NUTRIENT DYNAMICS IN NEOTROPICAL STREAM FOOD WEBS: EFFECTS OF STREAM PHOSPHORUS LOADING ON CONSUMER PHYSIOLOGY, AND THE ROLE OF CONSUMERS IN RECYCLING NUTRIENTS

by

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(Under the Direction of Catherine M. Pringle)

ABSTRACT

Humans have dramatically altered global biogeochemical cycles, resulting in widespread nutrient pollution in freshwater ecosystems. The harmful effects of eutrophication are well-documented in lakes and estuaries, but less in known about the effects of sustained nutrient pollution in streams, mostly due to the short-term nature of most experiments. In this study, I use a series of streams in lowland Costa Rica that naturally vary in dissolved phosphorus (P) levels due to inputs of solute-rich groundwater to investigate the effects of sustained, high levels of P-loading on stream food webs. I ask two major questions: (1) What are the effects of sustained P-loading on consumer physiology? and (2) What role do consumers play in supplying limiting nutrients in these stream ecosystems? In the naturally high-P streams, invertebrate consumers increased two-fold in body P-content, showing that the elemental composition of

consumers can reflect their environmental conditions as much as their phylogeny. For one important group of invertebrate detritivores, chironomid larvae, I found that P-demand reflected food quality in streams across this heterogeneous landscape. As a result, chironomids from low-P streams circumvented P-limitation, and did not respond to P-enriched food resources with increased growth rates, which may act to stabilize this detritus-based food web against perturbations caused by nutrient enrichment. I found that all fish species in high-P streams excreted P at high rates, but in a low-P stream, P-excretion rates for species with higher body P-content were negligible. In the low-P focal stream, one species accounted for 90% of P recycled by the fish assemblage, even though it only represented 18 % of the total fish biomass, due to its high-P diet and low body P-demand. Finally, I quantified the potential importance of terrestrial insects as a nutrient subsidy in rainforest streams, showing that this nutrient pathway may supply a significant fraction of nutrient demand in small headwater streams. This dissertation improves our understanding of how rates of nutrient retention and recycling by organisms are affected by ecosystem nutrient availability, which ultimately determines the resilience of ecosystems to anthropogenic nutrient pollution.

INDEX WORDS: Stream, Ecological Stoichiometry, Food web, Phosphorus, Resource subsidy, Insect physiology, Keystone species, Consumer-driven nutrient cycling, Costa Rica

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DEDICATION

To the exceptional scientists and educators who have inspired me along the way, including Star Ellis, Nancy Wynne, Bill Bailey, Martha Groom, Nick Haddad, Patricia Ligon, and Frank Golley.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

We are, indeed, earthborn, but yet not altogether common clay.—Lotka (1925)

General context—Living organisms are composed of multiple elements that occur in characteristic ratios. Some of these elements, such as carbon, nitrogen, and phosphorus, are found in living cells at much higher concentrations than in the Earth's surface (Lotka 1925). Ratios of elements required for life are determined by the chemical composition of the biomolecular building blocks of life: proteins, lipids, carbohydrates, and nucleic acids, as well as by the proportion of these different biomolecules in a given organism (Sterner and Elser 2002). Life on Earth is constrained by the availability of limiting elements in the biosphere, but living organisms simultaneously affect the chemistry of the biosphere, for example, with the evolution of photosynthesis leading to a buildup of oxygen in Earth's atmosphere (Wilkinson 2006), or the balance between the microbial metabolic processes of nitrogen fixation and denitrification regulating the ratios of carbon, nitrogen, and phosphorus in the global ocean (Redfield 1958).

The field of ecological stoichiometry—the study of the elemental ratios characteristic of life on Earth—has roots in Liebig's studies of limiting nutrients (1840), Lotka's comparison of the elemental composition of the lithosphere and the human body (1925), and Redfield's insight into mechanisms regulating nutrient ratios in the ocean (1958). This area of research has

advanced rapidly in the past two decades, with Sterner and Elser (2002) demonstrating connections between the biomolecular composition of organisms, their nutrient demand, maximal growth rates, and rates of nutrient recycling. Because animals (and some microbes) maintain a relatively fixed elemental composition (homeostasis), we can use elemental ratios of their food resources to predict the ratios at which they recycle excess nutrients. This relatively simple concept allows ecological stoichiometry to make predictions linking biomolecules to the biosphere.

Despite rapid advances in recent years, fundamental questions in ecological stoichiometry remain unanswered. For example, to what degree do animals remain homeostatic when feeding on nutrient-enriched food? Can assemblages of consumers become adapted to high-nutrient environments, either through shifts in species composition or through microevolution of populations within species? How do an organism's dietary nutrient supply and body nutrient demand interact to determine nutrient recycling rates? The predictive power of ecological stoichiometry theory hinges on the answers to these questions, among others.

Understanding how living organisms affect, and are affected by, nutrient availability in the biosphere is more than an academic exercise. Humans have dramatically altered the global biogeochemical cycles of nitrogen and phosphorus (Vitousek et al. 1997, Carpenter et al. 1998). Under pristine conditions, these two elements commonly limit productivity in freshwater ecosystems (Elser et al. 2007), but due to fertilizer runoff from agricultural and urban areas, wastewater inputs, and (in the case of nitrogen) atmospheric deposition, high levels of these nutrients make their way into inland waters. Harmful consequences of anthropogenic eutrophication in lakes, reservoirs, and estuaries (e.g., cyanobacteria blooms, fish kills, "dead

zones") are well-documented (Smith and Schindler 2009), but we have less understanding of the effects of nutrient pollution in stream ecosystems.

Ecological stoichiometry at the organismal level is an important component of nutrient dynamics within ecosystems. The elemental composition of organisms affects the storage and fluxes of nutrients within the food web, and the degree to which organisms maintain homeostasis influences the rates at which limiting nutrients are retained and excess nutrients are mineralized. Differences in elemental composition among different taxa are well-documented in stream ecosystems (e.g., Cross et al. 2003, Evans-White et al. 2005), due to variation among different species in body nutrient demand and dietary nutrient supply. While the P-content of vertebrate consumers is largely a function of "structural biomass" (i.e., bones and scales) (Vanni et al. 2002), nearly all body P in invertebrate consumers is found in "metabolic biomass" (sensu Allen and Gillooly 2008). Ribosomal RNA and other intra-cellular pools of P may vary based on dietary P availability (Sterner and Elser 2002), potentially causing large changes in invertebrate body P content within a taxon. However, stoichiometric theory has assumed that, within a species, animals maintain a fixed elemental composition (i.e., strict homeostasis) that is independent of diet quality (e.g., Andersen and Hessen 1991, Urabe and Watanabe 1992, Sterner and Hessen 1994). A growing body of evidence has demonstrated that the P content of some invertebrate taxa can vary substantially depending on dietary P content (e.g., DeMott et al. 1998, Carrillo et al. 2001, Fink and Von Elert 2006, Shimizu and Urabe 2008). These deviations from strict homeostasis, observed for invertebrates feeding on high-P food, have been attributed to increased levels of RNA (Schade et al. 2003, Acharya et al. 2004, Elser et al. 2005) as well as P stored in hemolymph (Woods et al. 2002). However, there are few data on the extent to which ecosystem nutrient loading affects the elemental composition of entire consumer assemblages.

Only one study to date has examined the effects of a multi-year, whole stream nutrient enrichment on the elemental composition of an entire invertebrate consumer assemblage. In that study, moderately elevated levels of N and P led to increased P-content in a subset of the invertebrate assemblage, showing that at least some taxa (notably Trichoptera) deviated from strict homeostasis (Cross et al. 2003). Higher levels of nutrient loading, sustained over many years, could potentially lead to more pronounced effects on consumer stoichiometry, or, conversely, homeostatic regulation by consumers could preclude greater effects.

Most primary production enters food webs via detrital pathways (Moore et al. 2004), and detritivores face a unique stoichiometric challenge due to the characteristically high C:P ratios of this food resource (Enriquez et al. 1993, Cross et al. 2003). Many invertebrate detritivores use a combination of physiological strategies to decrease their P-demand, including having high body C:P ratios (decreasing the imbalance between their food and their own biomass) (Frost et al. 2006) and slow growth rates (resulting in a greater fraction of ingested carbon being lost through respiration) (Anderson et al. 2005). However, other invertebrate detritivores, such as some chironomid larvae, maintain fast growth rates (e.g. Huryn 1990, Hauer and Benke 1991, Benke 1998) and low body C:P ratios (Cross et al. 2003), suggesting that these taxa may be especially susceptible to P-limitation. Because animals tend to be homeostatic, they may respond to P-enriched food resources through increased growth rates if they are P-limited or through increased P-excretion rates if P-intake exceeds their requirements for growth (Sterner & Elser 2002). Accordingly, production of chironomids and other short-lived taxa increased during a multi-year experimental whole-stream nutrient enrichment (Cross et al. 2005, 2006), eventually destabilizing the stream food web (Davis et al. 2010). Little is known, however, about the potential for adaptation of consumer assemblages to sustained nutrient loading. Chronic P-

loading could potentially lead to selection for consumers with higher P-demands, either within species (through microevolution) or at the community level (through increased abundance of species with higher P-demand) (Hall 2009).

Nutrient recycling through consumer excretion has been shown to be an important biogeochemical flux, and a source of nutrients that limit primary productivity, in a wide range of aquatic ecosystems (e.g., Meyer et al. 1983, Grimm 1988, Vanni et al. 2002, McIntyre et al. 2008). In most of these cases, the importance of consumer-driven nutrient recycling is due to high levels of consumer biomass rather than to high mass-specific excretion rates (e.g., Caraco et al. 1997, Hall et al. 2003, McIntyre et al. 2007). However, stoichiometric variables unique to individual species, such as diet nutrient content and body elemental composition, could result in some taxa playing disproportionately important roles in the recycling or retention of nutrients, thereby affecting ecosystem nutrient availability (e.g., Elser et al. 1988). N-recycling rates tend to be similar among similar-sized species, due to a limited variation among the N-content of different food resources and in body N content across different species (Vanni et al. 2002). The contribution of individual species to total N recycling is therefore largely dependent on the total biomass of each species (McIntyre et al. 2007). In contrast, vertebrate consumers (such as fishes) can vary greatly in body P-content (due to different amounts of bones or scales) (Vanni et al. 2002), and diets can range widely in P (e.g., Pilati and Vanni 2007), leading to a wide range of mass-specific P-excretion rates among different species. As a result of species-specific differences in P-excretion rates, ecosystem-level P-recycling (more so than N-recycling) has the potential to be influenced by species identity, and allows for relatively rare species to potentially play disproportionately important roles in these ecosystems. In addition to recycling nutrients within stream ecosystems, some fishes may play important roles in facilitating terrestrial

subsidies through feeding on terrestrial invertebrates, and ultimately making those nutrients available through excretion.

Project overview—My dissertation research took place in the streams of La Selva Biological Station in lowland Costa Rica. Geomorphological features of this landscape result in regional groundwater inputs emerging in some La Selva streams, enriching them in phosphorus to levels comparable to P levels downstream from wastewater treatment plant inputs. Two decades of research on these streams has focused on the ecological implications of this additional P in an otherwise P-limited landscape; in particular, measuring the effects of P on leaf litter breakdown and microbial activity, and on separating the bottom-up effects of P availability from the top-down effects of macroconsumers on algal growth and leaf litter breakdown. Simultaneously, a second line of research has focused on the biogeochemistry of these streams through nutrient uptake experiments (Triska et al. 1993, Triska et al. 2006). Through my dissertation, I have tried to bridge the gap between the ecological and biogeochemical research programs through the conceptual framework of ecological stoichiometry. I have focused on two general questions: what are the effects of phosphorus loading on consumer physiology, and what role do consumers play in recycling nutrients in stream ecosystems?

Chapter 2: Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a Neotropical stream

A central tenet of Ecological Stoichiometry is that the elemental composition of consumers is relatively independent of food resource nutrient content. Although the P-content of some invertebrate consumer taxa has been shown to increase as a consequence of P-enriched food resources (Cross et al. 2003, Bowman et al. 2005, Evans-White et al. 2005), little is known

about how high levels of P-loading over long periods of time can affect the elemental composition of entire consumer assemblages. The purpose of this chapter is to use the natural phosphorus gradient of La Selva streams to test the effects of high levels of P-loading over millennia on stoichiometric relationships in stream food webs. Models central to advancing stoichiometric theory (e.g. Sterner and Hessen 1994, Frost et al. 2006) are based on the assumption that consumers maintain strict homeostasis (i.e., their elemental content is invariable) when feeding on food resources that differ in P-content. However, an increasing number of empirical studies show that at least some invertebrate taxa can become enriched in P when feeding on high-P food (e.g., DeMott et al. 1998, Shimizu and Urabe 2008). This chapter is an important test of the degree to which entire invertebrate assemblages maintain homeostasis when feeding on P-enriched food resources.

Chapter 3: Differences in phosphorus demand among detritivorous chironomid larvae reflect inter-stream differences in food resource stoichiometry

Nutrient loading in aquatic ecosystems can affect stoichiometric relationships between consumers and their food resources, which in turn could lead to adaptive responses in consumer physiology, either through evolution within species, or through shifts in species composition of consumers. This chapter explores the potential adaptations to food P-content for chironomid larvae, which are an important group of invertebrate detritivores in many tropical streams. Detritivores feed on characteristically low-P food resources, and fast-growing chironomids should have a high potential for P-limitation in low-P streams. Building on previous research that found increasing chironomid growth rates with increases in stream SRP (Rosemond et al. 2002, Ramírez and Pringle 2006), I explicitly measured P-limitation for chironomids from both a

high-P stream and a low-P stream (using growth rates, P-excretion rates, and RNA content), and used genetic analysis to determine whether differences in P-demand were due to shifts in species composition or to genotypic differences within species.

Chapter 4: Emergent role of the fish, Astyanax aeneus (Characidae), as a keystone nutrient recycler in low-nutrient Neotropical streams, Costa Rica

Nutrient recycling by consumers is a potentially important biogeochemical process in both terrestrial and aquatic ecosystems. Stoichiometric traits of individual species may result in some taxa playing disproportionately important roles in the recycling of nutrients relative to their biomass, acting as keystone nutrient recyclers (*sensu* Power et al. 1996). The objective of this chapter is to investigate the interaction between food P-content and consumer P-demand in controlling P recycling rates by the fish assemblage. Mass-specific excretion rates of nitrogen vary little among species, but P-excretion rates can vary greatly depending on the dietary supply and physiological demand of this nutrient. Previous studies have shown that differences in the P-content among different fish species are an important control of P-recycling rates. I compared P-excretion rates among twelve species that vary in body P-content and diet (from algivores to insectivores). Moreover, individual food items vary in P-content with stream dissolved P levels. We were therefore able to use this unique series of streams to tease apart the relative importance of these factors, and to measure the potential for species to emerge as disproportionately important P-recyclers in low-P streams.

Chapter 5: Are terrestrial insects an important nutrient subsidy in lowland Neotropical rainforest streams?

The importance of terrestrial inputs into streams is well-documented in temperate ecosystems (reviewed in Baxter et al. 2005), but little is known about the magnitude of these subsidies in tropical rainforests. In tropical rainforests, terrestrial insects falling from the forest canopy may represent an important nutrient flux in nutrient-poor headwater streams, if these insects are efficiently captured by fishes. In addition, whereas terrestrial insect inputs tend to be negligible for much of the year in temperate streams (e.g., Cloe and Garman 1996), tropical rainforests potentially maintain high rates of terrestrial insect inputs year-round. In this chapter, we follow the pathways of nitrogen and phosphorus from terrestrial insects through the food webs of a pair of headwater streams. I use a combination of methods to measure the input rate of terrestrial insect biomass (in terms of N and P) into streams and the rate of consumption of terrestrial insects by fishes. I used gut content analyses to partition terrestrially- and aquatically-derived N and P excretion, and using fish biomass measurements, calculate the proportion of stream nutrient demand that is supplied by terrestrially-derived fish nutrient excretion. The purpose of this study is to provide the first estimate of one potentially important linkage between riparian and stream ecosystems in a rapidly changing tropical landscape.

CHAPTER 2

DEVIATION FROM STRICT HOMEOSTASIS ACROSS MULTIPLE TROPHIC LEVELS IN AN INVERTEBRATE CONSUMER ASSEMBLAGE EXPOSED TO HIGH CHRONIC PHOSPHORUS ENRICHMENT IN A NEOTROPICAL STREAM[1]

[1] Small, G. E., and C. M. Pringle. *Oecologia.* 162:581-590.
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Abstract

A central tenet of ecological stoichiometry is that consumer elemental composition is relatively independent of food resource nutrient content. Although the phosphorus (P) content of some invertebrate consumer taxa can increase as a consequence of P-enriched food resources, little is known about how ecosystem nutrient loading can affect the elemental composition of entire consumer assemblages. Here we examine the potential for P-enrichment across invertebrate consumer assemblages in response to chronic high P-loading. We measured elemental ratios in invertebrate consumers and basal food resources in a series of streams in lowland Costa Rica that range widely in P-levels (2-135 μg L^{-1} soluble reactive phosphorus). Streams with high P-levels receive natural long-term (over millennia) inputs of solute-rich groundwater while low-P streams do not receive these solute-rich groundwater inputs. Phosphorus content of leaf litter and epilithon increased four-fold across the natural P-gradient, exceeding basal resource P-content values reported in the literature from other nutrient-rich streams. Invertebrate consumers from the high-P study stream were elevated two-fold in P-content across multiple taxonomic and functional feeding groups, including predators. Our results strongly support the hypothesis that elevated P-content in consumers feeding on P-enriched food resources is a consequence of deviation from strict homeostasis. In contrast to prior studies, we found that between-stream variation in P-content of a given taxon greatly exceeded within-stream variation among different taxa, suggesting that environment may be as important as phylogeny in controlling consumer stoichiometry. Relaxing the assumption of strict homeostasis presents challenges and opportunities for advancing our understanding of how nutrient limitation affects consumer growth. Moreover, our findings may provide a window into

the future of how chronic anthropogenic nutrient loading can alter stoichiometric relationships in food webs.

Introduction

Ecological stoichiometry at the organismal level is an important component of nutrient dynamics within ecosystems. The elemental composition of organisms affects the storage and fluxes of nutrients within the food web. Likewise, the degree to which organisms maintain homeostasis influences the rates at which limiting nutrients are retained and excess nutrients are mineralized.

Differences in elemental composition among taxonomic and functional feeding groups are well-documented (e.g., Cross et al. 2003, Evans-White et al. 2005), due to variation among different consumers in body nutrient demand and dietary nutrient supply. While the P-content of vertebrate consumers is largely a function of "structural biomass" (e.g., bones and scales; Vanni et al. 2002), nearly all body-P in invertebrate consumers is found in "metabolic biomass" (*sensu* Allen and Gillooly 2008). Ribosomal RNA and other intra-cellular pools of P may vary based on dietary P- availability (Sterner and Elser 2002), potentially causing large changes in invertebrate body P-content within a taxon. However, stoichiometric theory has assumed that, within a species, animals maintain a fixed elemental composition (i.e., strict homeostasis) that is independent of diet quality (e.g., Andersen and Hessen 1991, Urabe and Watanabe 1992, Sterner and Hessen 1994). Despite these assumptions of strict homeostasis, a growing body of evidence has demonstrated that the P-content of some invertetebrate taxa can vary substantially with diet P-content (e.g., DeMott et al. 1998, Carrillo et al. 2001, Fink and Von Elert 2006, Shimizu and Urabe 2008). These deviations from strict homeostasis, observed for invertebrates feeding on high-P food, have been attributed to increased levels of RNA (Schade et al. 2003, Acharya et al. 2004, Elser et al. 2005) as well as P stored in haemolymph (Woods et al. 2002). However, there are few data on the extent to which ecosystem nutrient loading affects the elemental composition

of entire consumer assemblages (e.g., Singer and Battin 2007). Only one study to date has examined effects of a multi-year, whole-stream nutrient enrichment on the elemental composition of invertebrate consumers. In that study, moderately elevated levels of N and P led to increased P-content in a subset of the invertebrate assemblage, showing that at least some taxa (notably Trichoptera) deviated from strict homeostasis (Cross et al. 2003).

Phosphorus-loading is a ubiquitous problem in many freshwater ecosystems (Carpenter et al. 1998) and can greatly increase the P-content of both algal epilithon and leaf litter (e.g., Cross et al. 2003), which constitute the base of stream food webs. If deviation from strict homeostasis is a widespread trait in aquatic invertebrates, then chronic high stream P-loading could lead to the entire benthic consumer assemblage becoming P-enriched as a consequence of feeding on high-P food resources.

To better understand how P-loading affects stoichiometric relationships throughout a stream food web, we measured the elemental composition of invertebrate consumers and basal food resources in streams in lowland Costa Rica that vary widely in P-levels due to natural, long-term (over millennia) inputs of solute-rich groundwater. We analyzed (1) assemblage-level patterns between streams, (2) paired comparisons of similarly-sized, conspecific taxa (in order to evaluate deviation from strict homeostasis), (3) comparisons between streams by insect order (to examine phylogenetic patterns in homeostasis), and (4) comparisons between streams by functional feeding group (in order to examine the effects of diet on homeostasis). We predicted that the historically high concentrations of dissolved-P in our study streams would result in high P-content across all taxa in the invertebrate consumer assemblage.

Methods

Study site—La Selva Biological Station (10°26'N, 84°01'W) is situated on the Caribbean Slope of Costa Rica at the gradient break between the mountains and coastal plain, receiving almost 4000 mm of rainfall each year (Sanford et al. 1994). Geomorphological features of the La Selva landscape result in natural interbasin transfers of solute-rich groundwater entering some streams (Pringle et al. 1990). These groundwater inputs are characterized by high solute concentrations (e.g. P, Na^+, Cl^-, HCO_3^-; Pringle et al. 1993). Dense riparian vegetation results in light-limited algal communities (Pringle and Triska 1991) and high inputs of allochthonous material into streams.

We chose seven streams that differed widely in average SRP concentration (2–135 μg L^{-1}) due to differential inputs of solute-rich groundwater (Table 2.1). These sites are 2^{nd}–3^{rd} order streams, within close proximity (<2 km apart), and surrounded by dense forest. Channel widths range from 1–3 m, and the dominant substrata are detritus, silt, and clay, with boulders present at some sites. Our seven study streams are a subset of sites used in a long-term study of the physicochemistry of La Selva streams (Pringle and Triska 1991; Triska et al. 2006), for which continuous monthly data are available since 1997. We analyzed the elemental composition of leaf litter and epilithon at all seven sites. Insect assemblages vary widely among streams at La Selva, largely due to differences in geomorphology (Ramírez et al. 2006). In order to maximize the number of comparisons between similar taxa, we selected one high-P stream (Arboleda; SRP = 135 μg L^{-1}) and one low-P stream (Sura-60; SRP = 2.8 μg L^{-1}) to compare the elemental composition of insect consumers, based on their largely overlapping insect assemblages (Ramírez et al. 2006), as well as similarities in discharge and substrate (Table 2.1).

Sample collection and analysis—Leaf litter and epilithon were collected from our seven study streams in February and July 2006. At each sampling date, we collected three replicate grab samples of submerged leaves, so that individual leaf species were represented roughly in proportion to their abundance in the stream. Epilithon was collected from unglazed ceramic tiles after four weeks of incubation in each stream. Epilithon was scrubbed from the tiles, filtered (0.45 μm GF/F), and dried. In the Arboleda and Sura-60, we also sampled fine particulate organic matter (FPOM) and filamentous algae. FPOM samples consisted of deposited material from the upper 5 cm of substrate, and was collected with a syringe. Filamentous algae was collected by hand from submerged rocks.

Invertebrates were collected in the Arboleda and Sura-60 in May 2007 from all major stream habitats (leaf packs, boulders, depositional areas). Insects were sorted live under a dissecting microscope, length was measured to the nearest mm, and specimens were identified to family or genus. Guts were not removed prior to stoichiometric analysis due to the small size of many individuals (see discussion). For smaller taxa, composite samples consisting of 3-50 individuals of a similar size class (within 2 mm) were used for C, N, and P analysis; otherwise, individual organisms were used. Some low-mass samples were analyzed only for P. Functional feeding groups (FFGs) were designated according to Merritt and Cummins (1996) and Jackson and Sweeney (1995). FFGs are based on mouthpart morphology and behavioral characteristics and include scrapers (scrape biofilm from hard surfaces), shredders (consume primarily leaf material), collector-gatherers (gather fine organic particles), collector-filterers (filter fine particles) and predators (consume other animals).

All samples were dried at 50° for 48 hours and homogenized. For C and N analysis, samples were analyzed on a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan, Italy). For

P analysis, samples were acid-digested (Aqua Regia double acid; Jones et al. 1991), and analyzed spectrophotometrically (ascorbic acid method). Ground pine needles (US National Institute of Standards and Technology, 1575a) and poplar leaves (Analytical Chemistry Laboratory, University of Georgia) were used as external standards for P and N analyses. In each stream, nutrient concentrations were measured from filtered water samples (0.045 μm Millipore filters) for SRP (ascorbic acid method), NO_3-N (Cadmium reduction method), and NH_4-N (phenate method; APHA 1998).

Statistical analyses—Relationships among leaf litter and epilithon nutrient content and stream SRP were determined using regression analysis. Mean SRP values for each stream were calculated from monthly samples from January 2006-August 2007. For each stream, we calculated mean %P, C:P, and N:P for leaf litter and epilithon. The relationship between SRP and leaf litter or epilithon %P was fit with a linear model. Because %C and %N showed no relation to SRP, C:P and N:P were inverse-transformed (i.e., to P:C and P:N, respectively) and fit with a linear model.

Overall differences in the elemental composition of insect assemblages between the high-P Arboleda and low-P Sura-60 were assessed using *t*-tests ($\alpha = 0.05$), based on all samples collected for each stream (where each sample represents a certain size class of a species, *sensu* Cross et al. 2003). We compared 33 samples consisting of 16 taxa from the Arboleda and 41 samples consisting of 16 taxa from the Sura-60. Thirteen taxa overlapped between the two streams in this analysis, 3 taxa were found only in the Arboleda (Baetidae, Naucoridae, and Tricorythiidae), and 3 taxa were found only in the Sura-60 (Calamoceratidae, Gomphidae, and Lutrochidae).

Differences in elemental composition between samples of similarly-sized (within 2 mm), conspecific taxa between the two streams were analyzed using a paired *t*-test. Differences among FFGs and orders were analyzed with two-way analysis of variance (ANOVA). Where stream identity and the interaction term (stream × FFG or stream × order) were both significant, Tukey's HSD was used to compare differences between streams for each FFG or order. All analyses were conducted in SAS (SAS Institute 2001). All data are presented as either %C, N, and P of dry mass or as molar ratios.

