important to consider that aquatic ecosystems vary greatly among elevations, and latitudes, as well as temporally, and these conditions may be important drivers in determining which sampling techniques prove to be most effective and logistically suitable. We provide a summary of the sampling techniques, our initial goals and our dry sediment experiment in Table 3.1.

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Table 3.1. Summary of invertebrate attempted sampling methods, targeted habitats, stream site, sampling season and expected taxa.

Sampling method	**Habitat type**	**Stream**	**Sampling season**	**Expected invertebrate taxa**
Emergence traps	riffles, pools, streambanks, boulders	perennial, intermittent	wet, dry	Trichoptera, Ephemeroptera, Diptera,
Soil corer	hyporheic zone	perennial, intermittent	wet, dry	Diptera larvae, Plecoptera, burrowers
Reactivation experiment	dry sediment	perennial, intermittent	dry	Ceratopogonidae, Chironomidae, Plecoptera,

CHAPTER 4

CONCLUSION AND SUMMARY OF FINDINGS

Tropical stream ecosystems are exposed to many ecological drivers, one of which is disturbance of the natural flow regimes. Although studies of aquatic invertebrates in tropical streams have increased in recent decades, many important gaps in knowledge remain (Ramírez & Gutíerrez-Fonseca, 2014). Results from our study suggest that tropical streams may vary greatly even when located in close proximity, with similar riparian vegetation, abiotic factors and surrounding land use; therefore, it can be challenging to make assumptions or generalizations about the ecological integrity and biotic responses of highly varied tropical streams to disturbances. However, studies of macroinvertebrate community responses to hydrological alterations across different tropical streams are important for understanding variations in ecosystem structure and function in these diverse systems.

Costa Rica is a unique neotropical country in that it has an unusually high degree of biodiversity which is protected by governmental policy. Aquatic macroinvertebrates are part of this biodiversity, and are well studied, relative to other tropical countries, (Springer, 2008). However, climate change models indicating alterations in precipitation and increasing water demand due to growing tourism and agriculture (DeFries & Rosenzweig, 2010) in Costa Rica may affect aquatic ecosystems with complex consequences that can be difficult to predict. Our findings of declines in macroinvertebrate richness and abundance during both wet and dry seasons, as well as possible explanations from functional feeding group characteristics and survival strategies, contribute to knowledge about the natural history of macroinvertebrate taxonomic groups in the Neotropics. Furthermore, macroinvertebrates in Costa Rica are commonly used as indicators of water quality (Gutiérrez-Fonseca & Lorion, 2014) and our findings are useful for comparing how macroinvertebrate communities naturally respond to hydrological variations at reference stream sites, without additional stressors of pollution or urbanization.

Tropical aquatic systems are changing rapidly, not only impacted by multiple anthropogenic stressors, but also intense atmospheric events (e.g. tropical cyclones) and cyclical ENSO events. For example, during our study, Hurricane Otto affected the Caribbean slope of Costa Rica in November 2016 and four months after we completed sampling, Tropical Storm Nate caused catastrophic flooding and landslides throughout the country. In San Luis de Monteverde, torrential rains associated with Tropical Storm Nate caused large amounts of debris flow to change the course of our perennial stream. Atmospheric events of this magnitude have not been common in the Pacific slope of Costa Rica in the past. Our study provides information on how macroinvertebrate communities in these systems respond to high and low flow events and potentially recover from drought or flood. The monthly resolution of macroinvertebrate communities from our study provides nuanced insights into immediate biotic responses to disturbance in both perennial and intermittent reference streams, information that is critical for identifying the ways in which hydrological alterations may impact tropical freshwater streams.

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APPENDICES

Appendix A: List of total abundance of all taxa found in each stream type (perennial and intermittent) and sampled habitats (benthic and leaf packs).

Order	Genus	FFG	Abundance		Abundance	
			Benthic		Leaf	
			Perennial	Intermittent	Perennial	Intermittent
Ephemeroptera	*Baetis*	CG		2		
Ephemeroptera	*Baetodes*	Sc	46	60	32	21
Ephemeroptera	*Moribaetis*	CG	29	22	11	19
Ephemeroptera	*Camelobaetidius*	CG		6		
Ephemeroptera	*Thraulodes*	CG	8	6		
Ephemeroptera	*Farrodes*	CG	164	56	28	41
Ephemeroptera	*Leptohyphes*	CG	225	192	295	299
Ephemeroptera	*Tricorythodes*	CG		1		1
Ephemeroptera	*Allenhyphes*	CG		2		
Ephemeroptera	*Euthyplocia*	CG		1		
Ephemeroptera	*Haplohyphes*	CG		6		
Plecoptera	*Anacroneuria*	Pr	93	107	273	166
Odonata	*Brechmorhoga*	Pr	1	16		
Odonata	*Erythrodiplax*	Pr	3	9		2
Odonata	*Perithemis*	Pr		1		

