FOOD WEB RESPONSE TO LONG-TERM EXPERIMENTAL ENRICHMENT OF A DETRITUS-BASED STREAM ECOSYSTEM

by

JOHN M. DAVIS

(Under the Direction of Amy D. Rosemond)

ABSTRACT

Nutrient enrichment of freshwater ecosystems is occurring on a global scale with significant effects on their structure and function. However, our current understanding of these effects is limited because of the paucity of long-term experimental manipulations in detritus-based food webs. This study assessed the effects of enrichment in a detritus-based headwater stream. Using a paired watershed design, macroinvertebrate abundance, biomass, and production were compared in a treatment and reference stream during a five-year continuous enrichment. I examined whether the effects of nutrient enrichment varied between primary consumers and predators and whether consumer body size mediated consumer responses. To determine how changes in community structure affected nutrient fluxes, I also quantified the nutrient assimilation and excretion rate of a dominant primary consumer, *Pycnopsyche* spp. (Trichoptera). I also tested whether enrichment altered subsidies to riparian consumers via increased aquatic insect emergence from the nutrient-enriched stream.

During the fourth and fifth year of enrichment, nutrient enrichment stimulated primary consumer, but not predator, production and biomass. The increased dominance of large-bodied primary consumers that were predator-resistant likely attenuated the positive nutrient effect on higher trophic levels. Consumer response to nutrient enrichment also varied with body size, but this body size effect varied with trophic level. Specifically, enrichment increased the abundance and biomass of large-bodied primary consumers, but not large-bodied predators. Nutrient enrichment also accelerated the rate that *Pycnopsyche* assimilated and excreted nitrogen and phosphorus. Because *Pycnopsyche* disproportionately increased their rate of phosphorus assimilation relative to nitrogen, *Pycnopsyche* facilitated phosphorus sequestration at the stream-level. Despite nutrient enrichment doubling aquatic emergence biomass, it did not increase the biomass or abundance of riparian spiders, likely because of the increased dominance of predator-resistant prey. Enrichment increased the relative abundance of Trichoptera and the individual body size of emerging adults; two groups of prey that are not readily eaten by spiders. Thus, shifts in the primary consumer composition reduced the positive effects of nutrient enrichment on instream and riparian predators. Because consumer body size was an important factor determining how nutrient enrichment affected this stream food web, such species-specific traits may be key determinants in predicting ecosystem-level responses to nutrient enrichment.

INDEX WORDS: Headwater stream, Invertebrate, Detritus, Predator, Prey, Predator resistance, Food web efficiency, Nutrient enrichment, Body size, Species-specific trait, Assimilation, Resource subsidy, Spider, *Pycnopsyche*, Coweeta, Southern Appalachian

FOOD WEB RESPONSE TO LONG-TERM EXPERIMENTAL ENRICHMENT OF A DETRITUS-BASED STREAM ECOSYSTEM

by

JOHN M. DAVIS

B.S. The University of Florida, 2001

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2009

FOOD WEB RESPONSE TO LONG-TERM EXPERIMENTAL ENRICHMENT OF A DETRITUS-BASED STREAM ECOSYSTEM

by

JOHN M. DAVIS

Major Professor:	Amy D. Rosemond
Committee:	J. Bruce Wallace Darold Batzer Mark Bradford Jacqueline Mohan

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2009

DEDICATION

To my parents, who made sure that I knew that Florida was more than strip malls and asphalt parking lots.

ACKNOWLEDGEMENTS

My dissertation research would not have been possible without the contributions of numerous people over the years. First, I have to acknowledge my major professor, Amy Rosemond, who was willing to transform an avian ecologist into a stream ecologist. She has always treated me as a colleague, while continually challenging me and helping me to develop as an ecologist. I am also grateful to J. Bruce Wallace for giving me a solid grounding in the natural history of aquatic insects and southern Appalachian headwater streams. The quality of my dissertation research also benefited from the guidance of my dissertation committee, Darold Batzer, Mark Bradford, and Jacqueline Mohan. Also, I appreciate the input of my 'honorary' committee members, Wyatt Cross and Susan Eggert. It was only through Wyatt Cross's quality data collection during the early years of the experimental enrichment that I was able to address many of my research questions during the longer-term enrichment. The majority of the data collected in the reference stream during the later years of the enrichment can also be attributed to the efforts of Susan Eggert. Her important contributions in the field and laboratory over the years improved the quality of my dissertation research. Furthermore, their willingness to share their expertise and advice during my initial arrival in Athens helped to ground me in this ecosystem. I am also indebted to my previous undergraduate advisor, Kathryn Sieving, for her invaluable guidance during my early scientific career. Her mentoring provided me with many research opportunities and skills that helped to facilitate my dissertation research and have continued to shape my scientific perspective.

This research would not have been possible without the 20+ laboratory assistants who have helped me over the years, but most especially Erin Baker, Amanda Ely, Jessica Hoehn, and Jessica Holland. Much of the data contained within this dissertation would have suffered without their strong commitment to a mostly monotonous and wearisome laboratory job. As my dissertation research was part of a larger collaborative project, I have to recognize the various insights and contributions of my collaborators, Amy Rosemond, Deanna Conners, Cynthia Tant, Wyatt Cross, Jennifer Greenwood, J. Bruce Wallace, Susan Eggert, Keller Suberkropp, Vlad Gulis, and Namil Chung. This larger project would never have succeeded without the quality data provided by these researchers. This project also benefited from the commitment of the various project managers over the years—Jon Benstead, Roger Hilten, Nathan Taylor, and Susan Dye—who made sure that the field and laboratory work ran smoothly, even under difficult field and financial conditions. I also thank the past and present ecology staff —Renee Baughens, Janice Sand, Tom Maddox, Anisa Sullivan, Thelma Richardson, and Jeremy Sanderlin—for keeping the wheels of ecology greased over the years and preserving the spirit of the 'Institute.'

Moreover, I am grateful for the support of Sue Dye these past four years because her encouragement and understanding gave me the confidence to finish, especially during the most trying times. I also received much support over the years from my officemates in Ecology 199B and the South Carrels who were always willing to listen and provide insightful advice on a range of problems. Countless friends over the years at the University of Georgia and in Athens continually reminded me that there was more to life than dissertations, experimental nutrient enrichments, and *Pycnopsyche gentilis*. A final thank you goes to Cynthia Tant, my fellow SNAX compatriot, who provided me with advice these past five years as we navigated our way through the 'dark' world of southern Appalachian headwater streams.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

General context— Increased nutrient mobilization associated with human activities represents one of the greatest threats to global freshwater ecosystems (Smith et al. 1999). In fact, because of increased urban development, fertilizer production and run-off, and exhaust from fossil fuel consumption, humans now represent the dominant source of nitrogen (N) and phosphorus (P) loading to freshwater ecosystems (Smith et al. 1999, Bennett et al. 2001, Smith and Schindler 2009). As primary productivity in global freshwater ecosystems is largely limited by nutrient availability (N and P) (Schindler 1977, Gruner et al. 2008, Schindler et al. 2008); increased nutrient loading can significantly alter the functioning, stability, and overall productivity of impacted ecosystems (Rosenzweig 1971, Smith et al. 1999). However, these effects are difficult to predict because few large-scale experimental manipulations have been conducted (but see Slavik et al. 2004, Cross et al. 2006) and ecosystem-level responses are difficult to predict from small-scale experimental approaches (Carpenter 1996).

Our predictive ability is further limited because the overall effects of increased nutrient availability on ecosystem processes can depend on food web structure and ecological context (e.g., Abrams 1993, Chase 1999). Initially, because early food web models (e.g., Oksanen et al. 1981) assumed that resources were efficiently transferred between trophic levels, the positive effects of nutrient enrichment were similarly predicted to flow efficiently to higher trophic levels (Abrams 1993). Accordingly, these efficient trophic transfers were predicted to result in the

addition of higher trophic levels and increased food chain length (Oksanen et al. 1981). Previous evidence from large-scale experimental enrichments have largely supported these earlier predictions as nutrient enrichment stimulated both primary consumer and predator production (Slaney et al. 2003, Slavik et al. 2004, Cross et al. 2006).

However, mounting evidence from other theoretical and empirical studies show that food web structure can reduce trophic transfer efficiency because of the increased dominance of defended taxa, suggesting that nutrient enrichment may not always stimulate productivity of higher order consumers (Abrams 1993, Chase 1999, Diaz and Rosenberg 2008). For instance, increased nutrient loads to marine coastal zones can reduce trophic transfer efficiencies between algae and primary consumers, generating excess algal production that is not consumed by primary consumers and is ultimately decomposed by heterotrophic microbes (Diaz and Rosenberg 2008). Because this increased algal production does not stimulate primary consumer or predator production, the positive effects of enrichment may not always be efficiently transferred through food webs. Small-scale mesocosm experiments have also shown similar reductions in trophic efficiency as enrichment decreased predator production, even with sustained increases in primary consumer productivity (e.g., Bohannan and Lenski 1999, Stevens and Steiner 2006). In contrast to earlier food web models that predicted a positive relationship between food chain length and ecosystem productivity (Oksanen et al. 1981), a large-scale regional comparison of lake ecosystems indicated that food chain length was related to ecosystem size, not ecosystem productivity (Post et al. 2000). Because trophic distance can attenuate the positive effects of enrichment on higher trophic levels, nutrient enrichment may not always stimulate predators because they are more top-down controlled (Brett and Goldman 1997). These contrasting responses of consumers to nutrient enrichment show our current

inability to adequately predict how increased nutrient availability may alter the structure, function, and overall stability of freshwater ecosystems.

Even in ecosystems where enrichment has largely increased overall food web productivity, responses of component populations may be difficult to predict, due to species-specific variation in response (e.g., Slavik et al. 2004, Cross et al. 2006). For instance, nutrient enrichment of a detritus-based headwater stream mostly stimulated those consumers that fed primarily on leaf detritus (i.e., shredders) compared to other functional feeding groups (Cross et al. 2006). However, even taxa within the same functional feeding groups can exhibit variation in response to an environmental driver such as nutrient enrichment. A 16-year seasonal enrichment of an arctic stream increased the abundance of a common filtering trichopteran (*Brachycentrus* spp.), but not black fly larvae (Diptera: Simulidae), another common filterer in this ecosystem (Peterson et al. 1993, Slavik et al. 2004). As the enrichment responses of consumers have also been related to larval development time (Cross et al. 2006), body phosphorus content (Singer and Battin 2007), and consumer body size (Bourassa and Morin 1995), this suggests that ecosystem-level effects of enrichment can vary depending on life history, physiological, or functional diversity of organisms within recipient ecosystems.

The prevalence of intentional artificial nutrient enrichments of freshwater ecosystems aimed at stimulating predator production (i.e., fish) (Slaney et al. 2003, Compton et al. 2006) suggests that the potential drawbacks of excess nutrients may not be fully appreciated. The vast uncertainties in how ecosystems and consumers respond to nutrient enrichment also reveal our current inability to adequately predict how specific ecosystems may respond to nutrient enrichment. Thus, despite the 30+ years since the initial recognition of the negative impacts of excess nutrients on freshwater ecosystems (Schindler 1974, 1977), we still lack sufficient long-

term experimental evidence to fully understand and predict the effects of nutrient enrichment on the structure and function of a diversity of freshwater ecosystems (Schindler et al. 2008, Smith and Schindler 2009). Accordingly, the main objective of this study was to increase our understanding of how detritus-based headwater streams, an often overlooked ecosystem, responded to long-term enrichment. Given the broad distribution of streams similar to my study streams, it also sought to improve our general understanding of how functional diversity and food web structure can alter the effects of nutrient enrichment on aquatic food webs.

Importance of detrital-food web pathways— The series of studies comprising this dissertation were conducted under the aegis of a larger-scale collaboration that assessed the effects of nutrient enrichment on the production of stream-dwelling organisms and consequent effects on nutrient and carbon dynamics in a detritus-based ecosystem. This larger study focused on a detritus-based food web because most primary production enters food webs via detrital pathways (Moore et al. 2004), but the effects of nutrient enrichment on these detrital pathways are not well-known (but see Rosemond et al. 2001, Rosemond et al. 2002, Benstead et al. 2005). With respect to aquatic ecosystems, understanding these nutrient enrichment effects will increase our overall predictive ability because of the wide geographic distribution of these detritus-based food webs. For instance, many stream and river food webs are net heterotrophic, such that inputs of allochthonous organic matter exceeds *in situ* primary production (Mulholland et al. 2001). Even within lake ecosystems that exhibit high levels of autotrophic production, consumer production can still be largely reliant upon terrestrially derived organic matter (Pace et al. 2004, Cole et al. 2006). Despite this overall importance of detrital food web pathways in aquatic ecosystems, previous large-scale experimental enrichments have largely focused on algal-based food webs (Peterson et al. 1985, Peterson et al. 1993, Slavik et al. 2004). Therefore, our

ecosystem-level nutrient enrichment of a detritus-based headwater stream sought to increase our understanding of how nutrients alter the structure and function of this important food web type.

Importance of headwater streams— While these studies were aimed at improving our ability to predict the ecosystem-level responses of detritus-based ecosystems to enrichment, their findings also have practical implications for the eastern United States because of the high freshwater diversity in this region (Abell et al. 2000, Meyer et al. 2007). Partly because the freshwater ecosystems in this region were not glaciated during the previous glacial maximum (Isphording and Fitzpatrick 1992), they serve as global diversity hotspots for some freshwater taxa (e.g., mollusks, fishes, and amphibians) (Dodd 1997, Morse et al. 1997, Warren et al. 1997, Abell et al. 2000). Furthermore, even within these highly diverse river networks, headwater streams can represent important biotic diversity refugia because these small streams dominate overall stream miles in river networks and possess a diversity of habitat types (Huryn and Wallace 1988, Morse et al. 1997, Meyer and Wallace 2001, Meyer et al. 2007). Headwater streams can also represent important biogeochemical hotspots because of their small surface area to volume ratios that can accelerate carbon and nutrient transformations (Meyer and Wallace 2001, Peterson et al. 2001). As headwater streams are directly linked to downstream food webs through material, energy, and macroinvertebrate transport (Vannote et al. 1980, Wipfli and Gregovich 2002, Romaniszyn et al. 2007), changes in the functioning of these upstream ecosystems have the potential to substantially alter downstream food web dynamics. Despite their relatively small size, headwater streams can represent important biological and biogeochemical hotspots for maintaining the overall function and biotic integrity of stream networks. Thus, understanding the enrichment response of these biologically important

ecosystems will help improve our ability to predict the response of overall river networks to nutrient enrichment.

Project overview— These studies were focused on long-term responses (years four and five) of a headwater stream to nutrient enrichment and followed-up on results from the first two years of enrichment (see Cross 2004, Greenwood 2004). Evidence from the first two years of enrichment indicated that nutrient enrichment stimulated the production of heterotrophic microbes on detritus, which improved detritus quality (lower carbon to nutrient ratios) and stimulated the production of macroinvertebrate consumers (Gulis and Suberkropp 2003, Cross et al. 2006). Associated with these increases in consumer production, enrichment also accelerated organic matter processing rates and reduced overall leaf litter standing crops, which subsequently lengthened periods of low resource availability (Gulis and Suberkropp 2003, Cross et al. 2006, Greenwood et al. 2007, Benstead et al. 2009). Thus, despite the increased resource quality associated with enrichment, reductions in resource quantity (quantity of detrital carbon) were hypothesized to reduce positive bottom-up effects on macroinvertebrate consumers over a longer-term period of enrichment. We predicted that declines in organic matter standing crop would eventually lead to carbon limitation and suppress the positive nutrient effects on macroinvertebrate production. As we also saw concurrent increases in the production of heterotrophic microorganisms and changes in organic matter dynamics (Gulis and Suberkropp 2003, Benstead et al. 2009), we also examined whether these microorganisms were dominant drivers of ecosystem-level processes within the nutrient-enriched stream. Thus, the overall focus of this five-year nutrient enrichment was to assess the effects of chronic enrichment on microorganisms and higher-order consumers, and to determine the subsequent effects on ecosystem-level processes such as carbon and nutrient flux.

Experimental design— In the context of this larger-scale collaboration, the objective of my dissertation was to assess the long-term effects of nutrient enrichment on macroinvertebrate community structure and function within these stream food webs. Specifically, we applied a paired-watershed approach in two forested headwater catchments (C53 and C54) with similar physiochemical properties (i.e., catchment area, slope, elevation, discharge, temperature, and pH). We conducted this study at the USDA Forest Service Coweeta Hydrologic Laboratory, a Long-Term Ecological Research site in Macon County, North Carolina, USA. Coweeta is a heavily-forested experimental watershed (2185 ha) located in the southern Appalachians Mountains. Prior to the experimental enrichment, the reference (C53) and treatment (C54) streams did not differ in nutrient concentrations; however, from July 2000 to August 2005 (ca. 1877 days), we experimentally enriched a 150-m reach of the treatment stream with N and P. This enrichment successfully increased nutrient concentrations in the treatment stream to a realistic, low-level enrichment, while the reference stream concentrations did not change. Specific details of the nutrient enrichment methods are described elsewhere (Chapter 2, Gulis and Suberkropp 2003, Rosemond et al. 2008).

On a monthly basis, macroinvertebrates were sampled in both streams during a two year pretreatment period and a five year experimental enrichment to assess the macroinvertebrate response to enrichment (Chapter 2). Results from the pretreatment period and the initial two years of enrichment have been previously reported (Cross 2004, Greenwood 2004, Cross et al. 2006, Greenwood et al. 2007). Based on the results from Chapter 2, I also conducted a body size-specific analysis to assess the relationship between consumer body size and consumer response to nutrient enrichment (Chapter 3). Because consumers are important drivers of ecosystem processes in these headwater streams (Wallace et al. 1991, Wallace and Hutchens

2000), I also conducted laboratory-based and field-based experiments to assess the role of a dominant stream consumer in the stream-level elemental transformations associated with nutrient enrichment (Chapter 4). Finally, because aquatic insect emergence can represent a significant subsidy to terrestrial predators (Baxter et al. 2005), I sampled aquatic emergence and terrestrial predators (i.e., riparian spiders) to determine the effect of stream nutrient enrichment on the surrounding riparian community (Chapter 5).

Dissertation objectives

Chapter 2: Long-term nutrient enrichment decouples predator and prey production

The objective of Chapter 2 was to determine the long-term effects of nutrient enrichment on the production and composition of the macroinvertebrate community within a detritus-based headwater stream. Results from the first two years of enrichment showed that nutrient enrichment stimulated the production of heterotrophic microbes, primary consumers, and predators (Gulis and Suberkropp 2003, Cross et al. 2006). Although this and other studies suggested an efficient flow of resources through this stream food web (Wallace et al. 1997, Cross et al. 2006), results from the second year of enrichment indicated an attenuation of the positive enrichment response of predators (Cross 2004, Cross et al. 2006). Therefore, my continued sampling during the fourth and fifth year of enrichment assessed whether nutrient enrichment would continue to stimulate multiple trophic levels, or whether potential shifts in the structure of the macroinvertebrate community might alter these flows and reduce the positive effects on higher trophic levels.

Chapter 3: Nutrient enrichment differentially affects body sizes of primary consumers and predators in a detritus-based ecosystem

Results from Chapter 2 and the first two years of enrichment (Cross 2004, Cross et al. 2006) indicated that the response of consumers to nutrient enrichment was not homogenous across taxonomic groups. This suggested that consumers may possess species-specific traits that alter their nutrient enrichment response. Therefore, the objective of Chapter 3 was to assess whether the nutrient response of consumers was related to consumer body size, an important species response- and effect-trait within aquatic food webs (sensu Naeem and Wright 2003). Specifically, predation risk is related to consumer body size (Crowl and Covich 1990, Emmerson and Raffaelli 2004, Woodward and Warren 2007); thus, the response of prey to any changes in predation pressure associated with nutrient enrichment may be mediated by consumer body size. Because consumer body size scales allometrically with metabolic rate (Brown et al. 2004), shifts in consumer body size distributions may also alter nutrient and energy flows via changes in consumer respiration and excretion rates (Poff et al. 1993, Hall et al. 2007). Thus, this chapter attempted to elucidate the relationship between species-specific traits and consumer response to nutrient enrichment because it would allow us to better predict ecosystem-level effects.

Chapter 4: Nutrient enrichment affects stream nutrient transformations via increased consumer assimilation and excretion rates

Results from concurrent studies showed that enrichment significantly increased organic matter processing rates and carbon export during the first two years of enrichment (Greenwood 2004, Greenwood et al. 2007, Benstead et al. 2009). Within headwater stream ecosystems, consumers can represent an important driver of these processes (Cuffney et al. 1990, Wallace et

al. 1991, Cross et al. 2005c). Therefore, my fourth chapter examined the importance of a dominant stream consumer, *Pycnopsyche* spp., in driving these observed changes in ecosystem processes. I used laboratory-based assimilation experiments and field-based excretion experiments to assess the effects of enrichment on *Pycnopsyche* assimilation and excretion rates. By applying the results from the laboratory- and field-based experiments to known *Pycnopsyche* standing stocks and production (Chapter 2), I examined the effects of nutrient enrichment on these nutrient transformations at the ecosystem-scale. Because the rate that consumers ingest and assimilate nutrients is the first metabolic step in converting basal resources into consumer biomass, shifts in these rates may have important implications for meeting overall consumer nutrient demand and maintaining nutrient flows through aquatic food webs.

Chapter 5: The effects of stream nutrient enrichment on aquatic to terrestrial subsidies along a forested headwater stream

Aquatic ecosystems are linked to their surrounding riparian zone via aquatic emergence subsidies to terrestrial predators (Sanzone et al. 2003, Baxter et al. 2005). Furthermore, secondary production of aquatic emergence can be ca. 25% of benthic secondary production and the two likely covary over benthic productivity gradients (Jackson and Fisher 1986). This suggests that if nutrient enrichment stimulated the secondary production of stream consumers (Chapter 2), it may have similarly increased the export of aquatic insect emergence to terrestrial predators. Therefore, my fifth chapter examined the effects of nutrient enrichment on aquatic insect subsidies to the surrounding terrestrial predator community (i.e., riparian spiders). I sampled riparian spiders along the reference and treatment streams to assess whether potential increases in aquatic emergence stimulated the abundance and biomass of terrestrial spiders. By

isotopically enriching both streams with a ^{15}N stable isotope tracer, I also quantified the flow of N from these streams to the surrounding terrestrial community. This quantification allowed me to test whether nutrient enrichment increased the reliance of terrestrial predators on aquatic subsidies because of greater emergence availability. Understanding the effects of nutrient enrichment on these linkages would determine whether the effects of nutrient enrichment extended to adjacent food webs that were not directly enriched.