Results

Phosphorus content of basal food resources—Leaf litter %P increased with SRP from 0.05–0.22 %P ($r^2 = 0.91$, $P = 0.0008$), and led to subsequent decreases in C:P from 1986–466 ($r^2 = 0.88$, $P = 0.0016$) and N:P from 61–17 ($r^2 = 0.91$, $P = 0.0007$; Fig. 2.1). Leaf litter %C, %N, and C:N did not differ among streams.

The P-content of epilithon was more variable than leaf litter but %P increased significantly with SRP from 0.12–0.47 %P ($r^2 = 0.83$, $P = 0.0042$). Epilithon decreased in C:P from 245–41 ($r^2 = 0.90$, $P = 0.001$) with increasing stream SRP (Fig. 2.1). Epilithon N:P ranged from 5.4–28.9 but was not related to stream SRP. Epilithon %C, %N, and C:N did not differ among streams.

The P-content of basal food resources was elevated in the high-SRP Arboleda relative to the low-SRP Sura-60. P-content by dry weight increased 4-fold for leaf litter (0.22 %P vs. 0.05 %P), > 2-fold for epilithon (0.47 %P vs. 0.18 %P), 6-fold for FPOM (0.18 %P vs. 0.03 %P), and 5-fold for filamentous algae (0.30 %P vs. 0.06 %P).

Phosphorus content of invertebrate consumers—Phosphorus content in invertebrate consumer assemblage averaged two-fold higher in the high-P Arboleda (mean %P = 1.15) relative to the low-P Sura-60 (mean %P = 0.51; Fig. 2.2). In the Arboleda, 79% of invertebrates sampled had a P-content > 1.0%. In contrast, in the Sura-60, only 5% of invertebrates sampled had a P-content exceeding 1.0%. The Arboleda consumer assemblage was significantly higher in %P (t = 8.05, df = 40.4, $P < 0.0001$), and lower in C:P (t = 6.10, df = 66, $P < 0.0001$) and N:P (t = 6.19, df = 68, $P < 0.0001$; Fig. 2.2) relative to the Sura-60 assemblage. Invertebrate %C, %N, and C:N did not differ among streams ($P > 0.05$).

Paired comparisons of similar-sized conspecific taxa between the two streams showed that these taxa deviate from strict homeostasis, with a two-fold average increase in P-content in the high-P Arboleda. Invertebrates in the high-P Arboleda had significantly higher %P (t = 6.70, df = 16, $P < 0.0001$; Fig. 2.3) and lower C:P (t = 7.04, df = 13, $P < 0.0001$) and N:P (t = 7.72, df = 13, $P < 0.0001$) compared to the low-P Sura-60. Paired taxa did not differ in %C, %N, and C:N between the two streams ($P > 0.05$).

When grouped by order, %P was significantly higher among Arboleda invertebrates (mean 1.17) compared to Sura-60 invertebrates (mean 0.52, $F_{1,58} = 57.25$, $P < 0.0001$; Fig. 2.4). Mean %P differed among orders ($F_{8,58} = 3.15$, $P = 0.005$), ranging from 0.68 (Odonata) to 1.16 (Plecoptera). The difference in insect %P between streams varied by order (stream × order interaction: $F_{8,58} = 3.42$, $P < 0.01$), with significant differences between streams observed in Diptera, Ephemeroptera, and Plecoptera. Insect C:P was significantly lower for orders in the Arboleda (stream effect: $F_{1,52} = 13.53$, $P = 0.0006$). No differences in C:P were observed among different orders ($F_{8,52} = 1.02$, $P = 0.43$), and differences between streams in C:P did not vary by order (stream × order interaction: $F_{7,52} = 0.93$, $P = 0.49$). Likewise, insect N:P was lower for

orders in the Arboleda ($F_{1,53} = 13.17$, $P = 0.0006$), but no differences were detected among different orders ($F_{8,53} = 0.70$, $P = 0.69$) and stream effects did not vary among orders (stream × order interaction: $F_{8,53} = 0.95$, $P = 0.48$).

Insect %C varied among orders ($F_{7,52} = 47.04$, $P < 0.0001$), ranging from 43.0 (Trichoptera) to 48.6 (Hemiptera), but did not vary between streams ($F_{1,52} = 0.30$, $P = 0.58$), nor was there a significant stream × order interaction ($F_{5,52} = 1.37$, $P = 0.25$). Insect %N did not vary between streams ($F_{1,53} = 0.36$, $P = 0.55$) or among orders ($F_{8,53} = 1.60$, $P = 0.15$), and there was no stream × order interaction ($F_{8,53} = 1.32$, $P = 0.25$). Insect C:N varied among orders ($F_{8,52} = 2.61$, $P = 0.02$), ranging from 3.38 (Diptera) to 5.61 (Trichoptera). Insect C:N did not vary between streams ($F_{1,52} = 0.10$, $P = 0.75$), nor was there a significant stream × order interaction ($F_{7,52} = 1.10$, $P = 0.37$).

When grouped by FFG, insects were again significantly higher in %P ($F_{1,64} = 30.52$, $P < 0.001$) in the high-P Arboleda (mean = 1.11) relative to the low-P Sura-60 (mean = 0.51; Fig. 2.5). There was no difference in %P among different FFGs ($F_{4,64} = 0.17$, $P = 0.95$), nor was there a significant stream × FFG interaction ($F_{4,64} = 0.16$, $P = 0.96$). Likewise, insect C:P was lower across insect FFGs in the Arboleda ($F_{1,57} = 17.12$, $P = 0.001$) but did not vary among different FFGs ($F_{4,57} = 0.24$, $P = 0.91$), and there was no stream × FFG interaction ($F_{4,57} = 0.73$, $P = 0.57$). Insect N:P was also lower in the Arboleda (mean = 22.2) relative to the Sura-60 (mean = 45.8; $F_{1,59} = 21.93$, $P < 0.0001$). There were no differences in N:P among different FFGs ($F_{4,59} = 0.62$, $P = 0.65$), nor was there a stream × FFG interaction ($F_{4,59} = 0.62$, $P = 0.65$).

There was no difference in insect %C between streams ($F_{1,57} = 0.04$, $P = 0.85$) or among FFGs ($F_{4,57} = 1.91$, $P = 0.12$), and the stream × FFG interaction term was not significant ($F_{4,57} = 1.09$, $P = 0.37$). Insect %N did not differ between streams ($F_{1,59} = 2.50$, $P = 0.12$), but there

were differences among FFGs ($F_{4,59}$ = 10.45, P < 0.0001), as %N ranged from 8.8 (shredders) to 11.0 (predators). There was a stream × FFG interaction for %N ($F_{4,59}$ = 3.09, P = 0.02), although there we no significant differences in %N within a given FFG between the two streams. Likewise, there was no difference in C:N between streams ($F_{1,57}$ = 3.51, P = 0.07), but differences among FFGs were significant ($F_{4,57}$ = 6.35, P = 0.0003), as mean C:N ranged from 4.88 (predator) to 5.84 (shredder). The stream × FFG interaction term was not significant for C:N ($F_{4,57}$ = 1.12, P = 0.36).

Discussion

Observed high P-levels in basal resources and elevated P-levels across the invertebrate assemblage indicate a general deviation from strict homeostasis among invertebrate consumers. Our results are unique in the generality of the observed response of all invertebrate consumers to P-enrichment (i.e., across orders and FFGs). Even taxa identified as predators showed elevated P-content in the high-P stream, the first such evidence for altered elemental composition in higher trophic levels. In contrast, other studies have reported highly variable invertebrate P-content among taxa in nutrient-enriched streams, or no relationship between elevated stream P and invertebrate P-content. For example, in a comparison of streams above and below municipal wastewater treatment plant inputs (3-29 μg L^{-1} total P), Bowman et al. (2005) found an increase in the P-content of heptageniid mayflies (Ephemeroptera) at only one of four high-nutrient sites, despite a 10-fold increase in epilithon P-content. In a survey of streams ranging in SRP from 3-99 μg L^{-1}, Evans-White et al. (2005) found no relationship between stream SRP and invertebrate P-content. After two years of an experimental N+P addition in a detritus-based headwater stream (increasing SRP from 7-46 μg L^{-1}), Cross et al. (2003) only found significant increases in

P-content in one group of primary consumers (Trichoptera). In contrast, our findings show that insect consumers were enriched in P across all taxonomic and functional feeding groups in the high-P Arboleda.

The generality of P-enrichment across invertebrate consumers in the naturally high-P Arboleda is a consequence of high historic levels of P-loading, resulting in extremely high P-content across all basal food resources. Basal food resources in the Arboleda were highly enriched in P relative to low-P La Selva streams and are higher than values reported from other nutrient-enriched streams. Mixed-species leaf litter collected from streams across the SRP gradient showed a four-fold increase in P-content, similar to values reported by Ardón et al. (2006) for single-species leaf packs incubated in the same study streams. Leaf litter P-content in the Arboleda (0.22 %P) greatly exceeded the only other published values for leaf litter from a nutrient-enriched stream (0.05 %P; Cross et al. 2003). Increased microbial biomass on leaf litter in our high-P study stream likely accounts for this difference (Cross et al. 2003, Ardón et al. 2006). Epilithon P-content increased four-fold over the SRP range exhibited by our seven study streams, and values from our high-P study streams exceed reported values from other P-enriched streams (Stelzer and Lamberti 2001, Cross et al. 2003, Bowman et al. 2005), which could result from luxury P-uptake by algal cells or increased densities of P-rich heterotrophic bacteria.

Our findings support the hypothesis that deviation from strict homeostasis may be a general trait among invertebrate consumers, observable when food resources are sufficiently P-enriched. The paired comparison of taxa between the Arboleda and Sura-60 shows that similar-sized individuals of the same taxon were uniformly enriched in P in the high-P stream. All 17 samples of similar-sized taxa increased in P-content in the high-P stream, from 1.3 to 4.7-fold (mean = 2.7; Appendix A). In contrast, Cross et al. (2003) found a more limited response, with

15 of 24 comparable samples (of similar-sized taxa) enriched in P, from 1.1 – 4.0-fold (mean = 2.1). The difference in responses between these two studies is likely due to the much greater degree of basal resource P-enrichment in our high-P study streams. Primary consumers in high-P La Selva streams consume food resources that are 2–6 times greater in P-content relative to those in low-P streams, and invertebrate predators in high-P streams consume prey items that are 2-fold greater in P in high-P streams. By contrast, primary consumers in the nutrient-enriched stream studied by Cross et al. (2003) consumed food resources that were on average 1.5 times higher in P, and invertebrate predators fed on prey items that were enriched only 1.2 times in P over reference conditions.

The strength of homeostasis can be evaluated as the change in consumer body elemental composition relative to the change in the elemental content of its food resources, according to the equation:

$$\log(y) = \log(c) + \frac{\log(x)}{\mathrm{H}}$$

where y = consumer stoichiometry, x = resource stoichiometry, c is a constant, and H (eta) represents strength of homeostatic regulation (Sterner and Elser 2002). Values of H > 1 are consistent with some degree of homeostasis (i.e., the increase in consumer P-content is smaller than the increase in food resource P-content), and very large H values reflect strict homeostasis. While controlled feeding experiments are required to accurately determine H for a given taxon, we can estimate this value based on field data. Across invertebrate taxa in our study, we estimated values of H ranging from 0.4 – 5.4 (Fig. 6, Table S1). For comparison, among those taxa that exhibited increased P-content in the study by Cross et al. (2003), we calculated H values that ranged from 0.3 – 11.0. Taxa classified as shredders had the lowest H values (i.e., they were the least homeostatic) among primary consumers in our study and among all

consumers in the Cross et al. (2003) study. The most striking difference between the two datasets is that invertebrate predators showed a high degree of P-enrichment and had the lowest calculated H values in our study (range 0.4 – 1.4), whereas most predators in Cross et al. did not increase in P-content in the nutrient-enriched stream. We suspect that this difference is due to the higher degree of P-enrichment in food resources of invertebrate predators in the high-P La Selva stream, rather than a higher degree of homeostasis among predators analyzed by Cross et al. (2003). These values should be interpreted with caution, however, since we assume that our measurements of food resource stoichiometry accurately represent the nutrient content of food ingested by the consumer (e.g., no selective grazing). In a parallel study, the P-content of chironomid larvae from both the Arboleda and Sura-60 increased from 0.5 to 1.0 %P with increasing P-content of leaf litter when groups of larvae from each source stream were reared on leaf litter conditioned in streams across the natural P-gradient (Chapter 3). These results add weight to our contention that differences in invertebrate P-content between streams are due to deviation from strict homeostasis.

Another potential mechanism for how the primary consumer assemblage could increase in response to P-enriched food resources is through a community shift towards species with higher P-demand (Singer and Battin 2007). This is unlikely since our two study streams were purposely selected for their similarity in invertebrate species composition (Ramírez et al. 2006). It is also possible that populations of invertebrate consumers in naturally high-P streams could have evolved to have a higher P-content. However, some degree of genetic isolation would also be required, which is very unlikely in this landscape. Our study sites are all within close proximity (within 2 km), and low-P streams become high-P at the gradient break (at 35 m above sea level) where high-solute springs emerge. In order to maintain genetic isolation sufficient for

adaptation to very local conditions, ovipositing female insects would have to show high site fidelity, and invertebrate drift would have to be low. In contrast, lowland tropical streams of La Selva are characterized by large numbers of drifting invertebrates (Ramírez and Pringle 1999, 2001). Similarly, Peckarsky et al. (2005) found that phenotypic plasticity, rather than genetic differentiation, was the primary explanation for different invertebrate responses between adjacent streams with contrasting environmental conditions. Thus, while it is possible that invertebrate populations between streams may differ in their P-demands, our results support the conclusion that increased P-content in consumers is primarily due to deviation from strict homeostasis.

A fraction of the elevated P-content measured in invertebrates in the high-P Arboleda is due to the elevated P-content of food in their guts. To minimize the effect of undigested food, some studies have reported consumer biomass nutrient values that were based on measurements made after guts were removed (e.g., Cross et al. 2003) or after consumers were allowed time to clear their guts (e.g., Evans-White et al. 2005). Potential effect of actual gut contents on our nutrient measurements may be evaluated by a simple mixing model:

$$(GC_{\text{mass}} \times GC_{\%\text{P}}) + (B_{\text{mass}} \times B_{\%\text{P}}) = S_{\text{mass}} \times S_{\%\text{P}}$$

where GC is gut contents, B is body (i.e., insect tissue without guts), and S is the composite sample which includes gut contents. For example, a consumer feeding on leaf litter, whose gut contents constitute 20% of its dry mass (Cain et al. 1995), ingests food that is 0.22 %P in the Arboleda and 0.05 %P in the Sura-60. If similar-sized individuals (consumer tissue + gut contents) are 0.97 %P in the Arboleda and 0.62%P in the Sura-60, then the consumer body %P (with gut contents excluded) would be 1.16 %P and 0.76 %P, respectively. Even with basal food resources increasing up to five-fold in P-content, the total-P represented in consumer gut

contents is a small fraction of the total-P in the organism and should be a relatively small source of error in our measurements.

Primary consumers in many aquatic ecosystems face P-limited growth due to the large discrepancy between their body C:P ratio and the C:P ratio of their food resources (Cross et al. 2003). In contrast, the high degree of P-enrichment in basal food resources in the high-P Arboleda is likely to release consumers from P-limitation. Leaf litter C:P decreased from approximately 2000 (molar ratio) in our low-P study streams to 500 in the high-P Arboleda, much lower than Threshold Elemental Ratio ($TER_{C:P}$) values (i.e., the optimal value of food C:P for a given consumer) calculated for detritivorous aquatic invertebrates (mean ~ 1000; Frost et al. 2006). Similarly, epilithon C:P values ranged from 200 in low-P streams to 50 in the highest-P stream, relative to a mean $TER_{C:P}$ of 200 for grazing invertebrates (Frost et al. 2006). This alleviation from P-limitation in primary consumers is likely the cause of elevated growth rates that have been recorded for some primary consumers in high-P La Selva streams (Rosemond et al. 2002, Ramírez and Pringle 2006).

In contrast to prior studies, our study found that between-stream variation in P-content within each taxon greatly exceeded within-stream variation among different taxa, suggesting that environment may be as important in controlling consumer stoichiometry as phylogeny. In fact, P-content of some invertebrate taxa from our high-P stream was similar to values from some vertebrates reported by Vanni et al. (2002). Stoichiometric theory, under the assumption of strict homeostasis, has attributed variation in measured C:P values among taxa solely to differences in P-demand by these organisms (Sterner and Elser 2002). However, if consumers are not strictly homeostatic, then high C:P values measured in invertebrate consumers could also reflect P-limitation by these organisms (Hillebrand et al. 2008, Shimizu and Urabe 2008). The increase in

P-content of the entire consumer assemblage that we observed in the high-P Arboleda is consistent with this hypothesis, and adds to the weight of evidence that deviation from strict homeostasis may be a common trait in invertebrate consumers. Additionally, models predicting nutrient limitation in consumers typically assume strict homeostasis (Sterner and Elser 2002), and calculations of $TER_{C:P}$ values are highly sensitive to input values for consumer elemental composition (Frost et al. 2006). Relaxing the assumption of strict homeostasis presents challenges and opportunities for advancing our understanding of how nutrient limitation affects the growth of consumers.

The most significant ecological effects of P-enriched food resources would be expected for primary consumers, which are most likely to be P-limited (Sterner and Elser 2002). Secondary production of primary consumers in an experimentally nutrient-enriched stream was between 1.2-3.3 times higher than in a reference stream, and resulted in greater secondary production of invertebrate predators (Cross et al. 2006). Combined with increases in secondary production, elevated P-content in primary consumers would have a multiplicative effect on the amount of P moving through the food web. While nutrient limitation may be common in primary consumers, it is not limited to this trophic level (Boersma et al. 2008). Invertebrate predators, feeding on relatively low-P aquatic insects, also have the potential to be P-limited in low-P streams. The increased %P observed in invertebrate predators in the Arboleda suggests that any P-limitation affecting this trophic level was alleviated in the high-P stream. If predator taxa are ingesting excess-P in high-P streams, they should excrete this excess-P at elevated rates. Elevated P-content in aquatic invertebrates also suggests that P-export to the terrestrial ecosystem through insect emergence may be elevated, especially if combined with increases in secondary production.

In this study, we document a two-fold increase in P-content in invertebrate consumers across multiple taxonomic and functional feeding groups in a stream which has received high P-loading over millennia. Anthropogenic nutrient loading is a ubiquitous problem that is becoming exacerbated in many rivers due to the combination of increased effluent discharge and increased water withdrawals. If, as our results suggest, deviation from strict homeostasis is a common trait in freshwater invertebrates, then our study may represent a window into the future indicating how stoichiometric relationships throughout food webs may be altered by continuing anthropogenic nutrient loading in aquatic ecosystems.

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Table 2.1: Means (and ranges) of physicochemical variables in the 7 study streams along the natural P-gradient from monthly samples collected from January 2006 through August 2007. Dissolved inorganic nitrogen (DIN) = NO_3-N + NH_4-N.

Stream	DIN:SRP (molar)	SRP (µg/L)	NO_3-N (µg/L)	NH_4-N (µg/L)	Temp. (°C)	Discharge (m^3/s)	Cond. (µS/cm @ 25°C)	pH
Arboleda	2.39	135 (27-397)	126 (63-162)	20 (7-42)	25.6 (24.7-26.4)	0.17 (0.09-0.21)	257 (173-310)	6.2 (5.9-6.5)
Sura-30	4.82	83 (39-150)	163 (60-277)	18 (0-87)	25.4 (24.7-26.7)	0.61 (0.43-0.86)	157 (73-188)	6.1 (5.8-6.8)
Saltito-60	7.84	33 (1.6-87)	98 (35-170)	19 (0-64)	25.0 (24.0-25.9)	0.11 (0.04-0.20)	110 (37-170)	6.1 (5.9-6.9)
Salto-60	44.2	10 (4.3-21)	180 (101-261)	20 (0-59)	24.7 (23.7-25.6)	0.45 (0.07-0.93)	32 (28-44)	5.9 (5.6-6.6)
Saltito-100	143	3.1 (0-7.2)	163 (78-460)	37 (18-60)	24.3 (23.6-25.6)	0.03 (0.02-0.06)	19 (17-24)	5.7 (5.3-6.7)
Sura-60	174	2.8 (0-9.0)	199 (58-353)	21 (0-51)	24.8 (24.0-26.2)	0.19 (0.05-0.55)	20 (16-26)	5.6 (4.7-6.5)
Piper	219	2.3 (0-6.8)	188 (99-404)	40 (6-166)	25.0 (24.3-25.9)	0.03 (0.01-0.10)	22 (19-26)	5.5 (4.8-6.2)

Figure Legends:

Figure 2.1: Changes in mean phosphorus (P), carbon to phosphorus molar ratio (C:P), and nitrogen to phosphorus molar ratio (N:P) (± SE) of leaf litter (a,c,e) and epilithon (collected from tiles; b,d,f) from seven streams along a natural P gradient. Stream soluble reactive phosphorus (SRP) values are means from each stream studied in La Selva Biological Station, Costa Rica from January 2006–August 2007. For C:P and N:P, linear regressions were plotted for P:C and P:N, respectively, see text for explanation. Regression statistics: (a) $r^2 = 0.91$, $P = 0.0008$, (b) $r^2 = 0.83$, $P = 0.0042$, (c) $r^2 = 0.88$, $P = 0.0016$, (d) $r^2 = 0.90$, $P = 0.001$, (e) $r^2 = 0.91$, $P = 0.0007$, (f) $r^2 = 0.15$, $P = 0.46$

Figure 2.2: Frequency histograms of invertebrate body P, C, N, C:P, N:P, and C:N for high-P Arboleda (135 µg SRP L^{-1}, shaded bars) and low-P Sura-60 (2.8 µg SRP L^{-1}, open bars) streams. Summary statistics (n, mean ± SD): (a) Arboleda, 33, 1.1 ± 0.4; Sura-60, 41, 0.5 ± 0.2; (b) Arboleda, 29, 124.0 ± 83.8; Sura-60, 38, 238.8 ± 71.3; (c) Arboleda, 30, 44.4 ± 2.9; Sura-60, 37, 42.8 ± 7.7; (d) Arboleda, 30, 24.4 ± 17.6; Sura-60, 39, 47.2 ± 13.2; (e) Arboleda, 30, 10.3 ± 1.4; Sura-60, 38, 10.2 ± 0.9; (f) Arboleda, 29, 5.1 ± 0.4; Sura-60, 38, 5.1 ± 0.5

Figure 2.3: Phosphorus content (%P) of paired invertebrates of similar size from high-P Arboleda (solid circles) and low-P Sura-60 (open circles) streams. Capital letters indicate insect order: C = Coleoptera, E = Ephemeroptera, P = Plecoptera, O = Odonata, T =

Trichoptera, D = Diptera, H = Hemiptera. Numbers in parentheses indicate length of larvae in mm; taxa are arranged in order of increasing size

Figure 2.4: Phosphorus (P), carbon (C), nitrogen (N), C:P, N:P, and C:P (mean ± 1 SE) for invertebrates of four dominant insect orders in the Arboleda (shaded bars) and Sura-60 (open bars) streams. EPHE = Ephemeroptera, ODON = Odonata, PLEC = Plecoptera, TRIC = Trichoptera. Overall statistical differences in elemental composition between streams ($P < 0.05$) indicated by double asterisks (**). Differences in elemental composition within an order indicated by a single asterisk (*)

Figure 2.5: Phosphorus (P), carbon (C), nitrogen (N), C:P, N:P, and C:P (mean ± 1 SE) for invertebrates from five functional feeding groups (FFG) in the Arboleda (shaded bars) and Sura-60 (open bars) streams. SCRA = scraper, SHRE = shredder, COL-G = gathering collector, COL-F = filtering collector, PRED = predator. Overall statistical differences in elemental composition between streams ($P < 0.05$) indicated by double asterisks (**)

Figure 2.6: Comparison of strength of homeostasis (H) for primary consumers and predators from a. La Selva Biological Station (LSBS) and from a nutrient enrichment experiment at b. Coweeta Hydrologic Laboratory (CWT, Cross et al. 2003). Higher H values denote strict homeostasis, whereas low, positive values of H reflect a high sensitivity of consumer stoichiometry relative to changes in food nutrient content. Negative values

result from consumers decreasing in nutrient content in the presence of nutrient-enriched food resources. Details on H calculations are provided in Appendix A.