Odonata	*Argia*	Pr	21	53		11
Odonata	*Heteragrion*	Pr		22		
Odonata	*Cora*	Pr		1		
Odonata	*Hetaerina*	Pr	16	14	4	7
Megaloptera	*Corydalus*	Pr		3		2
Coleoptera	*Heterelmis (A)*	CG	7	6	118	59
Coleoptera	*Heterelmis (L)*	CG	91	70	145	66
Coleoptera	*Neocylloepus (A)*	CG	13	26	14	2
Coleoptera	*Neocylloepus (L)*	CG	54	20		
Coleoptera	*Hexanchorus (A)*	CG				
Coleoptera	*Hexanchorus (L)*	CG	6			1
Coleoptera	*Macrelmis (A)*	CG	1	6	5	6
Coleoptera	*Macrelmis (L)*	CG	2	41	1	7
Coleoptera	*Phanocerus (A)*	CG			2	
Coleoptera	*Phanocerus (L)*	CG		1		1
Coleoptera	*Pharceonus (A)*	CG				
Coleoptera	*Pharceonus (L)*	CG		1	2	
Coleoptera	*Disersus (A)*	CG				
Coleoptera	*Disersus (L)*	CG	9	9	5	21
Coleoptera	*Austrolimnius (A)*	CG	3	15	6	5

Coleoptera	*Austrolimnius (L)*	CG	8	25		
Coleoptera	*Pseudodisersus (A)*	CG	3		4	
Coleoptera	*Pseudodisersus (L)*	CG	0	2	2	
Coleoptera	*Onychelmis (A)*	CG				
Coleoptera	*Onychelmis (L)*	CG			1	
Coleoptera	*Bledius*	Pr		17	60	212
Coleoptera	*Anchytarsus*	Sh-Dt	222	283	360	138
Coleoptera	*Psephenus*	Sc	1	29		1
Coleoptera	*Dytiscidae*	Pr				1
Coleoptera	*Lutrochus*	Sh-Dt	1			
Trichoptera	*Leptonema*	Ft	271	203	61	34
Trichoptera	*Calosopsyche*	Ft	316	60	68	5
Trichoptera	*Macronema*	Ft				
Trichoptera	*Chimarra*	Ft	5	24		1
Trichoptera	*Atopsyche*	Pr	2	2	23	19
Trichoptera	*Nectopsyche*	Sh-Hb	4	24	1	14
Trichoptera	*Phylloicus*	Sh-Dt	6	30	2	19
Trichoptera	*Polycentropus*	Pr		7		
Trichoptera	*Cyrnellus*	Ft		4		
Trichoptera	*Cernotina*	Pr	1	8	5	7

Trichoptera	*Centromacronema*	Ft		3		
Trichoptera	*Limnephilus*	Sh-Dt				
Trichoptera	*Marilia*	Sh		1		13
Trichoptera	*Austrotinodes*	Ft		1		
Trichoptera	*Xiphocentron*	CG				
Trichoptera	*Culoptila*	Sc		1		
Trichoptera	*Lepidostoma*	Sh-Dt			1	1
Diptera	Tanypodinae	Pr	33	257	876	2147
Diptera	Non-Tanypodinae	CG	399	788	2726	2675
Diptera	*Probezia*	Pr	2			1
Diptera	*Hexatoma*	Pr		6	2	1
Diptera	*Tipula*	Sh-Dt	6	6	18	65
Diptera	*Limonia*	Sh-Hb	1			
Diptera	*Simulium*	Ft	128	152	1040	1084
Diptera	*Tabanidae*	Pr	1		4	
Diptera	*Limonicola*	Sc	0			
Diptera	*Dixella*	CG	2		3	6
Diptera	*Rhaphium*	Pr				1
Diptera	*Chelifera*	Pr				2
Diptera	*Culicidae*	Ft	1			

Diptera	*Limnophora*	Pr		1		
Hemiptera	*Limnocoris*	Pr	1	2		
Hemiptera	*Rhagovelia*	Pr	60	25		
Hemiptera	*Microvelia*	Pr	2	0		
Hemiptera	*Halobates*	Pr	1	1		
Hemiptera	*Hebrus*	Pr		1	5	8
Hemiptera	*Eurygerris*	Pr	5	2		
Lepidoptera	*Petrophila*	Sc	2		1	
Oligochaeta	Oligochaeta	CG	24	9	3	2
Tricladida	*Planariidae*	Pr	11	23	1	5
Amphipoda	Hyallelidae	Sh	5	16	68	9
Gastropoda	Planorbidae	Sc		1		
Decapoda	*Ptychophallus*	CG	33	9	2	