CHAPTER 2

LONG-TERM NUTRIENT ENRICHMENT DECOUPLES PREDATOR AND PREY PRODUCTION[1]

[1] Davis, J. M., A. D. Rosemond, S. L. Eggert, W. F. Cross, and J. B. Wallace. To be submitted to *Proceedings of the National Academy of Sciences, U.S.A.*

Abstract

Increased nutrient mobilization by human activities represents one of the greatest threats to global ecosystems, but its effects on ecosystem productivity can differ depending on food web structure. When this structure facilitates efficient energy transfers to higher trophic levels, evidence from previous large-scale enrichments suggests that nutrients can stimulate the production of multiple trophic levels. Here we report results from a five-year continuous nutrient enrichment of a forested stream that increased primary consumer production, but not predator production. Because of strong positive correlations between predator and prey production (evidence of highly efficient trophic transfers) under reference conditions, we originally predicted that nutrient enrichment would stimulate energy flow to higher trophic levels. However, enrichment decoupled this strong positive correlation and produced a non-linear relationship between predator and prey production. By increasing the dominance of large-bodied predator-resistant prey, nutrient enrichment truncated energy flow to predators and reduced food web efficiency. This unexpected decline in food web efficiency indicates that nutrient enrichment, a ubiquitous threat to aquatic ecosystems, may have unforeseen and unpredictable effects on ecosystem structure and productivity.

Introduction

By shifting species dominance and energy pathways, nutrient enrichment from human activities represents one of the greatest threats to global ecosystems with significant consequences for ecosystem structure and function (Smith et al. 1999). However, these effects are difficult to predict because of few large-scale experimental manipulations (e.g., Slavik et al. 2004, Cross et al. 2006) and the potential difficulties in predicting ecosystem-level responses from small-scale experimental approaches (Carpenter 1996). Despite this uncertainty and limited knowledge of how aquatic ecosystems respond to nutrient enrichment, a large number of restoration projects artificially enrich streams and rivers to stimulate fish production (Slaney et al. 2003, Compton et al. 2006). These practices are largely based on early food web models and empirical studies showing that nutrient enrichment can have positive bottom-up effects that extend to top predators (e.g., Oksanen et al. 1981, Slavik et al. 2004). Thus, when the entire primary consumer assemblage is equally vulnerable to predators (Oksanen et al. 1981), increased primary consumer production is predicted to be efficiently transferred to higher trophic levels (i.e., high trophic efficiency) where it stimulates predator production (e.g., Slavik et al. 2004).

However, mounting evidence indicates that nutrient enrichment can frequently have unintended consequences as resources are diverted into alternate food web pathways that are relatively unavailable to higher trophic levels. For instance, high levels of nutrient enrichment in marine coastal zones can reduce trophic transfer efficiencies between algae and primary consumers, generating excess algal production that is not consumed by primary consumers and is ultimately decomposed by heterotrophic microbes (Diaz and Rosenberg 2008). In extreme cases, nearly 100% of primary productivity may be diverted to microbial respiration, resulting in increasingly prevalent anoxic ‘dead-zones’ (Diaz and Rosenberg 2008). Food web models also

predict that nutrient enrichment can decrease food web stability as it can amplify variability in predator-prey cycles and even extirpate predator populations (i.e., 'the paradox of enrichment') (Rosenzweig 1971).

More recent models predict that nutrient enrichment can further alter predator-prey interactions by increasing the dominance of predator-resistant primary consumers, diverting energy flow to predator-resistant pathways that are relatively inaccessible to top predators (e.g., Abrams 1993, Chase 1999). Small-scale mesocosm experiments have shown that such a reduction in trophic efficiency can ultimately decrease predator production, even with sustained increases in primary consumer productivity (i.e., resulting in a trophic decoupling) (e.g., Bohannan and Lenski 1999, Stevens and Steiner 2006). Thus, if nutrient enrichment disproportionately stimulates predator-resistant prey, it may reduce positive nutrient effects on predators and inhibit predator production.

Despite these results from small-scale manipulations, there is no ecosystem-level evidence that enrichment can decouple predator production from primary consumers. While nutrient enrichment of coastal zones can reduce the production of higher trophic levels, this effect results not from a decoupling of primary consumer and predator production, but rather from a diversion of energy flow between basal resources and primary consumers that result in anoxic conditions (e.g., Diaz and Rosenberg 2008). In fact, other large-scale experimental nutrient enrichments have largely stimulated both primary consumer and predator production (e.g., Deegan and Peterson 1992, Slaney et al. 2003, Slavik et al. 2004). This suggests that such trophic decouplings may be sampling artifacts of small-scale manipulations using species-depauperate food webs and may be unlikely in diverse natural food webs. Because the effectiveness of anti-predator defenses depend on the foraging strategies used by predators

(Power et al. 1992), food webs with a diversity of predators and foraging strategies may increase the predation risk of predator-resistant prey, maintain efficient energy flow to higher trophic levels, and reduce the likelihood of an enrichment-induced trophic decoupling.

Here we report the results from an ecosystem-level manipulation of a detritus-based headwater stream that is dominated by ca. 20 taxa of gape-limited invertebrate and salamander predators (Stevens and Steiner 2006). Primary consumer production in these stream food webs is based on seasonal inputs of terrestrial leaf detritus because stream algal production is light limited by a dense forest understory (Wallace et al. 1997). As both the invertebrate and salamander predators in these stream food webs predominantly eat small-bodied primary consumers, these two predator groups occupy a similar trophic position (Davic 1991, Hall et al. 2000, Johnson and Wallace 2005).

For five years, we experimentally enriched a treatment stream with low levels of dissolved nitrogen and phosphorus and compared the food web response in the treatment stream to a reference stream. Previous work in these streams showed that nutrient enrichment increased microbial production at the base of the food web, where it subsequently stimulated primary consumer and predator production (Gulis and Suberkropp 2003, Cross et al. 2006). There was also a strong linear relationship between predator and prey production under reference conditions (Wallace et al. 1997), which suggested a relatively efficient flow of energy between heterotrophic microbes, primary consumers, and predators. Therefore, based on our earlier results from the first two years of enrichment (Cross et al. 2006) and from a similar long-term enrichment that showed positive effects of nutrient enrichment on predators and primary consumers (Deegan and Peterson 1992, Slavik et al. 2004), we hypothesized *a priori* that in

subsequent years of nutrient enrichment (years four and five), primary consumer and invertebrate predator production would continue to be positively correlated.

Methods

We conducted this study at the USDA Forest Service Coweeta Hydrologic Laboratory, a Long-Term Ecological Research site in Macon County, NC, USA. Coweeta is a heavily-forested experimental watershed (2185 ha) located in the southern Appalachians. The forest is dominated by mixed hardwoods (oak, maple, and tulip poplar) with a dense understory dominated by *Rhododendron maximum* that results in heavy stream shading. This light limitation decreases autotrophic production and increases the food web's reliance on heterotrophic microbes that colonize inputs of terrestrial leaves (Wallace et al. 1997, Cross et al. 2007).

To test the long-term effects of nutrient enrichment on macroinvertebrate food webs, we used a paired-watershed approach in two forested headwater catchments (C53 and C54) with similar physiochemical properties (i.e., catchment area, slope, elevation, discharge, temperature, and pH). Both streams were fishless and were dominated by over 20 taxa of gape-limited invertebrate (e.g., *Beloneuria* [Plecoptera], Ceratopogonidae [Diptera], *Cordulegaster* [Odonata], *Hexatoma* [Diptera], and *Lanthus* [Odonata]) or vertebrate (e.g., *Eurycea wilderae* [Plethodontidae] and *Desmognathus quadramaculatus* [Plethodontidae]) predators. Further descriptions of the study sites are reported elsewhere (Lugthart and Wallace 1992).

The reference (C53) and treatment (C54) streams did not differ in nutrient concentrations prior to the experimental enrichment (mean ± SE, C53: DIN: 23.2 ± 8.5 μg L^{-1}, SRP: 6.8 ± 3.0 μg L^{-1}; C54: DIN: 29.3 ± 4.9 μg L^{-1}, SRP: 9.5 ± 2.3 μg L^{-1}). From July 2000 to August 2005 (ca. 1877 days), we experimentally enriched a 150-m reach of the treatment stream with nitrogen

(NH_4NO_3) and phosphorus (K_2HPO_4 and KH_2PO_4). We dripped nutrients continuously along the entire 150-m length of the treatment stream using an irrigation line running down the center of the stream. Details of the nutrient-delivery system have been previously reported (2003). This flow-proportional delivery system increased nutrient concentrations in the treatment stream to a realistic, low-level enrichment (DIN: 506.2 ± 36.3 µg L^{-1}, SRP: 80.0 ± 5.6 µg L^{-1}), while the reference stream concentrations during this same time period were comparable to the pretreatment period (DIN: 31.0 ± 3.4 µg L^{-1}, SRP: 8.0 ± 1.3µg L^{-1}). We monitored stream nutrient concentrations every two weeks at three points along the 150-m reach of the treatment stream and at the weir of the reference stream (APHA 1998). Water temperature was measured every 30 minutes in both streams with Optic StowAway temperature probes (Onset Computer, Pocasset, Massachusetts, USA). We measured stream discharge at 5-minute intervals with an ISCO data logger.

We sampled the benthic macroinvertebrate fauna in both streams during an initial pretreatment period (September 1998 to June 2000) followed by a five-year experimental period (July 2000 to August 2005). On a monthly basis, we collected four mixed-cobble substrate samples per stream using a stovepipe corer (400 cm^2) and processed them according to established protocols (Cross et al. 2006). We identified most taxa to genus; however, we only identified Chironomidae to either Tanypodinae (predators) or non-Tanypodinae (non-predators), and non-insects (e.g., oligochaetes, nematodes, copepods, etc.) to the lowest possible taxonomic level. We measured the length of each individual to the nearest millimeter and then applied previously published length-mass regressions (Benke et al. 1999) to quantify ash-free dry mass (AFDM). For most taxa, we calculated secondary production with the size-frequency method corrected for cohort production intervals (Benke 1979, Wallace et al. 1999). However, we used

the instantaneous growth rate method to calculate the secondary production of non-Tanypodinae chironomids (Cross et al. 2005b). For a few taxa that lacked sufficient data to calculate secondary production with these two methods, we estimated their annual production by multiplying their annual standing stock biomass by their average production to biomass ratio (e.g., oligochaetes, nematodes, copepods). We classified all taxa as either predators or primary consumers based on literature values (Merritt and Cummins 1996) and on previous research conducted in our study streams (Wallace et al. 1999).

We evaluated the trophic level response to nutrient enrichment with community biomass and secondary production. However, because it integrates multiple metrics in assessing taxonomic response to nutrient enrichment (i.e., abundance, biomass, growth rate, survivorship, and development time) (Benke 1993), we believed that secondary production was the best metric to quantify the overall response.

Because large-bodied primary consumers were relatively predator resistant (Davic 1991, Hall et al. 2000, Johnson and Wallace 2005), we also conducted an additional size-specific comparison of the primary consumer production response. This comparison allowed us to separate the overall response of preferred prey (i.e., small-bodied individuals) from the response of predator-resistant primary consumers (i.e., large-bodied individuals). First, we selected the ten most dominant taxa in both streams, which represented 70-90% of total primary consumer production in a given year (*Pycnopsyche* spp. [Trichoptera], *Tipula* sp. [Diptera], *Fattigia* sp. [Trichoptera], *Lepidostoma* spp. [Trichoptera], *Tallaperla* spp. [Plecoptera], *Molophilus* sp. [Diptera], *Leuctra* spp. [Plecoptera], *Diplectrona* sp. [Trichoptera], non-Tanypodinae Chironomidae [Diptera], Copepoda). We then categorized each individual within these ten taxa based on body size. We did not assign a single body size to each taxon (i.e., an average or

maximum body size) because early instars of typically large-bodied prey are likely more vulnerable to predation than later instars of the same taxon. Instead, we classified each individual within a given taxon as either large-bodied (> 10 mm in total length) or small-bodied primary consumers (≤ 10 mm in total length) because this delineation categorized the preferred prey taxa (i.e., non-Tanypodinae chironomids and copepods) as small-bodied individuals. Based on this body size grouping, we then summed the secondary production of all individuals within both of these body size categories, regardless of taxonomic affiliation. We repeated this process for each year and stream and compared the trends graphically.

To determine the short- versus long-term responses of macroinvertebrates, we divided the study into three time periods: pretreatment (PRE 1 and PRE 2; July 1998-August 2000), short-term response (ENR 1 and ENR 2; September 2000-August 2002), and long-term response (ENR 4 and ENR 5; September 2003-August 2005). Within this notation, the number following the abbreviations (PRE: pretreatment year or ENR: enrichment year) corresponded with the treatment year (e.g., ENR 1 represented the first year of nutrient enrichment). The third year of enrichment (ENR 3; September 2002-August 2003) was not included in the analysis because samples were lost due to inadequate preservation. Bias in our analysis due to the exclusion of ENR 3 is extremely unlikely because any trends associated with ENR 3 would likely be captured by the final two years of enrichment (ENR 4 and ENR 5). These time periods were selected because 2+ years of enrichment allowed many of the taxa to reach new population levels, as 90% of these taxa have life-cycles of one year or less, and only two taxa have larval periods longer than two years (*Anchytarsus* [1095 days], *Cordulegaster* [1140 days]) (Wallace et al. 1999). Thus, by the fourth and fifth year of enrichment, 90% of taxa would have produced > 4

generations under nutrient-enriched conditions. Moreover, those taxa with a life-cycle of one year or less represent ca. 90-95% of the total secondary production in any given year.

We used randomized intervention analysis (RIA) to analyze the effects of nutrient enrichment on macroinvertebrate biomass (Table 1). By comparing the differences in the treatment (C54) and reference stream (C53) during the pretreatment and nutrient enrichment periods, RIA assessed the null hypothesis that macroinvertebrate biomass in the treatment stream did not change relative to the reference stream during the nutrient enrichment (Carpenter et al. 1989). To isolate the long-term and short-term responses over time, we conducted three separate RIA analyses on both primary consumer and predator biomass (Table 1). Short-term responses of macroinvertebrate biomass to nutrient enrichment were assessed by comparing the short-term response (ENR 1 and 2; n = 26 months) to the pretreatment period (PRE 1 and 2; n = 22 months) (Cross et al. 2006). To evaluate the longer-term responses, we compared ENR 4 and 5 (n = 24 months) to the pretreatment period (PRE 1 and 2). The third analysis compared the long-term response (ENR 4 and 5) to the short-term response (ENR 1 and 2) and assessed whether long-term nutrient enrichment continued to have a positive effect on biomass, or if its effect had leveled off after an initial short-term response. Based on these contrasts, we calculated probabilities of change for each pairwise comparison using 1000 random permutations of interstream differences (Carpenter et al. 1989).

Results

Unexpectedly, during the fourth and fifth year of enrichment, nutrient enrichment produced a trophic decoupling whereby enrichment continued to stimulate primary consumer production with no concomitant increase in invertebrate predators (Figs. 2.1A-2.1D, Table 2.1).

In addition, this primary consumer and predator response varied with time. Two years of nutrient enrichment stimulated the production and biomass of both primary consumers and predators (Cross et al. 2006), which agreed with other nutrient enrichment manipulations (e.g., Deegan and Peterson 1992, Slavik et al. 2004). However, this short-term response contrasted sharply with our longer-term results showing that predator biomass and production did not respond positively to nutrient enrichment, despite continued stimulation of primary consumer biomass ($P < 0.001$) and production relative to the pretreatment period (Fig. 2.1A-2.1D, Table 2.1). Thus, predators initially increased with short-term enrichment, but then declined to pretreatment levels with a longer-term enrichment, even as primary consumer production continued to increase in the treatment stream (Fig. 2.1A-2.1D).

This trophic decoupling reduced overall food web efficiency during the long-term enrichment (Fig. 2.2) and contrasted with previous studies showing evidence of highly efficient energy transfer from primary consumers to predators in similar stream food webs (e.g., Wallace et al. 1997, 1999). Because of this reduction, we observed dramatically different relationships between primary consumer and predator production in the treatment and reference streams (Fig. 2.2). Predator production varied linearly and steeply with primary consumer production during all years in the reference stream (Cross et al. 2006, this study) and in a similar Coweeta stream (Wallace et al. 1999). In the treatment stream, primary consumer and predator production also varied linearly throughout the pretreatment period (Fig. 2.2) (Lugthart and Wallace 1992, Cross et al. 2006). These strong linear relationships suggested an efficient energy transfer between primary consumers and predators under reference conditions (Fig. 2.2). Conversely, during the enrichment period, primary consumer production continued to respond positively to long-term enrichment, but predators declined to pretreatment levels in a non-linear trajectory over time

(Fig. 2.2). In the fourth year of enrichment, the reference and treatment streams (represented by E4 in Fig. 2.2) had comparable levels of predator production, despite ca. 2.2x greater primary consumer production in the treatment stream relative to the reference stream (Fig. 2.2). These contrasting responses of predators and primary consumers strongly reduced the contribution of predators to overall macroinvertebrate biomass. Prior to enrichment, predators and primary consumers each contributed approximately 50% of the total biomass (Fig. 2.1A and 2.1C). However, during the final two years of enrichment, predator contribution declined to 18% in the treatment stream, but remained at approximately 40% in the reference stream. Taken together, our results provide evidence that long-term nutrient enrichment did not stimulate predator production and reduced the efficiency of energy flow from primary consumers to predators.

The alteration of the predator-prey relationship during the long-term enrichment was largely driven by changes in the relative dominance of large- versus small-bodied primary consumers (Fig. 2.3). Because predators from our study streams primarily eat small-bodied primary consumers and seldom eat large-bodied prey (Davic 1991, Hall et al. 2000, Johnson and Wallace 2005), large-bodied primary consumers are likely more resistant to predation by these gape-limited predators. Thus, as the predation risk of prey can decline with increased body size (Crowl and Covich 1990, Chase 1999), the wide variation in primary consumer body sizes in our streams (< 1 mm to 65 mm) likely increased the variation in the relative predation risk of primary consumers. The reference and treatment streams initially did not differ in the production of large- or small-bodied primary consumers during the pretreatment period (Fig. 2.3). Enrichment increased the production of both large- and small-bodied primary consumers in the first two years of enrichment, but only increased the production of large-bodied primary consumers in the treatment stream during years four and five (Fig. 2.3). Since small-bodied prey

declined to pretreatment levels by the fourth year of enrichment, long-term nutrient enrichment primarily stimulated large-bodied primary consumers that were relatively resistant to predation (Fig. 2.3). Thus, enrichment did not stimulate predator production because the increase in predator-resistant (i.e., large-bodied) taxa likely did not benefit instream-predators.

Discussion

Our results provide strong evidence that low levels of nutrient enrichment reduced energy flow to predators and decreased the trophic transfer efficiency between primary consumers and predators. Thus, even within a diverse food web with 20 predator taxa, long-term nutrient enrichment decoupled primary consumer and predator production, as most primary consumer production was relatively unavailable to predators. Nutrient enrichment of natural food webs may not always increase predator production, but instead can produce unintended 'ecological surprises' as resources are diverted to alternate trophic pathways. Our results further demonstrate our limited ability to predict how higher trophic levels in aquatic ecosystems will respond to nutrient enrichment, and highlight the difficulties in predicting long-term food web responses from few large-scale experimental manipulations.

The lack of a significant positive predator response to nutrient enrichment suggests that the majority of the increased ecosystem productivity in our study was confined to the lower trophic levels. This suggests that increased nutrient supplies do not always propagate up food webs to increase the productivity or biomass of higher trophic levels. These findings largely agree with an earlier regional comparison of food chain lengths showing that increased ecosystem size, but not productivity, lengthened food chains (Post et al. 2000). However, as we fully quantified changes in energy flow within these trophic levels, our results indicate a likely

mechanism explaining why increased ecosystem productivity does not increase the trophic position of predators or add additional trophic levels. Specifically, nutrient enrichment resulted in inefficiencies at lower trophic levels that limited the transfer of energy to higher trophic levels and attenuated the positive effects of nutrients on higher trophic levels. Therefore, if increased ecosystem productivity is confined to lower trophic levels and does not stimulate predator production, it likely diminishes the ability of enrichment to support additional trophic levels regardless of any increase in the productivity of basal resources. Overall, these results add to the mounting empirical evidence that the positive effects of nutrient enrichment can be attenuated by trophic distance (e.g., Brett and Goldman 1997).

These results suggest that trophic decouplings due to nutrient enrichment, as well as other types of natural or anthropogenic disturbance, may be more likely to occur in food webs dominated by gape-limited predators. When predators are gape-limited, primary consumers may be able to obtain predator size-refugia and divert prey production away from predators. However, a long-term nutrient enrichment of the Kuparuk River in Alaska did not lead to a trophic decoupling of the top fish predator in this ecosystem, arctic grayling (*Thymallus arcticus*). Nutrient enrichment of this ecosystem continued to stimulate arctic grayling production even after 16 years of seasonal enrichment (Slavik et al. 2004). Although they are gape-limited predators, arctic graylings are substantially larger than predators found in our study and could more easily consume larger prey (e.g., Golden and Deegan 1998). Thus, they could maintain a positive response to nutrient enrichment (Slavik et al. 2004).

However, even in food webs dominated by fish predators, large-bodied primary consumers can still reduce their relative predation risk through predator size-refugia or other anti-predator defenses (e.g., Bremigan and Stein 1994, Power et al. 2008) and potentially lead to

a similar diversion of resources under nutrient-enriched conditions. For instance, larval gizzard shad (*Dorosoma cepedianum*) eat primarily small-bodied zooplankton; thus, the increased dominance of large-bodied zooplankton may reduce prey availability and threaten the recruitment of this common lake fish (Bremigan and Stein 1994). In addition, during drought years on the South Fork Eel River, CA, the increased dominance of a large-bodied, case-building caddisfly (*Dicosmoecus gilvipes*) reduced energy flow to steelhead (*Oncorhynchus mykiss)* as algal production was diverted into a predator-resistant *Dicosmoecus* pathway (Wootton et al. 1996, Power et al. 2008). Because increased light availability can stimulate algal production and likely accelerates *Dicosmoecus* dominance (Power et al. 1996), it indicates that greater ecosystem productivity associated with nutrient enrichment may strengthen this diversion. Thus, given the prevalence of gape-limited predators and predator-resistant prey in a variety of aquatic ecosystems, our results suggest that trophic decouplings due to nutrient enrichment, as well as other types of natural and anthropogenic disturbances, may potentially be of wide-scale occurrence.

Although several large-bodied taxa responded positively to enrichment, the relative dominance of a large-bodied caddisfly, *Pycnopsyche* spp., steadily increased throughout the experimental enrichment (Fig. 2.4). This suggests that enrichment beyond our five-year manipulation would have likely continued to decouple predator production because of several factors that would have maintained conditions conducive to this common consumer's dominance. *Pycnopsyche* spp. are competitive dominants in these stream ecosystems (Creed et al. 2009)and eat leaf detritus (Cross et al. 2007), which exhibited larger increases in resource quality than other basal resources during our experimental enrichment (Cross et al. 2005a, Greenwood et al. 2007). *Pycnopsyche*'s period of peak production is earlier than many other

leaf-eating taxa and occurs before periods of low resource availability (e.g., Huryn and Wallace 1988, Suberkropp et al. *In press*). They also construct rigid stone cases and obtain a larger maximum body size than other leaf-eating taxa (22 mm vs. 14 mm), which may reduce their predation risk. The combination of these traits likely allowed this taxon to better exploit the positive enrichment effects on resource quality. Because prolonged enrichment would likely strengthen, not weaken, these benefits, the observed trophic decoupling is unlikely to be easily reversed with continued enrichment.