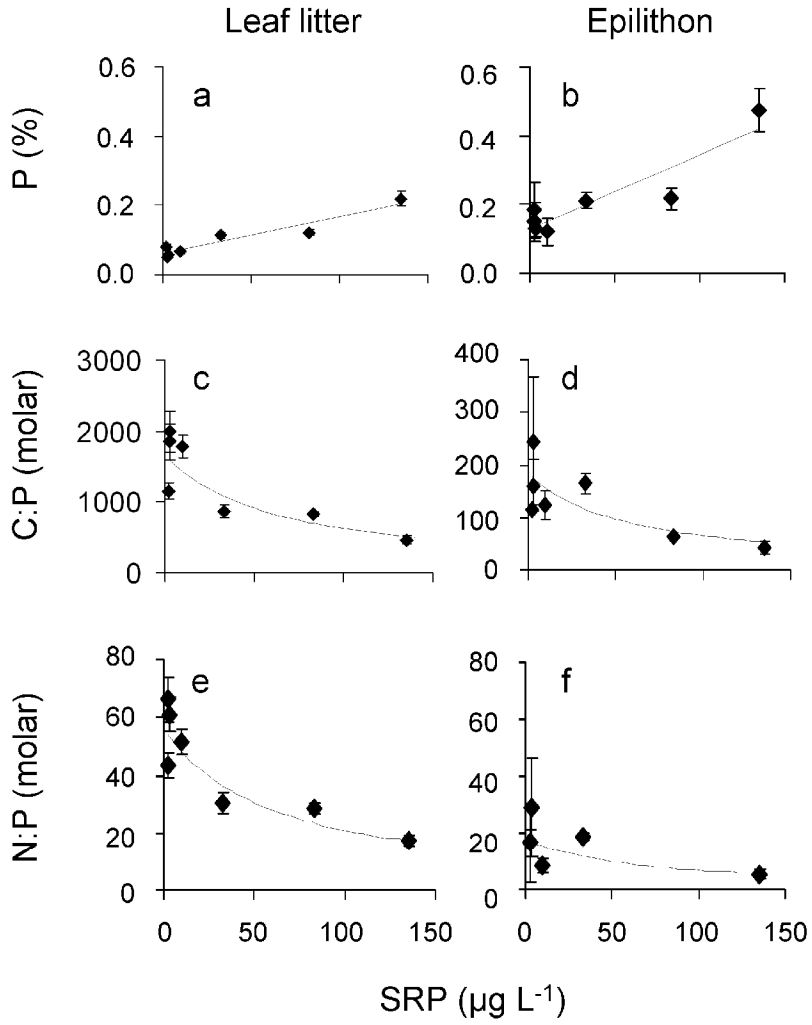
Leaf litter
Epilithon
P (%)
C:P (molar)
N:P (molar)
SRP (µg L-1)
a
b
c
d
e
f
0.6
0.4
0.2
0.0
3000
2000
1000
0
400
300
200
100
80
60
40
20
0
50
100
150

Fig. 2.1

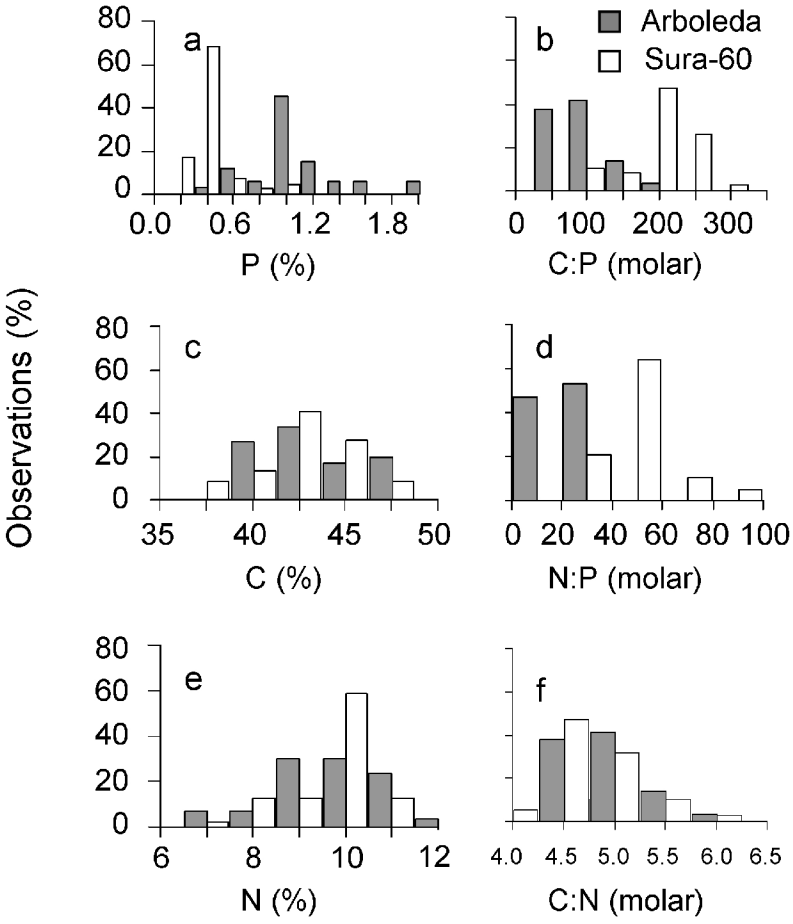
Observations (%)
a
b
c
d
e
f
Arboleda
Sura-60
P (%)
C:P (molar)
C (%)
N:P (molar)
N (%)
C:N (molar)

Fig. 2.2

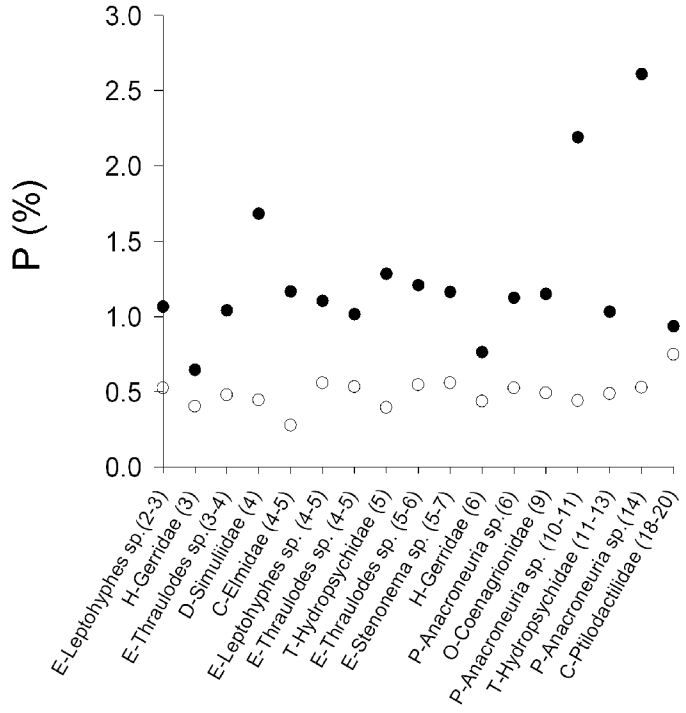
P (%)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
E-Leptohyphes sp.(2-3)
H-Gerridae (3)
E-Thraulodes sp.(3-4)
D-Simulidae (4)
C-Elmidae (4-5)
E-Leptohyphes sp. (4-5)
E-Thraulodes sp. (4-5)
T-Hydropsychidae (5)
E-Thraulodes sp. (5-6)
E-Stenonema sp. (5-7)
H-Gerridae (6)
P-Anacroneuria sp.(6)
O-Coenagrionidae (9)
P-Anacroneuria sp. (10-11)
T-Hydropsychidae (11-13)
P-Anacroneuria sp.(14)
C-Ptilodactilidae (18-20)

Fig. 2.3

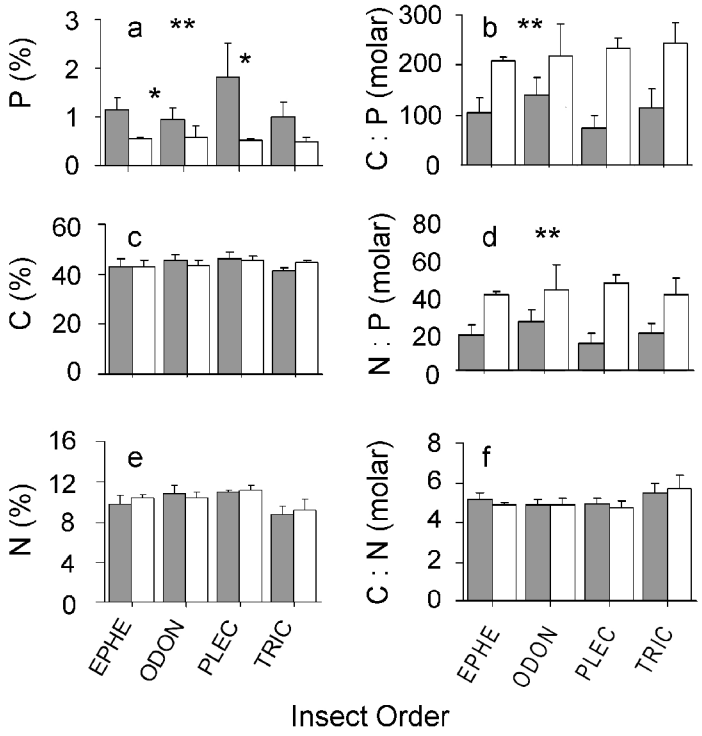
a
b
c
d
e
f
P (%)
C : P (molar)
C (%)
N : P (molar)
N (%)
C : N (molar)
EPHE
ODON
PLEC
TRIC
Insect Order

Fig. 2.4

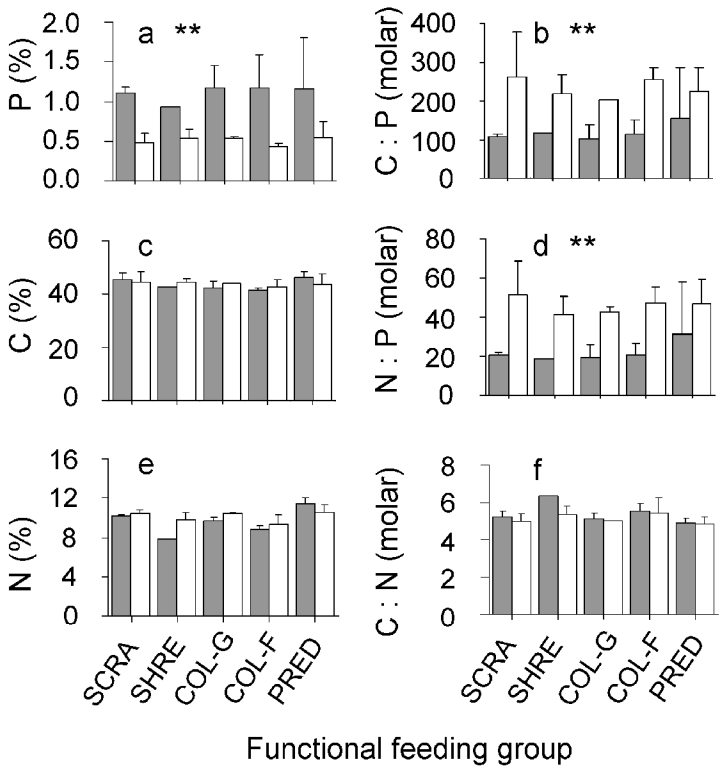
a **
b **
c
d **
e
f
P (%)
C : P (molar)
C (%)
N : P (molar)
N (%)
C : N (molar)
SCRA
SHRE
COL-G
COL-F
PRED
Functional feeding group

Fig. 2.5

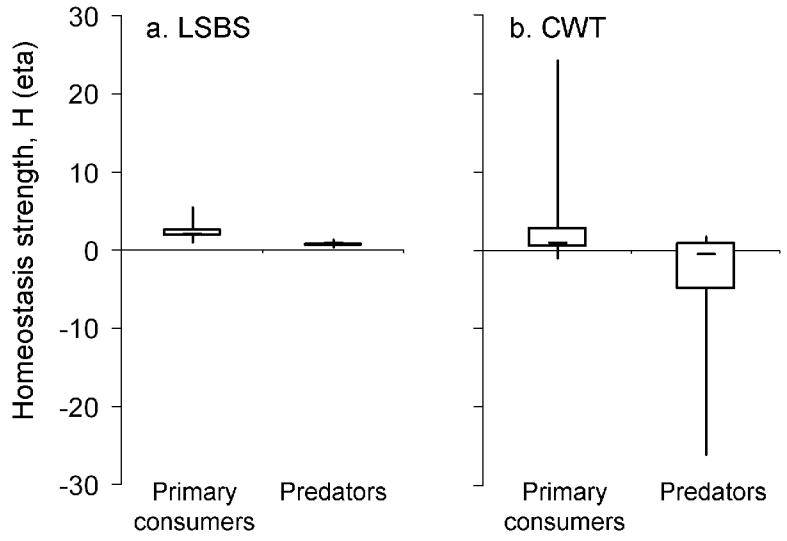
a. LSBS
b. CWT
Homeostasis strength, H (eta)
30
20
10
0
-10
-20
-30
Primary consumers
Predators
Primary consumers
Predators

Fig. 2.6

CHAPTER 3

DIFFERENCES IN PHOSPHORUS DEMAND AMONG DETRITIVOROUS CHIRONOMID LARVAE REFLECT INTER-STREAM DIFFERENCES IN FOOD RESOURCES STOICHIOMETRY [2]

[2] Small, G. E., J. P. Wares, and C. M. Pringle. To be submitted to *Limnology & Oceanography*.

Abstract

Variable nutrient loading rates across a landscape could result in inter-stream differences in the nutrient demand of primary consumers, either through shifts in species composition or by intra-specific physiological adaptations to local food quality. We tested whether larval chironomid (Chironomidae: Diptera) assemblages varied in P-demand based on local nutrient conditions using a series of streams in lowland Costa Rica that naturally range in dissolved P due to inputs of solute-rich groundwater. Chironomids collected from three high-P streams, feeding on high-P detritus, had growth rates and P-excretion rates that were similar to, or lower than, those of to chironomids collected from four low-P streams. Chironomids from a naturally low-P stream that was experimentally-ammended with P over eight years showed an increase in P-excretion rates but not growth rates, indicating an inability of these chironomids to use additional dietary P. Chironomids from a high-P stream showed greater evidence of P-limitation (lower growth rates, P-excretion rates, and RNA content) when fed low-P detritus compared to chironomids from a low-P stream. Our findings support the hypothesis that chironomid assemblages are adapted to local food quality in this heterogeneous landscape, thereby circumventing P-limitation. Genetic analysis indicates that chironomid assemblages across the study streams are similar in species composition, suggesting that differences in P-demand are due to microevolution. The lower P-demand among chironomids from low-P streams appears to limit their ability to respond to nutrient-enriched food, effectively stabilizing the food web in response to changes in nutrient availability.

Introduction

Variable rates of phosphorus (P) loading across landscapes can lead to differences in food resource P-content among streams in a watershed (e.g., Stelzer and Lamberti 2001, Cross et al. 2003, Bowman et al. 2005), creating a heterogeneous template for resource-consumer stoichiometric relationships. In low-P environments, species with lower P-requirements should be favored in competitive interactions (Tilman 1982). Simultaneously, within species, genotypes that lead to increased P-use efficiency should be favored (Jeyasingh et al. 2007). As a result of either of these mechanisms, the optimal dietary elemental ratios ($TER_{C:P}$; Frost et al. 2006) of stream consumer assemblages could reflect inter-stream differences in food resource P-content. However, despite an increasing number of studies measuring the short-term responses of individual consumer species to changes in the P-content of food resources (e.g., Frost & Elser 2002, Kyle et al. 2006, He & Wang 2007), we know relatively little about how the P-demand of entire consumer assemblages may be altered due to chronic P-loading in stream ecosystems.

Detritivores face a unique nutritional challenge because detritus is typically a nutrient-poor food resource, with carbon:phosphorus (C:P) ratios among the highest experienced by all consumers (Enriquez et at. 1993, Cross et al. 2003). Many invertebrate detritivores use a combination of physiological strategies to decrease their P-demand, including having high body C:P ratios (decreasing the imbalance between their food and their own biomass; Frost et al. 2006) and slow growth rates (resulting in a greater fraction of ingested carbon being lost through respiration; Anderson et al. 2005). Stoichiometric theory predicts that taxa with slower growth rates should have a low P-demand (and therefore be less susceptible to P-limitation) because of the smaller required investment in P-rich RNA required for protein synthesis (Sterner & Hessen 1994, Elser et al. 1996).

Chironomid larvae, which can be important detritivores in many freshwater ecosystems (Armitage et al. 1995), may be especially prone to P-limitation due to their fast growth rates (e.g. Huryn 1990; Hauer & Benke 1991; Benke 1998) and low body C:P ratios (Cross et al. 2003). As a result, selection pressure due to dietary P-availability should be high for chironomids. Although detritus is a nutrient-poor food resource in general, the P-content of detritus varies in response to stream P-availability (Cross et al. 2003, Small & Pringle 2010). Consistent with this prediction of general P-limitation, chironomid growth rates have been shown to increase in response to an experimental whole-stream nutrient enrichment (Cross et al. 2005) and along a natural P-gradient (Rosemond et al. 2001, Ramírez and Pringle 2006). However, no study to date has explicitly tested for potential differences in P-demands for larval chironomid assemblages from high- and low-P streams. Moreover, because chironomid larvae are notoriously difficult to identify to species using morphological characters, relatively little is known about how sustained P-loading may alter chironomid species composition.

To better understand how the P-demand of consumer assemblages may reflect local food quality in a heterogeneous landscape, we assessed P-limitation for larval chironomid assemblages across eight streams in lowland Costa Rica that vary widely in P levels due to natural groundwater inputs and an eight-year experimental P addition. We also measured P-demand for chironomid assemblages from high- and low-P focal streams, and evaluated whether species composition in these two streams shifts in response to P-availability. We predict that chironomid P-demand increases with stream P-levels due to a combination of species shifts and intra-specific genetic variation.

Methods

Site Description— La Selva Biological Station (10°26'N, 84°01'W) is situated on the Caribbean Slope of Costa Rica and receives almost 4000 mm of rainfall annually (Sanford et al. 1994). Geomorphological features of this landscape result in natural interbasin transfers of solute-rich groundwater entering some La Selva streams (Pringle et al. 1993). Groundwater is modified by volcanic activity at high elevations, cools as it moves downhill, and emerges at the base of ancient lava flows. These groundwater inputs are characterized by high solute concentrations (e.g. P, Na^{+}, Cl^{-}, HCO^{3-}; Pringle et al. 1993).

We chose seven streams that varied widely in average SRP concentration (2-135 μg L^{-1}) due to differential inputs of groundwater (Table 1). These streams are 2^{nd}-3^{rd} order, are all within close proximity (<2 km), and are surrounded by dense forest. Channel widths range from 1-3 m, and the dominant substrata are detritus, silt, and clay, with boulders present at some sites. These seven streams are a subset of sites used in a long-term study of the physicochemistry of La Selva streams (Pringle & Triska 1991; Triska et al. 2006), for which continuous monthly data are available since 1997.

Experiment 1: Growth and excretion rates of chironomid assemblages across a natural P gradient

From February-June 2006, we measured growth rates and phosphorus excretion rates for chironomid assemblages collected from the sites described above, feeding on detritus conditioned in those respective sites. Methods for measuring growth rate were modified from Ramírez & Pringle (2006). Chironomid growth rates were measured for each stream site in the laboratory using four chambers (8 × 10 cm) with 90-μm mesh windows that were placed in a 12-

L plastic tub filled with stream water. Stream water was collected from each source stream on the day each experiment was initiated, and was filtered through 1 mm mesh to remove large particles. Water was continuously aerated and maintained at ambient temperature throughout the experiment. We collected a water sample from each site for SRP analysis and measured pH, conductivity, and water temperature in each plastic tub during each growth trial.

Larval chironomids for growth experiments were obtained by placing *Ficus insipida* leaf packs in each of the study streams. Leafpacks were removed after 6-7 days, and chironomids (excluding the predator subfamily Tanypodinae) were removed. Length was measured to the nearest 0.1 mm under a dissecting microscope using a reference grid, to obtain initial biomass based on a length-mass regression for chironomid larvae from these streams ($r^2 = 0.68$). A group of ca. 20 larvae with an initial length of 2–4 mm were placed in a growth chamber. Thirty conditioned *Ficus insipida* leaf disks (1.5 cm) were cut from leaves incubated in the source stream for 15 days before the experiment to use as food in each chamber. After 3 days, chironomid larvae were recovered from the chambers and measured. Instantaneous growth rates (IGR) for each experimental unit (chamber) were estimated using the equation

$$IGR = (\ln Wf - \ln Wi) / t$$

Where *Wi* and *Wf* are the initial average biomass and final average biomass for chironomids in each chamber, respectively, and t is the incubation time.

Immediately following final length measurements, we measured P-excretion rates of chironomids from each growth chamber. Chironomids recovered from each growth chamber were rinsed in distilled water and placed into an acid-washed beaker containing 30 mL of distilled water. Two controls (distilled water with no chironomids added) were used with every four beakers containing chironomids. SRP values from the two controls were averaged for each

set of excretion experiments. After a 3-hour incubation period, a 20 mL water sample was removed, filtered through a 0.45 μm Millipore filter into a scintillation vial, and frozen until later SRP analysis. SRP was measured spectrophotometrically using the ascorbic acid method (APHA 1998) at the Analytical Chemistry Laboratory, University of Georgia. Excretion rate was calculated as the increase in SRP per unit chironomid biomass divided by the incubation time. While these rates likely do not accurately represent ambient chironomid excretion rates due to the potentially confounding effects of fasting and stress (Whiles et al. 2009), the excretion values should be comparable among treatments within each experiment to indicate relative P excretion rates.

After leaf disks were removed, the remainder of leaf material was dried at 50°C and analyzed for C:P ratios. For C analysis, samples were analyzed on a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan, Italy). For P analysis, samples were acid-digested (Aqua Regia double acid) and analyzed spectrophotometrically. All C:P values are reported as molar ratios.

Growth rate experiments were performed using chironomid and leaf disks from the seven sites along the natural P-gradient in both March and July 2006. Four replicate chambers were used at each site on two separate sampling dates. For statistical analysis, each sampling date at each site was treated independently. We used multiple regression analysis to examine relationships between the mean growth rate measured for each chamber and detritus C:P, with average initial mass as a covariate. P-excretion rate was also regressed against detritus C:P, with average chironomid mass as a covariate.

Experiment 2: growth and excretion of a chironomid assemblage across an experimental P gradient

Using the methodology described above, we measured growth rates and excretion rates for chironomid assemblages from six sites along a naturally low-P stream that was experimentally P-enriched over eight years. A first-order stream, the Carapa, was experimentally enriched in P from July 1997-February 2006. Dissolved phosphate was added continuously from a Mariotte bottle to increase phosphorus concentrations from background levels of <5 $\mu g\ L^{-1}$ to ~300 $\mu g\ L^{-1}$ SRP over the study reach. The whole-stream P-enrichment is described in more detail in Ramírez & Pringle (2006) and Small et al. (2008). Growth rate experiments were conducted using chironomids, conditioned *Ficus* leaves, and water from six sites along this stream (10 m upstream of the injection site, and 10 m, 50 m, 100 m, 200 m, and 500 m below the injection site) in February 2006 (during P-enrichment) and June 2006 (4 months after P-enrichment ended), creating an experimental P gradient. As described above, the four growth rate and excretion rate measurements from each site at each sampling date were regressed against detritus C:P, with chironomid mass as a covariate.

Experiment 3: Response of chironomid assemblages from a naturally high- and low-P stream to range of litter C:P

In April-June 2007, we measured the growth rates of chironomids from the highest-P site (Arboleda) and a low-P site (Sura-60) when feeding on detritus conditioned in streams across the natural P-gradient. These two streams are similar in discharge, substrate, and invertebrate assemblage (Ramírez et al. 2006), but detritus P-content is > 4-fold higher in the high-P stream (Small & Pringle 2010). Growth rate experiments were conducted as described above, except that instead of using chironomids from each site along the P-gradient, four chambers of chironomids collected from the high-P stream and 4 chambers of chironomids from the low-P

stream were incubated in water from each site, feeding on *Ficus* leaf disks conditioned in those sites. Fifteen different leaf C:P values were used, achieved by incubating *Ficus* leafpacks for 15 days in stream water ranging in dissolved P. Incubation times were decreased from 3 days to 2 days in Experiment 3, which still resulted in an average 3-fold increase in biomass but prevented pupation during the study. Leaf C and P analyses were conducted as described above.

P-excretion rates were measured for eight of the treatments from Experiment 3 using methods slightly modified from Experiment 1 and 2. Instead of distilled water, filtered water collected from the low-solute Sura-60 was used for all excretion trials, and the length of incubation time in excretion trials was decreased from 3 hours to 1 hour. Following the excretion measurements, most chironomids were analyzed for body C:P ratios. Due to the small size of individuals, chironomids from each treatment were combined in a single composite sample.

RNA content was measured as % RNA by dry weight for 8 individual chironomids ranging in size for each of 8 different food C:P treatments. Nucleic acids were measured on frozen individual insects using protocols modified from methods used for zooplankton (Wagner et al. 1998; Gorokhova & Kyle 2002). DNA and RNA were quantified by extraction with N-laurylsarcosine, followed by sonication and staining with Ribogreen® (Molecular Probes, Eugene, OR). Nucleases were used to determine DNA and RNA separately.

Multiple regression was used to test for the effects of detritus C:P, average initial size, and chironomid identity (i.e. collected from high-P stream or low-P stream) on growth rates, P-excretion rate, chironomid C:P, and RNA content. All statistical analyses were conducted using the PROC GLM procedure in SAS (version 9.2), with Type III Sums of Squares.

Genetic Characterization of Chironomid Assemblage

In June 2007, chironomid larvae from *Ficus* leafpacks in each of the two focal streams (14 from Sura-60 and 5 from Arboleda) were reared to adults for taxonomic identification. In March 2009, approximately 80 chironomids from each of the two focal streams were collected from *Ficus* leafpacks and were preserved in molecular-grade ethanol for DNA sequencing. DNA was extracted from these larvae, as well as from adult specimens that had been previously identified to species, following the methods used to sequence chironomid DNA by Sinclair and Gresens (2008). A 650-bp fragment of the *COI* mitochondrial gene was amplified using the primers 911 (5'-TTTCTACAAATCATAAAGATATTGG-3') and 912 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Guryev et al. 2001). DNA was amplified in the following 25 µL reaction: 5 µL of PCR buffer, 5 µL of $MgCl_2$, 2 µL of DNTP, 1 µL of each primer, 0.15 µL of *Taq* polymerase, and 1 µL of template DNA. The PCR thermocycling program used an annealing temperature of 40°C. PCR products were verified by electrophoresis, and were sequenced in one direction (using the 911 primer) on an ABI 3730xl automated sequencer at the Georgia Genomics Facility. Quality-trimmed nucleotide sequences of ca. 550-bp were aligned using CodonCode ALIGNER. 2.0.4 (CodonCode Corporation). Sites with a PHRED score (Ewing and Green 1998) <20 were coded as ambiguous.

Genetic distances were calculated using the Kimura-2-parameter (K2P) distance model (Kimura, 1980). Neighbor-joining and maximum parsimony trees of K2P distances were created using PAUP* 4.0b10 (Swofford 2002). Nonparametric bootstrap analysis was performed with 100 replicates for maximum parsimony. We identified species as clades with K2P distances <0.06, a criterion previously used in other chironomid taxa (Sinclair and Gresens 2008). We

then used the χ^2 statistic to test for differences in species distributions between the two focal streams.

Intraspecific genetic variance for each of the common species was analyzed within and between the two study sites using the Snn "nearest neighbor" statistic of Hudson (2000). Snn is a sequence-based statistic that measures how often "nearest neighbor" sequences (haplotypes that are most similar to one another) are found in the same geographic location. Significance of the nearest-neighbor statistic was determined via permutational test (1,000 replicates) using DNASP v.5.0 (Librado and Rozas 2009). We also calculated summary statistics including haplotype diversity (Hd) and nucleotide diversity (π).

Results

Experiment 1: Growth and excretion rates of chironomid assemblages across a natural P gradient

Across the natural P-gradient in the 2006 experiments, *Ficus* leaf litter C:P ranged from 268-1397 (Fig. 3.1). Chironomid growth rates were high, with a mean of 0.39 mg mg^{-1} d^{-1} (range: 0.18–0.42 mg mg^{-1} d^{-1}). Average initial chironomid mass in each chamber explained 16% of variance in growth rates. Chironomids from low-P streams feeding on low-P food grew at a marginally faster rate ($F_{1,43} = 3.75$, $p = 0.059$, $r^2 = 0.23$; Fig. 2A) and excreted P at a higher rate ($F_{1,43} = 6.40$, $p = 0.0153$, $r^2 = 0.14$; Fig. 2B) compared to chironomids from high-P streams feeding on high-P detritus. Pupae and adult chironomids were observed in some of the growth chambers at the end of the 3-day growth trials (mean < 1, but a maximum of 7 out of 20 in a chamber). The number of pupae and adult chironomids observed in growth chambers increased

with increasing litter P-content ($F_{1,43} = 30.76$, $p < 0.001$, $r^2 = 0.42$), while average initial larval size was not a significant factor ($F_{1,43} = 0.28$, $p = 0.6023$).

Experiment 2: growth and excretion of a chironomid assemblage across an experimental P gradient

Across the experimental P-gradient, *Ficus* leaf litter C:P ranged from 417-1933 (Fig. 3.1). Chironomids had a mean growth rate of 0.27 mg mg^{-1} d^{-1} (range: 0.19–0.37 mg mg^{-1} d^{-1}). Initial size explained 11% of variance in measured growth rates. Litter P-content had no effect on growth rates ($F_{1,44} = 1.18$, $p = 0.28$, $r^2 = 0.18$; Fig. 3A) after accounting for average initial size ($F_{1,44} = 7.93$, $p = 0.0073$). However, chironomid P-excretion increased significantly with increasing leaf litter P-content ($F_{1,42} = 48.67$, $p < 0.0001$, $r^2 = 0.54$) after accounting for variation in average larval biomass ($F_{1,42} = 1.21$, $p = 0.28$; Fig. 3B). The number of pupae and adults at the end of the three-day growth trials decreased with increasing leaf litter P-content ($F_{1,44} = 3.87$, $p = 0.035$, $r^2 = 0.18$) after accounting for initial average biomass ($F_{1,44} = 3.87$, $p = 0.056$).

Experiment 3: Response of chironomid assemblages from a naturally high- and low-P stream to range of litter C:P

Ficus leaf litter C:P ranged from 426-1884 across all treatments in the 2007 experiment (Fig. 3.1). Chironomid growth rates in this experiment had an overall mean of 0.21 mg mg^{-1} d^{-1} (range: 0.05–0.35 mg mg^{-1} d^{-1}). Initial size of larvae in each chamber explained 44% of variance in growth rates ($F_{1,112} = 98.13$, $p < 0.0001$).

The chironomid assemblage from the high P stream showed increasing growth rates with increasing litter P-content, while the chironomid assemblage from the low-P stream showed no

response (chironomid identity × leaf litter C:P: $F_{1,112} = 4.15$, $p = 0.044$; Fig. 3.4A). Measured P-excretion rates in Experiment 3 were highly variable and showed no statistical relationship to leaf litter C:P ($p > 0.05$; $r^2 = 0.15$). However, for all leaf litter C:P values > 700, chironomids from the high-P stream showed lower rates of P excretion compared to chironomids from the low-P stream (Fig. 3.4B).

Chironomid biomass C:P increased with increasing detritus C:P ($F_{1,22} = 6.24$, $p = 0.02$, $r^2 = 0.23$; Fig. 3.5A), but there was no difference in this relationship between chironomids from the high-P stream and low-P stream (chironomid identity $p = 0.87$; identity × leaf C:P $p = 0.91$). However, RNA content was higher for chironomids from the low-P stream across the range of detritus C:P in this study ($F_{1,120} = 22.24$, $p < 0.0001$, $r^2 = 0.48$; Fig. 3.5B). Chironomid RNA content was negatively related to size of the individual ($F_{1,120} = 32.77$, $p < 0.001$) and leaf C:P ($F_{1,120} = 15.18$, $p = 0.0002$) but the chironomid source × leaf C:P interaction term was non-significant ($F_{1,120} = 1.77$, $p = 0.1857$). When data from both chironomid assemblages were considered together, chironomid RNA content was a significant predictor of size-corrected growth rates ($F_{1,14} = 6.4014$, $p = 0.0021$, $r^2 = 0.31$; Fig. 3.6).

Genetic Characterization of Chironomid Assemblage

Three chironomid species were identified from the high-P Arboleda and low-P Sura-60 streams: *Polypedilum pterospilus* Townes (1 in Arboleda, 2 in Sura-60), *Endotribelos* cf. *hesperium* (Sublette) (1 in Arboleda, 7 in Sura-60), and an undescribed species of *Endotribelos* (3 in Arboleda, 5 in Sura-60; J. H. Epler, personal communication).

We successfully sequenced mtCOI DNA from 146 chironomid larvae from the two focal streams, as well as 5 adult chironomids that were identified to species (Genbank GU565707–

GU565917). 752 total characters were evaluated in PAUP from the 650 bp fragment (some sites had multiple substitutions). Of these, 511 characters were constant, 41 characters were parsimony-uninformative, and 200 characters were parsimony-informative.

Across the 146 chironomid larvae sequenced, 129 (88%) fell into four species (defined by monophyletic groups of divergence <6%; Fig. 7). We were unable to provide unambiguous associations between sequenced adults that had been identified based on morphological characteristics, and the phylogeny-defined species. For this reason, we only refer to the phylogeny-defined species by letters rather than by species names.

Based on these sequencing data, community composition is similar between these two focal streams ($\chi^2 = 3.77$, 2 d.f., $p > 0.25$). Of the 69 chironomid larvae sequenced from the low-P Sura-60, 5 (7%) were identified as Species A, 8 (12%) as Species B, 12 (17%) as Species C, and 34 (49%) as Species D. Of the 77 larvae sequenced from the high-P Arboleda, 6 (8%) were identified as Species A, 14 (18%) as Species B, 21 (27%) as Species C, and 29 (38%) as Species D. Within each of these dominant species, we found no evidence of phylogenetic separation between the two focal streams. For Species B, C, and D, the Snn statistic has p-values > 0.25 (Table 2). A single haplotype was found for Species A, so Snn cannot be calculated for this group.

Discussion

Our results support the hypothesis that chironomid P-demand reflects local food quality across a landscape characterized by nutrient heterogeneity. Chironomids from a naturally high-P stream showed evidence of P-limitation when fed low-P detritus, while chironomids from naturally low-P streams showed no evidence of P-limitation. Our results appear to contrast with

prior studies in streams at La Selva that reported increases in chironomid growth rates with increasing stream P-levels (Rosemond et al. 2001, Ramírez and Pringle 2006). However, these studies used chironomid larvae collected from a single stream (intermediate in dissolved P levels) and did not measure the P-content of food resources. In our study, we explicitly considered the potential for variation in chironomid P-demand across a stoichiometrically heterogeneous landscape.

Several lines of evidence from our study indicate that chironomids from naturally low-P streams are not P-limited when feeding on high C:P detritus. First, chironomid assemblages from low-P streams, feeding on high C:P detritus, showed marginally *higher* growth rates and P-excretion rates compared to chironomids from high-P streams that fed on low C:P detritus (Experiment 1). These results suggest that the chironomids in our low-P study streams that feed on high C:P detritus are ingesting more P than is required for biomass production. Chironomid larvae in the experimentally enriched stream showed no change in growth rates, but a sharp increase in P-excretion rates, when feeding on low C:P detritus (Experiment 2), suggesting that the chironomids in this historically low-P stream are not capable of converting the extra P into new biomass. Finally, the chironomid assemblage from the low-P focal stream showed no changes in growth rates over a wide range of detritus C:P (Experiment 3). The fact that chironomid growth rates in Experiment 3 were consistent across the 4-fold range in detritus C:P also indicates that this chironomid assemblage that typically feeds on low-P detritus can tolerate high-P detritus with no apparent negative effects (*sensu* Boersma and Elser, 2006).

Our results also indicate that P-demand is higher among chironomids in naturally high-P streams. The relatively low growth rates and P-excretion rates observed for chironomids from high-P streams feeding on high-P detritus (Experiment 1) suggest that their P-intake may have

been insufficient for the demands of biomass production, even while feeding on high-P food resources. Additionally, compared to chironomids from the low-P focal stream, chironomids from the high-P focal stream showed decreases in growth rates and lower P-excretion rates when fed detritus with C:P > 800 (Experiment 3).

The differences if P-demands among chironomids from high- and low-P streams appear to be due to altered P-allocation. Within each assemblage, RNA content declines with increasing food C:P, consistent with other studies on invertebrate consumers (Schade et al. 2003, Elser et al. 2005), and, for chironomids of a given size, RNA content is a good predictor of growth rate, consistent with the Growth Rate Hypothesis (Elser et al. 1996). However, across the range of detritus C:P examined in this study, chironomids from the low-P stream had consistently higher levels of RNA (Fig. 3.5).

To illustrate how P is differentially allocated with changes in dietary P-content for chironomids from high- and low-P streams, we modeled the contribution of RNA and DNA to the total P-content of a chironomid with a mass of 0.05 mg, typical for our study (Fig. 3.8), based on regressions of total body P, %RNA, and %DNA versus detritus C:P (with body size as covariate) for both chironomid assemblages. %RNA and %DNA were converted to RNA-P and DNA-P by assuming that nucleic acids are 8.7% P by mass (Sterner & Elser 2002). For a chironomid from the high-P stream feeding on high-P detritus, RNA accounts for 30% of its body P, compared to 35% when feeding on low-P food. For chironomids from the low-P focal stream, RNA-P increases proportionately with body P, accounting for approximately 46% P across all food P-levels. In all cases, DNA accounts for approximately 3% of body P.

The differences in P-allocation between chironomid assemblages suggests that chironomids from the low-P stream have a lower non-RNA P-demand, so that for a given food

C:P value, these chironomids would have more P remaining to allocate to RNA. Kyle et al. (2006) found that different zooplankton taxa have different maintenance costs of RNA and P; similarly, chironomids in low-P La Selva streams may have higher P-use efficiency due to lower baseline P-demands. In addition to altered P-allocation, it is also possible that chironomids in low-P streams have adopted a combination of behavioral and physiological strategies to mitigate the elemental imbalance in their food, such as selectively feeding on patches of leaves colonized by nutrient-rich microbes (Arsuffi & Suberkropp 1985), compensatory feeding to increase total P-intake, higher assimilation of P (He & Wang 2007), or compensatory respiration to burn off excess C (He & Wang 2008).

The apparent higher P-demand for chironomids from high-P streams could be due to differences in P-storage. For example, elevated levels of non-nucleic acid P have been documented in other invertebrates feeding on P-enriched food as a result of P-storage in hemolymph (Woods et al. 2002) or in metal-containing granules along the digestive tract (Hopkin 1989). The higher levels of non-RNA P-storage in chironomids from high-P streams could lead to increased fitness in that environment. For example, even though larval growth rates were marginally lower for chironomids in high-P streams (Experiment 1; Fig. 3.2A), we found indirect evidence of more rapid larval development (higher numbers of pupae and adults found at the end of the three-day growth trials), as has been observed in other insect taxa feeding on P-enriched food resources (Perkins et al. 2004).

Differences in P-demand among chironomid assemblages could potentially be attributable to differences in species composition, intra-specific genetic differences, or phenotypic plasticity, but our results allow us to eliminate several possible mechanisms. The similar species composition between the two focal streams leads us to rule out species shifts as

the primary driver of the different physiological responses to food P-content. At the individual level, another possible mechanism is differences in gene expression during early developmental stages (i.e., before larvae were collected for use in our experiments) determining P-demand in later instars (Jeyasingh and Weider 2007), but this explanation is inconsistent with the lack of response by chironomids in the experimentally P-enriched stream. Transgenerational phenotypic effects of food quality (through maternal nutrition) have been documented in some invertebrates (Frost et al. 2010), but this explanation is also inconsistent with our results, as it would imply that chironomid larvae from high-P streams would generally be less prone to P-limitation.

By ruling out alternative explanations, our results suggest that genetic adaptations within populations may be responsible for the observed differences. Many populations have considerable standing genetic variation for P-physiology, and microevolutionary responses to spatial or temporal P-supply have been observed in other systems (reviewed in Jeyasingh et al. 2007). For example, a similar genotype × environment interaction creates a competitive tradeoff in *Daphnia*, based on genetic variation at the phosphoglucose isomerase locus (Jeyasingh et al. 2009). Although we found no phylogenetic divergence between populations based on the (presumably neutral) mitochondrial DNA sequences, a small amount of gene flow between populations would be sufficient to maintain this homogeneity, and is not unlikely given that the sample sites are separated by only 1500 m. Local genetic adaptation can occur despite gene flow between populations, for example, as a result of genotype-specific mortality (e.g., Schmidt and Rand 2001). If microevolution explains the differences among chironomid assemblages in the naturally high- and low-P streams, the fact that no differences in P-demand were observed after eight years of experimental P-enrichment in the Carapa (corresponding to hundreds of generations) would seem to suggest a low heritability of this trait. However, the lack of genetic

response by chironomids in the Carapa could also be explained by the fact that, due to high rates of uptake of dissolved P (Small et al. 2008), only ~100 m of the stream experienced consistently high-P conditions, effectively decreasing the selection pressure on this population. In order to unequivocally show intra-specific genetic variation for P-demand, and that such P-linked traits determine the spatial assortment of genotypes as a result of ecosystem P-supply, future research should examine polymorphic loci in these populations, and test whether genotypes within each species from high- and low-P streams are competitively dominant within their respective environments.

Regardless of the exact mechanism relating chironomid P-demand to local food resource quality, our finding that chironomid larvae in the low-P streams were not P-limited has important ecological implications. The correspondence between chironomid P-demand and local food quality across this heterogeneous landscape could serve to stabilize the food web against perturbations caused by nutrient loading. In streams where primary consumers are P-limited, nutrient loading can have important effects at multiple trophic levels. For example, in a detritus-based temperate stream, a two-year nutrient (N+P) addition resulted in a 1.5-fold increase in growth rates and a three-fold increase in production of larval chironomids (Cross et al. 2005). This increased production of chironomids, along with other short-lived primary consumers, led to a decrease in leaf litter standing stock, and increased abundance and biomass of predators during the initial years of the experiment (Cross et al. 2006). By contrast, our study found that chironomids from low-P streams did not respond to increased P-availability. In both experiments 2 and 3, chironomids from naturally low-P streams showed no increase in growth when feeding on low C:P detritus. Furthermore, no increases in chironomid biomass were observed in the experimentally P-enriched stream over the course of the eight-year study

(Ramírez & Pringle 2006). The lower P-demand of chironomids in low-P streams appears to limit the ability of these consumers to respond to nutrient-enriched food. Because chironomid larvae are the dominant group of detritivores in these study streams, this limited physiological response may act to stabilize the detritus-based food web against changes in nutrient availability.

In summary, in spite of the apparent elemental imbalance between fast-growing chironomids and low-P detritus, assemblages of larval chironomids in low-P streams showed no evidence of P-limitation under ambient conditions, as a result of their lower P-demand relative to chironomid assemblages in nearby high-P streams. While ecological stoichiometry theory has considered a consumer's nutrient demand to be a species-level property (Frost et al. 2006), our results showing differences in P-demand among similar assemblages suggest that microevolution may cause differences in nutrient demand among populations. Anthropogenic nutrient pollution, through land-use change, fertilizer runoff, and wastewater discharge, can all lead to landscape-scale heterogeneity in stream basal food resource nutrient content, and, over time, may lead to similar responses in other stream consumers.

Acknowledgements

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Table 3.1: Physical and chemical properties of streams used in this study. Values are means (and ranges) from monthly data collected in 2006-2007. Dissolved phosphorus in streams is reported as soluble reactive phosphorus (SRP). The Carapa-60 was experimentally enriched in P from 1998-2006. Background SRP is reported for this site; SRP concentrations for the P-enriched reach varied with distance from the injection site, with a maximum concentration of 91 μg L^{-1} during the current study.

Stream	SRP (μg/L)	NO3-N (μg/L)	NH4-N (μg/L)	Temp. (°C)	Discharge (m^3/s)	Cond. (μS/cm @ 25°C)	pH
Arboleda-30	135 (27-397)	126 (63-162)	20 (7-42)	25.6 (24.7-26.4)	0.17 (0.09-0.21)	257 (173-310)	6.2 (5.9-6.5)
Sura-30	83 (39-150)	163 (60-277)	18 (0-87)	25.4 (24.7-26.7)	0.61 (0.43-0.86)	157 (73-188)	6.1 (5.8-6.8)
Saltito-60	33 (1.6-87)	98 (35-170)	19 (0-64)	25 (24.0-25.9)	0.11 (0.04-0.2)	110 (37-170)	6.1 (5.9-6.9)
Salto-60	10 (4.3-21)	180 (101-261)	20 (0-59)	24.7 (23.7-25.6)	0.45 (0.07-0.93)	32 (28-44)	5.9 (5.6-6.6)
Saltito-100	3 (0-7)	163 (78-460)	37 (18-60)	24.3 (23.6-25.6)	0.03 (0.02-0.06)	19 (17-24)	5.7 (5.3-6.7)
Sura-60	3 (0-9.0)	199 (58-353)	21 (0-51)	24.8 (24.0-26.2)	0.19 (0.05-0.55)	20 (16-26)	5.6 (4.7-6.5)
Piper-30	2 (0-6.8)	188 (99-404)	40 (6-166)	25.0 (24.3-25.9)	0.03 (0.01-0.10)	22 (19-26)	5.5 (4.8-6.2)
Carapa-60	6 (0-17)	176 (131-220)	23 (2.5-132)	25 (23.0-26.0)	0.02 (0.01-0.073)	18.7 (12.8-55.5)	5.58 (5.13-6.46)

Table 3.2: Number of chironomid larvae sequenced (n), haplotype diversity (Hd), and nucleotide diversity (π) for each of the four common species detected in the low-P Sura-60 stream and the high-P Arboleda stream. The non-parametric Snn statistic, and accompanying P-value, is shown for Species B-D. Because we found a single haplotype of Species A, Snn cannot be calculated for this species.

	n	Hd	π	Snn	P
Species A					
Sura-60	5	0.000	0.000		
Arboleda	6	0.000	0.000		
Species B					
Sura-60	8	0.429	0.001	0.515	0.543
Arboleda	14	0.143	0.000		
Species C					
Sura-60	12	0.000	0.000	0.546	0.204
Arboleda	21	0.338	0.016		
Species D					
Sura-60	34	0.731	0.017	0.52	0.263
Arboleda	29	0.564	0.014		

Figure Legends:

Fig. 3.1: Relationship between stream soluble reactive phosphorus (SRP) levels and detritus C:P used in the three experiments.

Fig. 3.2: (A) Size-corrected chironomid growth rates (residual of instantaneous growth rate vs. average initial larval size for each treatment) vs. detritus C:P for *in situ* chironomid assemblages along the natural-P gradient in Experiment 1. (B) Size-corrected P-excretion rates (residual of P-excretion rate vs. average size of larvae in each replicate) vs. detritus C:P along the natural P-gradient in Experiment 1. Error bars represent 1 SE.

Fig. 3.3: (A) Size-corrected chironomid growth rates (residual of instantaneous growth rate vs. average initial larval size for each treatment) vs. detritus C:P along the experimental P-gradient in Experiment 2. (B) Size-corrected P-excretion rates (residual of P-excretion rate vs. average size of larvae in each replicate) vs. detritus C:P along the experimental P-gradient in Experiment 2. Error bars represent 1 SE.

Fig. 3.4: (A) Size-corrected chironomid growth rates (residual of instantaneous growth rate vs. average initial larval size for each treatment) vs. detritus C:P for chironomids collected from a high-P stream (shaded diamonds) and a low-P stream (open squares) in Experiment 3. (B) Size-corrected P-excretion rates (residual of P-excretion rate vs. average size of larvae in each replicate) vs. detritus C:P for chironomids collected from a high-P and low-P stream. Error bars represent 1 SE.

Fig. 3.5: Chironomid (A) body C:P and (B) size-correced RNA content vs. detritus C:P for chironomids collected from a high-P stream (shaded diamonds) and low-P stream (open squares) in experiment 3. Size-corrected RNA content is calculated as residual of % RNA by dry mass vs. mass of individual larvae. Error bars represent 1 SE.

Fig. 3.6: Size-corrected growth rate vs. size-corrected RNA-content for chironomids collected from high-P stream (shaded diamonds) and low-P stream (open squares) in Experiment 3. Error bars are not shown for clarity.

Fig. 3.7: Phylogram of 146 chironomid larvae from low-P Sura-60 (open symbols) and high-P Arboleda (shaded symbols) based on mitochondrial COI DNA sequences. Species are defined by K2P divergence of <6%.

Fig. 3.8: Estimated contribution of RNA and DNA to total P content of a chironomid (mass 0.05 mg) from (A) high-P stream and (B) low-P stream, across range of detritus C:P, based on modeled relationships between %RNA, %DNA, and total %P vs. detritus C:P for each assemblage.

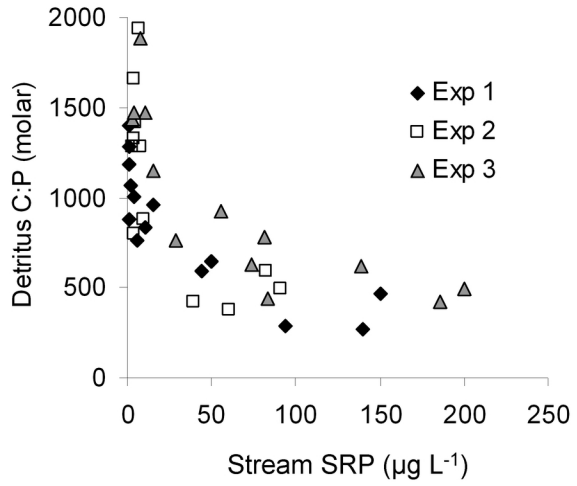
2000
1500
1000
500
0
Detritus C:P (molar)
0
50
100
150
200
250
Stream SRP (µg L-1)
Exp 1
Exp 2
Exp 3

Fig. 3.1

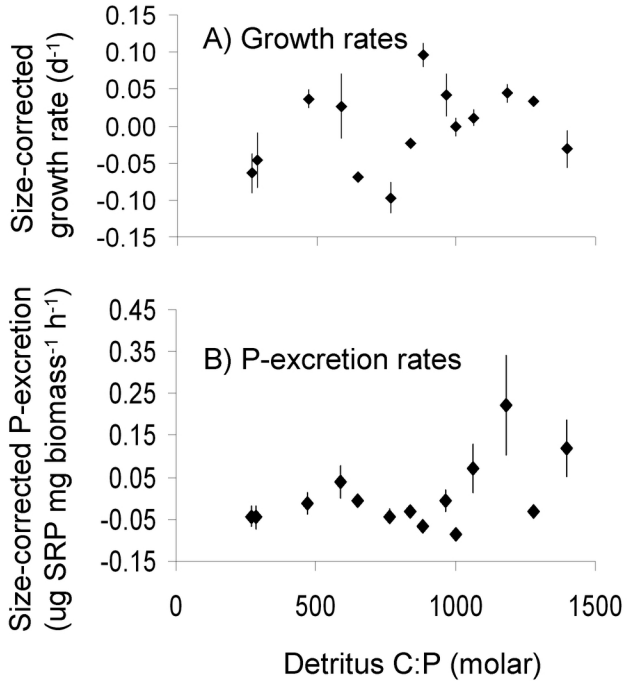
A) Growth rates
Size-corrected growth rate (d-1)
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
B) P-excretion rates
Size-corrected P-excretion (ug SRP mg biomass-1 h-1)
0.45
0.35
0.25
0.15
0.05
-0.05
-0.15
0
500
1000
1500
Detritus C:P (molar)

Fig. 3.2

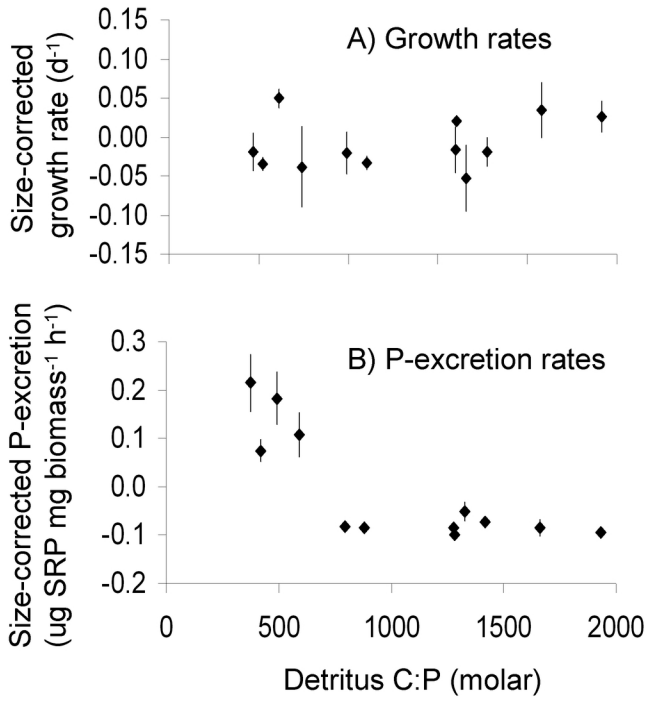
A) Growth rates
Size-corrected growth rate (d-1)
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
B) P-excretion rates
Size-corrected P-excretion (ug SRP mg biomass-1 h-1)
0.3
0.2
0.1
0.0
-0.1
-0.2
0
500
1000
1500
2000
Detritus C:P (molar)