This decoupling of the predator-prey relationship observed in our study may have ecosystem-level effects that extend beyond our particular study streams. Headwater streams similar to our study streams dominate overall stream miles and are a common landscape feature within an ecosystem type that has a worldwide distribution (Meyer and Wallace 2001). Thus, our results indicate an important nutrient enrichment response that is applicable to globally-distributed aquatic food webs and helps increase our understanding of how such stream networks may respond to enrichment. Streams similar to our study streams are also important sites for carbon and nutrient transformations within river networks (Meyer and Wallace 2001, Peterson et al. 2001) and are directly linked to downstream food webs through material, energy, and macroinvertebrate transport (Vannote et al. 1980). As macroinvertebrate consumers are important drivers of many of these processes (Cuffney et al. 1990), our observed decoupling of the predator-prey relationship has the potential to alter the functioning of overall river networks through changes in these downstream subsidies. In fact, a concurrent study showed that nutrient enrichment increased organic matter processing and downstream carbon export because of associated changes in consumer production (Greenwood et al. 2007, Benstead et al. 2009). Because aquatic emergence can also represent an important subsidy to terrestrial predators

(Baxter et al. 2005), nutrient enrichment may have also increased aquatic-terrestrial subsidies, as the excess primary consumer production not consumed by instream predators is exported to the surrounding terrestrial community as adult emergence. Therefore, the various linkages between these biologically-active ecosystems and associated food webs suggest an important pathway by which our observed changes in the structure and function of these nutrient-enriched ecosystems may indirectly alter the function of a variety of food webs not directly experiencing enrichment.

In summary, low-level nutrient enrichment dramatically shifted the primary consumer assemblage in this stream food web to larger-bodied, predator-resistant taxa. As this shift decoupled predator and prey production, nutrient enrichment ultimately diverted energy flow into predator-resistant pathways that reduced overall food web efficiency. Humans are intentionally (e.g., salmon restoration) and unintentionally (e.g., land-use change and agricultural run-off) increasing nutrient inputs to a variety of aquatic ecosystems (Smith et al. 1999, Slaney et al. 2003); thus, nutrient enrichments similar to our experimental manipulation are a frequent global occurrence. Given the prevalence of this environmental change in a diversity of ecosystems that include predator-resistant prey and gape-limited predators, our results suggest that nutrient-stimulated resource flows can be diverted into predator-resistant pathways and thereby truncate predator responses. As we did not originally predict these trophic efficiency declines, our results also show our current inability to fully assess *a priori* how ecosystems will respond to enrichment. Therefore, even in ecosystems where energy flow is predicted to be relatively efficient, low-level nutrient enrichment may still increase the production of non-target taxa (e.g., predator / grazer resistant prey), decrease the production of higher trophic levels, or lead to unintended consequences that may compromise the productivity of freshwater ecosystems.

Acknowledgments

We thank S. Dye, N. Taylor, R. Hilten, J. Benstead, and C. Tant for help in the field and maintaining field equipment. We also thank all the technicians who helped process samples, but especially E. Baker, A. Ely, J. Hoehn, and J. Holland. This experiment was made possible by the contributions of our collaborators, D. Conners, V. Gulis, K. Subberkropp, and C. Tant. Logistical support was provided by the staff at the Coweeta Hydrologic Laboratory. Water chemistry analyses were conducted by T. Maddox at the University of Georgia (UGA) Analytical Chemistry Lab. The Rosemond lab group, S. Dye, R. Hall, J. Hutchens, and E. Rosi-Marshall provided insightful comments on an early draft of this manuscript. Funding was provided by NSF (DEB-0318063, DEB-9629268, and DEB-0212315). J. Davis was supported by a NSF Graduate Research Fellowship and a UGA Presidential Fellowship.

Table 2.1: Probabilities of change for the randomized intervention analysis (RIA) that tested for differences in macroinvertebrate biomass between the reference and treatment streams. Probabilities were based on 1000 random permutations of interstream differences. To assess the changes over time, we divided the study into three sampling periods: pretreatment (PRE 1 and PRE 2; July 1998-August 2000; n = 22), short-term response (ENR 1 and ENR 2; September 2000-August 2002; n = 26), and long-term response (ENR 4 and ENR 5; September 2003-August 2005; n = 24). The short-term vs. pretreatment response was previously reported (Cross et al. 2006).

	Short-term vs. Pretreatment*	Long-term vs. Pretreatment	Long-term vs. Short-term
Primary consumers	< 0.001 (+)	< 0.001 (+)	NS
Predators	< 0.001 (+)	NS	< 0.001 (-)

* From Cross *et al.* 2006

Fig. 2.1: Average annual biomass (mean ± 1SE) and secondary production of primary consumers (**A** and **B**) and predators (**C** and **D**) during the seven year experiment. The arrow indicates the beginning of nutrient enrichment. Each year represents an average of twelve monthly samples with four samples per stream. Note difference in scales between primary consumers and predators. AFDM is ash-free dry mass.

.

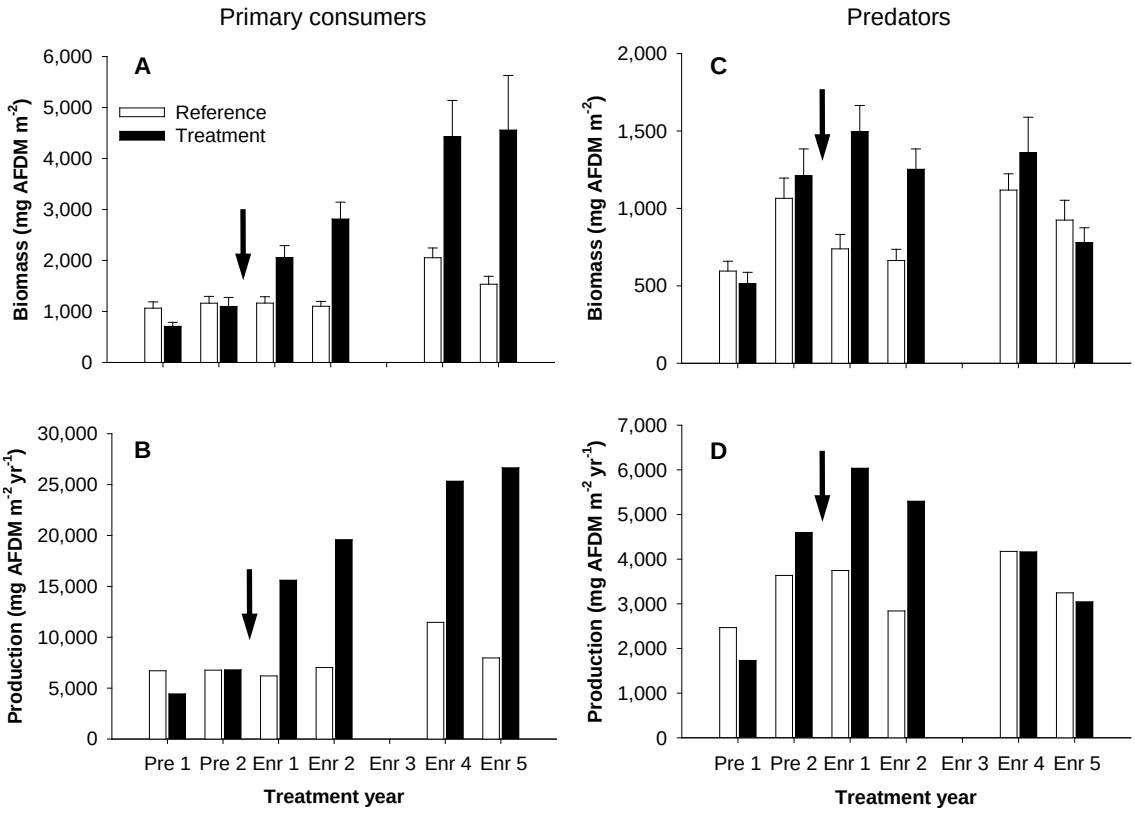

Primary consumers
Predators
A
B
C
D
Reference
Treatment
Biomass (mg AFDM m-2)
Production (mg AFDM m-2 yr-1)
6,000
5,000
4,000
3,000
2,000
1,000
0
2,000
1,500
1,000
500
30,000
25,000
20,000
15,000
10,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
Pre 1 Pre 2 Enr 1 Enr 2 Enr 3 Enr 4 Enr 5
Treatment year

Fig. 2.2: Relationship between primary consumer and predator secondary production for the reference stream (grey circles), the treatment stream (black circles), and previously published data (open circles). The arrows represent the temporal trajectory of the treatment stream starting with the two years of pretreatment (P1 and P2) and ending with the fifth year of enrichment (E5). The data labels correspond to the sampling year for the treatment stream. The previously published data include five years of production data from the reference stream (C53) and a similar Coweeta stream (C55) that had experimentally reduced terrestrial leaf inputs during four of those years (Wallace et al. 1999). It also includes previously published data from an unmanipulated year that compared our current reference (C53) and treatment (C54) streams (Lugthart and Wallace 1992). AFDM is ash-free dry mass.

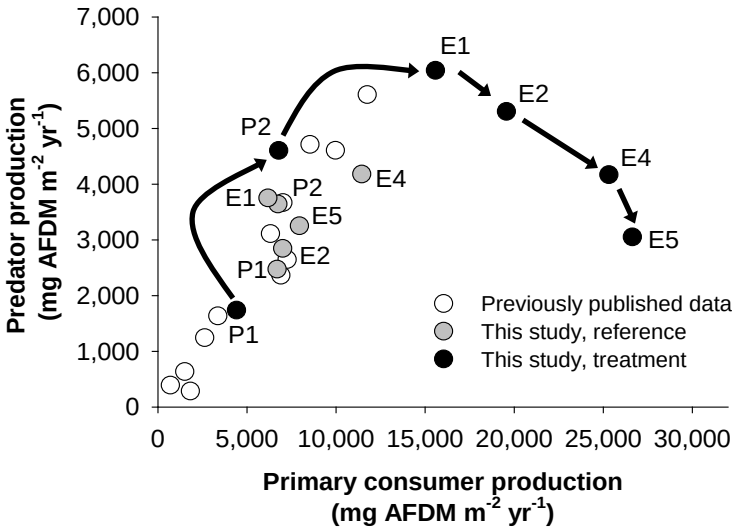

7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
0
5,000
10,000
15,000
20,000
25,000
30,000
Predator production
(mg AFDM m-2 yr-1)
Primary consumer production
(mg AFDM m-2 yr-1)
E1
E2
E4
E5
P2
P1
Previously published data
This study, reference
This study, treatment

Fig. 2.3: Size-specific secondary production of the ten dominant primary consumers in the reference and treatment streams. For any given year, the displayed secondary production represented 70-90% of total primary consumer production. Each individual within these ten taxa was classified as either small-bodied individuals (body length ≤ 10mm; circles) or large-bodied individuals (body length > 10mm; triangles), and their production was subsequently summed. Large-bodied individuals were relatively predator-resistant compared to small-bodied primary consumers. The arrow indicates the beginning of nutrient enrichment. AFDM is ash-free dry mass.

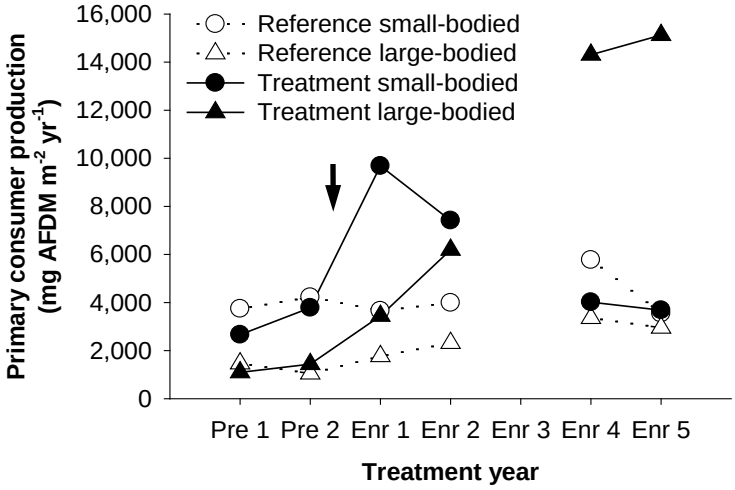
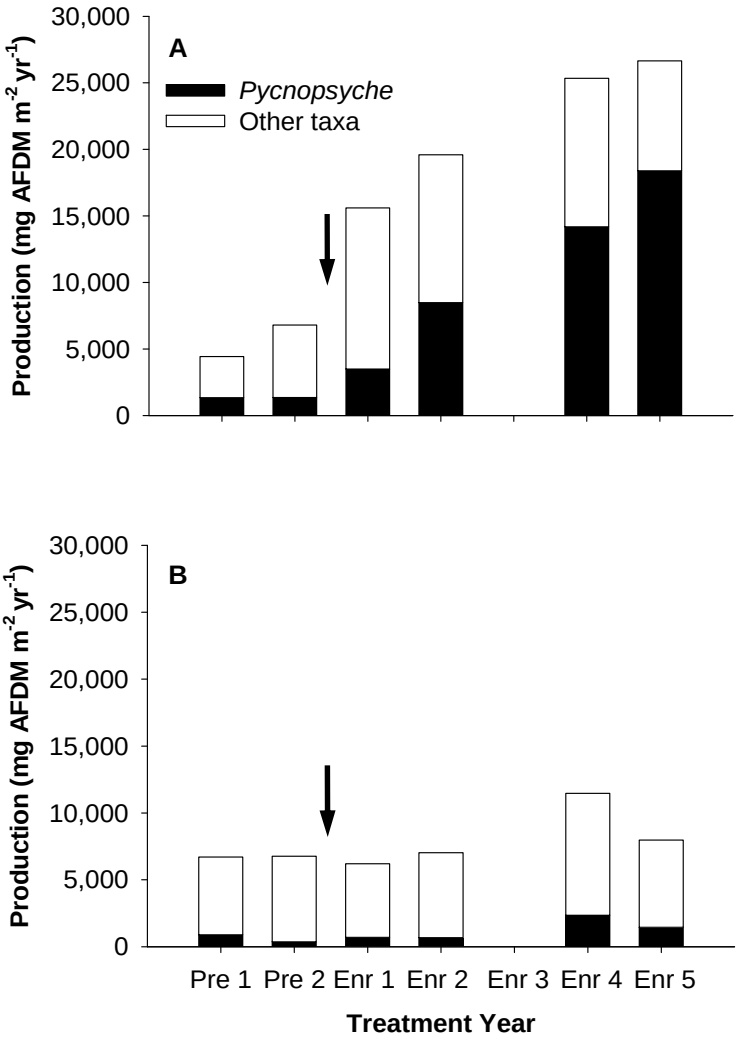

Reference small-bodied
Reference large-bodied
Treatment small-bodied
Treatment large-bodied
Primary consumer production
(mg AFDM m-2 yr-1)
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
Pre 1
Pre 2
Enr 1
Enr 2
Enr 3
Enr 4
Enr 5
Treatment year

Fig. 2.4: Relative contributions of *Pycnopsyche* spp. and all other primary consumer taxa to overall primary consumer production in the treatment (**A**) and reference (**B**) streams. The arrow indicates the beginning of nutrient enrichment. AFDM is ash-free dry mass


A
Pycnopsyche
Other taxa
Production (mg AFDM m-2 yr-1)
30,000
25,000
20,000
15,000
10,000
5,000
0
B
Production (mg AFDM m-2 yr-1)
30,000
25,000
20,000
15,000
10,000
5,000
0
Pre 1
Pre 2
Enr 1
Enr 2
Enr 3
Enr 4
Enr 5
Treatment Year

CHAPTER 3

NUTRIENT ENRICHMENT DIFFERENTIALLY AFFECTS BODY SIZES OF PRIMARY CONSUMERS AND PREDATORS IN A DETRITUS-BASED ECOSYSTEM[2]

[2] Davis, J. M., A. D. Rosemond, S. L. Eggert, W. F. Cross, and J. B. Wallace. To be submitted to *Limnology and Oceanography*.

Abstract

Nutrient enrichment of freshwater ecosystems can affect size distributions of organisms, frequently via positive effects on large-bodied organisms. However, these models and empirical findings are largely derived from autotrophic-based ecosystems, where enrichment can increase the quantity and quality of basal resources. Enrichment of detritus-based aquatic food webs increases resource quality, but can also decrease resource quantity because of increased detrital-processing rates. These reductions in resource quantity may subsequently minimize any potential positive effects of enrichment on consumer body size in detritus-based food webs. Here we assessed how a five-year nutrient enrichment affected the responses of different size classes of primary consumers and predators in a detritus-based headwater stream. Specifically, we determined if enrichment had positive effects on large-bodied consumers and whether that effect differed for primary consumers and predators. Two years of enrichment increased the biomass and abundance of all consumers regardless of body size. However, during the fourth and fifth year of enrichment, the abundance and biomass of large-bodied primary consumers continued to increase, while small-bodied primary consumers returned to pretreatment levels. Large-bodied predators did not respond to long-term enrichment during this same experimental period, indicating that the positive effects of enrichment did not propagate up to higher trophic levels. Thus, long-term enrichment increased the dominance of large-bodied primary consumers in these detritus-based ecosystems. As consumer body size can be an important species-specific trait determining population dynamics and ecosystem processes, the observed change in consumer body size composition suggests an important pathway for nutrient enrichment to alter stream food web structure and function.

Introduction

Within aquatic ecosystems, nutrient enrichment not only alters the biomass and production of consumers (Slavik et al. 2004, Cross et al. 2006), but it can also substantially alter their community composition and body size distributions (Sprules and Munawar 1986, Bourassa and Morin 1995). These enrichment-induced shifts in body size distributions have largely been attributed to top-down and bottom-up effects via size-selective predation and exploitative competition for resources (Brooks and Dodson 1965, Finlay et al. 2007). Specifically, when predators preferentially consume large-bodied prey, increased ecosystem productivity associated with nutrient enrichment can increase the abundance of smaller bodied prey (Brooks and Dodson 1965, Finlay et al. 2007). Conversely, when predators do not preferentially consume large-bodied prey, nutrient enrichment can increase consumer body size in autotrophic-based food webs because of increased resource quality (lower C:N and / or C:P) and quantity (Bourassa and Morin 1995). This greater resource quality and quantity can increase consumer growth rates and body sizes (e.g., Lurling and Van Donk 1997, Boersma and Kreutzer 2002), which may shift the community toward dominance by large-bodied consumers (e.g., Romanovsky and Feniova 1985, Sprules and Munawar 1986, Bourassa and Morin 1995).

Although the effects of nutrient enrichment on primary consumer body size are mixed, these earlier autotrophic-based studies have largely linked primary consumer body size and enrichment response through associated increases in resource quantity and quality. Less is known about the effects of enrichment on primary consumer body sizes in detritus-based ecosystems, where enrichment can increase resource quality but decrease resource quantity. Specifically, enrichment stimulates the production of heterotrophic microbes on detritus, which can improve detritus quality (lower carbon to nutrient ratios) and stimulate the production of

primary consumers and predators (Gulis and Suberkropp 2003, Cross et al. 2006). Stimulation of heterotrophic microorganisms can also increase detrital processing rates and reduce overall detrital-standing crop (Greenwood et al. 2007, Benstead et al. 2009), which may lengthen periods of low resource quantity. Thus, despite the increased resource quality associated with enrichment, reductions in resource quantity may minimize the potential positive bottom-up effects on the dominance of large-bodied primary consumers and on individual body size.

Here we report results from a five-year experimental nutrient enrichment that assessed the effects of enrichment on the body sizes of primary consumers and predators in a detritus-based headwater stream. We tested whether consumer response to nutrient enrichment varied with body size by grouping organisms into body size classes and assessing which size classes responded to nutrient enrichment. Because enrichment can increase detrital processing rates and reduce detrital-standing crops (Greenwood et al. 2007, Benstead et al. 2009), we expected that this reduced resource quantity would likely offset any potential increases in resource quality. Therefore, we predicted that these declines in resource quantity would minimize the positive effects of enrichment on the abundance and biomass of large-bodied primary consumers.

Body size responses of higher trophic levels were more difficult to predict. Because predators in these stream food webs prefer small-bodied prey (e.g., Davic 1991, Hall et al. 2000, Johnson and Wallace 2005), they may not benefit from increases in large-bodied primary consumers. Thus, the effects of nutrient enrichment on predator body size may be dependent upon the potential shifts in primary consumer body size distributions that may alter prey availability. If enrichment increases the relative dominance of smaller-bodied primary consumers because of decreased resource quantity, this greater availability of smaller-bodied prey may increase the predator's prey base. These increases may subsequently stimulate

predator growth rates and shift the community towards dominance by larger-bodied predators. Conversely, if enrichment increases the relative dominance of large-bodied primary consumers because of greater resource quality, prey resources may be sequestered in larger primary consumers that are not readily eaten by predators. This reduced prey availability may limit predator growth rates and shift the community towards dominance by small-bodied predators. Because these predator responses are largely contingent upon the primary consumer response, it reduces our ability to accurately predict *a priori* the effects of nutrient enrichment on large-bodied predators and overall predator body size distributions.

To further assess the potential effects of resource limitation on primary consumer body size, we also evaluated the effect of nutrient enrichment on the average individual body mass of *Pycnopsyche* spp., a dominant consumer in many temperate forested headwater streams (Herbst 1980, 1982, Cross et al. 2006, Creed et al. 2009). A previous experimental manipulation that reduced leaf litter inputs to an adjacent headwater stream decreased the individual body size of *Pycnopsyche* and indicated that they were particularly sensitive to reductions in resource quantity (Wallace et al. 1999, Eggert and Wallace 2003). Thus, we believed the individual body mass response of this taxon would be an effective indicator of the overall changes in resource quality and quantity in our study.

Methods

Study site— We conducted this study at the USDA Forest Service Coweeta Hydrologic Laboratory, a Long-Term Ecological Research site located in the southern Appalachian Mountains (Macon County, North Carolina). Coweeta is a heavily-forested experimental watershed (2185 ha) that is comprised of mixed hardwoods (oak, maple, tulip poplar) with a

dense understory dominated by *Rhododendron maximum* that limits light availability. This light limitation reduces autotrophic production and increases the food web's reliance on heterotrophic microbes that colonize terrestrial leaf inputs (Wallace et al. 1997, Hall et al. 2000, Cross et al. 2007).