Fig. 3.3

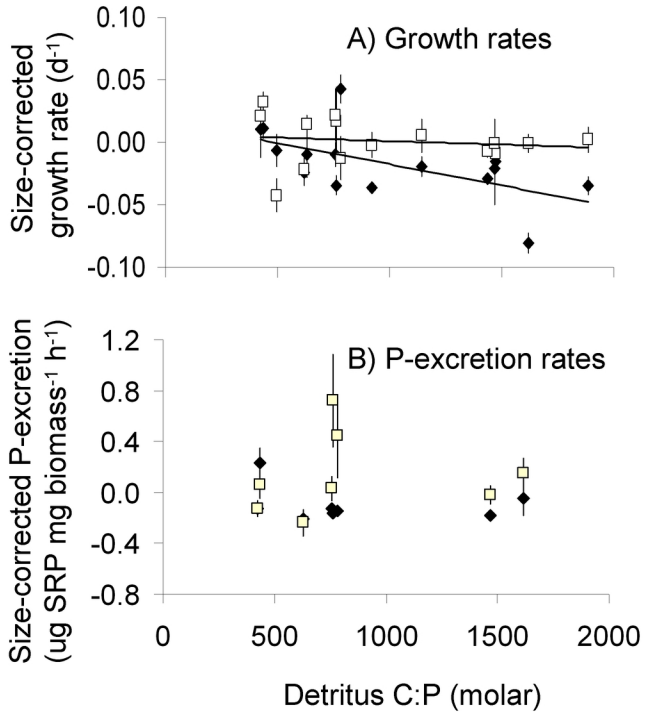
A) Growth rates
Size-corrected growth rate (d-1)
0.10
0.05
0.00
-0.05
-0.10
B) P-excretion rates
Size-corrected P-excretion (ug SRP mg biomass-1 h-1)
1.2
0.8
0.4
0.0
-0.4
-0.8
0
500
1000
1500
2000
Detritus C:P (molar)

Fig. 3.4

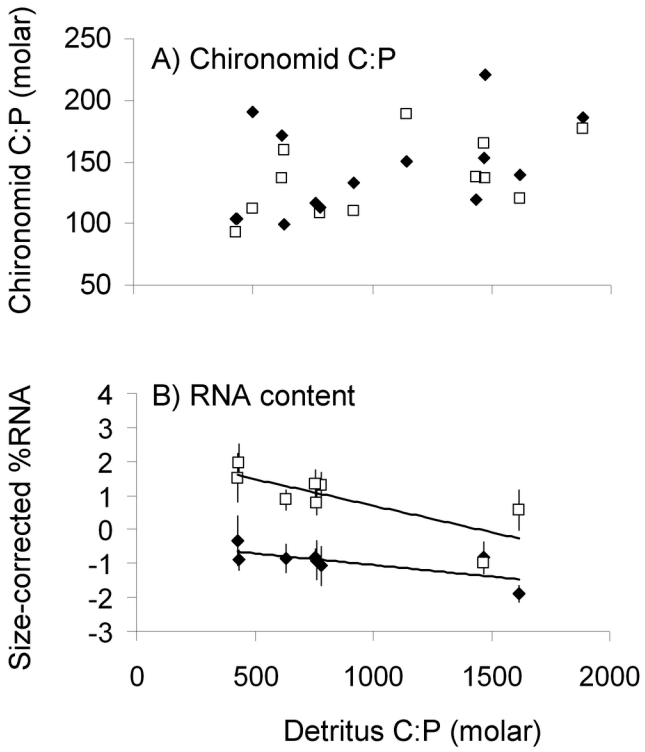
A) Chironomid C:P
Chironomid C:P (molar)
250
200
150
100
50
B) RNA content
Size-corrected %RNA
4
3
2
1
0
-1
-2
-3
0
500
1000
1500
2000
Detritus C:P (molar)

Fig. 3.5

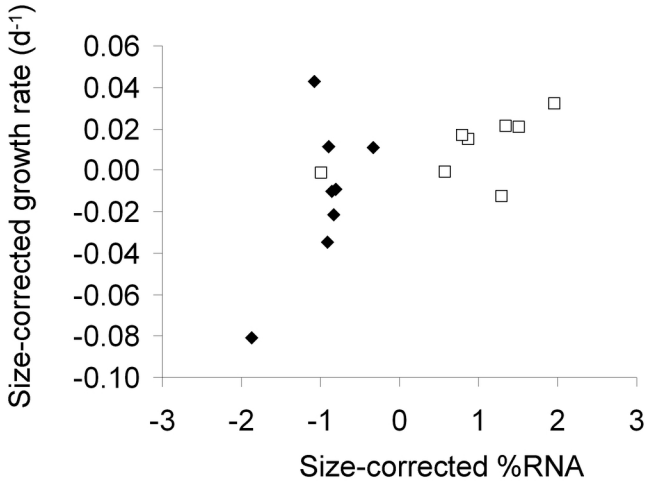
Size-corrected growth rate (d-1)
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-3
-2
-1
0
1
2
3
Size-corrected %RNA

Fig. 3.6

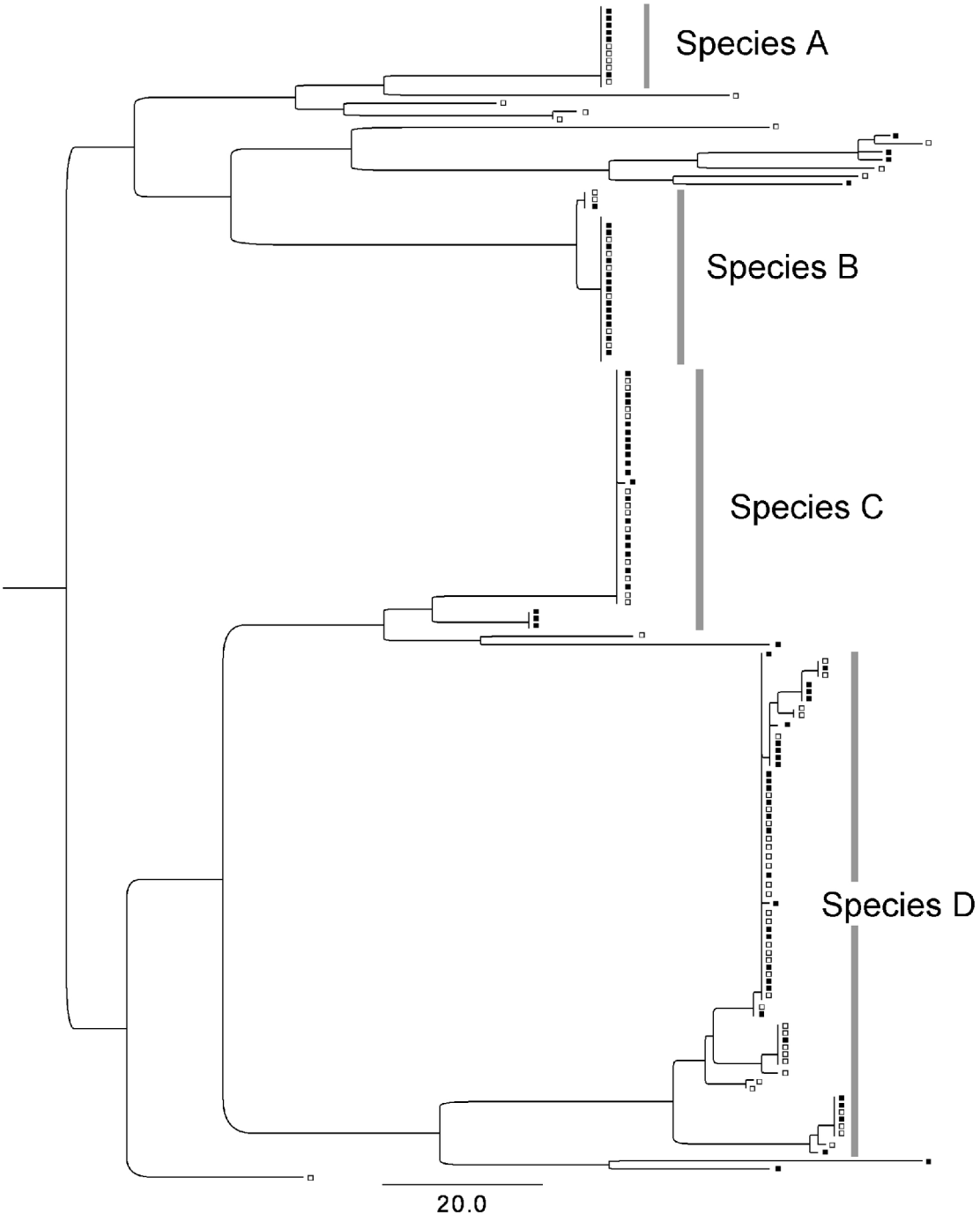

Fig. 3.7

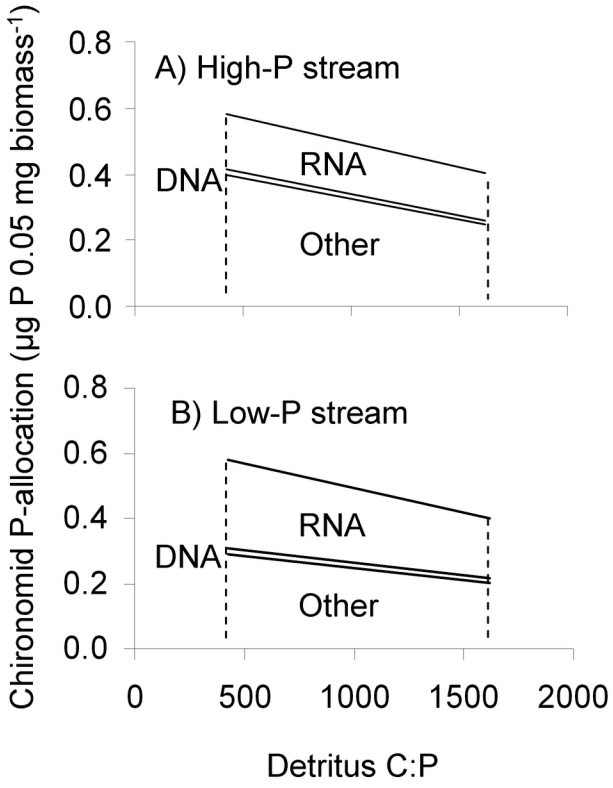
A) High-P stream
B) Low-P stream
RNA
DNA
Other
Chironomid P-allocation (µg P 0.05 mg biomass-1)
Detritus C:P
0.0
0.2
0.4
0.6
0.8
0
500
1000
1500
2000

Fig. 3.8

CHAPTER 4

EMERGENT ROLE OF THE FISH *ASTYANAX AENEUS* (CHARACIDAE) AS A KEYSTONE NUTRIENT RECYCLER IN LOW-NUTRIENT NEOTROPICAL STREAMS, COSTA RICA[3]

[3] Small, G. E., M. Pyron, J. H. Duff, and C. M. Pringle. Submitted to *Ecology*, 1/13/2010.

Abstract

Nutrient recycling by consumers is a potentially important biogeochemical process in both terrestrial and aquatic ecosystems. Stoichiometric traits of individual species may result in some taxa playing disproportionately important roles in the recycling of nutrients relative to their biomass, acting as keystone nutrient recyclers. Here we examine factors controlling the relative contribution of twelve Neotropical fish species to nutrient recycling in four streams spanning a range of phosphorus (P) levels. In high P conditions (135 μg L^{-1} soluble reactive phosphorus, SRP), most species showed similarly high P excretion rates. In low P conditions (3 μg L^{-1} SRP), most species had greatly reduced P excretion rates. However, *Astyanax aeneus* (Characidae), which represented only 12% of the total population and 18% of the total biomass of the fish assemblage in our focal study stream, had P excretion rates > 10-fold higher than other abundant fishes. As a result, we estimate that P excretion by *A. aeneus* accounted for 90% of the P recycled by this fish assemblage, and also supplied approximately 90% of the stream P demand in this P-limited ecosystem. Nitrogen excretion rates showed little variation among species and the contribution of a given species to ecosystem N recycling was largely dependent on the total biomass of that species. Our results indicate that the importance of species identity in determining nutrient recycling rates is context dependent since *A. aeneus* only appear to be a major contributor to P recycling under low P conditions. Species that have relatively low body P content and feed on high P diets are most likely to maintain high P excretion rates in low P environments, and thus play a disproportionately important role in ecosystem P recycling. Because of the high variability in P excretion rates among fish species, ecosystem-level P recycling could be particularly sensitive to changes in fish community structure in P-limited

systems. By contrast, consumer-driven N recycling is less sensitive to species identity and therefore should be more buffered against species losses.

Introduction

Ecologists have identified a number of species that can have disproportionately large effects on certain ecosystems, relative to their biomass (keystone species, *sensu* Power et al. 1996). However, most research in this area has focused on species' roles in trophic interactions (e.g., Paine 1966) rather than in recycling nutrients (but see Molvar et al. 1993, Knapp et al. 1999). Within aquatic ecosystems, consumer-driven nutrient recycling can constitute an important biogeochemical flux and supply nutrients that limit primary productivity (e.g., Meyer et al. 1983, Grimm 1988, Vanni et al. 2002, McIntyre et al. 2008). In most of the cases where excretion by a given consumer species is an important component of ecosystem nutrient dynamics, these effects are explained by high levels of consumer biomass rather than by high mass-specific excretion rates (e.g., Caraco et al. 1997, Hall et al. 2003, McIntyre et al. 2007). However, stoichiometric variables unique to individual species, such as diet nutrient content and body elemental composition, may result in some taxa playing disproportionately important roles in the recycling or retention of nutrients, thereby affecting ecosystem nutrient availability (e.g., Elser et al. 1988).

Aquatic ecosystems are commonly limited by phosphorus (P) and nitrogen (N) (Elser et al. 2007), but the stoichiometric variables controlling rates of P and N excretion by different species could lead to differential importance in the roles that individual species play in the cycling of these two elements. Phosphorus (P) recycling rates are known to vary widely among species due to differences in body elemental composition. Vertebrate species with high body P content (e.g., due to bones and scales) excrete P at lower rates compared to species with lower levels of body P (Vanni et al. 2002). Similarly, diets higher in P can contribute to higher levels of P excretion for a given species (e.g., Pilati and Vanni 2007). A fish assemblage that varies in

diet and body elemental composition could include species with a wide range of P excretion rates, with the potential for a species represented at relatively low biomass levels to account for a high proportion of ecosystem-level P recycling. In contrast to P, N recycling rates tend to be similar among similar-sized species, due to smaller variation both among N content of different food resources and in N demand by different consumer species (Vanni et al. 2002). The contribution of individual species to total N recycling is therefore largely dependent on the total biomass of each species (McIntyre et al. 2007). Thus, as a result of highly variable mass-specific P excretion rates among species, ecosystem-level P recycling (more so than N recycling) has the potential to be influenced by species identity.

Phosphorus excretion rates for homeostatic consumers reflect a balance between P supply in food resources and the P demand by the consumer for growth and maintenance (Sterner 1990, Elser and Urabe 1999), so that species with a low body P demand feeding on a high P diet should recycle excess P at a high rate. In addition to the type of food resources consumed, the P content of a given food resource may vary according to levels of dissolved phosphorus in a lake or stream. Algae, detritus, and several invertebrate taxa have increased P content in high P aquatic environments (e.g., Stelzer and Lamberti 2001, Cross et al. 2003, Elser et al. 2005), so that fishes in a high P system may ingest more P, potentially resulting in higher P excretion rates across the fish assemblage. In low P systems where food resources are generally lower in P content, fish species that maintain high P excretion rates through a combination of low P demand and a relatively high P supply in their food resources (e.g., by feeding on terrestrial insects, which are independent of stream P levels) could become disproportionately important in supplying the limiting nutrient to the ecosystem.

To better understand how species-specific nutrient recycling rates are affected by ecosystem nutrient availability, we measured N and P excretion rates for fish assemblages in four lowland Neotropical streams that had a wide range in dissolved P levels due to natural inputs of solute-rich groundwater. Across this P gradient, algae and aquatic invertebrates increase in P content by five-fold and two-fold, respectively (Chapter 2), so that most fishes in high P streams ingest more P per unit of food consumed compared to fishes in low P streams. We predicted that fish P excretion rate will be elevated in high P streams relative to low P streams. Moreover, while most fish species will have low rates of P excretion in low P streams, we predict that some taxa (e.g., insectivores with relatively low body P content) will maintain high P excretion rates, thereby playing a disproportionately important role in the recycling of this element within the stream ecosystem.

Methods

Study sites—This study took place at La Selva Biological Station in the lowlands of Costa Rica's Caribbean Slope. Some streams at La Selva receive natural inputs of solute-rich groundwater (Pringle et al. 1993), resulting in high soluble reactive phosphorus (SRP) concentrations, whereas other streams do not receive solute-rich groundwater and are P limited (Pringle & Triska 1991). We selected four 3^{rd}-4^{th} order streams that range in SRP from 2-135 μg L^{-1} (Table 4.1). These sites are a subset of streams monitored as part of a long-term study on the physicochemistry of La Selva streams (Pringle & Triska 1991; Triska et al. 2006) for which continuous monthly data are available since 1997. Daily and seasonal stream temperature has low variation, ranging from 24-26°C.

The P content of aquatically-derived food resources increases across the natural P gradient in our study streams. Comparing the low P Sura-60 and the high P Arboleda, the P content increases more than two-fold for epilithon (0.18 – 0.47 % P by dry mass), five-fold for filamentous algae (0.06 – 0.30 % P), and two-fold for aquatic invertebrates (0.5 – 1.1 %; Chapter 2). Terrestrial invertebrates, collected from pan traps along the study streams, have a mean P content of 0.5 % which is independent of stream chemistry (G. Small, unpublished data).

Forty-three species of fishes have been documented in the rivers and streams of La Selva Biological Station (Bussing 1994). Our study streams contain a subset of these species; Burcham (1988) documented nineteen species in the Sura-30 and twenty-six species in the Sabalo. In our study, we measured N and P excretion rates for twelve fish species (within four different families) that are sufficiently abundant in our study sites to be collected by seine (Table 4.2).

Quantification of nutrient recycling rates by fishes—We quantified N and P excretion rates for these twelve fish species across our study streams during June-July 2007. Fishes were collected by seine and were placed immediately into plastic bags (1 individual per bag) containing 250 mL of water for smaller individuals (<3 g wet weight) or 500–1000 mL of water for larger individuals. Water for all excretion trials was collected from the low-P Sura-60 and filtered to remove suspended particles using Whatman Grade No. 1 filter paper (11 μm pore size). During incubations, bags were kept in shallow water along the stream margins to maintain constant temperature and to minimize stress. Any individuals showing visible signs of stress during the incubation were not used in the study. After one hour, water samples were collected from each bag and filtered through a 0.45 μm Millipore filter to remove feces and other particles. Water samples were also collected from five control bags (incubated for one hour with no fish)

for every round of measurements. Water samples were immediately frozen and transported on ice to the University of Georgia's Analytical Chemistry Laboratory for analysis. Soluble Reactive Phosphorus (SRP) was measured using the ascorbic acid method, and NH_4 was measured using the phenate-hypochlorite method (APHA 1998).

We measured excretion rates for 531 individual fish. Not all species were present in all four streams, and the number of excretion replicates varied among species roughly in proportion to species abundances (Table 4.2). Per capita N and P excretion rates were calculated as the increase in NH_4-N and SRP, relative to controls, during the one-hour incubation.

Fish total lengths were measured in the field to the nearest 0.1 mm, and wet weight was estimated using regressions established for each species from individuals collected for body nutrient content. Collected individuals were weighed, guts were removed and preserved in formalin for later analysis, and specimens were then dried and ground to a powder. Samples of ground animals were analyzed for carbon (C) and N with a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan, Italy). For P analysis, samples were acid-digested (aqua-regia double acid; Jones et al. 1991), and analyzed spectrophotometrically (ascorbic acid method). Gut contents were examined using a dissecting microscope: food items were classified as terrestrial insect, aquatic insect, terrestrial plant, or aquatic plant. Material in guts was spread over a grid of 1 mm squares, and diet proportion was estimated based on the relative area covered by each food category.

Body nutrient composition and gut contents were measured for 235 individuals (a subset of the 531 fish used for excretion measurements; typically 5 individuals of each species in each stream). Body N:P and diet for each species did not vary significantly across body sizes and between streams.

Of 531 fish excretion measurements, 96 measurements of NH_4 and 124 measurements of SRP were equal to or slightly less than mean values from corresponding control bags. We assigned a nutrient increase in these samples of 0.1 μg/L, essentially a minimum detection limit for excreted nutrients, which ensured that all values were positive before log-transformation.

Statistical analyses—We used multiple regression to quantify relationships between rates and ratios of nutrient recycling and body mass, fish body nutrient content, proportion of diet made up of insects, and stream SRP, with individual fish as the unit of observation (n = 531). For the 271 individuals for which we directly measured diet and body nutrient content, we used these data in the model. For the other individuals, we used a mean value for that species in each stream to estimate diet and body nutrient content. Excretion rates (N and P) and ratios (N:P), as well as wet mass, were log-transformed. Species identity *per se* was not included in the model, because our goal was to identify those species attributes (body elemental composition and diet) that determine nutrient recycling rates.

To evaluate the importance of the three stoichiometric variables (body nutrient content, % insects in diet, and stream SRP) on excretion rates, we compared a null model using only mass with the results of a backwards stepwise regression model using the stoichiometric variables. We calculated standardized regression coefficients (β_{std}) to compare the relative importance of body nutrient composition, diet, and stream identity in controlling rates of nutrient recycling (Neter et al. 1996). The standardized regression coefficient is defined as the change in the dependant variable (in terms of standard deviation) resulting from a change of 1 SD in the corresponding independent variable.

To test the hypothesis that effects of stream identity on fish P excretion would be greatest for species with low body P content that feed primarily on aquatic insects, we constructed a

second set of statistical models predicting N and P excretion rates, and N:P excretion ratio, for each species separately as a function of stream SRP, with wet mass as a covariate. All statistical analyses were conducted in SAS using PROC GLM (SAS Institute 2001). Variables were evaluated for significance using type III sums of squares.

Contribution of fish excretion to nutrient recycling in a low-P stream—In March 2008, population sizes were estimated for the five most abundant fish species (*Alfaro cultratus* [Poeciliidae], *Archocentrus septemfasciatus* [Cichlidae], *Astatheros alfari* [Cichlidae], *Astyanax aeneus* [Characidae], and *Priapicthys annectens* [Poeciliidae]) in our focal low-P study stream, the Sura-60. Fish were collected by electrofishing along a 50 m reach. Each individual was marked, and population estimates were calculated based on the number of recaptures over a two-day period using the program NOREMARK (http://welcome.warnercnr.colostate.edu/~gwhite/software.html). We estimated total N and P recycling rates for these five species using the size distribution of individuals captured from each species, and calculating per capita N and P excretion rates based on mass relationships calculated for each species in this stream (mean $r^2 = 0.56$). For each species, we then scaled this flux of excreted nutrients to the stream reach by multiplying by the ratio (estimated total population/number of captured individuals), and then divided by reach area to estimate area-specific excretion rates.

These five most abundant species make up an estimated 85% of the total fish population in this reach (87 out of 102 captured individuals). Eight other species were captured: *Anguilla rostrata* (Anguillidae), *Awaous tajasica* (Gobiidae), *Brycon guatemalensis* (Characidae), *Gymnotus cylindricus* (Gymnotidae), *Melanirus hubbsi* (Atherinidae), *Parachromis dovii* (Cichlidae), *Rhamdia guatemalensis* (Heptapteridae), and *Rivulus isthmensis* (Aplocheilidae).

We have insufficient data to accurately estimate population densities of these species (most were represented by a single individual), although densities are likely less than 0.05 m^{-2}, if we assume capture probabilities similar to the abundant species. Based on their low densities, we assume that each of these taxa were minor contributors to total N and P recycling by the fish assemblage.

In March 2006, we measured stream nutrient demand in our focal low P study stream, Sura-60, using a short-term addition of NH_4^+ and PO_4^{3-}. The reactive solutes were injected along with a conservative tracer (rhodamine WT) for 6 hours to measure plateau concentrations at three downstream stations (at 260, 360, and 460 m). At plateau, NH_4^+ was elevated to 55 μg L^{-1} and SRP was elevated to 63 μg L^{-1}. The rate of decline in dilution-corrected nutrient concentration over distance was converted into an areal uptake rate (U) by multiplying by stream velocity, mean depth, and background nutrient concentration (Stream Solute Workshop 1990). We calculated 95% confidence intervals for U using confidence intervals for the slope of the uptake regression.

Results

Fish nutrient content and diet—Across the twelve species, body N content was essentially constant, with species means ranging from 10.1 – 10.2 % N by dry mass. Species means for body P content ranged from 3.6 – 5.0 % P by dry mass, and as a result, mean body N:P molar ratios ranged from 5.0 – 6.7 (Table 4.3). Within individual species, fish elemental composition did not vary significantly with body size or across streams. Species in the family Cichlidae had the highest body P-content, while the families Atherinidae and Poeciliidae were intermediate. The characid, *Astyanax aeneus*, had the lowest body P-content among species in this study.

The twelve fish species ranged in diet from herbivory to insectivory, with most species consuming food items from multiple categories (Table 4.3). Individual species did not show differences in diets among streams. Aquatic insects constituted the majority of the diets for the atherinid, *Melaniris hubbsi*, and for the cichlids *Archocentrus septemfasciatus*, *Astatheros alfari*, and *Hypsophrys nicaraguensis*. The characid *Astyanax aeneus* and poeciliids *Alfaro cultratus*, and *Brachyraphis parismina* fed primarily on terrestrial insects. The diet of *Priapicthys annectens* (Poeciliidae) was evenly divided between terrestrial and aquatic insects. The cichlid *Neetroplus nematopus* and poeciliids *Neoheterandria umbratilis*, *Phallichthys amates*, and *Poecilia gillii* fed primarily or exclusively on aquatic plants (filamentous algae or diatoms). Terrestrial plant material was only a minor contribution to the diets of any fish species in our study (Table 4.3).

Relative importance of nutrient supply and demand in affecting excretion rates—The null model for N excretion (n = 531), using only individual fish mass to predict per capita N-excretion rates, explained 20% of variance among individuals. Adding % insects in diet improved the model slightly (explaining 22% of variance), with higher levels of insectivory associated with higher N-excretion rates. Body %N and stream SRP are not related to measured rates of N excretion (Table 4.4). The coefficient for mass was not significantly different than 1 (based on 95% confidence intervals), indicating that mass-specific N-excretion rates in this dataset do not vary with the size of the fish (Fig. 4.1).

For per capita P-excretion rates, the null model with mass alone explained 20% of variance. Adding the stoichiometric variables body %P, % insects in diet, and stream SRP improved the model such that 28% of variance was explained. The parameter estimate for mass was not significantly different than 1, consistent with an isometric relationship between mass-

specific P-excretion rates and fish body size (Fig. 4.2). Rates of P-excretion were negatively related to fish body %P, and positively related to % insects in diet and stream SRP (Table 4.4). Based on the standardized regression coefficients (β_{std}), the effects of fish P-demand (based on body P-content) and the effects of dietary P supply (% insects in diet and stream SRP) are similar in magnitude in controlling P excretion rates. In the high-SRP Arboleda stream, nearly all measurements of P-excretion rates were well above the detection limit. As stream SRP decreased, an increasing number of individuals had P-excretion rates near zero, and this was especially pronounced for cichlids (Fig. 4.2).