To assess how consumer response to nutrient enrichment varied with body size, we used a paired-watershed approach. We selected two forested first-order catchments (C53 and C54) that did not differ in their physiochemical properties (see Lugthart and Wallace 1992 for further description of study streams). Both study streams were fishless. The primary consumer community was comprised of ca. 40 taxa, while the predator community was dominated by over 20 taxa of size-selective invertebrate predators (e.g., *Beloneuria* [Plecoptera], Ceratopogonidae [Diptera], *Cordulegaster* [Odonata], *Hexatoma* [Diptera], and *Lanthus* [Odonata]) or gape-limited vertebrate predators (e.g., *Eurycea wilderae* [Plethodontidae] and *Desmognathus quadramaculatus* [Plethodontidae]). Both predator groups in these stream food webs occupy a similar trophic position because they predominantly eat small-bodied primary consumers (e.g., Davic 1991, Hall et al. 2000, Johnson and Wallace 2005)

The reference (C53) and treatment (C54) streams did not differ in nutrient concentrations prior to the experimental enrichment (mean ± SE, C53: DIN: 23.2 ± 8.5 μg L^{-1}, SRP: 6.8 ± 3.0 μg L^{-1}; C54: DIN: 29.3 ± 4.9 μg L^{-1}, SRP: 9.5 ± 2.3 μg L^{-1}). From July 2000 to August 2005 (ca. 1877 days), we experimentally enriched a 150-m reach of the treatment stream with nitrogen (NH_4NO_3) and phosphorus (K_2HPO_4 and KH_2PO_4). We added nutrients continuously along the entire 150-m length of the treatment stream using an irrigation line running down the center of the stream. See Gulis and Suberkropp (2003) for further descriptions of the nutrient-delivery system. This delivery system increased nutrient concentrations in the treatment stream to a

realistic, low-level enrichment (DIN: 506.2 ± 36.3 µg L^{-1}, SRP: 80.0 ± 5.6 µg L^{-1}), while the reference stream concentrations during this same time period were comparable to the pretreatment period (DIN: 31.0 ± 3.4 µg L^{-1}, SRP: 8.0 ± 1.3µg L^{-1}). We monitored stream nutrient concentrations every two weeks at three points along the 150-m reach of the treatment stream and at the weir of the reference stream (APHA 1998). Water temperature was measured every 30 minutes in both streams with Optic StowAway temperature probes (Onset Computer, Pocasset, Massachusetts, USA). We measured stream discharge at 5-minute intervals with an ISCO data logger.

Macroinvertebrate sampling— The macroinvertebrate communities in both streams were sampled monthly during an initial two-year pretreatment period (September 1998 to June 2000) followed by a five-year experimental period (July 2000 to August 2005). We sampled mixed-cobble substrates according to Cross et al. (2006). Using a stovepipe corer (400 cm^2), four mixed-cobble substrate samples were randomly collected in each stream on each date. The corer was firmly placed on the stream substrate and all material was removed by hand to a depth of 15 cm. We transported the samples back to the laboratory and processed them within 48 hours of collection. Each sample was rinsed onto nested sieves (1-mm and 250-µm mesh size) and elutriated to remove inorganic material. We divided the remaining organic matter into large (> 1 mm) and small (250 µm – 1 mm) size fractions, and preserved them using 6-8% formalin. Because of the large number of macroinvertebrates in the small size fraction, we subsampled this size fraction using a sample splitter (Waters 1969). All macroinvertebrates were removed from both the large and small size fractions using a dissecting scope at 15x magnification. Each organism was identified to genus; however, Chironomidae were identified to either Tanypodinae (predators) or non-Tanypodinae (non-predators), and most non-insects were identified to the

order level or higher (e.g., oligochaetes, nematodes, copepods, etc). All individuals were enumerated and measured to the nearest millimeter under 12x magnification. We calculated consumer biomass using length-mass regressions established for Coweeta streams (Benke et al. 1999, J. B. Wallace unpubl. data). We classified all taxa as either primary consumer or predator according to Merritt and Cummins (1996) and based on our previous knowledge from working in this ecosystem (Wallace et al. 1999).

Body size analysis— To first determine potential effects of nutrient enrichment on coarse-scale changes in the body size of the stream macroinvertebrate community, we first compared the abundance and biomass response of the entire macroinvertebrate community (primary consumers plus predators). Divergent results in these responses (total biomass and abundance) would suggest potential changes in body sizes. Increased biomass without a concomitant increase in abundance would imply that nutrient enrichment increased the average individual body size of at least some groups of consumers.

We then assessed whether the effects of nutrient enrichment varied with consumer body size by conducting a body size-specific analysis of consumer response. Macroinvertebrate body size is largely indeterminate and can change under varying environmental conditions (e.g., Peckarsky et al. 2001); thus, we did not assign a single body size to a taxon (i.e., an average or maximum body size). Instead, we assigned each individual within a particular taxon to an appropriate size class based on its $\log_{10}$ transformed individual body mass (mg). Early instars of large-bodied taxa were assigned to the same size class as similar-sized individuals of small-bodied taxa. Accordingly, we first $\log_{10}$ transformed the body mass (mg) of each individual within each taxon for a given month. Based on this transformed body size, we then grouped individuals into one of the 22 separate $\log_{10}$ body size classes (see Web Appendix 1 for further

descriptions of the specific upper and lower limits for body mass used for each body size class). Then for all individuals classified in a specific $\log_{10}$ body size class, we summed either their biomass or abundance regardless of taxonomic classification. Because we repeated this grouping and summation for each month, we created a taxon-independent monthly time-series that followed changes in biomass or abundance within each of the 22 size classes over time. We conducted this body size analysis separately for primary consumers and predators.

Statistical analysis— We first evaluated whether nutrient enrichment differentially affected total macroinvertebrate biomass and abundance (predators and primary consumers combined), because this would indirectly suggest a shift in body size distributions for the entire macroinvertebrate community. Randomized intervention analysis (RIA, Carpenter et al. 1989) was applied to the monthly time-series of total macroinvertebrate biomass and abundance. To assess the size-specific effects of nutrient enrichment on primary consumers and predators, we also applied RIA to the monthly time series of biomass and abundance data that were grouped by body size into the 22 separate body size classes. Analyzing each of the body size classes separately allowed us to evaluate how the effects of nutrient enrichment varied between body size classes. Because the effects of nutrient enrichment have been previously shown to vary during the first two years of enrichment (ENR 1 and 2) versus the fourth and fifth years of enrichment (ENR 4 and 5) (Davis et al. *In review-a*), we analyzed the short- and long-term responses separately for each of the RIA comparisons listed above (i.e., total abundance, total biomass, size-specific biomass, size-specific abundance). Specifically, we divided the study into three time periods: pretreatment (PRE 1 and PRE 2; July 1998 - August 2000), short-term response (ENR 1 and ENR 2; September 2000 - August 2002), and long-term response (ENR 4 and ENR 5; September 2003 - August 2005). For each of the above comparisons, RIA then

compared the short-term response (26 months) to the pretreatment period (22 months), and the long-term response (24 months) to the pretreatment period. Based on these contrasts, we calculated probabilities of change for each pairwise comparison using 1000 random permutations of interstream differences (Carpenter et al. 1989). These separate analyses allowed us to isolate the short- and long-term effects of nutrient enrichment on the parameter of interest. The third year of enrichment (ENR 3; September 2002 - August 2003) was not included in our analyses because samples were lost due to inadequate preservation. Bias in our analysis due to the exclusion of ENR 3 is extremely unlikely because any trends associated with ENR 3 would likely be captured by the final two years of enrichment (ENR 4 and ENR 5).

By comparing the differences in the reference and treatment stream during the pretreatment and post-treatment periods, RIA tested the null hypothesis that biomass or abundance in the treatment stream did not change relative to the reference stream during the nutrient enrichment. However, because RIA only established if there was a significant change in the treatment stream post-treatment versus pre-treatment, we examined the monthly time series data to determine the direction of the change (positive or negative) for the biomass and abundance responses within each size class. Briefly, for the biomass or abundance within each individual size class *j*, we first calculated the difference between the reference and treatment streams (e.g., $\text{reference}_{ij} - \text{treatment}_{ij}$) for each month *i*. We then averaged these monthly differences for each of the three sampling periods: pretreatment (22 months), short-term (26 months), and long-term (24 months), to obtain an average period difference for each size class *j* (Δ_j). Using these average period differences, we applied the following equation (Osenberg et al. 1994) to calculate the short-term and long-term effect sizes for each size class *j*.

$$\text{Effect Size}_j = \Delta_{\overline{PREj}} - \Delta_{\overline{POSTj}} \qquad (1)$$

To calculate the short-term enrichment effect size for each size class *j*, we compared the short-term difference to the pretreatment difference, while the long-term effect size compared the long-term difference to the pretreatment difference. We repeated this calculation for both the abundance and biomass estimates within each of the 22 size classes. These calculated effect sizes helped assess the direction and magnitude of the nutrient enrichment response within each size class.

Based on our RIA results, we observed that the effects of nutrient enrichment varied with primary consumer body size (see Results). Because we detected a breakpoint in the long-term primary consumer response at a body size threshold of 1.778 mg (Tables 3.1 and 3.4), we categorized macroinvertebrates into two representative groups: small-bodied individuals (individual body size $\leq$ 1.778 mg) and large-bodied individuals (body size > 1.778 mg). We graphed these overall trends to better illustrate the generalized response of small- and large-bodied consumers to nutrient enrichment.

Finally, we evaluated the effects of nutrient enrichment on the individual body mass of the dominant primary consumer in these stream food webs (*Pycnopsyche* spp.) because we assumed changes in its individual body mass would be a good indicator of the net effect of shifts in resource quality and quantity. We first calculated the maximum body mass that *Pycnopsyche* obtained in a given year, which would indicate larval body mass at pupation. We also calculated the average individual body mass of *Pycnopsyche* in each stream during a particular year. We plotted these changes as maximum and average individual body size.

Results

Total abundance vs. total biomass trends— Total macroinvertebrate (primary consumer plus predators) biomass was significantly higher in the treatment versus reference stream through both the short- (ENR 1 and 2) (Cross et al. 2006) and long-term enrichment (ENR 4 and 5) (RIA, $P < 0.05$) (Fig. 3.1A). However, total abundance increased during the short-term enrichment (RIA, $P < 0.05$) (Cross et al. 2006), but was not significantly different from the pretreatment years during the long-term enrichment (Fig. 3.1B). These sustained increases in total macroinvertebrate biomass, but lack of a total abundance response to long-term nutrient enrichment, indicated that nutrient enrichment increased the average individual body size of stream consumers.

Size-specific analysis— The body size-specific analyses showed that the effect of nutrient enrichment on consumers was related to body size. Due to insufficient sample sizes, we excluded two size classes for primary consumers and four size classes for predators (represented by ND in Tables 3.1 and 3.2). This analysis categorized biomass and abundance estimates into 22 separate body size classes that ranged in body mass from < 0.001 mg to 169.360 mg (Table 3.3).

Primary consumer size-specific response— During the short-term enrichment (ENR 1 and 2), the response of primary consumer biomass and abundance mostly did not vary with body size (Tables 3.1 and 3.4). Specifically, short-term enrichment largely increased the biomass and abundance of primary consumers across the range of body sizes tested (with the exception of two size classes [1.778 to 3.162 and 17.783 to 31.623 mg] that did not respond to nutrient enrichment) (Tables 3.1 and 3.4). However, during the long-term enrichment (ENR 4 and 5), primary consumer response varied with body size (Tables 3.1 and 3.4). In this case, the biomass

and abundance of large-bodied primary consumers (defined here as body size > 1.778 mg) continued to increase with the long-term enrichment, but most small-bodied primary consumers did not (defined here as body size ≤ 1.778 mg) (Tables 3.1 and 3.4). There were two exceptions to this general lack of response of small-bodied primary consumers, as the abundance and biomass of two smaller body size classes (0.001 to 0.002 and 0.056 to 1.000 mg) continued to positively respond to long-term nutrient enrichment (Tables 3.1 and 3.4).

Because nutrients disproportionately stimulated the abundance and biomass of large-bodied primary consumers (body size > 1.778 mg) during the fourth and fifth years of enrichment, the relative dominance of these primary consumers increased (Figs. 3.2A and 3.2B). Prior to enrichment, large-bodied primary consumers contributed 56% of primary consumer community biomass in the treatment stream, but this percentage increased to 82% during the final two years of enrichment in the treatment stream. This substantially greater response of large-bodied primary consumers skewed the community biomass composition towards these larger-bodied primary consumers during the longer-term enrichment (Fig. 3.2A and 3.2B).

Predator size-specific response— In accordance with the short-term primary consumer response, the biomass and abundance of predators in most body size groups increased with short-term nutrient enrichment, which suggested that the effects of nutrient enrichment did not vary with predator body size (Tables 3.2 and 3.5). In contrast, during the long-term enrichment, predator response varied with body size. The biomass and abundance of small predators (body size ≤ 0.018 mg) increased during the long-term enrichment, but larger predators did not (body size > 0.018 mg) (Tables 3.2 and 3.5). The abundance and biomass of predators in two of the larger-body size classes (0.316 to 0.562 and 3.162 to 5.623 mg, respectively) were the only body

size classes (primary consumer or predator) that declined significantly below pretreatment levels (Tables 3.2 and 3.5).

In comparison to primary consumers, where the enrichment responses diverged at a body size of 1.778 mg (Table 3.1), predator response diverged at a smaller body size threshold of 0.018 mg (Table 3.2). Because we were primarily interested in comparing the responses of predators and primary consumers with similar body size, we elected to use the same 1.778 mg body size division for both trophic levels. However, even when the smaller threshold (0.018 mg) was used, we observed similar graphical trends because of the relatively low contribution of small-bodied predators to overall community biomass.

Using the above threshold, we found that similar to the primary consumers, the biomass of small-bodied (body size $\leq$ 1.778 mg) and large-bodied predators (body size > 1.778 mg) increased during the short-term enrichment (Figs. 3.2C and 3.2D). Conversely, the biomass of large-bodied predators did not increase during the long-term enrichment (Figs. 3.2C and 3.2D). Thus, although primary consumers and predators of both body sizes exhibited similar positive responses during the short-term enrichment, long-term enrichment did not increase the biomass of large-bodied predators, despite continued increases in the biomass of large-bodied primary consumers (Tables 3.1 and 3.2; Figs. 3.2A-D).

Effects on Pycnopsyche individual body mass—Nutrient enrichment also altered the population size structure of *Pycnopsyche* spp. (Fig. 3.3), a dominant primary consumer that has previously exhibited sensitivity to declines in resource quantity (Wallace et al. 1999, Eggert and Wallace 2003). During the fifth year of enrichment, the maximum individual body mass of *Pycnopsyche* was 61% greater in the treatment stream relative to the reference stream (37.4 vs. 23.2 mg, respectively) and was 42% larger than the maximum size observed during the

pretreatment period (26.3 mg). Because of these increases in larger size classes that shifted *Pycnospsyche*'s population size distribution to the right, nutrient enrichment also increased the annual average body mass of individual *Pycnopsych*e during the five year enrichment relative to the reference stream and pretreatment period (Fig. 3.3).

Discussion

Differences between primary consumer and predator response— The results from our five-year ecosystem-level manipulation provided convincing evidence that consumer response to chronic nutrient enrichment was related to body size. This response also differed for primary consumers and predators, and was only evident after two years of continuous enrichment. One possible mechanism to explain the contrasting body size response of primary consumers and predators was the potential existence of predator size-refugia that reduced the predation risk of large-bodied prey. The streams used for this study were dominated by over 20 taxa of invertebrate and vertebrate predators that primarily eat small-bodied primary consumers (e.g., Davic 1991, Hall et al. 2000, Johnson and Wallace 2005). Thus, rather than top-down effects of predation diminishing the positive response of larger prey (if predators preferentially eat them) and increasing the abundance of small prey (e.g., Brooks and Dodson 1965), predation likely facilitated the increased dominance of large-bodied prey in our streams. Energy flow from prey to predators in these stream food webs can also be highly efficient (Wallace et al. 1997). This suggests that if predators primarily eat smaller-bodied prey, these prey may be more limited by top-down predator control. Conversely, the production of large-bodied primary consumers may be more coupled to changes in resource quality because they may obtain predator size-refugia that reduce their predation risk. These trends largely agree with an earlier enrichment of an

autotrophic-based stream (e.g., Bourassa and Morin 1995), suggesting that body size may help mediate consumer response to nutrient enrichment in a variety of food web types.

Size-selective predation can also help explain why large-bodied predators did not respond to enrichment because predators frequently eat prey smaller than themselves (Emmerson and Raffaelli 2004, Woodward and Warren 2007). The increased dominance of large-bodied primary consumers may reduce the vulnerability of the prey community, subsequently reducing prey availability and minimizing the positive effects of enrichment on these larger-bodied predators. It is less clear why the abundance and biomass of small-bodied predators increased with enrichment in both the short- and long-term experimental periods because the lack of a significant response of small-bodied prey during the longer-term period should have minimized any positive effects on small predators. However, as these small-bodied predators were dominated by predatory mites (Acari), Ceratopogonidae (Diptera), and Tanypodinae chironomids (Diptera), these taxa may have been eating substantially smaller meiofauna that were not adequately sampled by our 250 µm sieves.

Short- vs. long-term responses— The abundance and biomass of primary consumers and predators within most body size classes initially increased due to nutrient enrichment, but longer-term enrichment positively affected only the abundance and biomass of large-bodied primary consumers and small-bodied predators. This suggests that the importance of consumer body size in affecting primary consumer and predator responses may have been delayed and driven in part by shifts in stream habitat dynamics. Specifically, declines in leaf litter standing crop during the experimental enrichment (e.g., Suberkropp et al. *In press*) may have reduced stream consumer habitat and increased predation risk. Within stream ecosystems, leaf litter can provide important food resources and habitat complexity to consumers, such that reductions in debris dams can

reduce macroinvertebrate production (Smock et al. 1989, Wallace et al. 1999). Accordingly, increased leaf litter standing crop may also reduce predation risk, as increased habitat complexity can provide spatial refugia (Power et al. 2004, Beaty et al. 2006). Since predators primarily eat small-bodied prey, these prey may benefit more from leaf litter refugia because larger prey may reduce their predation risk independent of leaf litter availability (i.e., body size refugia). Although the short-term enrichment reduced leaf litter standing crop (e.g., Benstead et al. 2009, Suberkropp et al. *In press*), there may still have been adequate spatial refugia that reduced predation risk and allowed small-bodied prey to positively respond to enrichment. Leaf litter standing crop declined even more precipitously during the long-term enrichment (Suberkropp et al. *In press*), which may have decreased habitat complexity beyond a threshold that could no longer provide adequate spatial refugia for small-bodied prey. Thus, this decline in spatial refugia may have disproportionately increased the predation risk of small-bodied prey, counteracted their positive nutrient response, and resulted in our body size-mediated consumer response during the long-term enrichment.

Because these declines in leaf-litter standing crop occurred during the winter and spring months (Suberkropp et al. *In press*) when many stream consumers increase their body mass in preparation for emergence (e.g., Huryn and Wallace 1988), the positive nutrient response of large-bodied primary consumers was unexpected. We initially predicted that these seasonal declines in resource quantity would reduce the positive effects of nutrient enrichment on consumer body size. However, consumers likely obtained this larger body size during the short time that resources were abundant (fall and early-winter), which may have allowed them to survive through later periods of low resource availability. For instance, invertebrate body size can be positively related to their lipid and energy content (Otto 1974). Lipid content is also

positively related to the body size, starvation resistance, and survival of *Daphnia* (Tessier et al. 1983). This suggests that the larger body size of consumers in our treatment stream may have subsequently increased their lipid storage capacity and allowed them to maintain their positive nutrient response despite the later seasonal reductions in resource quantity. Indeed, we observed more robust individuals from several taxa that appeared to have greater fat stores in the treatment stream during the fourth and fifth years of enrichment (J. Davis and S. Eggert, pers. obs.). Overall, these results contribute to the growing empirical evidence indicating the relatively greater importance of resource quality versus quantity in stimulating consumer production within aquatic ecosystems (e.g., Boersma and Kreutzer 2002).

Contrast with previous growth rate experiment — The increased dominance of large-bodied consumers contrasted with previous evidence that predicted an increased dominance of small-bodied consumers with enrichment (Cross et al. 2005b). Specifically, two years of enrichment increased the growth rate of a small-bodied primary consumer (non-Tanypodinae chironomid [Diptera]), but not a large-bodied primary consumer (*Tallaperla* spp. [Plecoptera]) (Cross et al. 2005b). Thus, based on these earlier results, we expected that nutrient enrichment would have stimulated small-bodied primary consumers and increased their relative dominance. Despite this previous evidence, our results and those from other studies have shown that enrichment can disproportionately stimulate large-bodied consumers (e.g., Sprules and Munawar 1986, Vanni 1986, Bourassa and Morin 1995). These contrasting results are likely due to differences in experimental scale. This linkage between consumer growth rate and nutrient enrichment response, which predicted the increased dominance of small-bodied consumers, was measured at the individual level without predation. Thus, it likely represents the consumer's 'potential' nutrient enrichment response. However, our increased dominance of large-bodied

primary consumers were due to changes in population / community-level responses that are related to both top-down and bottom-up forces (Brooks and Dodson 1965, e.g., Brett and Goldman 1997). Despite their potential for faster individual growth rates under nutrient-enriched conditions, the greater predation risk of small-bodied prey may minimize their population-level responses because of decreased survival. In addition, predation risk may have directly reduced individual growth rates of small-bodied prey at the stream-level, as it can reduce the foraging activity of predator-vulnerable prey (Peacor and Werner 2000). When predation risk is greater for small-bodied prey, it may reduce their foraging activity, allowing large-bodied prey to more fully exploit the improved resource quality and to increase their community dominance.

Effects on ecosystem processes— These shifts toward greater dominance of large-bodied primary consumers indicate a little-recognized pathway for nutrient enrichment to alter stream function because consumer size structure can alter ecosystem processes (Poff et al. 1993, Hall et al. 2007). Because body size is negatively related to mass-specific metabolic rates (Brown et al. 2004), food webs dominated by larger-bodied organisms may have lower community-level respiration rates and slower biomass turnover rates (Poff et al. 1993, Huryn and Benke 2007). Consumer assemblages that possess similar levels of consumer biomass, but differ in body size distributions, can also have substantially different consumer nutrient excretion rates (Hall et al. 2007). Thus, enrichment may alter energy and nutrient flows within nutrient-enriched food webs through shifts in body size distributions. Given the overall importance of consumer body size in determining consumer functional roles, these changes in body size distributions facilitated by nutrient enrichment may further alter aquatic ecosystem function.

In summary, consumer response to nutrient enrichment depended on body size. Contrary to our original prediction that long-term nutrient enrichment would not stimulate large-bodied consumers because of potentially greater resource limitation, enrichment increased the biomass and abundance of large-bodied primary consumers throughout our five-year manipulation. Enrichment also increased the individual body mass of a dominant stream consumer that had previously exhibited sensitivity to resource limitation. However, the consumer body size response was not homogeneous across trophic levels, because large-bodied predators did not respond to the long-term enrichment. Thus, despite reduced detrital resource availability, long-term enrichment continued to stimulate large-bodied primary consumers likely because of associated increases in resource quality and reductions in predation pressure.

Acknowledgments

S. Dye, N. Taylor, R. Hilten, J. Benstead, and C. Tant helped in the field and maintained field equipment. We are also grateful to our numerous laboratory assistants, but especially E. Baker, A. Ely, J. Hoehn, and J. Holland. Contributions from our collaborators, D. Conners, V. Gulis, K. Subberkropp, and C. Tant, made this experiment possible. The Coweeta Hydrologic Laboratory provided logistical support. Water chemistry analyses were conducted by T. Maddox at the University of Georgia (UGA) Analytical Chemistry Lab. S. Dye, C. Anderson, R. Creed, J. Allgeier, J. Frisch, A. Helton, A. Mehring, S. Norlin, C. Small, C. Tant, and A. Trice provided insightful comments on an early draft of this manuscript. Funding was provided by NSF (DEB-0318063, DEB-9629268, and DEB-0212315). J. Davis was supported by a NSF Graduate Research Fellowship and a UGA Presidential Fellowship.