Ratios of N:P excretion were not related to fish mass or % insects in diet, but are negatively related to body N:P ratio and stream SRP. However, this model explains only 5% of variance in this dataset, so these results should be interpreted with caution. The large variance among individuals in excretion N:P is due to samples at the detection limit of either N or P, leading to a large range in measured excretion N:P across the dataset.

When individual species were modeled separately to test for the effects of stream SRP on per capita P-excretion rates (using mass as a covariate), four of the twelve species showed significant decreases in P-excretion rates with decreasing stream SRP (r^2 0.14 – 0.46; Table 4.5). These four species consist of two cichlids, *Archocentrus septemfasciatus* and *Astatheros alfari*, and two poeciliids, *Alfaro cultratus* and *Brachyraphis parismina*.

Contribution of fish excretion to nutrient recycling in a low-SRP stream—Estimated population densities for the five fish species that were most abundant in the Sura-60 ranged from 0.4 – 2.5 individuals m^{-2}. Mean wet mass for individuals of these abundant species ranged from 0.9 g to 36.6 g, and estimated total biomass ranged from 0.3 – 17.1 g wet mass m^{-2} (Table 4.6). Mean P excretion rates for the two cichlids, *Archocentrus septemfasciatus* and *Astatheros alfari*,

were essentially at the detection limit (~0.2 µg SRP individual^{-1} h^{-1}). In contrast, the characid *Astyanax aeneus* had the highest per capita P excretion rate among the five abundant fishes in this stream (81.0 µg SRP individual^{-1} h^{-1}), nearly ten-fold higher than the next highest species. As a result, *Astyanax aeneus* supplied an estimated 90 % of the P recycled by the five abundant fish species (40.5 out of 45.2 µg SRP m^{-2} h^{-1}), while accounting for only 9 % of the total population (9 out of 102 individuals collected in this reach during population survey) and 19 % of the total biomass of the fish assemblage (5.5 g wet weight m^{-2} out of a total fish biomass of 29.4 g wet weight m^{-2}; Table 4.6). By contrast, variation among N excretion rates for these five abundant species is largely due to differences in body size, so that estimated total N recycled by each species was proportionate to its total biomass (Table 4.6).

From the nutrient addition experiment in this low P focal study stream, we estimated areal uptake rates (U) of 755 (95% C.I., 499 – 999) µg NH_4^+ m^{-2} h^{-1} and 45 (17 – 74) µg PO_4^{3-} m^{-2} h^{-1} for N and P, respectively. Based on these measurements, fishes appear to be important nutrient recyclers in this low nutrient stream. The fish assemblage in our study reach may supply 100% or more of stream NH_4^+ and PO_4^{3-} demand. P excretion by *A. aeneus* supplied an estimated 90% of stream P demand, while P excretion by the other four abundant species in this reach supplied a combined 10% of stream P demand.

Expressing nutrient excretion in volumetric units and accounting for stream velocity (*sensu* McIntyre et al. 2008) indicates that, assuming constant conditions downstream, nutrient recycling by the entire fish assemblage in this stream would be sufficient to turn over the pools of dissolved NH_4^+ and PO_4^{3-} in 1.3 km and 3.4 km, respectively. P excretion by *A. aeneus* alone would be sufficient to turn over the dissolved P pool over 3.8 km, compared to a distance of 33

km that would be required for turnover due to the combined P excretion of the other four most abundant species.

Discussion

Role of Astyanax aeneus in ecosystem-level nutrient recycling—Our calculations indicate that *Astyanax aeneus* acts as a keystone nutrient recycler in the low SRP Sura-60 because it maintained a relatively high P excretion rate in an ecosystem in which other abundant fish species excreted P at much lower levels. Our results highlight the fact that the importance of species identity in ecosystem processes is context-dependent. In higher-SRP streams, P excretion rates across the fish assemblage tended to be uniformly high as a result of P-enriched food resources; in low SRP streams where aquatically-derived food resources were depleted in P, species that fed on aquatically-derived food resources had much lower P excretion rates. However, *A. aeneus*, due to its relatively low P demand and moderately high-P diet (predominantly terrestrial insects), maintained a high P excretion rate that was >10-fold higher than other species that had similarly high levels of biomass. *Astyanax aeneus* represented 18% of the total biomass of the five abundant fish species, yet contributed 90% of the P recycled by these species while also supplying an estimated 90% of stream P demand.

Our results clearly indicate that *A. aeneus* dominates P recycling by fishes in our low P focal stream. We suggest that the dissolved P recycled by the fish assemblage (and *A. aeneus* in particular) may be important in ecosystem functioning, given previous studies that have found that microbial respiration (Ramírez et al. 2003) and leaf decomposition (Rosemond et al. 2002) increase rapidly with slight increases in dissolved P availability in these streams. Nonetheless, these values should be interpreted with some caution, because we did not measure P excretion

for all fish species (i.e., our estimates of total P excreted by the entire fish assemblage are conservative). Direct measurements of ambient nutrient excretion rates are subject to error from effects of fasting and stress; the hour-long incubation times used in our study are likely long enough to compensate for initial stress effects, but could cause underestimates of excretion rates due to fasting, especially for insectivores with faster gut passage times (Whiles et al. 2009). The higher excretion rates documented for insectivores are therefore likely conservative. We cannot rule out the possibility that placing fish from a high solute (high SRP) stream into low solute water (from the low SRP stream) during the incubation could contribute to additional stress, but the fact that P excretion, but not N excretion, was higher in the high SRP streams suggests that this effect was due to food nutrient content rather than physiological stress. Additionally, our estimates of stream N and P uptake are based on a single nutrient addition experiment (so we cannot estimate temporal variability in uptake rates), and estimates of stream N and P demand from our short-term nutrient addition most probably underestimated ambient uptake rates (Mulholland et al. 2002). The measured nutrient uptake rates are integrated over a 200 m reach, whereas the fish census represented a 50 m subset of this reach that tended to be good fish habitat, so reported fish densities (and therefore per-area excretion rates) are likely to be higher in our study reach than for the entire stream. In spite of these qualifications, our data provide robust evidence that P excretion by *A. aeneus* is a large flux relative to the rest of the fish assemblage and to stream P demand.

The stoichiometric traits of a given fish species can be used to predict its potential role in nutrient recycling. Insectivorous (and piscivorous) fish species should excrete excess P at high rates, which increase with the P-content of their food, since they are rarely P limited (Schindler and Eby 1997). Herbivorous and detritivorous fishes may be P limited (Hood et al. 2005), and P

excretion rates should be low. Rates of P excretion by fishes feeding on aquatically-derived food resources are affected by stream nutrient levels (via dietary P content). However, because terrestrial subsidies are independent of stream nutrient status, subsidized species may be more likely to play disproportionately important roles in stream P recycling. The effects of diet stoichiometry are compounded by a species' P demand (Vanni et al. 2002). In low P La Selva streams, three species (*A. aeneus*, *Melanirus hubbsi*, and *A. cultratus*) maintained high mass-specific P excretion rates (>9 μg SRP g^{-1} biomass h^{-1}) due to primarily insectivorous (P-rich) diets and relatively low body P content (N:P > 6.0). Of these three species, only *A. aeneus* had sufficient abundance for their nutrient recycling to represent an appreciable ecosystem flux in our focal study stream (Sura-60), and as a result, this species dominated consumer-driven P recycling. While the importance of *A. aeneus* in nutrient recycling is highly context dependant, as is typical of keystone species (Power et al. 1996), we note that low nutrient, forested streams are common across the range of this widespread taxon, and it is likely that *Astyanax* plays similarly important roles in many of these stream ecosystems.

Effect of stream phosphorus levels, diet, and body nutrient content on nutrient recycling rates—As expected, individual fish mass accounts for most of the variance in per capita N and P excretion rates explained by the model. Metabolic theory predicts that smaller organisms have higher mass-specific metabolic rates, and therefore higher mass-specific excretion rates (Hall et al. 2007). However, both our N and P excretion models have mass coefficients that are not significantly different than one (based on 95% confidence intervals), indicating that size structure of the fish assemblage may not be an important factor controlling ecosystem nutrient recycling rates by the fish assemblage in these streams.

Nitrogen excretion rates of fishes in this study were minimally influenced by the stoichiometric variables that we measured. Fish body N content showed little variation among all individuals (coefficient of variation: 0.04) and was not related to N excretion rates. Of the stoichiometric variables, only % insects in diet was significantly related to N excretion rates. However, despite the fact that insects have an N content ca. 10-fold higher than epilithon (Chapter 2), adding this variable led to a minimal improvement in the amount of variance explained by the model. As with the dataset analyzed by McIntyre et al. (2007), in our dataset, ecosystem-level N excretion by the fish assemblage can be predicted with reasonable accuracy using only the total biomass of fish in the ecosystem.

In contrast to N excretion, P excretion rates were dependant upon dietary P supply and body P demand. Differences among these variables led to differences in P excretion rates among species, and, for some species, intraspecific differences among the study streams. Our findings are consistent with the conclusions of Vanni et al. (2002) that fish body P content is an important factor controlling P excretion rates, with species with higher body P recycling P at a lower rate. The importance of body P content was high even though the range among species in our study (molar N:P ratio: 5.0 – 6.7) was much lower than that of the fish and tadpole assemblage measured by Vanni et al. (2002), in which body N:P ranged from 4 – 23.

Our analysis also indicates that dietary P-content is as important as fish P-demand in determining P excretion rates. Insectivorous fishes excrete P at higher rates than algivores, consistent with the fact that insects have 2-fold higher P-content compared to epilithon across these streams (Chapter 2). Furthermore, stream SRP is an important predictor of P-excretion across the entire dataset. Aquatically-derived food resources increase in P-content > 2-fold with increasing SRP across these four study streams (Chapter 2).

The effect of stream SRP on P excretion rates varied by species when each species was analyzed separately, with four species having a significant positive relationship between these variables. The insectivorous cichlids, *A. alfari* and *A. septemfasciatus*, had ~20-fold decreases in mass-specific P excretion rates between highest and lowest P streams. These two species have some of the lowest mean body N:P ratios of all species considered in this study (5.13 and 5.45, respectively). They also both feed primarily on aquatic insects, indicating that P-ingestion may decline by 50% in the low P stream. This combination of diminished P-supply coupled with high P demand explains the very low levels of P excretion measured for these species in the Sura-60. Interestingly, the poeciliids *A. cultratus* and *B. parismina* also showed decreases in P excretion rates in low P streams, despite somewhat higher body N:P ratios and a greater reliance on terrestrial insects. The P excretion responses of the four species discussed above suggest that they excrete excess P in high P streams but not in low P streams. In contrast, other species in the fish assemblage did not show significant changes in P excretion rates with stream SRP. Herbivorous species, such as the cichlid *Neetroplus nematopus* and the poeciliids *Phallichthys amates*, *Neoheterandria umbratilis*, and *Poecilia gillii* maintained relatively low P excretion rates across all four streams, despite documented 3–5 fold increases in the P content of epilithon and filamentous algae. If algivorous fishes are P-limited (Hood et al. 2005), the additional P in algae in high SRP streams may result in increased growth rates, so that ingested P goes into biomass production rather than being excreted. Finally, *A. aeneus* (Characidae) and *M. hubbsi* (Atherinidae) maintained high P excretion rates across all four streams. Both of these species are characterized by insectivorous diets and relatively low body P content. Due to their lower P demand and the high amount of P ingested in their food resources, these species eliminate excess P at high rates in all four streams, regardless of ambient P levels.

Nutrient recycling rates may not always be completely explained by the nutrient imbalance between consumer and food resource. Species with a high P demand feeding on low P food resources could potentially compensate by ingesting more food, feeding selectively, or assimilating nutrients more efficiently. We did not measure rates of fish growth, ingestion, or assimilation; variation in these rates could decouple the relationship between diet P content and consumer P excretion rates. For example, food limitation could explain the low P excretion rates measured for *Priapicthys annectens*, an insectivore with a relatively high body N:P ratio. Besides excretion, fecal production is a significant nutrient flux (e.g., Grimm 1988, Rodehutscord et al. 2000, Hall et al. 2003) that we did not measure, although homeostatic regulation occurs primarily through excretion rather than egestion (Rodehutscord et al. 2000, Anderson et al. 2005).

When is species identity an important predictor of nutrient recycling?—Species-specific differences in P excretion rates, due to diet and body P content, create the potential for a species to have a disproportionately important role in ecosystem P cycling relative to its biomass. Freshwater ecosystems are losing species at an unprecedented rate (Bunn and Arthington 2002, Dudgeon et al. 2006), and the loss of an important P recycler (e.g., through over-harvesting or loss of critical habitat) could have a large effect on ecosystem processes in a P limited stream. Because of the species-specific traits that control P recycling rates, compensation by remaining species would be unlikely to buffer the decline in ecosystem P recycling rates.

Similarities in N recycling rates among species suggest two mechanisms that may stabilize ecosystem-scale N recycling against species losses. First, if rarity is a predictor of extinction risk, then the numerically dominant species with the largest role in N recycling should be less prone to extinction. Secondly, because mass-specific rates of N excretion are fairly

similar between species, there is a higher potential for species redundancy in this trait, so that if an important N recycler is extirpated, compensatory responses by other species would likely dampen ecosystem-level effects. The first of these mechanisms confers resistance, and the second resilience, potentially decreasing the sensitivity of consumer-driven N recycling to changes in community structure.

In summary, although *Astyanax aeneus* occurs at relatively low biomass (contributing approximately 18% of the total biomass of the fish assemblage) it dominates ecosystem P recycling by fishes in a low P Neotropical stream. Because of the disproportionately large effect that *Astyanax* has on ecosystem P recycling relative to its biomass, our study contrasts with other studies that have demonstrated that fish can be important nutrient recyclers. For example, while McIntyre et al. (2007) found that another characid, *Prochilodus mariae*, was the major contributor to N and P recycling in a Venezuelan river, its importance was due to its large individual size and high total biomass in this river rather than especially high mass-specific excretion rates. The important role of *Astyanax aeneus* in P recycling is due to its high P excretion rates in an ecosystem where other abundant consumers minimize their loss of this element, illustrating that the importance of species identity on nutrient cycling is context-dependent (i.e., it depends on background nutrient levels). In low P aquatic ecosystems, species that excrete P at high rates due to a low body P demand and a diet composed of high P food resources are most likely to play disproportionately important roles in P recycling. Our study supports the prediction that shifts in community composition in aquatic ecosystems, through extirpations or invasions, could dramatically alter ecosystem-scale P recycling rates and significantly affect ecosystem functioning.

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Table 4.1: Physical and chemical characteristics of the four streams used in this study, based on means of monthly samples (n = 20) collected from January 2006-August 2007.

Stream	Stream characteristics							
	Discharge ($m^3 s^{-1}$)	Cond. ($\mu S\ cm^{-1}$ @ 25°C)	pH	Temp. (°C)	DIN:SRP (molar)	NO_3-N ($\mu g\ L^{-1}$)	NH_4-N ($\mu g\ L^{-1}$)	SRP ($\mu g\ L^{-1}$)
Arboleda	0.17	257	6.2	25.6	2.4	126	20	135
Sura-30	0.61	157	6.1	25.4	4.8	163	18	83
Sabalo	0.28	47	6.0	25.5	21.3	137	29	8
Sura-60	0.19	20	5.6	24.8	173.7	199	21	3

Table 4.2: Number of individuals of fish species in the four study streams that were used to quantify N and P excretion rates.

	Stream			
	Arboleda	**Sura-30**	**Sabalo**	**Sura-60**
Atherinidae				
Melanirus hubbsi	--	20	13	20
Characidae				
Astyanax aeneus	15	16	14	12
Cichlidae				
Archocentrus septemfasciatus	3	5	22	18
Astatheros alfari	16	20	19	23
Hypsophrys nicaraguensis	--	10	9	--
Neetroplus nematopus	1	9	11	--
Poeciliidae				
Alfaro cultratus	19	23	20	20
Brachyraphis parismina	--	18	20	--
Neoheterandria umbratilis	--	20	19	--
Phallichthys amates	2	11	19	--
Poecilia gillii	--	13	20	--
Priapicthys annectens	2	15	--	16

Table 4.3: Body N and P content (% dry mass), N:P ratios, and diets (based on gut contents) for the twelve fish species examined in this study. Reported values are means for individuals across the four streams.

Species	% N (SE)	% P (SE)	Body N:P (molar) (SE)	Diet (% gut contents by area)			
				Aquatic insects	Terr. insects	Aquatic plants	Terr. plants
Atherinidae							
Melaniris hubbsi	10.6 (0.1)	3.8 (0.2)	6.0 (0.2)	62.1	19.1	18.6	0.2
Characidae							
Astyanax aeneus	10.6 (0.2)	3.6 (0.1)	6.7 (0.2)	20.8	65.4	10.6	3.2
Cichlidae							
Archocentrus septemfasciatus	10.5 (0.3)	4.3 (0.1)	5.4 (0.2)	80.2	15.5	4.4	0.0
Astetheros alfari	10.2 (0.1)	4.7 (0.3)	5.1 (0.2)	85.4	12.0	2.6	0.0
Hypsophrys nicaraguensis	10.1 (0.1)	5.0 (0.7)	5.0 (0.4)	60.7	10.1	29.2	0.0
Neetroplus nematopus	10.3 (0.1)	4.6 (0.3)	5.1 (0.3)	17.6	0.0	79.9	2.5
Poecilidae							
Alfaro cultratus	10.5 (0.1)	3.6 (0.1)	6.6 (0.1)	31.6	66.0	2.2	0.2
Brachyraphis parismina	10.2 (0.2)	4.1 (0.2)	5.6 (0.3)	13.3	80.0	6.7	0.0
Neoheterandria umbratilis	10.5 (0.1)	4.5 (0.5)	5.8 (0.5)	8.1	16.7	75.2	0.0
Phallichthys amates	10.5 (0.1)	4.3 (0.2)	5.5 (0.3)	0.0	0.0	100.0	0.0
Poecilia gillii	10.6 (0.1)	3.6 (0.2)	6.6 (0.3)	5.3	1.8	92.9	0.0
Priapicthys annectens	10.3 (0.1)	3.8 (0.1)	6.2 (0.2)	49.8	50.2	0.0	0.0

Table 4.4: Results of multiple regression to predict per capita N and P excretion rates, and N:P excretion ratios (n = 531). R^2 values for these models were 0.22, 0.28, and 0.05, respectively. All twelve fish species were included in these models.

Parameter	Estimate	(SE)	DF	Type III SS	*F*-ratio	*P*	β_{std}
Log N excretion rate							
Intercept	0.858	1.342					
Log wet body mass (g)	1.157	0.097	1	174.6	142.1	< 0.001	0.47
Insects in diet (%)	0.377	0.124	1	11.4	9.3	0.002	0.12
Log P excretion rate							
Intercept	0.451	0.256					
Log wet body mass (g)	0.976	0.09	1	124.1	118.4	< 0.001	0.37
Body P (%)	-0.174	0.053	1	11.2	10.7	0.001	0.13
Insects in diet (%)	0.351	0.114	1	9.9	9.4	0.002	0.11
Stream SRP	0.005	0.001	1	26.1	24.9	< 0.001	0.20
Log N:P excretion ratio							
Intercept	2.127	0.338					
Body N:P ratio	-0.116	0.055	1	8.1	4.5	0.034	0.09
Stream SRP	-0.005	0.001	1	31.1	17.3	<0.001	0.19

Table 4.5: Mean (and SE) mass-specific P excretion rates (µg SRP g wet mass^{-1} h^{-1}) for 12 fish species in four streams ranging in dissolved P levels from 3-135 µg SRP L^{-1}. Species with significant (P<0.05) increases in P excretion with increasing stream SRP are denoted with an asterisk (*).

		Stream							
		Arboleda (135 µg L^{-1} SRP)		**Sura-30** (83 µg L^{-1} SRP)		**Sabalo** (8 µg L^{-1} SRP)		**Sura-60** (3 µg L^{-1} SRP)	
Atherinidae									
Melanirus hubbsi				23.9	(4.9)	18.8	(7.6)	22.0	(5.1)
Characidae									
Astyanax aeneus		11.1	(2.0)	30.3	(5.9)	21.7	(4.4)	11.8	(1.9)
Cichlidae									
Archocentrus septemfasciatus	*	5.4	(1.3)	2.9	(2.5)	3.1	(1.5)	0.2	(0.1)
Astatheros alfari	*	9.8	(3.3)	21.3	(9.6)	1.3	(0.3)	1.1	(0.6)
Hypsophrys nicaraguensis				1.7	(0.6)	11.1	(5.6)		
Neetroplus nematopus		3.4		4.0	(2.0)	5.7	(1.7)		
Poeciliidae									
Alfaro cultratus	*	16.1	(4.8)	70.1	(18.0)	12.2	(2.8)	9.0	(2.8)
Brachyraphis parismina	*			26.3	(9.2)	1.7	(0.5)		
Neoheterandria umbratilis				0.6	(0.2)	2.2	(1.4)		
Phallichthys amates		5.8	(0.8)	2.5	(1.8)	1.9	(0.6)		
Poecilia gillii				5.7	(1.6)	3.5	(1.0)		
Priapicthys annectens		0.1	(0.1)	1.9	(0.9)			0.7	(0.4)

Table 4.6: Estimated contribution to stream N and P recycling for five common fish species in the low SRP Sura-60. 95% confidence intervals shown for stream N and P demand.

species	Popn. density (m^{-2})	Mean size (g wet weight)	Total biomass (g wet weight m^{-2})	Mean P-excretion rate (µg SRP fish $^{-1}$ h^{-1})	SRP excreted (µg SRP m^{-2} h^{-1})	Mean N-excretion rate (µg NH_4-N fish $^{-1}$ h^{-1})	NH_4 excreted (µg NH_4-N m^{-2} h^{-1})
Astyanax aeneus	**0.5**	**10.6**	**5.5**	**81.0**	**40.5**	**195.7**	**97.9**
Astetheros alfari	0.5	36.6	17.1	0.3	0.1	787.3	393.7
Archocentrus Septem.	0.6	5.9	3.7	0.2	0.1	392.6	235.6
Priapicthys annectens	2.5	1.8	4.3	2.2	1.1	247.5	123.8
Alfaro cultratus	0.4	0.9	0.3	8.6	3.4	32.2	12.9
				Total P excreted (µg SRP m^{-2} h^{-1})	45.2	**Total N excreted (µg NH_4-N m^{-2} h^{-1})**	863.9
				Stream P-demand (µg SRP m^{-2} h^{-1})	45 (17–74)	**Stream N-demand (µg NH_4-N m^{-2} h^{-1})**	755 (499–999)

Figure Legends:

Fig. 4.1: Per capita N-excretion rates versus mass of individual fishes in the four study streams.

Fig. 4.2: Per capita P-excretion rates versus mass of individual fishes in the four study streams. Data points for *Astyanax aeneus* in the Sura-60 are circled to illustrate that this species maintained relatively high P-excretion rates in the low-SRP stream.

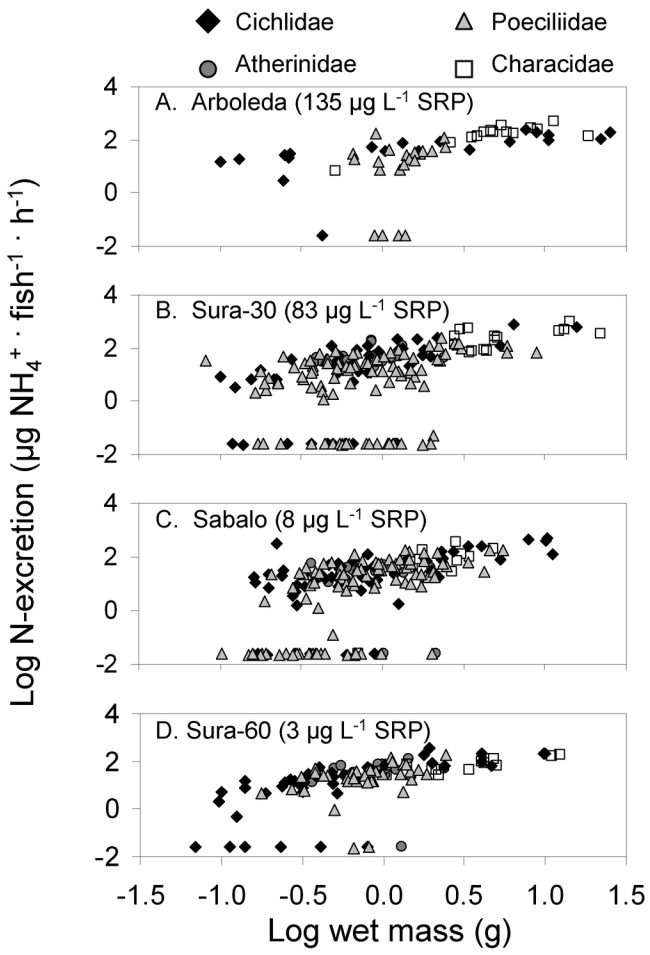
Cichlidae
Poeciliidae
Atherinidae
Characidae
A. Arboleda (135 µg L-1 SRP)
B. Sura-30 (83 µg L-1 SRP)
C. Sabalo (8 µg L-1 SRP)
D. Sura-60 (3 µg L-1 SRP)
Log N-excretion (µg NH4+ · fish-1 · h-1)
Log wet mass (g)
4
2
0
-2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5

Fig. 4.1

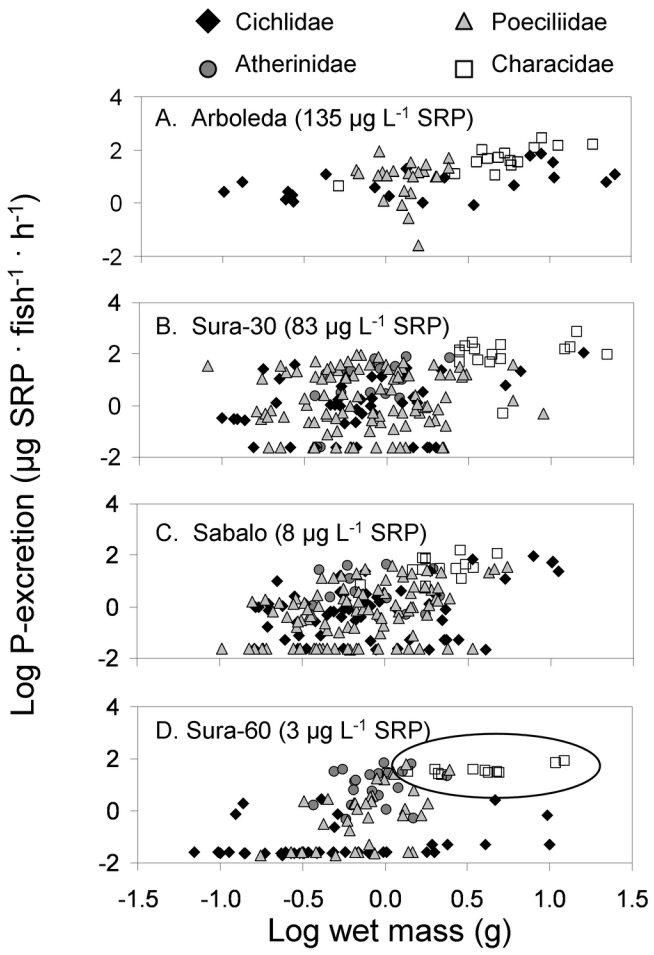
Cichlidae
Poeciliidae
Atherinidae
Characidae
A. Arboleda (135 µg L-1 SRP)
B. Sura-30 (83 µg L-1 SRP)
C. Sabalo (8 µg L-1 SRP)
D. Sura-60 (3 µg L-1 SRP)
Log P-excretion (µg SRP · fish-1 · h-1)
Log wet mass (g)
4
2
0
-2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5

Fig. 4.2

CHAPTER 5

ARE TERRESTRIAL INSECTS AN IMPORTANT NUTRIENT SUBSIDY IN LOWLAND NEOTROPICAL RAINFOREST STREAMS?[4]

[4] Small, G. E., P. J. Torres, L. M. Schweizer, J. H. Duff, and C. M. Pringle. To be submitted to *Ecosystems*.