Table 3.1: Primary consumer biomass effect sizes (mg AFDM m^{-2}) that indicate the direction and magnitude of the nutrient enrichment response (mean ± SE) within a given size class. Effect sizes are differences in grouped biomass, such that an effect size of 10 means that there was a difference of 10 mg in biomass between the reference and treatment streams for that size class. Negative values indicate higher biomass in the reference vs. treatment stream. Randomized intervention analysis (RIA) was applied to the monthly time-series within each size class. Asterisks represent a significant difference between the post-treatment period (either short-term [ENR 1 and 2] or long-term response [ENR 4 and 5]) and the two year pretreatment period (RIA, $P < 0.05$). Due to insufficient sample sizes, we excluded several body size classes from the analyses (represented by ND).

Body Size Range (mg) (> Min ≤ Max)	Short vs Pre	Long vs Pre
0.000 - 0.001	71.0 (13.1)*	-9.0 (10.7)
0.001 - 0.002	7.6 (1.7)*	4.5 (1.1)*
0.002 - 0.003	8.0 (2.9)*	-1.3 (1.9)
0.003 - 0.006	26.4 (8.8)*	-5.8 (5.5)
0.006 - 0.010	5.9 (2.3)*	-1.3 (1.7)
0.010 - 0.018	34.1 (10.0)*	1.7 (9.0)
0.018 - 0.032	61.9 (14.9)*	24.5 (15.4)
0.032 - 0.056	45.5 (13.3)*	22.2 (12.6)
0.056 - 0.100	85.5 (25.2)*	34.1 (12.1)*
0.100 - 0.178	63.3 (31.4)*	-15.2 (15.7)
0.178 - 0.316	58.2 (17.1)*	11.5 (19.8)
0.316 - 0.562	68.6 (26.2)*	-10.2 (25.9)
0.562 - 1.000	76.7 (20.1)*	7.1 (22.8)
1.000 - 1.778	113.8 (35.7)*	171.2 (105.8)
1.778 - 3.162	74.1 (40.1)	191.4 (98.3)*
3.162 - 5.623	137.3 (56.0)*	222.1 (115.2)*
5.623 - 10.000	119.2 (48.7)*	514.9 (188.3)*
10.000 - 17.783	259.1 (91.8)*	871.7 (318.0)*
17.783 - 31.623	114.4 (68.4)	540.0 (275.4)*
31.623 - 56.234	81.1 (37.3)*	223.8 (79.9)*
56.234 - 100.000	ND	ND
100.000 - 177.828	ND	ND

Table 3.2: Predator biomass effect sizes (mg AFDM m^{-2}) that indicate the direction and magnitude of the nutrient enrichment response (mean ± SE) within a given size class. Randomized intervention analysis (RIA) was applied to the monthly time-series of biomass data within each size class. Asterisks represent a significant difference between the post-treatment period (either short-term or long-term response) and the pretreatment period (RIA, $P < 0.05$). Due to insufficient sample sizes, we excluded several body size classes from the RIA analysis (represented by ND). Other designations as in Table 3.1.

Body Size Range (mg) (> Min ≤ Max)	Short vs Pre	Long vs Pre
0.000 - 0.001	2.4 (0.6)*	1.9 (0.6)*
0.001 - 0.002	ND	ND
0.002 - 0.003	10.3 (3.0)*	7.8 (2.5)*
0.003 - 0.006	6.0 (1.6)*	4.0 (1.3)*
0.006 - 0.010	3.6 (1.1)*	2.6 (1.2)*
0.010 - 0.018	36.0 (9.0)*	22.8 (7.5)*
0.018 - 0.032	9.1 (4.1)*	1.2 (3.0)
0.032 - 0.056	43.0 (14.5)*	27.8 (15.0)
0.056 - 0.100	43.9 (20.9)*	19.5 (14.6)
0.100 - 0.178	21.5 (17.3)	-20.6 (15.1)
0.178 - 0.316	75.1 (25.3)*	12.0 (21.2)
0.316 - 0.562	68.7 (20.5)*	-31.8 (12.5)*
0.562 - 1.000	78.6 (22.0)*	2.8 (11.3)
1.000 - 1.778	63.6 (16.9)*	29.7 (17.4)
1.778 - 3.162	57.4 (15.2)*	31.1 (22.1)
3.162 - 5.623	-19.6 (24.2)	-90.0 (21.6)*
5.623 - 10.000	27.5 (23.5)	-42.8 (22.4)
10.000 - 17.783	94.6 (40.5)*	-14.9 (38.0)
17.783 - 31.623	27.0 (64.5)	-3.3 (49.5)
31.623 - 56.234	ND	ND
56.234 - 100.000	ND	ND
100.000 - 177.828	ND	ND

Table 3.3: Breakdown of the 22 $\log_{10}$ body size classes used for categorizing primary consumers and predators by body size (mg). We based the $\log_{10}$ transformations on the upper limit of the body size class. Individual body masses of macroinvertebrates in our study streams ranged in body size from < 0.001 to 169.360 mg.

Log_{10} Body Size Class	Lower Limit (> mg)	Upper Limit (≤ mg)
-3.00	0.000	0.001
-2.75	0.001	0.002
-2.50	0.002	0.003
-2.25	0.003	0.006
-2.00	0.006	0.010
-1.75	0.010	0.018
-1.50	0.018	0.032
-1.25	0.032	0.056
-1.00	0.056	0.100
-0.75	0.100	0.178
-0.50	0.178	0.316
-0.25	0.316	0.562
0.00	0.562	1.000
0.25	1.000	1.778
0.50	1.778	3.162
0.75	3.162	5.623
1.00	5.623	10.000
1.25	10.000	17.783
1.50	17.783	31.623
1.75	31.623	56.234
2.00	56.234	100.000
2.25	100.000	177.828

Table 3.4: Primary consumer abundance effect sizes (no. m^{-2}) that indicate the direction and magnitude of the nutrient enrichment response (mean ± SE) within a given size class. Randomized intervention analysis (RIA) was applied to the monthly time-series of abundance data within each size class. Asterisks represent a significant difference between the post-treatment period (either short-term [ENR 1 and 2] or long-term response [ENR 4 and 5]) and the two year pretreatment period (RIA, $P < 0.05$). Due to insufficient sample sizes, we excluded several body size classes from the RIA analysis (represented by ND).

Body Size Range (mg) (> Min ≤ Max)	Short vs Pre	Long vs Pre
0.000 - 0.001	89,366.9 (15,912.2)*	-9,692.0 (13,445.5)
0.001 - 0.002	5,058.0 (1,101.3)*	2,984.0 (761.9)*
0.002 - 0.003	2,681.1 (1,128.5)*	-333.5 (673.9)
0.003 - 0.006	6,476.4 (2,099.4)*	-1,305.3 (1,327.8)
0.006 - 0.010	758.3 (297.0)*	-192.8 (220.6)
0.010 - 0.018	2,551.4 (766.8)*	198.4 (694.3)
0.018 - 0.032	2,656.2 (625.4)*	944.5 (644.0)
0.032 - 0.056	947.7 (283.2)*	413.4 (277.6)
0.056 - 0.100	1,220.3 (427.3)*	392.5 (159.2)*
0.100 - 0.178	490.3 (234.1)*	-124.8 (119.1)
0.178 - 0.316	267.2 (77.1)*	79.2 (95.4)
0.316 - 0.562	156.5 (62.7)*	-15.0 (62.0)
0.562 - 1.000	107.2 (28.0)*	8.6 (32.1)
1.000 - 1.778	86.4 (28.0)*	124.9 (73.1)
1.778 - 3.162	28.5 (15.8)	78.4 (39.4)*
3.162 - 5.623	30.2 (13.2)*	48.3 (26.0)*
5.623 - 10.000	14.9 (6.4)*	68.0 (25.8)*
10.000 - 17.783	18.5 (6.8)*	56.5 (25.3)*
17.783 - 31.623	5.1 (3.0)	23.9 (12.3)*
31.623 - 56.234	2.3 (1.0)*	5.8 (2.1)*
56.234 - 100.000	ND	ND
100.000 - 177.828	ND	ND

Table 3.5: Predator abundance effect sizes (no. m^{-2}) that indicate the direction and magnitude of the nutrient enrichment response (mean ± SE) within a given size class. Randomized intervention analysis (RIA) was applied to the monthly time-series of abundance data within each size class. Asterisks represent a significant difference between the post-treatment period (either short-term [ENR 1 and 2] or long-term response [ENR 4 and 5]) and the two year pretreatment period (RIA, $P < 0.05$). Due to insufficient sample sizes, we excluded several body size classes from the RIA analysis (represented by ND).

Body Size Range (mg) (> Min ≤ Max)	Short vs Pre	Long vs Pre
0.000 - 0.001	3,986.2 (1,073.4)*	3,122.6 (1,052.6)*
0.001 - 0.002	ND	ND
0.002 - 0.003	3,839.2 (1,092.7)*	2,891.1 (927.9)*
0.003 - 0.006	1,372.8 (359.9)*	906.4 (287.3)*
0.006 - 0.010	412.6 (125.7)*	339.9 (142.4)*
0.010 - 0.018	2,480.9 (620.9)*	1,571.6 (512.3)*
0.018 - 0.032	338.3 (143.5)*	44.2 (109.0)
0.032 - 0.056	1,073.9 (371.7)*	715.2 (384.7)
0.056 - 0.100	598.6 (273.4)*	221.4 (192.3)
0.100 - 0.178	171.0 (133.2)	-152.8 (116.5)
0.178 - 0.316	331.6 (116.9)*	47.8 (98.8)
0.316 - 0.562	165.1 (47.7)*	-70.4 (33.7)*
0.562 - 1.000	101.5 (28.1)*	4.6 (14.8)
1.000 - 1.778	48.9 (12.8)*	23.0 (13.2)
1.778 - 3.162	23.1 (6.4)*	14.1 (9.7)
3.162 - 5.623	-3.3 (5.3)	-18.4 (5.0)*
5.623 - 10.000	3.8 (3.0)	-5.2 (2.9)
10.000 - 17.783	6.7 (3.1)*	-1.4 (2.8)
17.783 - 31.623	0.2 (2.6)	-0.8 (2.0)
31.623 - 56.234	ND	ND
56.234 - 100.000	ND	ND
100.000 - 177.828	ND	ND

Fig. 3.1: Average annual biomass (**A**) and abundance (**B**) of all macroinvertebrates (primary consumers plus predators) (mean ± 1SE). The arrow indicates the beginning of nutrient enrichment. The short-term sampling period encompasses Enr 1 and 2, while the long-term sampling period encompasses Enr 4 and 5. Asterisks above each sampling period represent a significant difference between the post-treatment period (either short-term or long-term response) and the pretreatment period (Pre 1 and 2) (RIA, $P < 0.05$). AFDM is ash-free dry mass.

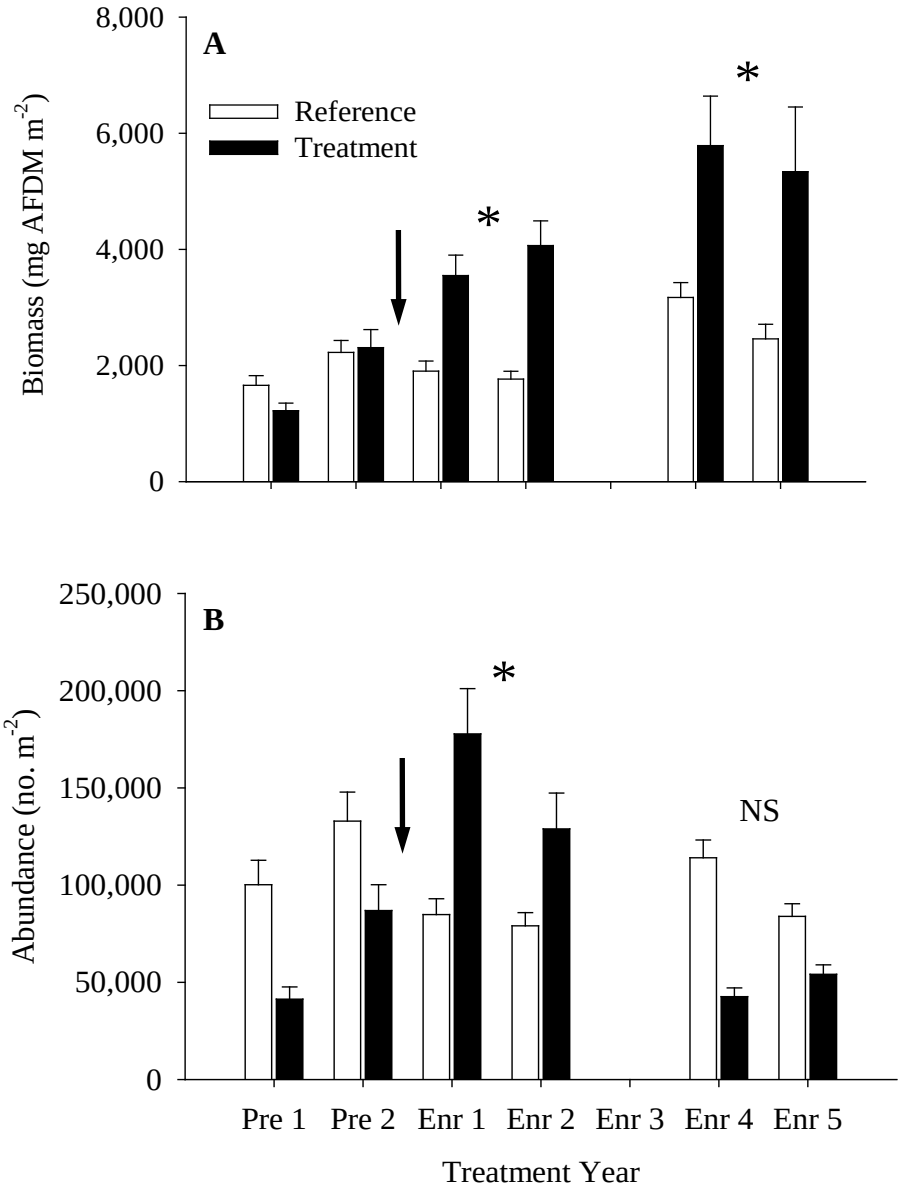

A
Reference
Treatment
Biomass (mg AFDM m-2)
8,000
6,000
4,000
2,000
0
*
*
B
Abundance (no. m-2)
250,000
200,000
150,000
100,000
50,000
0
*
NS
Pre 1
Pre 2
Enr 1
Enr 2
Enr 3
Enr 4
Enr 5
Treatment Year

Fig. 3.2: Average annual biomass (mean ± 1SE) of small- and large-bodied primary consumers (**A** and **B)** and predators (**C** and **D**). Small-and large-bodied individuals were classified based on the results of the randomized intervention analyses (RIA). Our RIA results indicated that primary consumer response to long-term nutrient enrichment diverged at a body size threshold of 1.778 mg (Tables 3.1 and 3.4). Thus, we defined small-bodied consumers as those individuals with a body size ≤ 1.778 mg, and large-bodied individuals as those individuals with a body > 1.778 mg. The arrow represents the beginning of nutrient enrichment. AFDM is ash-free dry mass.

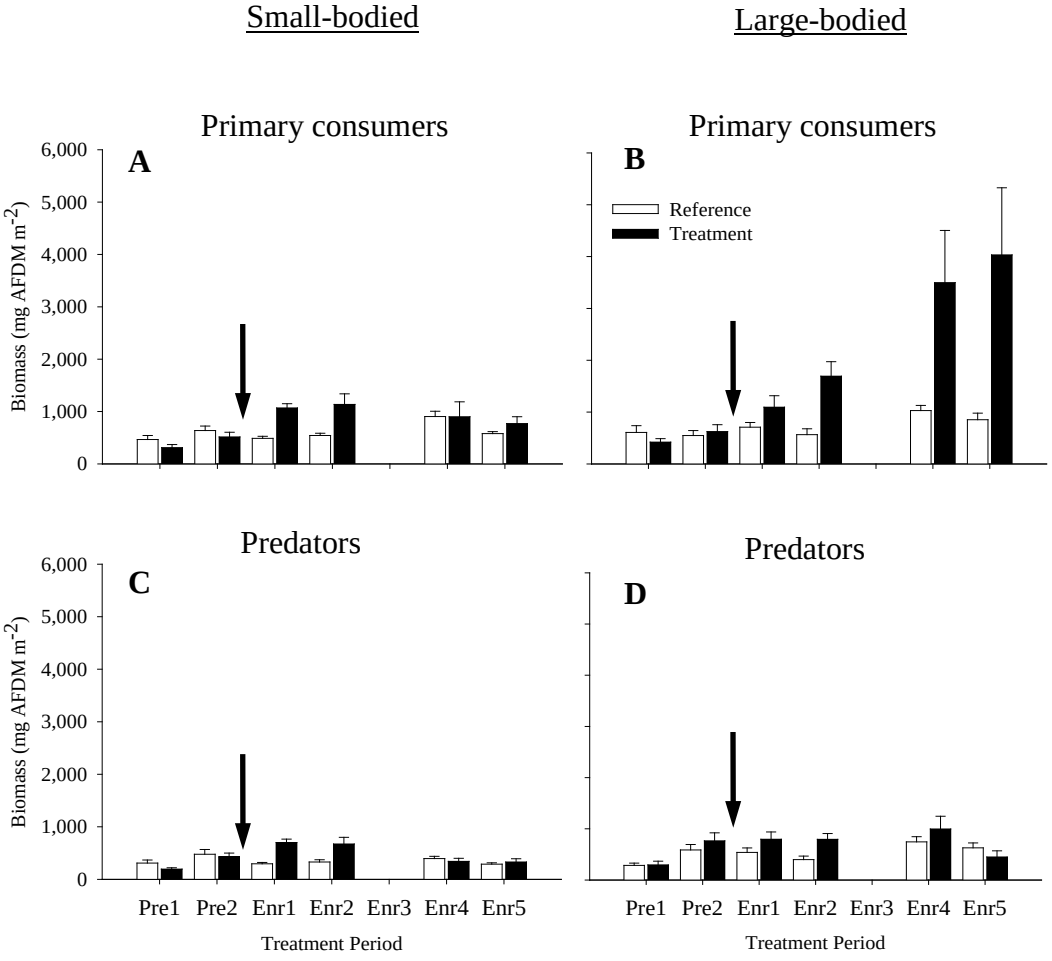
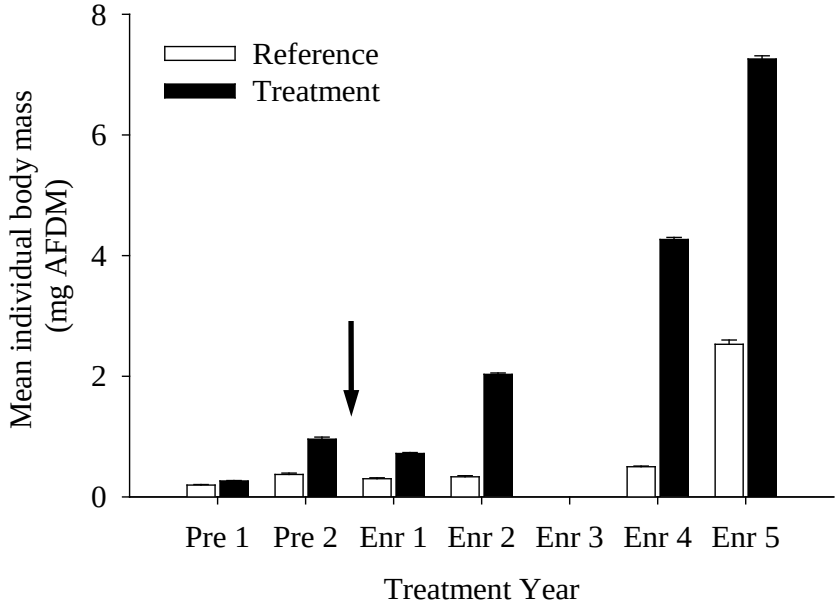

Small-bodied
Large-bodied
Primary consumers
Primary consumers
A
B
Reference
Treatment
Biomass (mg AFDM m-2)
6,000
5,000
4,000
3,000
2,000
1,000
0
Predators
Predators
C
D
Pre1
Pre2
Enr1
Enr2
Enr3
Enr4
Enr5
Treatment Period

Fig. 3.3: Mean body mass of individual *Pycnopsyche* spp. (mean ± SE) calculated on a yearly basis. AFDM is ash-free dry mass. The arrow represents the beginning of nutrient enrichment.


Reference
Treatment
Mean individual body mass
(mg AFDM)
0
2
4
6
8
Pre 1
Pre 2
Enr 1
Enr 2
Enr 3
Enr 4
Enr 5
Treatment Year

CHAPTER 4

NUTRIENT ENRICHMENT AFFECTS STREAM NUTRIENT TRANSFORMATIONS VIA INCREASED CONSUMER ASSIMILATION AND EXCRETION RATES[3]

[3] Davis, J. M., A. D. Rosemond, and V. Patel. To be submitted to *Ecosystems*.

Abstract

Within aquatic food webs, consumers can alter nutrient transformations through their rates of assimilation, excretion, and secondary production. Although the effects of nutrient enrichment on the stimulation of consumer biomass and production are well-studied, few studies have quantified the effects of enrichment on assimilation and excretion rates. Here we assessed whether nutrient enrichment would increase nutrient assimilation (determined in a laboratory study) and excretion rates (determined in the field) of *Pycnopsyche* spp., a leaf-shredding caddisfly common in eastern U.S. forested streams. Because enrichment can have significantly greater positive effects on the nutrient content of poor quality resources, we also tested whether enrichment effects on assimilation varied between detrital resources that differed in quality (initial C:N). By coupling our experimental results with known *Pycnopsyche* biomass, we scaled these results to the stream population level. Nutrient enrichment significantly increased *Pycnopsyche* nitrogen (N) and phosphorus (P) assimilation rates in the laboratory study and increased these rates the most for the poorest quality resource. However, carbon (C) assimilation showed less predictable responses to enrichment. Nutrient enrichment also stimulated N and P excretion rates. Assimilation N:P declined with enrichment, suggesting preferential sequestration of P relative to N. At the population-scale, enrichment increased all stocks and fluxes of N, P, and C, which occurred via both changes in rates of excretion and assimilation as well as consumer biomass. As consumers are important drivers of aquatic food web processes, these results illustrate that enrichment-induced shifts in consumer metabolic processes can alter elemental fluxes and storage at the stream level.

Introduction

Within aquatic ecosystems, consumers can alter the storage and flows of nutrients and energy through elemental consumption, assimilation, and excretion (Wallace and Hutchens 2000, Vanni 2002, Hall et al. 2003). However, their effects on elemental storage can be relatively weak because consumer standing stocks are small compared to basal resource standing crops (Cross et al. 2005c). Conversely, consumers can have a much larger effect on elemental transformations and flows when biomass turnover and secondary production are rapid compared to other fluxes (Vanni 2002, Cross et al. 2005c). During the ingestion of basal resources, primary consumers convert these stores of relatively inaccessible resources into consumer biomass as carbon and nutrients (Hall et al. 2000, Gonzalez and Graca 2003, Cross et al. 2007). Because this consumer biomass is available to predators, primary consumers can represent an important link between basal resources and predators, helping to stimulate energy and nutrient flows vertically through food webs (Hall et al. 2000, Cross et al. 2007).

Primary consumers not only increase resource and energy flows to higher trophic levels, but changes in their metabolic processes can also alter these flows laterally to other primary consumers or down to basal resources. Resources assimilated into consumer biomass are mostly re-released as excretion, which can provide a large proportion of primary producer nutrient demand (Vanni et al. 2006). Even unassimilated material (egested feces) can be a source of carbon (C), nitrogen (N), and phosphorus (P) for other consumers. Because of their inefficient assimilation during leaf processing, detritivorous consumers can be an important biotic pathway for converting leaf litter into fine particulate organic matter (FPOM) (Cuffney et al. 1990, Wallace et al. 1991). Thus, detritivores can increase FPOM egestion and export (Wallace et al.

1991), which can subsequently stimulate resource availability and the nutrient assimilation of downstream consumers (Short and Maslin 1977, Wallace and Webster 1996).

Primary consumers can substantially alter nutrient and energy flows through aquatic food webs; however, these overall effects can shift under varying environmental conditions. For instance, nutrient enrichment of detritus-based food webs can accelerate the rates at which consumers eat detritus (Cross et al. 2007), helping to increase detrital breakdown rates and reduce organic matter standing crops (Greenwood et al. 2007, Benstead et al. 2009). Enrichment can also increase consumer-driven nutrient recycling. By stimulating the biomass and excretion rates of gizzard shad (*Dorosoma cepedianum*), enrichment of a lake ecosystem increased the relative importance of consumer-driven nutrient recycling for primary production (Vanni et al. 2006). Because it can stimulate primary consumer production, enrichment can also increase the flow of nutrients up through the food web by increasing the availability of prey and stimulating the production of higher trophic levels (Peterson et al. 1993, Cross et al. 2007).

Despite these numerous studies addressing the effects of nutrient enrichment on consumer-driven nutrient recycling and secondary production, less is known about the relative importance and potential shifts in elemental assimilation rates associated with enrichment. However, increased resource quality can increase C assimilation efficiencies and turnover rates (Pandian and Marian 1986, Cebrian 1999, Hessen et al. 2004), which suggests that enrichment may similarly increase consumer nutrient assimilation rates. Because the rate that consumers ingest and assimilate nutrients is the first metabolic step in converting basal resources into consumer biomass, shifts in these rates may have important implications for meeting overall consumer nutrient demand and maintaining nutrient flows through aquatic food webs.

Here we assessed the effects of enrichment on the N, P, and C transformations of *Pycnopsyche* spp., a dominant primary consumer in eastern U.S. forested headwater streams (Wallace et al. 1999, Creed et al. 2009). Because of a dense forest understory that reduces light availability and autotrophic production, these detritus-based food webs are largely reliant upon heterotrophic microbes that colonize terrestrial leaf inputs (Wallace et al. 1997, Hall et al. 2000, Cross et al. 2007). Because nutrient enrichment of these stream food webs can stimulate the production of leaf-associated microbes (Gulis and Suberkropp 2003, Suberkropp et al. *In press*), it can increase detrital resource quality (carbon to nutrient ratios) and stimulate consumer production (Cross et al. 2007). As greater resource quality can increase C assimilation efficiency (Pandian and Marian 1986), the greater resource quality associated with enrichment may also stimulate nutrient flows via similar changes in consumer nutrient assimilation and excretion rates. Thus, we tested whether enrichment would significantly increase *Pycnopsyche* N, P, and C assimilation rates (measured in a laboratory study) and N and P excretion rates (measured in the field). Because enrichment can have greater effects on poorer quality substrates (high C:N) (Stelzer et al. 2003), we also tested whether potential shifts in assimilation rates varied between two leaf species that differed in initial C:N. Finally, applying these results from our assimilation and excretion experiments in conjunction with known areal-specific *Pycnopsyche* biomass and secondary production (Davis et al. *In review-a*), we constructed N, P, and C box models to determine the effects of nutrient enrichment on these elemental transformations at the population scale.

Methods

Study organism—To examine the effects of nutrient enrichment on stream elemental transformations, we selected *Pycnopsyche* spp. (Trichoptera: Limnephilidae), a leaf-shredding,

case-building caddisfly that is commonly found in eastern U.S. forested stream ecosystems (Wallace et al. 1999, Creed et al. 2009). This genus was an ideal study organism to assess the effects of enrichment on consumer-driven elemental transformations because it is a functional and community dominant in these stream ecosystems (Herbst 1980, 1982, Creed et al. 2009). Furthermore, nutrient enrichment disproportionately increased *Pycnopsyche* production and concentrated a large proportion of nutrient and energy flows through this single genus (Cross et al. 2007, Davis et al. *In review-a*).

Laboratory assimilation rate experiments—To quantify *Pycnopsyche*'s role in elemental transformations within these detritus-based ecosystems, we conducted a laboratory assimilation experiment during the spring of 2007. Leaf ingestion and elemental assimilation rates were measured for larvae fed leaf material of varying quality. Shortly after leaf fall, we collected recently abscised red maple (*Acer rubrum* L.) and rhododendron (*Rhododendron maximum* L.) leaves from the USDA Forest Service Coweeta Hydrologic Laboratory, a heavily-forested experimental watershed (2185 ha) located in the southern Appalachian Mountains (Macon County, North Carolina). Leaves were air dried and assembled into single species leaf packs using bags of 1 mm mesh size. Throughout the late winter and early spring, leaf packs were deployed in either a reference (C53) or nutrient-enriched (C54) headwater stream at the Coweeta LTER. Prior to the experimental enrichment, the reference and treatment streams did not differ in nutrient concentrations (mean ± SE, C53: DIN: 23.2 ± 8.5 μg L^{-1}, SRP: 6.8 ± 3.0 μg L^{-1}; C54: DIN: 29.3 ± 4.9 μg L^{-1}, SRP: 9.5 ± 2.3 μg L^{-1}). However, we increased nutrient concentrations in the enriched stream (DIN: 506.2 ± 36.3 μg L^{-1}, SRP: 80.0 ± 5.6 μg L^{-1}, 5 year average, n = 116), while the reference stream concentrations were comparable to the pretreatment period (DIN: 31.0 ± 3.4 μg L^{-1}, SRP: 8.0 ± 1.3μg L^{-1}, 5 year average, n = 106). These assimilation

experiments were conducted under the aegis of a broader-scale study that was assessing the effects of nutrient enrichment on the structure and function of the macroinvertebrate food web (see Davis et al. *In review-a*). The section of stream used for these incubations was in its sixth year of enrichment when the leaf packs were deployed.

Leaf pack deployments were staggered, such that rhododendron leaf packs were deployed in both streams for 90 and 60 d, while red maple leaf packs were deployed for 14 and 28 d. However, because of accelerated leaf breakdown rates in the nutrient-enriched stream (Greenwood et al. 2007, C. Tant unpubl. data), there was insufficient leaf material remaining in the rhododendron 90 d treatment for testing. These incubation intervals maximized microbial colonization and leaf conditioning, resulting in a broad range of detrital resource qualities (i.e., carbon to nutrient ratios). We had six leaf treatment types (M14+, M14-, M28+, M28-, R60+, R60-) where 'M' or 'R' indicates leaf species (red maple or rhododendron), the number represents the incubation period in d, and '+' or '-' indicates incubation in either the nutrient-enriched or reference stream.

Two weeks prior to the initiation of the feeding experiments (Feb. 2007), we collected ca. 100 *Pycnopsyche* from our reference stream at the Coweeta Hydrologic Laboratory. We collected similar-sized individuals that had already started constructing stone cases. Individuals were maintained for two weeks in a well-aerated aquarium with conditioned leaves from the reference stream. Aquaria and assimilation mesocosms were maintained in a walk-in incubator at 12.5 °C on a 12 hr light cycle. Average water temperature during the assimilation experiment was 9.20 ± 0.01 °C. After this two-week acclimation period, we placed individual *Pycnopsyche* in 100 ml plastic beakers (5.1 cm diameter x 7.2 cm) containing 80 ml of filtered stream water that was continuously aerated. Individuals were then starved for 48 hours prior to the initiation

of the feeding trials because a previous study showed that this was a sufficient amount of time for gut clearance (Eggert and Wallace 2007). After this initial starvation period, we changed the stream water, rinsed out any accumulated feces, and returned each *Pycnopsyche* to its appropriate beaker. To initiate the feeding portion of the assimilation experiments we randomly assigned ten individuals to each of the six leaf treatment types.

At the start of the feeding trials, leaf packs were collected from both streams at Coweeta and transported back to the lab in aerated stream water. We then cut leaf disks from each leaf type using a 1.1 cm diameter cork borer. To maintain intact biofilm communities, leaves were not rinsed prior to cutting. We randomly selected nine leaf disks from one of the treatment groups, gently blotted them dry, and weighed them to obtain the initial wet mass of leaf material. These nine leaf disks were then added to the plastic beaker containing 80 ml of filtered stream water and a single *Pycnopsyche*. To control for any microbial breakdown of leaf material that might occur during the trials, we also conducted five additional replicates for each leaf treatment type that were similarly processed, but did not contain macroinvertebrate larvae.

We calculated leaf disk wet weight to ash-free dry mass (AFDM) conversions by collecting five replicate samples of nine leaf disks from each leaf treatment type. Disks were blotted dry and weighed for initial wet mass, dried at 60°C, and then reweighed for dry mass. Disks were then ashed at 500°C for 6 hours and initial AFDM was calculated. From this pre-trial material, we also collected five composite samples per treatment type for later N, P, and C analyses (see below).

Assimilation trials were run for 72 hours. To minimize any microbial colonization of egested material, we collected egested material twice during the experiment; after 48 hours and at the end of the experiment. For the 48 hour collection, we temporarily removed the larvae and

any unconsumed leaf material from the mesocosms. The mesocosm was filtered through pre-weighed, ashed Gelman GFF glass-fiber filters (0.70 µm). For this 48 hour collection, the filtered stream water, larvae, and unconsumed leaf material were returned to the appropriate mesocosm. Assimilation trials were then run for an additional 24 hours after which all remaining unconsumed leaf material was removed. This unconsumed material was dried at 60°C, weighed, and ashed at 500°C to determine its AFDM. *Pycnopsyche* were starved for an additional 48 hours to allow for adequate gut clearance. *Pycnopsyche* were then sacrificed, dried at 60°C, and weighed to get *Pycnopsyche* dry mass. To calculate *Pycnopsyche* dry mass to AFDM conversion factors, we also sacrificed an additional twenty individuals that were not used in the feeding trials. After this starvation period, we filtered the mesocosm to collect the remaining egested material and combined it with the 48-hr egested material. This composite sample was dried and weighed. We then applied egestion dry mass to AFDM conversions that were previously determined from preliminary feeding trials. The filtrate from the final filtration was used for later dissolved organic carbon (DOC) analysis.

Elemental analysis—To determine the N, P, and C content of leaf material, the five composite samples of pre-trial material were dried at 60°C, ball-milled, and analyzed for N and C content with a Carlo-Erba NA 1500 CHN analyzer (Carlo Erba, Milan, Italy). For total P analysis, samples were dried, weighed into ceramic crucibles, ashed at 500°C, acid digested, and analyzed colometrically (APHA 1998). We applied the same analytical methods to egestion N, P, and C content. Filtrate DOC content was analyzed on a Shimadzu TOC-5000A total organic carbon analyzer (Shimadzu Scientific Instruments, Maryland, USA). Because we analyzed five replicate pre-trial DOC samples of filtered stream water, we could calculate the mass of DOC generated during the assimilation trials. We considered any DOC generated during the trials to

be C that was ingested, but not assimilated (i.e., it was egested C). However, some generated DOC was likely not consumed or egested by *Pycnopsyche* and was released directly from mechanical fragmentation of leaf material (Meyer and O'Hop 1983). Despite this potential underestimation of DOC released from mechanical fragmentation, this error likely did not affect our results because the mass of DOC generated during the assimilation trials represented a small proportion of the total egested C (average across all trials: 0.1%).

Assimilation rate calculations— Elemental assimilation efficiency (AE_X) was the proportion of N, P, or C that was ingested, but not egested as feces. It was calculated as follows:

$$AE_X(\%) = [(Ing * \%X_L) - (Ege * \%X_E)]/(Ing * \%X_L) \quad (1)$$

where Ing = AFDM of ingested material, Ege = AFDM of egested material, and X = either C, N, or P content for leaf (subscript L) or egested material (subscript E). The C assimilation efficiency formula had an additional value subtracted from the numerator (mass of DOC generated). Mass-specific leaf ingestion rate (IR_L) was calculated:

$$IR_L = Ing / M / T \quad (2)$$

where M = *Pycnopsyche* AFDM and T = time in days. Elemental ingestion rate (IR_X) was calculated by multiplying this leaf ingestion rate from equation (2) by the leaf elemental composition (i.e., %N, %P, or %C as a decimal). Elemental assimilation rates (AR_X) were then calculated (Golladay et al. 1983):

$$AR_X = IR_X * AE_X \quad (3)$$

This calculated the rate at which *Pycnopsyche* assimilated N, P, or C. We also calculated molar N:P assimilation ratios.

Threshold elemental ratio calculations— Applying our known assimilation efficiencies, we calculated threshold elemental ratios ($TER_{C:P}$) for *Pycnopsyche* in each treatment type. These ratios represent the C:P ratio of a food resource where consumer growth switches from C- to P-limitation (Frost et al. 2006). We calculated TERs for each *Pycnopsyche* in our assimilation trials according to Frost et al. (2006) using the following equation:

$$TER_{C:P} = \frac{A_P}{\frac{I_C A_C - R_C}{I_C}} * \frac{Q_C}{Q_P} \quad (4)$$

where A_P and A_C are the assimilation efficiencies of P and C, I_C is the mass-specific C ingestion rate, R_C is the mass-specific respiration rate, Q_C is *Pycnopsyche* body C content, and Q_P is *Pycnopsyche* body P content. Because we did not directly measure *Pycnopsyche* respiration in our experiment, we assumed that 40% of assimilated C was lost as respiration (i.e., $R_C = 0.40 \times A_C$) (Benke and Wallace 1997).

The $TER_{C:P}$ indicates whether a food resource will lead to C or P limitation for that consumer. It also predicts that consumers with higher $TER_{C:P}$ have relatively lower susceptibility to P-limitation because of a more efficient P assimilation capacity relative to their body C:P content. By comparing $TER_{C:P}$ between the reference and nutrient-enriched treatments, we could assess whether nutrient enrichment altered *Pycnopsyche*'s $TER_{C:P}$ and susceptibility to P-limitation. Accordingly, if nutrient enrichment disproportionately increased

the rate that *Pyncopsyche* were assimilating P compared to C, then it would have increased their $TER_{C:P}$ and decreased their susceptibility to P-limitation.

Field excretion experiments— To determine *Pycnopsyche*'s role in nutrient recycling, we also conducted a field-based excretion experiment during spring 2005 at the Coweeta LTER. These trials used the same reference and nutrient-enriched streams described above. We collected five *Pycnopsyche* from each stream, gently rinsed them with deionized water to remove any sediment, and then placed them into 50 ml beakers (3.9 cm diameter x 5.7 cm) containing 30 ml of water. Each beaker also had a 2.5 cm diameter piece of nitex mesh to allow the larvae to move around in the beaker. Because macroinvertebrates may reduce their excretion rates when they are not eating (J. B. Wallace *pers. comm.*), we provisioned each mesocosm with two leaf disks. This maintained the mesocosm at a state similar to the natural condition where *Pycnopsyche* would have food available. To correct for any microbial activity that might change water nutrient concentrations, we also conducted five additional microbial control trials per stream that contained leaf and nitex disks, but no *Pycnopsyche* larvae. These microbial controls were processed identically to the *Pycnopsyche* excretion trials.

Excretion mesocosms were incubated in the stream for 4 hours and were not aerated. At the conclusion of the excretion trials, the larvae, leaf material, and nitex mesh were removed from the mesocosm. The water was filtered through a 0.45 µm nitrocellulose membrane filter attached to a 60-cc syringe. Water samples and larvae were transported back to the lab on ice where they were frozen until analyzed for NH_4-N, NO_3-N, and soluble reactive phosphorus (SRP). NH_4-N and NO_3-N content were analyzed using an Alpkem Rapid Flow Analyzer and SRP was analyzed spectrophotometrically (ascorbic acid method, APHA 1998). Although *Pycnopsyche* would not excrete NO_3-N, we still analyzed for it because of potential nitrification

that might affect the measurement of low-level NH_4-N excretion. Therefore, we included NO_3-N in our calculations because in combination with NH_4-N it would indicate the mass of total dissolved inorganic nitrogen generated during the excretion trials. Larvae were sacrificed, dried at 60°C, and weighed to get larval dry mass. We then applied dry mass to AFDM conversions to calculate mass-specific excretion rates. We also calculated molar N:P excretion ratios.

Population level responses— Using values from the laboratory-based assimilation experiment and the field-based excretion trials, we calculated N, P, and C stocks and flows at the population-level by multiplying these excretion and assimilation rates by average daily areal-specific *Pycnopsyche* biomass and production (Davis et al. *In review-a*). These calculations represented the average daily elemental stocks and fluxes calculated on a per area basis for the fifth year of enrichment, the final year of the study for which comprehensive macroinvertebrate data were collected (Sept 2004 to Aug 2005). By incorporating *Pycnopsyche* biomass estimates, we assessed the mass of N, P, and C contained within *Pycnopsyche* standing stock on a daily basis. Furthermore, *Pycnopsyche* production estimates would indicate the proportion of the mass assimilated that was not lost as excretion and was incorporated into *Pycnopsyche* biomass. This would indirectly assess the mass of N, P, and C flowing to higher trophic levels via predation or emergence. Because we only used metabolic rates (assimilation and excretion rates) from the reference and enriched M treatments (M14- and M14+) for these calculations, these interstream differences were conservative relative to changes expected for R. *Pycnopsyche* biomass and production estimates were obtained for Sept 2004 to Aug 2005 from a concurrent study that sampled macroinvertebrates on a monthly basis in the reference and nutrient-enriched streams (see Davis et al. *In review-a*).

Because changes in areal-specific stocks and fluxes are a function of *Pycnopsyche* biomass, production, and metabolic rates, the relative importance of these pathways would be confounded if enrichment stimulated multiple factors. Therefore, to isolate the effects of increased metabolic rates, we calculated additional values for the nutrient-enriched stream that kept *Pycnopsyche* biomass and production constant at levels measured in the reference stream, but used metabolic processes measured under nutrient-enriched conditions. This allowed us to isolate the independent effects of changes in excretion and assimilation rates on stream-level transformations.

Statistical analysis— Pycnopsyche leaf ingestion rates, assimilation rates, assimilation N:P, and leaf elemental content were analyzed using a two-way ANOVA with a post-hoc Tukey's test. For the two-way ANOVA, the main effects were nutrients (reference vs. nutrient-enriched) and leaf type (leaf species grouped by incubation period: M14, M28, or R60). Including an interaction between the main effects (nutrients x leaf type) allowed us to assess whether the effects of nutrients varied by leaf type. We used the appropriate transformations when necessary to meet statistical assumptions. For the field-based excretion trials, we analyzed *Pycnopsyche* excretion rates (NH_4-N and SRP) and N:P with a t-test (reference vs. nutrient-enriched). However, NO_3-N excretion rates were analyzed using a nonparametric Wilcoxon rank sum test. All analyses were done using SAS (Version 9.1, SAS Institute Inc., Cary, NC).

Results

Laboratory assimilation experiment

Leaf nutrient content— Leaf N and P content from the assimilation experiment were significantly different between treatments based on nutrients and leaf type and there was a

significant nutrient × leaf type interaction (Table 4.1). This was due to the fact that positive effects of nutrient enrichment on N and P content were greatest for the poorest quality resource (R60). With nutrient enrichment, the N and P content of R leaves incubated for 60 d were comparable to M leaves incubated for 14 d, but under reference conditions these leaf types were dissimilar (Table 4.1). Enrichment also increased P content more than N content (mean increase for P, 3.6x greater; mean increase for N, 1.8x greater), which suggested that enrichment disproportionately stimulated detrital P sequestration. Because nutrient content varied with leaf type and nutrient-condition, we effectively created a broad range of resource quality (N content: 0.40% to 1.16%; P-content: 0.01% to 0.10%). However, leaf C content was not significantly different based on either main effect or their interaction (Table 4.1).

Leaf ingestion rates— Nutrients, but not leaf type, had a significant effect on ingestion rates and there was a significant nutrient × leaf type interaction (Fig. 4.1). Differences in leaf ingestion rates were primarily driven by differences between R60+ and R60- treatments, and R60+ and M28- (Fig. 4.1). One larva was inactive and did not eat any leaf material during the assimilation trials (M14-); therefore, we excluded this replicate from all analyses.

Elemental assimilation rates— Several replicates in the R60- treatment resulted in negative assimilation rates (5 N, 2 P, and 3 C out of 10 for each element). We interpreted these values as the assimilation rates being below the detection of our methods. Instead of excluding them, we set these negative rates to zero and included them in all analyses except for assimilation N:P calculations. All replicates for other treatments had measurable rates.

Nutrients and leaf type had significant effects on N assimilation rates and there was a significant nutrient × leaf type interaction (Fig. 4.2). Enrichment stimulated the assimilation of N (Fig. 4.2). Enrichment disproportionately increased N assimilation rates for rhododendron

relative to either M treatment. Thus, under reference conditions, N assimilation varied between leaf types and was the lowest for rhododendron (R60-), but they did not vary between leaf types under enriched conditions (Fig. 4.2).

P assimilation rates were driven by the same factors as N assimilation, with significant effects of nutrients, leaf type and their interaction (Fig. 4.2). Under reference conditions, P assimilation rates varied between leaf types and were lowest for rhododendron (R60-). Enrichment reduced these leaf type differences because P assimilation was comparable between enriched R (R60+) and enriched M (M14+). Despite these increases, P assimilation rates were still higher for the highest quality resource (M28+) (Fig. 4.2). Thus, N and P assimilation rates varied between leaf types under reference conditions. However, because enrichment disproportionately stimulated these rates for the poorest quality resources, N and P assimilation rates largely did not vary between leaf types with enrichment.

Carbon assimilation rates were significantly different between treatments based on nutrients, leaf type, and their interaction but the responses were less predictable (Fig. 4.2). Unlike N and P assimilation rates where enrichment stimulated these rates for all leaf types, enrichment only increased C assimilation for the poorest quality resource (R60-). The highest (M28+) and poorest quality leaf detritus (R60-) had comparable C assimilation rates (Fig. 4.2C).