Abstract

The importance of terrestrial inputs into streams is well-documented in temperate ecosystems, but little is known about the magnitude of these subsidies in tropical rainforests. In tropical rainforests, terrestrial insects falling from the forest canopy may represent an important nutrient flux in nutrient-poor headwater streams, if input rates of insects, and capture efficiency by fishes, are both high. We quantified the contribution of terrestrial insects to the nutrient budget of two first-order streams draining lowland tropical wet forest in Costa Rica, and we introduce a new method to quantify fish feeding efficiency by using an "ant tracer experiment", analogous to nutrient tracer experiments. Areal inputs of terrestrial insects constituted average nutrient fluxes of 123 μg N m^{-2} h^{-1} and 6.0 μg P m^{-2} h^{-1}. Capture efficiency of terrestrial insects by the dominant fish species, *Priapicthys annectens*, ranged from 32–93% across six different five-meter reaches. Terrestrially derived N-excretion by *P. annectens* averaged 32 μg NH_4^+ m^{-2} h^{-1}, and terrestrially derived P-excretion averaged 3.5 μg total dissolved P m^{-2} h^{-1}. Based on measured N-uptake rates in one of these streams, we estimate that the magnitude of terrestrially derived N-excretion is 79% of stream NH_4^+ demand. Annual terrestrial insect input rates in the study streams are similar to values from temperate forested streams, but unlike temperate streams, rates were relatively constant throughout the year. This aquatic-terrestrial linkage is being decoupled by deforestation in many tropical regions, and our study provides the first baseline data on the potential importance of terrestrial insect subsidies in a Neotropical rainforest stream.

Introduction

Stream ecosystems are closely linked to riparian zones through the exchange of organic and inorganic materials (Likens and Bormann 1974; Hynes 1975). Leaf litter inputs from terrestrial ecosystems are the dominant energy source in forested headwater stream ecosystems (Vannote et al. 1980; Wallace et al. 1997), and can also represent an important contribution to nutrient budgets of small streams (Meyer and Likens 1979; Meyer et al. 1981; Triska et al. 1984). However, leaf litter is characterized by high carbon:nutrient ratios and may serve as a sink for dissolved nutrients (Cross et al. 2005), as much of this material may be exported from the stream ecosystem as fine particulate organic material at lower carbon:nutrient ratios (Webster et al. 1999). Another nutrient pathway connecting forest and stream ecosystems is the input of terrestrial insects. Although insect biomass constitutes a smaller mass flux relative to plant material, insect biomass is relatively high in nutrient content (Sterner and Elser 2002), and insectivorous fishes are typically energy-limited and tend to excrete excess nutrients at relatively high rates (Schindler and Eby 1997). As a result of these factors, fish excretion derived from terrestrial insect inputs could be an under-appreciated source of dissolved nitrogen (N) and phosphorus (P) in small streams.

Studies quantifying the importance of terrestrial insect subsidies in streams have focused on the energetic rather than biogeochemical importance (reviewed in Baxter *et al.* 2005), and no studies to date have measured terrestrial insect subsidies in tropical streams. Some studies quantifying terrestrial insect inputs into temperate forested headwater streams have measured high influxes during the summer (up to 450 mg m^{-2} d^{-1}; Cloe and Garman 1996), and this input has been shown to be an important energetic subsidy to stream fishes during the summer when aquatic insect biomass is lowest (Nakano and Murakami 2001). However, terrestrial insect

inputs in temperate streams decrease during winter months (typically <1 mg m^{-2} d^{-1}; reviewed in Baxter *et al.* 2005).

In contrast to temperate forests, lowland tropical wet forests are characterized by high year-round productivity and the multi-stratal forest canopies typically have high insect biomass (particularly ants, with biomass 0.1 – 1 kg ha^{-1}; Leigh 1999), suggesting that terrestrial insects could potentially be an important subsidy. Polis et al. (1997) hypothesized that the degree and importance of spatial subsidies depends on the perimeter-to-area ratio, the relative productivity of the habitats, and the permeability of habitat boundaries. In small streams, the entire stream ecosystem is effectively edge habitat, due to the maximal perimeter-to-area ratio, and gravity-driven inputs from the highly productive tropical rainforest canopy exceed rates of within-stream primary productivity. Streams have high permeability for terrestrial insect subsidies if in-stream predators (insectivorous fishes) are efficient at capturing and processing terrestrial insect biomass. We predict that the biological availability of nutrients derived from terrestrial insects in stream ecosystems ultimately depends on the total input rate of terrestrial insects and the capture efficiency by fishes.

In this study, we used a combination of approaches to estimate the importance of terrestrial insects as a nutrient subsidy in nutrient-poor headwater streams draining a lowland Neotropical wet forest. We measured biomass and nutrient fluxes from terrestrial insect inputs into eight streams, ranging from 1^{st}-5^{th} order. For two first-order streams (hereafter referred to as focal streams), we also quantified the efficiency of insectivorous fishes in capturing drifting terrestrial insects. We then measured the proportion of terrestrial insects in the diet of the dominant fish species in the focal streams, and we determined the total terrestrially derived N- and P- excretion rates of this fish species relative to stream nutrient demand. We hypothesize

that nutrient excretion of terrestrially derived prey by insectivorous fishes supplies a significant proportion of stream ecosystem demand in headwater streams that drain highly-productive tropical forests.

Methods

Site description—This study was conducted at La Selva Biological Station, located in the lowlands of Costa Rica's Caribbean slope (10° 26' N, 84° 01' W). La Selva contains 1536-ha of lowland tropical wet forest, and receives nearly 4000 mm of rain annually, with a wet season (>400 mm/mo) from May-November (Sanford *et al.* 1994).

We measured terrestrial insect input along eight 1^{st}-5^{th} order streams that are a subset of sites used in a long-term study of the physicochemistry of La Selva streams (Pringle and Triska 1991; Triska *et al.* 2006), for which continuous monthly water chemistry data are available since 1997 (Table 5.1). Stream study sites are named by the watershed followed by a number indicating the approximate elevation (in m above sea level). We selected the two smallest of these streams (Carapa-60 and Saltito-100) as focal streams, to quantify the importance of terrestrially derived nutrient excretion by the fish assemblage, which is dominated in these two streams by the insectivorous poeciliid *Priapicthys annectens* (Burcham 1988).

The dense riparian vegetation along La Selva streams results in light-limited algal communities (Pringle and Triska 1991) and high inputs of allochthonous material. Canopy cover, based on spherical densitometer measurements, ranges from 81 – 88% across these sites. Leaf litter decomposition and microbial respiration are P-limited in these low-P streams (Rosemond *et al.* 2002; Ramírez *et al.* 2003).

Terrestrial insect input rates—We quantified input rates of terrestrial insects using pan traps placed along the stream margin. The pan traps consisted of plastic trays (0.32 m^2) filled with 5 cm of water (a mesh-covered hole prevented overflow), and a small amount of P-free surfactant to break surface tension. After 2-4 days, the contents of each pan trap were strained through 120 μm mesh. Pan traps were moved between sampling periods to integrate spatial heterogeneity in input rates. During June-July 2006, we collected pan trap samples in eight La Selva streams for a total of 195 trap-days, including 20 trap-days in the Carapa-60 and 22 trap-days in the Saltito-100. In June-July 2007, pan trap samples were collected along six streams for a total of 180 trap-days, including 30 trap-days along both focal streams. To measure seasonality of terrestrial insect inputs, monthly samples (8 trap-days $stream^{-1}$ $month^{-1}$) were also collected along three La Selva streams (Arboleda-30 for 26 months, and Piper-30 and Sura-60 for 13 months each), from January 2007-February 2009 (total 416 trap-days).

Terrestrial insects were separated from plant material, and were dried at 50°C for 48 hrs and homogenized. We measured the elemental composition of a composite sample of terrestrial insects for each stream site collected in June-July 2006. For C and N analysis, samples were analyzed on a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan, Italy). For P analysis, samples were acid-digested (Aqua Regia double acid; Jones *et al.*, 1991), and analyzed spectrophotometrically (ascorbic acid method). Ground pine needles (US National Institute of Standards and Technology, 1575a) was used as an external standard for P analysis. Isotope ratios for C and N are reported in delta notation (using Pee-Dee Belemnite as ^{13}C standard, and atmospheric N_2 as ^{15}N standard).

Consumption efficiency of insectivorous fishes feeding on terrestrial insects—We used two different approaches to quantify the feeding efficiency of the insectivorous fish assemblage

on drifting terrestrial insects. First, we performed ant addition experiments (because ants are a major component of drift samples as well as fish gut contents), analogous to nutrient addition experiments used to measure stream nutrient uptake rates (*sensu* Stream Solute Workshop 1990). In these experiments, 100 small ants were released at the top of a 5 m reach along with 100 small (1 cm) squares of waterproof paper, which served as a conservative tracer, behaving hydrologically like inedible ants. We used an invasive ant species, *Paratrechina longicornus*, in this experiment because it was easily collected in the laboratory building at La Selva, but is not present in the forest, so individuals of this species could be separated from native ant species present in the ambient drift. At the bottom of the reach, drift nets were set up to capture ants and paper "tracers". Consumption efficiency was calculated as 1 – (# ants recovered / # paper tracers recovered). Five minute trials that resulted in the recovery of at least 20% of the paper tracers were counted as successful. A total of 14 successful trials were run in two different 5-m reaches in the Carapa-60 and Saltito-100 streams (no more than one trial per day was performed on each reach to prevent feeding saturation). For validation of this method, an additional trial was run in one of the reaches after approximately 82% of fish were removed following multiple-pass depletion with dip nets. Because the large pulse of food into a small stream reach could potentially saturate the capacity of the fish to consume it, capture efficiency measured by this method is likely conservative.

We devised an additional method to estimate both capture efficiency and total area-specific consumption rates of terrestrial insects, based on the difference between expected and measured drift of terrestrial insects in a given reach. In this method, we completely blocked off a 5-m reach at the upstream and downstream ends using drift nets, to prevent drift from upstream to enter the study reach and to prevent the movement of fishes into or out of the study reach

during the experiment. We measured the area of this reach and estimated the total input of terrestrial insects during a 15-minute trial based on the area-specific input rate from pan trap measurements. We assume that, in the absence of insectivorous fishes, all insects falling into the 5 m reach would be captured in the downstream drift nets. Therefore, the expected drift value in the absence of fish consumption was calculated as the area-specific input rate × area of reach × duration of trial. The difference between the actual dry mass of terrestrial insects recovered in drift nets and the expected value was attributed to fish consumption. Capture efficiency was then calculated as: (Expected Drift – Actual Drift) / Expected Drift. The consumption rate of terrestrial insects by fish (in units mg dry mass m^{-2} h^{-1}) was calculated as: (Expected Drift – Actual Drift) × area of reach × duration of trial. A total of five to eight 15-minute trials were performed on three different 5 m reaches along both the Carapa-60 and Saltito-100 streams.

Terrestrial insects in fish diet—To determine the relative contribution of terrestrial and aquatic insects in the diet of *P. annectens*, we dissected guts from 42 individuals from Carapa-60 and Saltito-100. Gut contents were spread evenly over a grid of 1mm squares, and we recorded the number of squares covered by each food category. Additionally, we compared stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) for *P. annectens* to values for terrestrial insects collected in pan traps (described above) and to values for aquatic insects collected in the Carapa-60 and Saltito-100. Aquatic insects were sampled in the two focal streams from leafpacks and natural substrate, and were sorted by family and analyzed for nutrient and isotopic composition, as described above.

Terrestrially derived fish excretion—We estimated the population density of *P. annectens* in three, 5-m reaches on both the Carapa-60 and Saltito-100, using a triple-pass depletion method (Lockwood and Schneider 2000). In each pass, the reach was blocked with seines, and three researchers collected fish with dip nets for 5 min. A total length-wet mass relationship was

established using 36 individuals ($r^2 = 0.995$). To estimate total biomass of *P. annectens* in each reach, we multiplied the abundance estimate by the size distribution from all captured individuals, which we divided into size classes at 0.1 g intervals.

Excretion rates for N and P were measured by incubating individual fish in a plastic bag containing 250 mL filtered stream water for one hour. The increase in NH_4^+ and total dissolved phosphorus (TDP), relative to control bags (incubated without fish), was attributed to fish excretion. Excretion rates were measured for 44 individual *P. annectens* (21 from Carapa-60 and 23 from Saltito-100). Per-capita N-excretion was modeled as a function of fish wet weight, and total N-excretion for each of the six reaches was estimated by applying the N-excretion rate for each size class to the estimated abundance of that size class. To estimate ecosystem-level nutrient recycling rates, we used the measured abundance and size distribution data for each reach in combination with the size-specific N and P excretion rates measured for *P. annectens*. We estimated terrestrially derived N- and P-excretion based on the proportion of terrestrial insects in the average diet of *P. annectens*, given our observations of nearly identical %N and %P between aquatic and terrestrial insects, and our assumption that assimilation efficiency is equal for nutrients derived from both types of food resources.

Stream nutrient demand—In March 2006, we performed a short-term NH_4^+ addition experiment in the Carapa-60 to estimate stream nitrogen demand. The reactive solute NH_4Cl was injected along with a conservative tracer (rhodamine WT) for 6 hours to measure plateau concentrations at four downstream stations. We elevated NH_4^+ concentrations to 244 $\mu g\ L^{-1}$. The rate of decline in dilution-corrected nutrient concentration over distance (k_C, or 1/uptake length, S_W), was converted into an areal uptake rate (U) by multiplying by stream velocity, average depth, and background nutrient concentration (Stream Solute Workshop 1990).

Statistical analysis— Regression analysis was used to test for relationships between stream width and canopy cover (independent variables) and mean terrestrial insect input rates for the eight study streams. To test for the effects of seasonality on input rates of terrestrial insects, we analyzed the monthly pan trap samples using a two-way ANOVA, with stream and season as categorical variables. Each four-day pan trap sample mass was considered a replicate, and values were log-transformed to normalize the data. Wet season was defined as May-January, and dry season was defined as February-April. We set $\alpha = 0.05$ for all analyses, and analyses were conducted using SAS (SAS Institute 2001).

Results

Terrestrial insect input rates—Mean input rates of terrestrial insects across the eight study streams ranged from 4.9–40.5 mg dry mass m^{-2} d^{-1} (Table 5.1), and were not related to stream width ($F_{1,6} = 1.85$, $p = 0.22$) or canopy cover ($F_{1,6} = 0.01$, $p = 0.92$). Mean input rates for the two focal headwater streams were 22.6 ± 9.5 mg dry mass m^{-2} d^{-1} (mean ± SE) in Carapa-60 and 35.4 ± 17.8 mg dry mass m^{-2} d^{-1} in Saltito-100 (Table 5.1). Ants composed most or all of the insect biomass in most pan trap samples. Large insects were noted in less than 5% of samples.

Monthly pan trap samples from three of the study streams shows no differences in mean input rates between wet and dry seasons ($F_{1,88} = 0.40$, $p = 0.52$; Fig. 5.1). Samples collected over 26 months in the Arboleda-30 ranged from 0.5-191.0 mg dry mass m^{-2} d^{-1}, with a mean input rate of 29.9 mg dry mass m^{-2} d^{-1}. Samples collected over 13 months in the Piper-30 ranged from 2.2-75.2 mg dry mass m^{-2} d^{-1}, with a mean of 19.5 mg dry mass m^{-2} d^{-1}. In the Sura-60, monthly samples collected over 13 months ranged from 3.1-89.3 mg dry mass m^{-2} d^{-1}, with a mean of 40.5 mg dry mass m^{-2} d^{-1}.

Composite samples of terrestrial insects had an elemental composition of 49.6 %C, 10.0 %N, and 0.5 %P. These values are very similar to mean N- and P-content for aquatic insects from low-solute La Selva streams (mean %N = 10.2, mean %P = 0.5; Chapter 2). By applying these values to the fish consumption rates described above, we estimate that, in the two focal streams, fish ingest 11.2–17.6 mg C m^{-2} d^{-1}, 2.3–3.5 mg N m^{-2} d^{-1}, and 0.11–0.17 mg P m^{-2} d^{-1} in the form of terrestrial insect biomass.

Consumption efficiency of insectivorous fishes feeding on terrestrial insects—Experimental ant additions in four 5-m reaches in the Carapa-60 and Saltito-100 led to estimates of mean consumption efficiency (# consumed vs. # available) ranging from 44-74% across the four study reaches (Appendix B). For the reach in which we removed nearly all fish, we measured a consumption efficiency of 3%.

Using ambient drift measurements to measure fish consumption of terrestrial insects, we estimated values for consumption efficiency ranging from 35-93% in six 5-m reaches in the Carapa-60 and Saltito-100 (Appendix C). We estimated fish ingestion rates of terrestrial insects ranging from 0.33-1.28 mg dry mass m^{-2} h^{-1}. Based on the mean nutrient content of terrestrial invertebrates in pan trap samples, fish ingested 33.2-138.0 µg N m^{-2} h^{-1}, and 1.59-6.62 µg P m^{-2} h^{-1} via this source.

Terrestrial insects in fish diet—Mean gut contents (by volume) for *P. annectens*, across 42 individuals, were 50.0% for both aquatic and terrestrial insects. There was no relationship between fish size and diet. Ants were the most commonly observed terrestrial insect in fish guts, including the common genera *Atta* and *Pheidole*.

P. annectens had isotopic signatures of -26.00 ± 0.42 ‰ for $\delta^{13}C$ (mean ± SD) and 9.44 ± 0.25 ‰ for $\delta^{15}N$. Terrestrial insects from composite pan trap samples had a $\delta^{13}C$ value of -28.49

± 0.61 ‰ and a $\delta^{15}N$ value of 3.43 ± 1.47 ‰. Aquatic insects sampled from the Carapa-60 and Saltito-100 ranged in $\delta^{13}C$ from -26.80 to -32.43 (mean -28.95 ± 1.25) and in $\delta^{15}N$ from 3.91 to 8.64 (mean 6.58 ± 1.40). The $\delta^{13}C$ signatures between these two food resources overlap extensively and preclude the use of this isotope in partitioning the diet of *P. annectens*. Using mean $\delta^{15}N$ values for the two food resources and a mean $\delta^{15}N$ fractionation value of 1.4 ± 0.21 ‰ (for consumers feeding on invertebrate diets; McCutchan et al. 2003), no solutions exist in a simple mixing model; however, $\delta^{15}N$ values for *P. annectens* more closely match aquatic insects.

Terrestrially derived fish excretion—P. annectens accounted for an estimated 98% of the fish assemblage in the Carapa-60 and Saltito-100 (232 out of 236 captured individuals). Population densities for *P. annectens* in the six focal reaches ranged from 3.9-14.0 individuals m^{-2}. Mean wet weight for *P. annectens* was 0.472 g (± 0.107).

Per-individual N excretion rates were calculated as: log NH_4^+ excretion = 0.52 * log [wet mass] + 1.30 ($r^2 = 0.23$). Mass-specific P excretion rates were calculated as: -0.71 * log [wet mass] + 0.18 ($r^2 = 0.25$). Total N-excretion for *P. annectens* populations in the six study reaches ranged from 31.2–113.0 µg NH_4^+ m^{-2} h^{-1}, and total P-excretion ranged from 3.3–12.2 µg TDP m^{-2} h^{-1} (ranges based on differences in fish density among reaches).

Mean N- and P-content of terrestrial insects collected from pan traps were nearly identical to average values for aquatic insects collected from a low-solute La Selva stream (Chapter 2). Therefore, the amount of N and P ingested, and (assuming equal assimilation rates) excreted, by *P. annectens* from aquatic and terrestrial insects should depend on the proportion of these food resources in the diet (50 % for each category, based on gut content analysis). We estimate that terrestrially derived N-excretion rates averaged 32.4 ± 6.8 µg NH_4^+ m^{-2} h^{-1} (mean ±

SE) across the six study reaches in the focal streams. Rates of terrestrially derived P-excretion averaged 3.5 ± 0.7 μg TDP m^{-2} h^{-1} in the focal streams.

Stream nutrient demand—Measured NH_4^+ uptake length in the Carapa-60 was 262 m, corresponding to an areal uptake rate of 41.4 μg NH_4^+ m^{-2} h^{-1}. Based on this measurement, total NH_4^+ excreted by *P. annectens* (mean 64.7 μg NH_4^+ m^{-2} h^{-1}) exceeds stream NH_4^+ demand in the focal headwater streams, and estimated terrestrially derived N-excretion was 79% of the magnitude of stream NH_4^+ demand.

Discussion

Our results indicate that in Neotropical lowland rainforests streams, terrestrial insects can be an important nutrient input relative to stream nutrient demand due to a relatively high and consistent input rate throughout the year, combined with high capture efficiency by insectivorous fish. We used independent approaches to estimate total input rates of terrestrial insects, capture efficiency of terrestrial insects by fish, and terrestrially-derived nutrient excretion, and the resulting values for these processes were consistent (i.e., estimates do not violate mass-balance constraints; Fig. 5.2). Fish consumed an average of 65% of terrestrial insects entering a 5 m stream reach. Estimated terrestrially derived excretion of N and P accounted for 41% and 90%, respectively, of nutrients ingested as terrestrial insects. Terrestrially derived N-excretion was 79% of the magnitude of measured stream NH_4^+ demand (Fig. 5.2).

Input rates of terrestrial insects in these rainforest streams were fairly evenly distributed throughout the year (Fig. 5.1), in contrast to the strong seasonality of terrestrial subsidies reported in some temperate streams, where summer input rates of terrestrial insects can be up to 100-fold higher than winter rates (e.g., Cloe and Garman 1996, Nakano and Murakami 2001).

Mean influx rates in our study streams were lower than some of the reported summer values for temperate deciduous forest headwater streams, which range from 11 – 450 mg m^{-2} d^{-1} (Baxter et al. 2005). However, the relative lack of seasonality in our study streams results in annual inputs similar to the few temperate forest streams for which annual estimates are available. Annual inputs of terrestrial insect biomass from the three streams in which monthly samples were collected ranged from 7.1 – 14.8 g m^{-2} y^{-1}, similar to annual inputs reported for forested streams in Japan (8.7 g m^{-2} y^{-1}, Kawaguchi and Nakano 2001) and Scotland (11 g m^{-2} y^{-1}, Bridcut 2000). The relatively constant background input of terrestrial invertebrates that we documented contrasts with large, periodic inputs that have been documented in other aquatic ecosystems (e.g., Carlton and Goldman 1983, Pray et al. 2009). This continuous subsidy in tropical forest streams provides a substantial contribution to the diet of predatory stream consumers year-round. Correspondingly, excretion of terrestrially derived nutrients by fish acts as a nutrient source for the stream ecosystem throughout the year.

We selected the two first-order streams as focal streams in this study because shallow streams with high fish densities should have the greatest biogeochemical impact per gram of terrestrial insect that enters the stream. The high densities of the insectivorous fish *Priapicthys annectens* in the two focal streams explains the high measured consumption efficiency of terrestrial insects by fish, as well as the important role of this species in nutrient recycling. In these two first-order streams, *P. annectens* has densities 2 – 28 times higher than densities of the five most abundant fish species in the nearby, third-order Sura-60 stream (Chapter 4).

We estimated that half of the N and P excreted by *P. annectens* was terrestrially derived, based on observations that gut contents contained 50% terrestrial insects and 50% aquatic insects on average, and that N- and P-content was similar between terrestrial and aquatic insects. This

calculation also is based on the assumption that assimilation efficiency was similar for both food sources. However, terrestrial insects can have large amounts of N invested in cuticular chitin (Sterner and Elser 2002), which could be assimilated at a lower efficiency. Stable isotope signatures for *P. annectens* suggest that the fish biomass is composed of more aquatically-derived nutrients, which, given our observations of equal proportions of aquatic and terrestrial insects in the fish guts, implies a greater assimilation efficiency for aquatic insects. If so, our calculations have overestimated the fraction of fish excretion that originated as terrestrial insects. For example, if assimilation efficiency for terrestrial invertebrates were on average one-half as high as for aquatic invertebrates, then one-third, rather than one-half of total N-excretion should be attributed to terrestrial inputs. Conversely, a greater assimilation efficiency for aquatic insects would also imply that fecal material of *P. annectens* was dominated by terrestrial insects, and this nutrient pathway may still be an important subsidy in the detritus-based food web.

Our results suggest that insectivorous fishes play an important role in the nutrient dynamics of our study streams by capturing and processing terrestrially-derived nutrients. The actual importance of this role has been the subject of some debate, as Wurtsbaugh (2007) argued that once insects enter a body of water, they become part of the nutrient budget of that respective aquatic ecosystem, and, even in the absence of fishes, these nutrients would eventually be mineralized by microbes. However, in contrast to lakes (e.g., Mehner et al. 2005), terrestrial insects falling into high-gradient headwater streams drift rapidly downstream in the absence of fish, as illustrated by the very low consumption efficiency in our ant-release trial where most fish had been removed (Appendix 1), or may escape from the stream upon reaching the stream margin or a debris dam. Although fish may be redundant in their capacity to mineralize nutrients from terrestrial insect biomass, they are playing a unique role in their capacity to capture and

retain these nutrients within short distances, ultimately making them available to the local stream ecosystem.