Nutrients, but not leaf type affected assimilation N:P ratios (Fig. 4.3). Assimilation N:P ratios did not differ between leaf types experiencing similar nutrient regimes (for example, M14- vs. R60-). Enrichment significantly reduced these ratios relative to the reference (Fig. 4.3), suggesting that larvae were assimilating more P than N under nutrient-enriched conditions.

Threshold elemental ratios— Applying the values from our assimilation experiments to equation 4, we were able to calculate $TER_{C:P}$ for each individual *Pycnopsyche*. Compared to the

reference condition, *Pycnopsyche*'s $TER_{C:P}$ increased with nutrient enrichment (Table 4.2), which indicated a lower susceptibility to P-limitation under nutrient-enriched conditions. Similarly, *Pycnopsyche* consuming the highest quality resource (M28+) had the highest $TER_{C:P}$ (lowest susceptibility to P-limitation), while the poorest quality resource (R60-) had the lowest $TER_{C:P}$ (greatest susceptibility to P-limitation) (Table 4.2).

Field-based excretion experiment

Nutrient excretion rates— Several trials in the reference stream resulted in negative mass-specific excretion rates (3 NH_4-N, 1 NO_3-N, and 2 SRP out of 5 for each element). As these negative values only occurred in the reference trials, we interpreted these negative values to mean that the excretion levels were below our detection limit. Instead of excluding them from our analysis, we set these negative values to zero and included them in our final analyses, but excluded them from the excretion N:P ratio calculations.

Nutrient enrichment significantly increased *Pycnopsyche* mass-specific excretion rates for NH_4-N, NO_3-N, and SRP (Fig. 4.4). Despite these higher excretion rates, excretion N:P did not vary between the reference (17.2 ± 11.4) and nutrient-enriched (18.8 ± 4.9) streams (t-test, NS). Thus, *Pycnopsyche* assimilated relatively greater proportions of P than N under nutrient-enriched conditions (Fig. 4.3), but continued to excrete a relatively similar proportion of P and N.

Population-level N, P, and C standing stocks and fluxes

Population-level N, P, and C fluxes— By applying known *Pycnopsyche* areal-specific biomass and production estimates (Davis et al. *In review-a*) to our laboratory-based assimilation rates and field-based excretion rates, we were able to scale these small-scale experiments up to the population-level (Fig 4.5, Table 4.3). Enrichment stimulated all *Pycnopsyche* stocks and

fluxes because of associated increases in *Pycnopsyche* biomass (Davis et al. *In review-a*) and metabolic processes (assimilation and excretion rates). However, N and P stocks and fluxes exhibited relatively greater magnitude changes than C, which indicated that enrichment disproportionately stimulated N and P compartments (Fig. 4.5). Despite the dramatically greater magnitude changes in N and P stocks and fluxes, the total mass in C standing stocks and fluxes was still substantially higher than those observed for N and P. Thus, based on total mass, C dynamics dominated *Pycnopsyche* elemental flows, but N and P flows showed the greater sensitivity to enrichment.

Because enrichment increased P assimilation, production, and standing stocks more than N and C (i.e., greater magnitude changes), it suggested that enrichment facilitated P sequestration relative to N and C (Fig. 4.5, Table 4.3). However, despite this increased P sequestration, P egestion and excretion still increased with enrichment. Overall, enrichment significantly stimulated N, P, and C transformations at the population-scale level, but the relatively greater increase in P compartments meant that nutrient enrichment was likely facilitating P retention.

The isolated effects of metabolic processes— Because enrichment simultaneously increased *Pycnopsyche* biomass, production (Davis et al. *In review-a*), and metabolic activities (this study), all three of these factors affected population-level nutrient fluxes. To identify the contributions of changes in biomass and production vs. metabolic changes, we calculated additional nutrient fluxes by holding *Pycnopsyche* biomass and production constant at reference stream levels, but using metabolic processes measured under nutrient-enriched conditions. We were thus able to indirectly assess the relative effects of increased metabolic processes vs. increased *Pycnopsyche* biomass and production on nutrient transformations.

When *Pycnopsyche* biomass and production were held constant, enrichment still largely increased elemental fluxes because of faster metabolic processes under nutrient-enriched conditions relative to reference conditions (Table 4.3). Relative to the reference stream, P fluxes were at least 2x greater in the enriched stream, despite *Pycnopsyche* biomass and production being held constant. When we isolated metabolic processes, enrichment also increased N fluxes relative to the reference stream (Table 4.3). However, the magnitudes of these changes were attenuated relative to N fluxes calculated when *Pycnopsyche* biomass, production, and metabolic processes were allowed to increase (Table 4.3). Despite the positive effects of metabolic processes on N and P fluxes, enrichment had little effect on C dynamics when we isolated metabolic effects. These contrasting results suggest that enrichment likely stimulated N and P fluxes through a combination of increased *Pycnopsyche* biomass, production, and metabolic processes; but stimulated C fluxes primarily through increased *Pycnopsyche* biomass and production.

When comparing results from our N, P, and C box models, the outflows (excretion and secondary production) were greater than *Pycnopsyche* intake (assimilation) in some cases (Table 4.3). As direct measurement of excretion via mesocosms can sometimes miscalculate true consumer excretion rates because of handling stress or prolonged excretion trials (Whiles et al. 2009), this suggests that our excretion measurements may have overestimated true excretion rates and contributed to these potential errors. When excretion was calculated as the difference between assimilation and secondary production, values from these additional calculations exhibited similar interstream trends observed with our direct measurement of excretion (i.e., enrichment increased excretion relative to the reference condition). Therefore, given the diversity of methods used to calculate these values, these overestimations in elemental fluxes

were not surprising, but we don't think these errors affected the overall interstream trends indicating that enrichment stimulated metabolic processes.

Discussion

Nutrient enrichment stimulated *Pycnopsyche* assimilation and excretion rates in our laboratory-based and field-based experiments. When these results were scaled to the population-level, they showed an important consumer-driven pathway for enrichment to alter stream nutrient transformations. Because these positive results were relatively sustained when *Pycnopsyche* biomass and production were held constant, it suggests that these observed increases in consumer-driven fluxes were largely maintained by a combination of increased *Pycnopsyche* metabolic rates, biomass, and secondary production.

Ingestion rate response— Leaf ingestion rates were affected by nutrient enrichment (which affected detritus quality) but only for the poorest quality resource (R60-). This trend agrees with a previous feeding experiment that showed that *Pycnopsyche* reduced their ingestion of poor quality detritus (Hutchens et al. 1997). Other studies have also conclusively found reduced ingestion rates for low quality resources. For instance, *Pteronarcys* spp., a common leaf-shredding stonefly in eastern U.S. forested streams, reduced their ingestion rates of poor quality resources compared to high quality resources (Golladay et al. 1983). Presumably, these rates declined to increase gut retention time and to maximize the efficiency of elemental assimilation, which has been similarly shown for the caterpillar *Manduca sexta* (Reynolds 1990). Our results suggest that resource quality altered *Pycnopsyche* ingestion rates, but this occurred only for the food resource of lowest initial quality. We found that these leaves changed the most

in quality due to nutrient enrichment, suggesting that ingestion rates may only change with relatively large changes in resource quality.

Leaf litter composition effects on response to nutrients— Longer conditioning time can increase the biomass of detritus-associated microbes and increase the nutrient content of poor quality resources (Gulis and Suberkropp 2003, Greenwood et al. 2007). Thus, we originally predicted that nutrient assimilation rates would not vary between leaf types under reference conditions because the longer incubation of rhododendron detritus would increase its quality to a level comparable to M. However, despite this longer conditioning under reference conditions, R still had significantly lower nutrient content than M. Accordingly, larvae assimilated N and P faster from M vs. R leaves, even though R leaves had been incubated in the reference stream for a much longer period of time. This lower assimilation rate suggests that *Pycnopsyche* consuming R detritus may exhibit greater nutrient limitation and reduced secondary production than those consuming higher quality detritus. As consumer production in these stream food webs is largely dependent on terrestrial leaf inputs (Wallace et al. 1997), our large differences in the nutrient assimilative capacities between leaf species indicate an additional pathway for shifts in forest composition to alter stream consumer production.

Nutrient enrichment altered leaf functional diversity— Nutrient assimilation rates differed between leaf types under reference conditions, but they did not vary between leaf types under enriched conditions. Because enrichment increased the production of detritus-associated heterotrophic microbes (Gulis and Suberkropp 2003, Suberkropp et al. *In press*), it increased the quality of R detritus to a level comparable to M. This homogenization likely reduced the functional diversity of leaf litter in alleviating *Pycnopsyche* nutrient limitation. In fact, these results largely agree with a concurrent study that showed that enrichment reduced leaf litter

functional diversity because it reduced variation in breakdown rates due to species-specific litter traits (Rosemond et al. *In press*). Here, we found a similar homogenization of nutrient assimilation rates under nutrient-enriched conditions, indicating that enrichment may also alter the functional quality of leaf litter as it mediates nutrient assimilation by consumers.

Fate of assimilated nutrients— Despite enrichment significantly increasing *Pycnopsyche* elemental fluxes, enrichment may not necessarily stimulate the flow of nutrients to higher-order predators. Because predators in these stream food webs primarily consume small-bodied primary consumers (Davic 1991, Hall et al. 2000, Johnson and Wallace 2005), *Pycnopsyche* are relatively predator-resistant. Thus, this greater predator resistance may constrain these *Pycnopsyche*-assimilated nutrients to lower trophic levels and truncate nutrient flows to stream predators. Despite these reductions in vertical food web flows, nutrient enrichment may strengthen *Pycnopsyche*'s role in other transformation pathways (for example, egestion and excretion). For instance, N, P, and C stocks in these stream food webs are largely tied up in leaf litter standing crop, where it is relatively unavailable to most stream consumers (Cross et al. 2005c). Leaf litter processing by *Pycnopsyche* can transform these relatively unavailable resource pools into forms that are more readily available to other stream consumers (for example, FPOM). In fact, results from our ecosystem-level manipulation showed that nutrient enrichment significantly reduced leaf litter standing crop and increased FPOM export (Benstead et al. 2009, Suberkropp et al. *In press*). Although organic matter processing and fecal egestion by macroinvertebrates can be a dominant driver of this particulate N, P, and C export in headwater streams (Wallace et al. 1991, Cross et al. 2005c), other evidence suggests that macroinvertebrate egestion may only contribute ca. 22% toward these enrichment-induced increases in FPOM export (Benstead et al. 2009). This suggests that increased *Pycnopsyche*

biomass, production, and metabolic activities are likely important drivers of this consumer-driven FPOM export, but that there are still other undetected biotic interactions accounting for the rest of the FPOM export. However, despite these unaccounted pathways, nutrient enrichment likely increased the export of particulate N, P, and C via changes in *Pycnopsyche* egestion rates and may have stimulated flows through FPOM-dominated food web pathways.

Increased *Pycnopsyche* excretion rates also likely increased the export of dissolved nutrients to downstream habitats, but their effects on stream productivity were probably minor within the enriched stream. For instance, within open, enriched ecosystems, the importance of consumer-driven nutrient recycling can decline because it represents a small proportion of total available nutrients (Evans-White and Lamberti 2006). As enrichment also directly increased stream nutrient concentrations (Rosemond et al. 2008), it likely reduced the importance of consumer-driven nutrient recycling in meeting the nutrient demand of primary producers. Overall, these results suggest that despite increased nutrient excretion rates under nutrient-enriched conditions, these consumer-driven processes may actually have a reduced role due to increased availability of excess nutrients. However, increased N and P egestion under nutrient-enriched conditions likely increased nutrient flows through FPOM-dominated pathways, potentially stimulating productivity of consumers via particulate nutrient pathways.

Implications for ecological stoichiometry theory— The increases in assimilation N:P ratios, but not excretion N:P ratios, indicates that *Pycnopsyche* may have preferentially sequestered P relative to N. This result corroborates an earlier stoichiometric analysis of *Pycnopsyche* in which body P content, but not N content of field-collected individuals significantly increased with enrichment (Cross and others 2003). In the current study, enrichment significantly reduced *Pycnopsyche* assimilation N:P because they were assimilating

relatively greater proportions of P than N. Thus, the previously observed increase in P sequestration (Cross et al. 2003) was likely facilitated by *Pycnopsyche* maximizing their assimilation of P relative to N, while minimizing their P loss through excretion. Evidence suggests that heterotrophic microbes from these detritus-based ecosystems may also preferentially sequester P relative to N (Rosemond et al. 2008). These results suggest that heterotrophic microbes and production of at least this primary consumer was likely primarily P-limited and secondarily limited by N.

Although stream consumers were likely still P-limited in these stream food webs, our calculated threshold elemental ratios ($TER_{C:P}$) suggests that nutrient enrichment reduced this limitation. Consumers with higher $TER_{C:P}$ have relatively lower susceptibility to P-limitation because they exhibit a more efficient P assimilation capacity relative to their body C:P content. Accordingly, we found that *Pycnopsyche* eating the highest quality resources (M28+) had the highest $TER_{C:P}$ and lowest susceptibility to P-limitation. Also, compared to the larvae eating reference detritus, larvae eating nutrient-enriched detritus had a lower susceptibility to P limitation. Overall, this suggests the utility of using TERs for comparing consumer P-limitation. However, as our calculated $TER_{C:P}$ varied between treatment types, it indicates the risk of applying a single value to an individual taxon. It suggests that $TER_{C:P}$ are not necessarily an inherent attribute of an organism because *Pycnopsyche* appeared to adjust their C and P assimilation independently. As consumers divert C and P to largely divergent metabolic pathways (Elser et al. 1996), it is possible that C and P assimilation may similarly diverge to help alleviate potential C and P limitations.

In conclusion, enrichment significantly increased N, P, and C stocks and fluxes at the population-level through changes in *Pycnopsyche* assimilation and excretion rates. However,

these observed changes in fluxes were not homogeneous across N, P, and C. *Pycnopsyche* assimilated relatively greater proportions of P than N, reducing assimilation N:P ratios relative to reference conditions. As we did not see similar declines in excretion N:P ratios, it suggested that *Pycnopsyche* preferentially sequestered P within these nutrient-enriched food webs. Because *Pycnopsyche* is relatively predator-resistant, much of these assimilated nutrients were likely unavailable to higher trophic levels and subsequently reduced *Pycnopsyche*'s importance in transferring nutrients up the food web. Thus, enrichment likely strengthened *Pycnopsyche*'s role in consumer-driven nutrient transformations via storage and egestion. Overall, these effects of enrichment on N, P, and C transformations suggest that enrichment may alter the functioning of recipient ecosystems via changes in consumer metabolic processes and production.

Acknowledgments

We greatly appreciate J. Allgeier, S. Dye, A. Helton, A. Mehring, and C. Tant for invaluable laboratory assistance in the initiation of the feeding trials. D. Conners, W. Cross, S. Eggert, and J. B. Wallace provided advice in the design of the assimilation and excretion experiments. We would like to thank L. Dean and T. Maddox at the University of Georgia (UGA) Analytical Chemistry Lab, who analyzed all water samples and provided expert guidance in elemental analyses. A. Mehring provided helpful advice on data analyses. J. Benstead provided helpful comments on an earlier draft of this manuscript. Funding was provided by NSF (DEB-0318063). J. Davis was supported by a NSF Graduate Research Fellowship and a UGA Presidential Fellowship.

Table 4.1: Leaf N, P, and C content (mean ± SE) for the six treatments (n = 5). Leaf N and P content was significantly different between treatments (two-way ANOVA; nutrient effect: < 0.0001, leaf type effect: < 0.0001, nutrient x leaf type effect: < 0.0001). Leaf C content was not significantly different (two-way ANOVA, NS). Within an elemental comparison, different letters indicate significant pairwise differences (post-hoc Tukey's, $P < 0.05$).

%N Content	M14	M28	R60
Reference	0.578 (0.020)^{b}	0.637 (0.012)^{b}	0.397 (0.013)^{a}
Enriched	0.843 (0.018)^{c}	1.155 (0.026)^{d}	0.825 (0.031)^{c}

%P Content	M14	M28	R60
Reference	0.028 (0.001)^{b}	0.031 (0.001)^{b}	0.012 (0.001)^{a}
Enriched	0.062 (0.004)^{c}	0.096 (0.005)^{d}	0.063 (0.004)^{c}

%C Content	M14	M28	R60
Reference	49.074 (0.199)	47.386 (1.644)	45.517 (1.045)
Enriched	48.934 (0.317)	47.043 (0.997)	48.908 (0.527)

Table 4.2: Calculated *Pycnopsyche* threshold elemental ratios ($TER_{C:P}$) for C and P within each treatment (mean ± SE). These ratios represent the C:P ratio of a food resource where a consumer switches from C- to P-limitation. A higher $TER_{C:P}$ represents a lower susceptibility to P-limitation.

	Reference	Enriched
M14	614 ± 52	876 ± 60
M28	1299 ± 307	1561 ± 160
R60	491 ± 143	961 ± 88

Table 4.3: *Pycnopsyche* daily mean elemental stocks (mg N, P, or C m^{-2}) and fluxes (mg N, P, or C m^{-2} d^{-1}) in the reference and nutrient-enriched streams during the fifth year of enrichment (Sept 2004 to Aug 2005). Values for the nutrient-enriched stream were calculated two ways: (1) using actual *Pycnopsyche* biomass and production measured in the enriched stream, and (2) by keeping *Pycnopsyche* biomass and production constant at levels measured in the reference stream. Thus, values in the first enriched column (Bio/Prod + Metabolic) calculated stocks and fluxes when *Pycnopsyche* biomass, production, and metabolic activities increased with enrichment. Values from the second enriched column (Metabolic Only) isolated the effects of *Pycnopsyche* metabolic activities on population-level elemental dynamics. C excretion was not measured (represented by NC). We included an additional C flux, respiration (mg C m^{-2} d^{-1}) that was calculated by assuming a net growth efficiency of 40% (Benke and Wallace 1997). Values in the parentheses represent the magnitude change between the reference stream and that particular nutrient-enriched condition (i.e., nutrient-enriched / reference).

Nitrogen	Reference	Enriched (Bio/Prod + Metabolic)	Enriched (Metabolic Only)
Assimilation	0.30	7.43 (24.77)	0.64 (2.13)
Egestion	0.54	7.99 (14.80)	0.69 (1.28)
Excretion	0.05	6.71 (134.20)	0.58 (11.60)
Standing Stock	25.78	312.66 (12.13)	27.09 (1.06)
Production	0.38	5.04 (13.26)	0.40 (1.06)

Phosphorus	Reference	Enriched (Bio/Prod + Metabolic)	Enriched (Metabolic Only)
Assimilation	0.03	0.85 (28.33)	0.07 (2.33)
Egestion	0.01	0.27 (27.00)	0.02 (2.00)
Excretion	0.01	0.83 (83.00)	0.07 (7.00)
Standing Stock	0.75	18.56 (24.75)	1.61 (2.15)
Production	0.01	0.30 (30.00)	0.02 (2.00)

Carbon	Reference	Enriched (Bio/Prod + Metabolic)	Enriched (Metabolic Only)
Assimilation	24.68	274.25 (11.11)	23.76 (0.97)
Egestion	46.09	630.09 (13.67)	54.59 (1.18)
Excretion	NC	NC	NC
Standing Stock	142.39	1675.76 (11.77)	145.19 (1.02)
Production	2.10	27.01 (12.86)	2.14 (1.02)
Respiration	9.87	109.70 (11.11)	9.50 (0.96)

Fig. 4.1: Mass-specific *Pycnopsyche* leaf ingestion rates (mean ± SE) for the six treatments (n=10 or n=9 [M14-]). Red maple (M) was incubated under reference or nutrient-enriched conditions for either 14 or 28 days, while rhododendron (R) was incubated for 60 days. Ingestion rates were compared with a two-way ANOVA. Treatments with different letters indicate a significant pair-wise difference (Tukey's, $P < 0.05$). AFDM is ash-free dry mass.

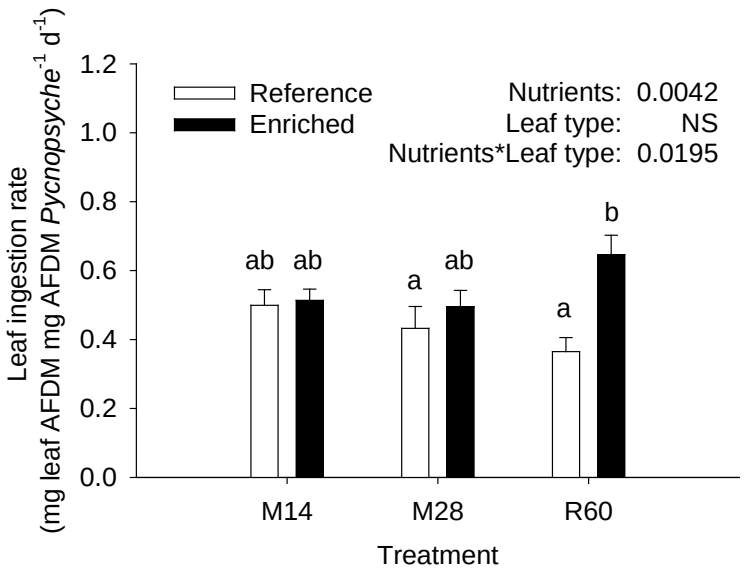

Leaf ingestion rate
(mg leaf AFDM mg AFDM Pycnopsyche-1 d-1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Reference
Enriched
Nutrients: 0.0042
Leaf type: NS
Nutrients*Leaf type: 0.0195
ab
ab
a
ab
a
b
M14
M28
R60
Treatment

Fig. 4.2: Mass-specific *Pycnopsyche* N, P, and C assimilation rates (mean ± SE) for the six treatments (n=10 or n=9 [M14-]). Red maple (M) was incubated under reference or nutrient-enriched conditions for either 14 or 28 days, while rhododendron (R) was incubated for 60 days. Rates were compared with a two-way ANOVA. Treatments with different letters indicate a significant pair-wise difference (Tukey's, $P < 0.05$). AFDM is ash-free dry mass. Note differences in scales between graphs.