Our results suggest that total N-excretion by *P. annectens* exceeds NH_4^+ demand in our focal streams, and that rates of terrestrially derived NH_4^+ excretion may represent 79% of the magnitude of stream NH_4^+ demand. La Selva streams have relatively low NH_4^+ concentrations and high nitrification rates (Triska et al. 1993), suggesting that much of the N entering the streams through terrestrial insects may ultimately be exported as NO_3^-. In addition to terrestrial insects providing an energetic subsidy to insectivorous fishes, microbial processing of terrestrially derived N (through nitrification) represents an additional energy subsidy. These uptake values should be interpreted with caution, however, as nutrient addition experiments overestimate uptake length and therefore may underestimate areal uptake rates (Payn et al. 2005). Additionally, we assume that stream NH_4^+ demand measured in the Carapa in March 2006 is representative of normal conditions in our focal streams. However, our finding that terrestrially derived N-excretion is fairly large relative to estimated stream N-demand suggests that this flux may be important, even after accounting for potential measurement errors.

Several lines of evidence suggest that the terrestrially derived P excreted by *P. annectens* is also an important stream nutrient subsidy. Low-solute headwater streams at La Selva are P-limited (Pringle and Triska 1991), and even small increases in dissolved-P can result in large increases in microbial respiration (Ramírez et al. 2003) and leaf decomposition (Rosemond et al. 2002). P-excretion by the omnivorous characid *Astyanax aeneus* accounts for 71% of the stream P-demand in one 50 m reach of the nearby, fourth-order Sura-60, and the diet of this species consists of 65% terrestrial insects (Chapter 4), suggesting that terrestrially derived P-excretion is

a significant nutrient subsidy in this larger stream. Most probably, terrestrial insects represent an important source of biologically-available P in our focal headwater streams as well.

Understanding cross-boundary food-web subsidies has been a focus of extensive ecological research in recent years. Our results show that terrestrial insects can be important in nutrient-poor tropical headwater streams due to year-round high input rates and efficient consumption and conversion to dissolved nutrients by insectivorous fishes. Extensive deforestation throughout the tropics is decoupling this subsidy and altering trophic pathways in stream food webs. To better understand the consequences of losing connections between different ecosystems, we must be able to accurately measure the baseline importance of these cross-boundary flows. Our study uses a combination of novel methods to provide the first baseline measurements of terrestrial insect subsidies and their importance to nutrient dynamics in lowland wet tropical forest streams.

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Table 5.1: Physical and chemical characteristics, and terrestrial insect input rates for study streams. Discharge (Q) and nutrient values are means from monthly samples taken from 2006-2008. Terrestrial insect input values represent mean (± SE) from pan trap samples collected in 2006-2007.

Stream	Stream order	Q ($L\ s^{-1}$)	Width (m)	Canopy cover (%)	SRP ($\mu g\ L^{-1}$)	NO_3-N ($\mu g\ L^{-1}$)	NH_4-N ($\mu g\ L^{-1}$)	Ter. insect input (mg DM $m^{-2}\ d^{-1}$)
Carapa-60	1	2	1.1	84.8	4	157	25	22.6 (9.5)
Saltito-100	1	3	1.0	83.7	3	163	37	35.4 (17.8)
Piper-30	3	30	2.9	82.8	2	188	40	19.5 (1.6)
Saltito-60	3	110	4.6	79.9	33	98	19	11.7 (2.9)
Arboleda-30	2	170	5.4	88.1	135	126	20	29.9 (27.2)
Sura-60	4	190	6.2	82.4	3	199	21	40.5 (30.2)
Salto-60	5	450	9.8	84.1	10	180	20	4.9 (1.7)
Sura-30	4	610	7.9	87.3	83	163	18	12.0 (3.1)

Figure Legends:

Fig. 5.1: Monthly input rates of terrestrial insects in three La Selva streams from 2007-2008. Values are means (± SE) of samples collected from two 0.33 m^2 pan traps that were set out for four days each month in the same locations to measure seasonal variation in terrestrial insect input rates.

Fig. 5.2: Estimated contribution of terrestrially derived N- and P-excretion by *P. annectens* to stream nutrient demand in the two focal headwater streams. Mean values and ranges are reported for each flux of N and P. Ranges of terrestrial insect inputs are based on means the two focal streams, and ranges for fish consumption and terrestrially derived excretion are based on means for the six study reaches within the two focal streams. Percentage values reported reflect the proportion of one flux that goes into the following flux (for the top two values), or the proportion of total stream nutrient demand that is represented by terrestrially derived nutrient excretion (for the bottom value). Stream N-demand was measured by an NH_4^+ addition experiment in the Carapa-60.

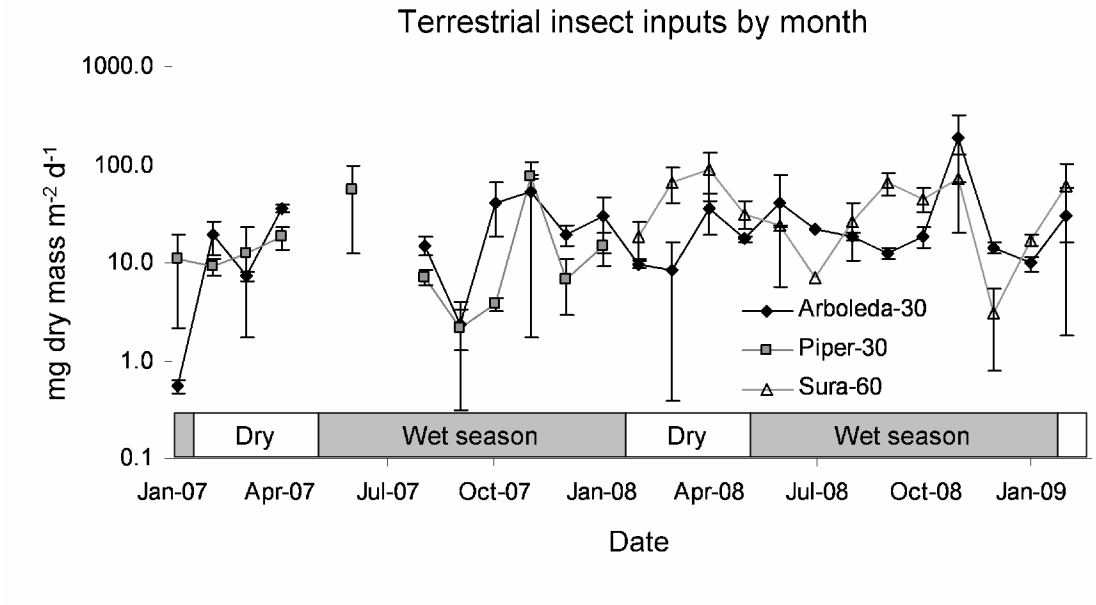
Terrestrial insect inputs by month
1000.0
100.0
10.0
1.0
0.1
mg dry mass m-2 d-1
Arboleda-30
Piper-30
Sura-60
Dry
Wet season
Dry
Wet season
Jan-07
Apr-07
Jul-07
Oct-07
Jan-08
Apr-08
Jul-08
Oct-08
Jan-09
Date

Fig. 5.1

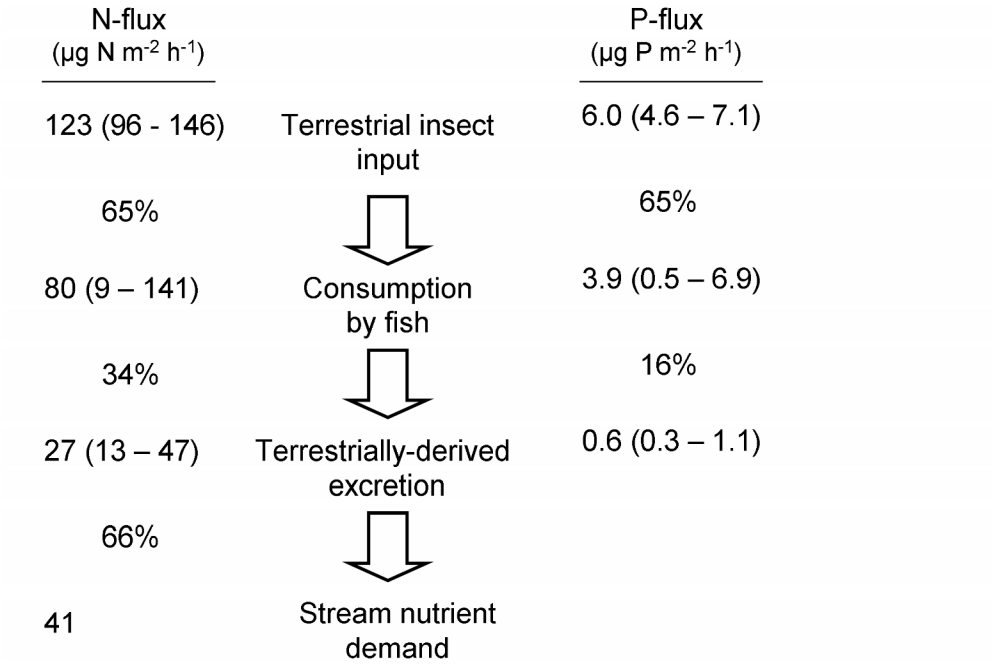
N-flux
(µg N m-2 h-1)
P-flux
(µg P m-2 h-1)
123 (96 - 146)
Terrestrial insect input
6.0 (4.6 – 7.1)
65%
65%
80 (9 – 141)
Consumption by fish
3.9 (0.5 – 6.9)
34%
16%
27 (13 – 47)
Terrestrially-derived excretion
0.6 (0.3 – 1.1)
66%
41
Stream nutrient demand

Fig. 5.2

CHAPTER 6

GENERAL CONCLUSIONS

Effects of phosphorus enrichment on stream ecosystems—Human activities have greatly increased the mobilization of nitrogen and phosphorus in the biosphere, which has led to the eutrophication of many freshwater ecosystems (Vitousek et al. 1997, Smith et al. 1999, Bennett et al. 2001, Smith and Schindler 2009). However, despite the threat that nutrient pollution causes to the functioning and stability of these ecosystems, there has been a mismatch between the spatial and temporal scales of anthropogenic pollution in aquatic ecosystems and the scale at which scientists study those problems. Stream ecologists commonly use short-term nutrient addition experiments to measure the capacity of streams to retain these nutrients (e.g., Stream Solute Workshop 1990), but we know little, for example, about how the capacity of streams to retain nutrients can change during years of nutrient loading, or how consumer assemblages can adapt to chronic high-P conditions. The streams at La Selva Biological Station that have received high levels of P-inputs over millennia offer a unique opportunity to measure the effects of chronic nutrient pollution on stream food webs, serving as a window into the future in predicting the effects of continued anthropogenic nutrient pollution.

Summary of dissertation objectives— The research presented in this dissertation explores the effects of long-term, high levels of P-inputs on trophic relationships in stream food webs, and in turn, the roles that consumers play in recycling nutrients. Chapter 2 examined the effects of long-term, high levels of P-loading on the elemental composition of the invertebrate assemblage. Prior studies have found inconsistent responses in the P-content of invertebrate consumers with

stream dissolved P levels (Cross et al. 2003, Bowman et al. 2005, Evans-White et al. 2005), but our study streams at La Selva have received P-inputs at higher levels, and over longer time periods, than other studies, allowing us to test the maximum effects of such high nutrient levels on food web stoichiometric relationships. Chapter 3 explored the physiological effects of high-P conditions on one important group of consumers, and tested whether these consumer assemblages become adapted to the local food quality across this heterogeneous landscape. In Chapter 4, I examined how stream nutrient levels interact with the diet and body nutrient content of the fish assemblages to control rates of N and P recycling. Finally, in Chapter 5, I quantified the potential importance of terrestrial insects as a nutrient subsidy in low-nutrient rainforest streams.

Chapter 2 Summary—The specific objective of Chapter 2 was to examine the effects of chronic stream P loading on the elemental composition of basal food resources and the invertebrate assemblage. According to stoichiometric theory, differences in P-content *among* different taxa indicate the relative P-demands of these organisms, but the P-content of a given taxon has generally been considered to vary minimally with diet (Sterner & Elser 2002). However, Cross et al. (2003) found that a whole-stream nutrient addition experiment led to increases in P-content of certain invertebrate taxa, raising questions about the assumption of strict homeostasis. I used the natural P gradient of La Selva streams to further explore this question, as the high-P La Selva streams receive ~3-fold higher concentrations of dissolved P, over much longer timescales, compared to the experimental enrichment of Cross et al. (2003). We found that, in the naturally high-P streams, epilithic algae and detritus were elevated ~4-fold in P-content compared to values from low-P streams, exceeding values reported from other nutrient-enriched streams (Stelzer and Lamberti 2001, Cross et al. 2003, Bowman et al. 2005).

The high levels of basal resource P-content resulted in a general enrichment of the P-content of invertebrate consumers, which increased from ~0.5 %P in the low-P study stream to ~1.0 %P in the high-P stream. This pattern of P-enrichment held across taxonomic groups and functional feeding groups. Even invertebrate predators had higher body P-content in the high-P stream, the first such evidence of deviation from strict homeostasis at higher trophic levels. Strictly speaking, these invertebrate consumers are still homeostatic, as their body elemental composition changed to a lesser degree compared to their food resources, when comparing across streams. However, our results show, for the first time, that environmental factors can be as important as phylogeny in controlling the elemental ratios of organisms. Ecosystem-scale implications of P-enriched invertebrate consumers include increased P-availability to higher trophic levels in the stream food web, and also potentially more P leaving the stream through the emergence of aquatic insects with terrestrial adult stages. The physiological effects of this P-enrichment on invertebrate consumers are explored in Chapter 3, and the effects on higher trophic levels of feeding on higher-P food resources were tested in Chapter 4.

Chapter 3 Summary—In Chapter 3, I explored whether consumer assemblages could become adapted to food resource quality across streams in a landscape characterized by nutrient heterogeneity. I extended the questions addressed in the previous chapter to focus on how P-enriched detritus affects growth and nutrient recycling by one important group of invertebrate consumers (chironomid larvae), and the capacity of these consumers to adapt to local differences in nutrient availability in a patchy landscape. Most detritivores deal with the problem of low-P food resources by having high body C:P ratios and low-growth rates, effectively decreasing dietary P-demand. By contrast, chironomid larvae, which dominate invertebrate biomass and production in many stream ecosystems, have relatively high body P-content and fast growth

rates, suggesting that they may be more susceptible to P-limitation, and therefore would show a greater response to increased dietary P-availability. Consistent with this prediction, prior results from studies at La Selva have found that chironomid growth rates increase with stream dissolved P levels (Rosemond et al. 2001, Ramírez and Pringle 2006), although both of these studies used chironomids collected from a single stream. When I measured growth rates of *in situ* chironomid assemblages, I was surprised to find no increase in either growth rates or P-excretion rates of chironomids from high-P streams feeding on high-P detritus, compared to chironomids from low-P streams feeding on low-P detritus. Also surprising was the lack of response in growth rates by chironomids in the experimentally-P-enriched stream to increases in food P. A third series of experiments helped clarify the previous results, showing that the chironomid assemblage from a naturally high-P stream has a higher P-demand compared the assemblage from an otherwise-similar low-P stream, due to apparent differences in non-RNA P-storage. Genetic analysis of these two assemblages showed that the observed physiological differences were not due to differences in species composition, and are most likely due to microevolution within species. The fact that chironomids from low-P habitats did not respond to additional dietary P has important ecological implications, potentially stabilizing the stream food web against perturbations due to changes in nutrient availability.

Chapter 4 Summary—The objective of Chapter 4 was to measure the relative effects of dietary P-supply and body P-demand in controlling P-recycling by the fish assemblage, and to understand the factors that could lead to one species playing a disproportionately important role in ecosystem-scale nutrient recycling. We found that, in high-P streams, most fishes feed on high-P food resources, and P-excretion rates are generally high across all species. In contrast, in low-P streams, many fishes feed on food resources that are lower in P, and especially for taxa

with higher body P-content, measured P-excretion rates drop to nearly zero. In low-P streams, fishes that feed on relatively high-P food resources (such as terrestrial insects), and have a slightly lower body P-demand, maintain fairly high rates of P excretion, which are disproportionately important contributors to P-recycling because of the low excretion rates of many of the other common species. These "keystone nutrient recyclers" appear to play important roles in supplying a limiting nutrient to the stream, based on their body stoichiometry and diet. Being able to predict which species play disproportionately important roles in ecosystems is critically important in directing conservation towards maintaining ecosystem functioning.

Chapter 5 Summary—The focus of Chapter 5 was to quantify the potential importance of terrestrial insects as a source of nutrients in low-nutrient headwater streams, mediated by ingestion and subsequent excretion by insectivorous fishes. I used a combination of methodologies to follow N and P from terrestrial insects through the stream food web, and produced the first estimated values of this nutrient pathway in tropical headwater streams. Our results show that this riparian subsidy is potentially important relative to stream nutrient demand in low-nutrient headwater streams. Changes in land use throughout the tropics are decoupling linkages between riparian and stream ecosystems, so our study provides important baseline data on the magnitude of one of these connections.

General conclusions—Each of these studies provided a new avenue to explore the interface between food webs, physiology, and biogeochemistry, and each has led to new insights as well as new questions. Taken together, this research shows that chronic high levels of P-loading in streams can have important effects on the flows of P through food webs, and that, in low nutrient streams, individual species can play important roles in supplying limiting nutrients.

In particular, the capacity of consumer assemblages to adapt to chronic nutrient loading is a surprising finding with important ecological implications. Productive avenues of future research include expanding the genetic analysis in Chapter 3 to find the alleles that cause differences in P-demand among chironomid assemblages, and, more generally, to expand the nutrient limitation experiments to other taxonomic groups to test whether the adaptation to local food quality that we documented in chironomids is widespread.

Measuring the extent to which species identity affects ecosystem functioning is a particularly urgent goal in the anthropocene era (Loreau et al. 2001). Results from these studies underscore the complexity of this issue. The apparent widespread plasticity in body elemental composition among stream invertebrates (Chapter 2) indicates that species identity (or higher taxonomic levels) may be less important than dietary P-content in determining consumer P-content. However, I also found that species identity is especially important in determining P-recycling rates by fishes, with large differences among species controlled by relatively small differences in body P-content. In light of emerging insights gained from Ecological Stoichiometry, ecologists should consider the stoichiometric contexts under which species play unique, or redundant roles in ecosystems.

Our finding that stream nutrient levels can greatly alter the elemental composition of invertebrate consumers has important implications on the role of the biota in stream nutrient dynamics. Retention of limiting nutrients by homeostatic consumers has the potential to lead to decouple the downstream movement of limiting and nonlimiting nutrients, resulting in greater ecosystem-scale retention of the limiting nutrient, but these effects are dampened with decreasing strength of homeostasis (Small et al. 2009). Our understanding of the linkages

between organism-scale stoichiometry and ecosystem-level nutrient dynamics is incomplete, and will undoubtedly evolve as new empirical data become available.

We quantified the importance of fish species in ecosystem-level nutrient recycling in Chapters 4 and 5, illustrating how a species can play a disproportionately important role in an ecosystem relative to its total biomass. We conclude that, if such a keystone nutrient recycler is extirpated, important changes in the availability of limiting nutrients could ensue. Exactly what those changes would be, however, depends on the complex dynamics of other species in the ecosystem. Such interconnectedness is illustrative of the challenges of integrating ecology and biogeochemistry.

Ecological stoichiometry is a powerful theory because of its success in linking organism-level retention and recycling of elements with ecosystem-level nutrient dynamics. Despite the inherent complexities, as we increase our understanding of the interactions between organisms and their environment, we move towards a deeper understanding of the consequences of human-induced changes in global nutrient cycles.

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APPENDIX A

COMPARISON OF CONSUMER AND DIET C:P VALUES, AND STOICHIOMETRIC REGULATION STRENGTH (H) FOR CHAPTER 2 AND CROSS ET AL. (2003)

Taxon	Size class (mm)	Order	Insect C:P		Diet	Diet C:P		H (eta)
			High-P stream	Low-P stream		High-P stream	Low-P stream	
This study								
Elmidae		Coleoptera	103	468	CPOM	466	1986	1.0
Ptilodactilidae		Coleoptera	120	157	CPOM	466	1986	5.4
Simuliidae		Diptera	77	306	FPOM	75	1517	2.2
Leptohyphes	2-3	Ephemeroptera	88	223	FPOM	75	1517	3.2
Leptohyphes	4-5	Ephemeroptera	64	204	FPOM	75	1517	2.6
Stenonema		Ephemeroptera	100	200	Epilithon	41	160	2.0
Thraulodes	3-4	Ephemeroptera	104	213	Epilithon	41	160	1.9
Thraulodes	5-6	Ephemeroptera	99	206	Epilithon	41	160	1.9
Thraulodes	4-5	Ephemeroptera	110	221	Epilithon	41	160	2.0
Gerridae	3	Hemiptera	198	312	insects	124	239	1.4
Gerridae	6	Hemiptera	158	295	insects	124	239	1.1
Coenagrionidae	9	Odonata	94	224	insects	124	239	0.8
Anacroneuria	14	Plecoptera	49	212	insects	124	239	0.4
Anacroneuria	6	Plecoptera	101	234	insects	124	239	0.8
Anacroneuria	10-11	Plecoptera	56	263	insects	124	239	0.4
Hydropsychidae	11-13	Trichoptera	104	235	insects	124	239	0.8
Hydropsychidae	5	Trichoptera	85	294	insects	124	239	0.5
Cross et al. 2003								
Lepidostoma		Trichoptera	218	877	CPOM	3063	4858	0.3
Tallaperla	5	Plecoptera	610	860	CPOM	3063	4858	1.3
Fattigia	15	Trichoptera	208	781	CPOM	3063	4858	0.3
Fattigia	11	Trichoptera	160	648	CPOM	3063	4858	0.3
Stenonema		Ephemeroptera	219	574	FPOM	673	1015	0.4
Pycnopsyche		Trichoptera	224	493	CPOM	3063	4858	0.6
Diplectrona	13	Trichoptera	359	467	25% FPOM, 25% CPOM, 50% chiron	991	1534	1.7
Diplectrona	8	Trichoptera	265	427	25% FPOM, 25% CPOM, 50% chiron	991	1534	0.9
Tipula		Diptera	229	373	CPOM	3063	4858	0.9
Epeorus		Ephemeroptera	322	369	FPOM	673	1015	3.0
Simuliium		Diptera	343	356	FPOM	673	1015	11.0
Parapsyche		Trichoptera	241	348	70% chiron, 15% FPOM, 15% CPOM	640	973	1.1
Isoperla		Plecoptera	164	305	100% chiron	120	145	0.3
Beloneuria		Plecoptera	287	279	70% chiron, 30% Parapsyche	151	183	-6.7
Lanthus	7	Odonata	249	246	100% Tallaperla	605	830	-26.1
Lanthus	16	Odonata	234	215	100% Tallaperla	605	830	-3.7
Hexatoma		Diptera	239	161	100% chironomids	120	145	-0.5
Leuctra		Plecoptera	156	159	CPOM	3063	4858	24.2
Tallaperla	8	Plecoptera	131	136	CPOM	3063	4858	12.3
midge	3-4	Diptera	113	131	FPOM	673	1015	2.8
midge	1-2	Diptera	80	123	FPOM	673	1015	1.0
tanypod	3-5	Diptera	112	102	midge (1-2)	85	135	-4.9
midge	5+	Diptera	141	95	FPOM	673	1015	-1.0
Dixa		Diptera	197	95	FPOM	673	1015	-0.6

Calculation of H *(eta).* Strength of homeostatic regulation (H) was calculated according to the equation

$$\log(y) = \log(c) + \frac{\log(x)}{\mathrm{H}}$$

where y = consumer C:P, x = resource C:P, c is a constant.

For invertebrate consumers from La Selva, diet was assigned based on functional feeding group (FFG). Predator taxa were assigned a food C:P value corresponding to the mean insect C:P for their respective stream. For invertebrate consumers from the Cross et al. (2003) study, diet was assigned based on quantitative gut contents values for primary consumers in Cross (2004), and for predatory taxa, based on energy-flow diagrams in Hall et al. (2000). In cases where consumers fed on multiple resources, a weighted average was used to calculate diet C:P. CPOM is coarse particulate organic matter (>1 cm), FPOM is fine particulate organic matter, and "chiron" is chironomid larvae.

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APPENDIX B

CONSUMPTION EFFICIENCY OF FISH ASSEMBLAGE FROM ANT TRACER EXPERIMENT

Mean values (± SE) for all successful trials, where at least 20 % of paper squares were recovered, are reported here for each study reach. One release was performed after nearly all fish were removed from the study reach.

Site	Successful trials	Paper squares recovered	Ants recovered	Consumption efficiency (%)
Carapa-60 A	3	33.0 (9.5)	10.0 (3.2)	60.4 (16.9)
Carapa-60 B	2	33.0 (2.0)	8.5 (1.5)	74.4 (3.0)
Saltito-100 A	5	49.6 (12.7)	18.8 (5.2)	50.5 (16.6)
Saltito-100 B	4	67.0 (7.5)	35.3 (7.5)	44.1 (15.2)
Saltito-100 B (no fish)	1	64.0	62.0	3.1

APPENDIX C

CONSUMPTION EFFICIENCY AND ABSOLUTE CONSUMPTION RATES FOR

PRIAPICTHYS ANNECTENS FEEDING ON TERRESTRIAL INSECTS

Values for mass of insects recovered represent means of 5-8 drift samples.

Site	Surface area (m^2)	Est. input during trial (mg/15 min)	Insects recovered (mg/15 min)	Fish consumption (mg/15 min)	Consumption efficiency (%)	Absolute consumption ($mg/m^2/h$)
Carapa A	7.50	1.77	0.39	1.38	78	0.73
Carapa B	5.25	1.24	0.80	0.44	35	0.33
Carapa C	4.25	1.00	0.90	0.10	10	0.65
Saltito 100 A	4.18	1.54	0.10	1.44	94	1.38
Saltito 100 B	4.88	1.80	0.76	1.04	58	0.85
Saltito 100 C	8.15	3.01	0.05	2.96	98	1.33