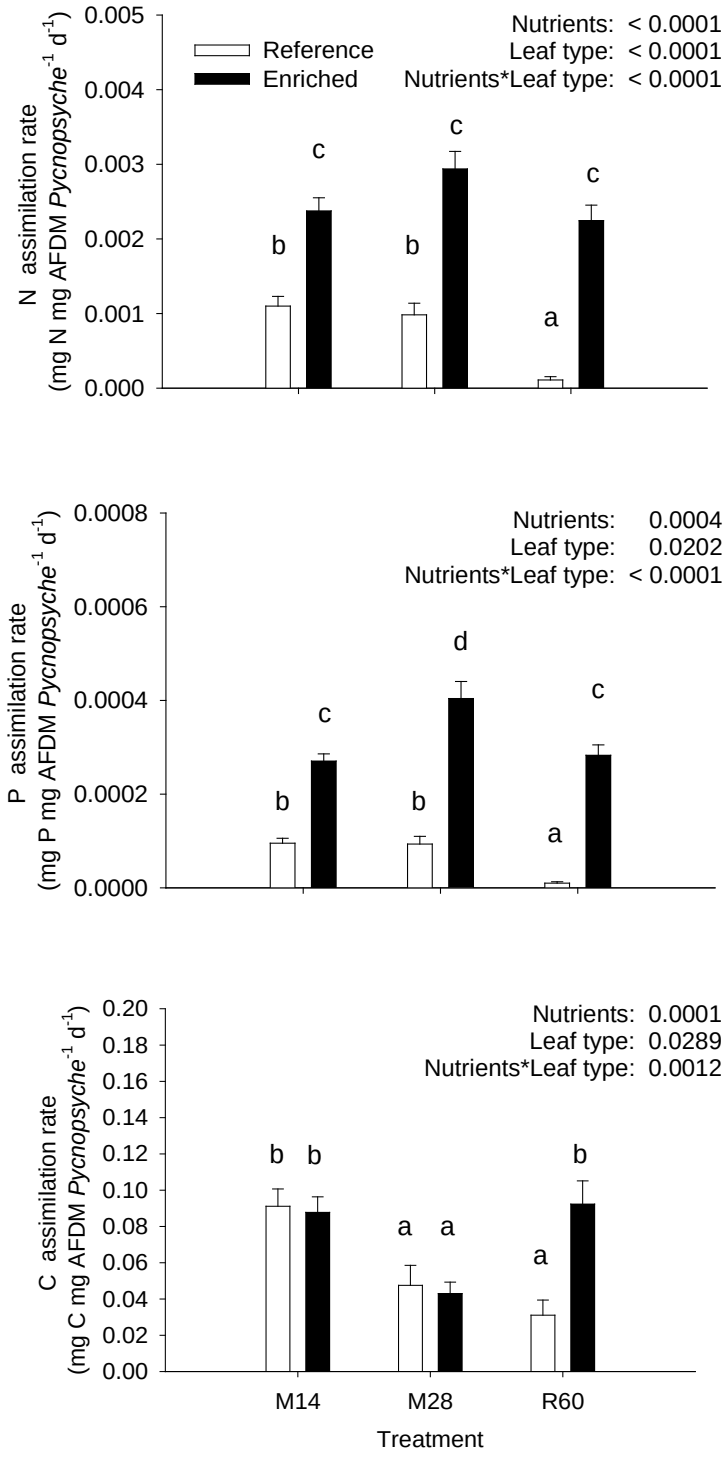

N assimilation rate
(mg N mg AFDM Pycnopsyche-1 d-1)
0.005
0.004
0.003
0.002
0.001
0.000
Reference
Enriched
Nutrients: < 0.0001
Leaf type: < 0.0001
Nutrients*Leaf type: < 0.0001
b
c
b
c
a
c
P assimilation rate
(mg P mg AFDM Pycnopsyche-1 d-1)
0.0008
0.0006
0.0004
0.0002
0.0000
Nutrients: 0.0004
Leaf type: 0.0202
Nutrients*Leaf type: < 0.0001
b
c
b
d
a
c
C assimilation rate
(mg C mg AFDM Pycnopsyche-1 d-1)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Nutrients: 0.0001
Leaf type: 0.0289
Nutrients*Leaf type: 0.0012
b b
a a
a
b
M14
M28
R60
Treatment

Fig. 4.3: Mass-specific *Pycnopsyche* assimilation rate N:P (mean ± SE) for the six treatments. Each bar represents an average of 10 replicates except for R60- (n = 4) and M14- (n = 9). All ratios are molar. Red maple detritus (M) was incubated under reference or nutrient-enriched conditions for either 14 or 28 days, while rhododendron detritus (R) was incubated for 60 days. Ratios were compared with a two-way ANOVA. AFDM is ash-free dry mass.

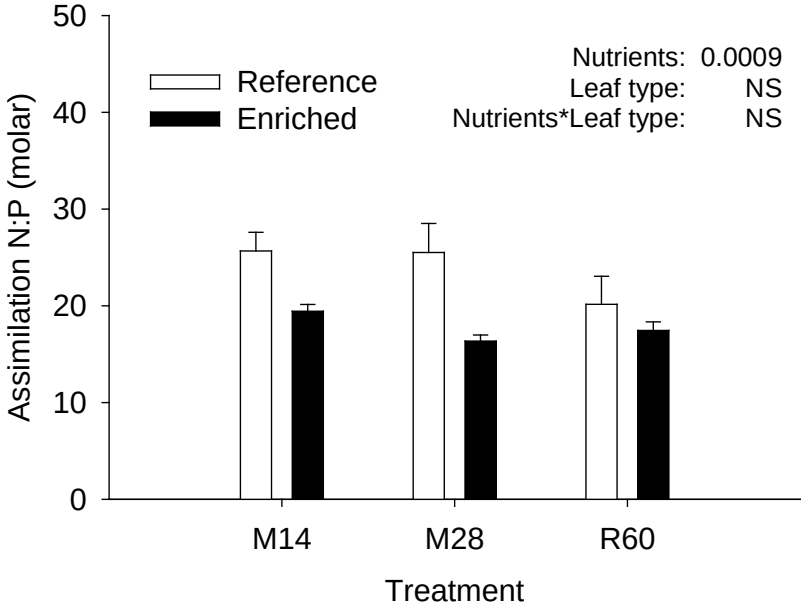

50
40
30
20
10
0
Assimilation N:P (molar)
Reference
Enriched
Nutrients: 0.0009
Leaf type: NS
Nutrients*Leaf type: NS
M14
M28
R60
Treatment

Fig. 4.4: Mass-specific *Pycnopsyche* excretion rates (mean ± SE) from the reference (n = 5) and nutrient-enriched (n = 5) streams. NH_4-N and SRP excretion rates were compared with a t-test, but NO_3-N excretion rates were compared with a Wilcoxon ranked sum test. Asterisks indicate a significant pairwise difference in *Pycnopsyche* excretion rates between the reference and nutrient-enriched stream for a given analyte ($P < 0.05$).

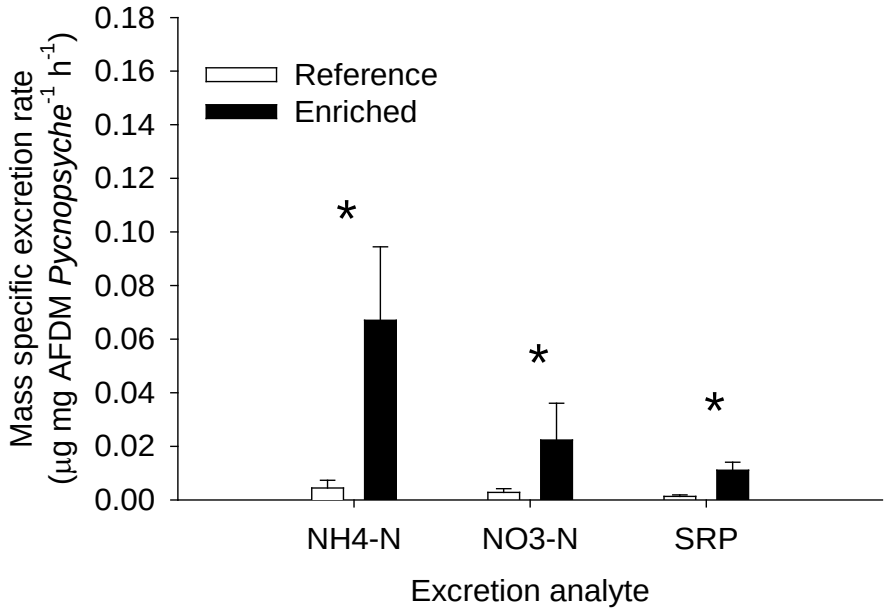
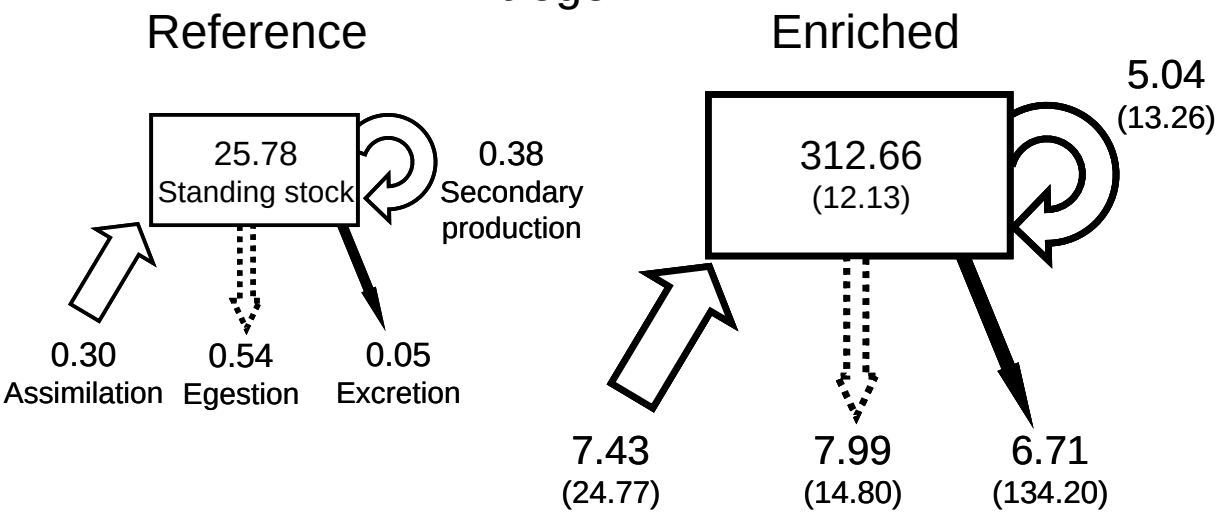
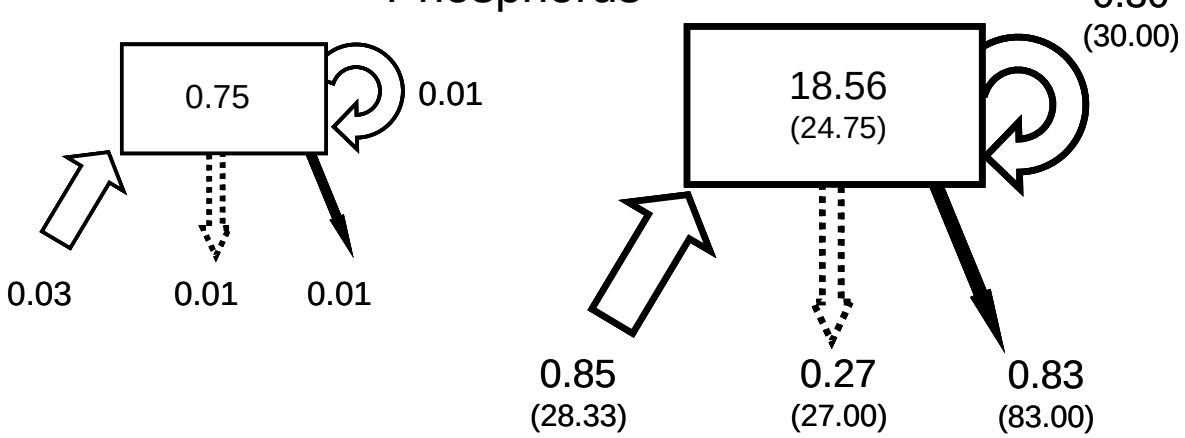

0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Mass specific excretion rate
(µg mg AFDM Pycnopsyche-1 h-1)
Reference
Enriched
*
*
*
NH4-N
NO3-N
SRP
Excretion analyte

Fig. 4.5: *Pycnopsyche* daily mean elemental stocks (mg N, P, or C m^{-2}) and fluxes (mg N, P, or C m^{-2} d^{-1}) in the reference (left panels) and the nutrient-enriched streams (right panels) during the fifth year of enrichment (Sept 2004 to Aug 2005). Nutrient-enriched values were based on the 'Bio/Prod + Metabolic' column (Table 4.2). The boxes represent *Pycnopsyche* standing stocks of N, P, or C. Arrows represent *Pycnopsyche* N, P, or C fluxes. C excretion was not measured. We included an additional flux, respiration (mg C m^{-2} d^{-1}), for the C budget (labeled 'r'). Values in the parentheses represent the magnitude change between the reference and nutrient-enriched condition for that particular stock or flow (i.e., nutrient-enriched / reference).

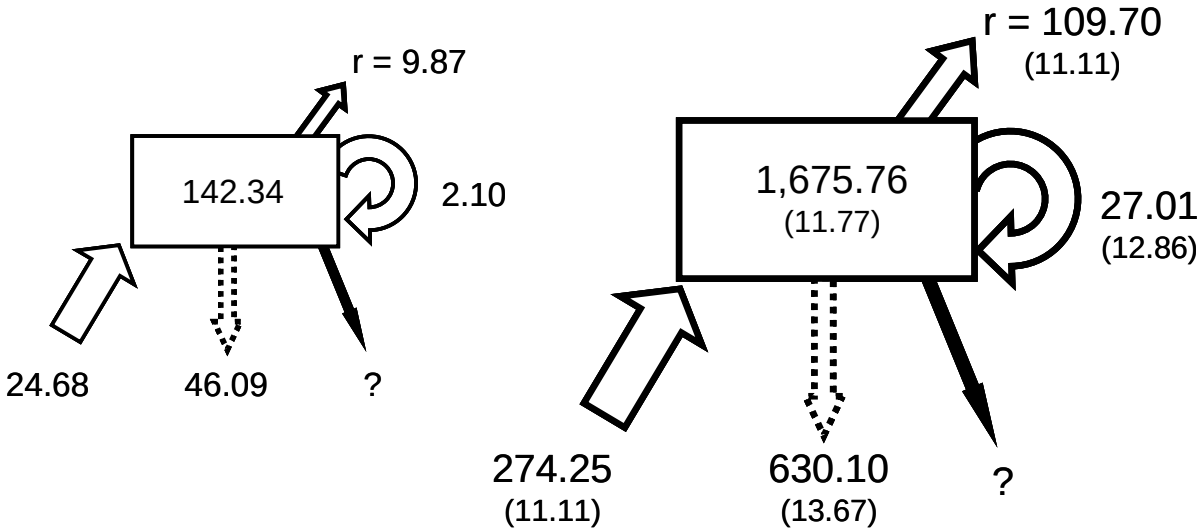
Nitrogen



Phosphorus



Carbon



CHAPTER 5

THE EFFECTS OF LONG-TERM NUTRIENT ENRICHMENT ON AQUATIC TO TERRESTRIAL SUBSIDIES ALONG A FORESTED HEADWATER STREAM[4]

[4] Davis, J. M., A. D. Rosemond, and G. E. Small. To be submitted to *Oecologia*.

Abstract

Aquatic emergence can represent an important resource for terrestrial predators found along stream corridors, affecting predator abundance and biomass. Because nutrient enrichment of stream food webs can stimulate instream secondary production, it may also increase aquatic emergence production. Thus, nutrient enrichment may indirectly stimulate cross-boundary flows and increase terrestrial predator populations. To assess the effects of nutrient enrichment on aquatic to terrestrial subsidies along a headwater stream, we quantified the biomass and abundance of aquatic emergence, arboreal spiders, and ground spiders along a reference and an adjacent treatment stream that had been continuously enriched with nitrogen and phosphorus for five years. We predicted that enrichment would increase aquatic emergence biomass and abundance, subsequently increasing the biomass and abundance of spiders, but especially those that specialize on aquatic emergence (e.g., Tetragnathidae). By adding a ^{15}N stable isotope tracer to both streams, we also quantified the flow of nitrogen from the stream into the riparian community. Enrichment increased emergence biomass, but not abundance. However, this stimulation of insect emergence largely did not increase the abundance or biomass of either arboreal or ground spiders along the treatment stream. The isotopic enrichment indicated that spiders along the treatment stream reduced their reliance on aquatic emergence, possibly due to shifts in the body size distributions and community composition of aquatic emergence. Despite enrichment significantly increasing consumer production in the treatment stream, these positive effects of nutrient enrichment were not readily transferred to riparian spiders. Our results indicate that the net effect of aquatic subsidies on terrestrial predators may not simply be a function of the magnitude of the subsidy, but also depends on the community-level characteristics of the subsidy that determine a predator's ability to utilize it.

Introduction

The flows of nutrients and energy across ecosystem boundaries can dramatically alter the structure and function of recipient food webs (Polis et al. 1997, Baxter et al. 2005). For example, inputs of terrestrial leaf detritus and insects into stream food webs can subsidize stream consumers, alter their community composition, and stimulate overall stream productivity (Wallace et al. 1997, Nakano et al. 1999, Kawaguchi et al. 2003, Johnson and Wallace 2005). However, because these aquatic-terrestrial linkages can also be bidirectional (Baxter et al. 2005), instream production, such as aquatic emergence, is frequently transferred to the surrounding terrestrial ecosystem (Henschel et al. 2001, Nakano and Murakami 2001, Kato et al. 2003, Sanzone et al. 2003). Because only about 3% of this insect emergence returns to stream food webs during oviposition (Jackson and Fisher 1986, Werneke and Zwick 1992), a significant proportion of aquatic insect production is available to the surrounding riparian zone where it can stimulate predator abundance and biomass (Sabo 2002a, 2002b, Kato et al. 2003, Sanzone et al. 2003, Marczak and Richardson 2007). Thus, because stream and riparian food webs are often inter-connected, aquatic emergence can be an important resource for terrestrial predators.

The importance of aquatic-terrestrial linkages along stream corridors has long been recognized (Hynes 1975), but more recently attention has focused on identifying factors that control the direction and magnitude of these flows (Marczak et al. 2007, Burdon and Harding 2008). It was initially predicted that the magnitude of cross-ecosystem subsidies were largely controlled by two factors: the overall productivity gradient between the donor and recipient ecosystems (Polis et al. 1997, Nakano and Murakami 2001, Ballinger and Lake 2006), and the physical characteristics of their boundary (Polis et al. 1997, Witman et al. 2004). Resources were predicted to flow from more productive to less productive ecosystems, and that their effect

on subsidized consumers was related to the productivity of the recipient food web (e.g., Nakano and Murakami 2001). However, a more recent meta-analysis suggests that the effects of subsidies on recipient ecosystems may be better correlated, albeit still weakly, with the magnitude of the actual subsidy rather than this productivity gradient (Marczak et al. 2007). Empirical evidence from a temperate rainforest further supports these large-scale trends because the reduction of aquatic insect emergence decreased spider abundance more than would be predicted from the productivity gradient alone (Marczak and Richardson 2007). Thus, the effects of resource subsidies, such as aquatic emergence, on recipient predators may be simply related to the magnitude of that flow.

This positive relationship between aquatic emergence and terrestrial predator biomass suggests that if environmental change alters aquatic emergence, it may also affect terrestrial predator populations (e.g., Baxter et al. 2004, Power et al. 2004). For example, the introduction of nonnative rainbow trout (*Oncorhynchus mykiss*) to a forested stream increased predation pressure on benthic invertebrates, subsequently decreasing aquatic emergence and spider abundance (Baxter et al. 2004). Along the South Fork Eel River, CA, algal mats can be hotspots of aquatic emergence that can subsidize and increase spider abundance (Power et al. 2004). However, damming can decrease the prevalence of these algal mats, potentially reducing aquatic emergence and its positive effects on spider abundance (Power et al. 2004). Conversely when environmental change increases stream productivity, it may increase aquatic emergence and terrestrial predator abundance. For instance, nutrient enrichment of freshwater ecosystems typically stimulates the production of stream consumers (Slavik et al. 2004, Cross et al. 2006, Davis et al. *In review-a*). Because aquatic emergence production can represent ca. 25% of benthic production (e.g., Jackson and Fisher 1986 and references cited therein), this increased

productivity may increase aquatic emergence subsidies and stimulate terrestrial predator populations.

To assess the effects of nutrient enrichment on aquatic emergence and associated resource flows to terrestrial predators, we sampled aquatic emergence and terrestrial spiders along a reference and nutrient-enriched headwater stream. Five years of nutrient enrichment more than doubled the secondary production of consumers in the treatment stream relative to the reference stream (Cross et al. 2006, Davis et al. *In review-a*). Thus, we predicted that enrichment would stimulate aquatic emergence, increase aquatic subsidies to terrestrial spiders, and increase spider biomass and abundance. We also predicted that positive nutrient effects on spider populations would be greatest for spiders relying predominantly on aquatic emergence (e.g., Tetragnathidae). By using a stable isotope tracer (^{15}N), we also quantified the spiders' reliance on aquatic emergence nitrogen (N). Because nutrient enrichment stimulated stream consumer production and nutrient flows within the aquatic food web (Cross et al. 2006, Cross et al. 2007, Davis et al. *In review-a*), we predicted that enrichment would increase the proportion of spider N originating from aquatic emergence. Because primary consumers and predators can be N limited due to their relatively greater body N content compared to their food resource (Sterner and Elser 2002, Fagan and Denno 2004), we also tested whether enrichment would increase the body N content of aquatic insect emergence and spiders.

Methods

We conducted this study at the USDA Forest Service Coweeta Hydrologic Laboratory, a Long-Term Ecological Research site in the southern Appalachian Mountains (Macon County, North Carolina). Coweeta is a heavily-forested experimental watershed (2185 ha) comprised of

mixed hardwoods (oak, maple, tulip poplar) with a dense understory dominated by *Rhododendron maximum* that limits light availability. This light limitation reduces autotrophic production and increases stream consumers' reliance on heterotrophic microbes that colonize terrestrial leaf inputs (Wallace et al. 1997, Hall et al. 2000, Cross et al. 2007).

To assess the effects of long-term nutrient enrichment on resource flows from aquatic to terrestrial food webs, we sampled aquatic emergence and terrestrial spiders (April to June 2005) along a reference (C53) and a treatment stream (C54). This study was conducted towards the end of a five-year ecosystem-level manipulation that examined the effects of nutrient enrichment on the production of stream-dwelling organisms and consequent effects on nutrient and carbon dynamics in a detritus-based ecosystem (see Cross et al. 2006, Benstead et al. 2009, Suberkropp et al. *In press,* Davis et al. *In review-a*). The reference and treatment streams did not differ in nutrient concentrations prior to the experimental nutrient enrichment (mean ± SE, C53: DIN: 23.2 ± 8.5 µg L^{-1}, SRP: 6.8 ± 3.0 µg L^{-1}; C54: DIN: 29.3 ± 4.9 µg L^{-1}, SRP: 9.5 ± 2.3 µg L^{-1}). From July 2000 to August 2005 (ca. 1877 days), we experimentally enriched a 150-m reach of the treatment stream with nitrogen (NH_4NO_3) and phosphorus (K_2HPO_4 and KH_2PO_4). We added nutrients continuously along the entire 150-m length of the treatment stream using an irrigation line running down the center of the stream. See Gulis and Suberkropp (2003) for further descriptions of the nutrient-delivery system. This delivery system increased nutrient concentrations in the treatment stream to a realistic, low-level enrichment (DIN: 506.2 ± 36.3 µg L^{-1}, SRP: 80.0 ± 5.6 µg L^{-1}), while the reference stream concentrations during this same time period were comparable to the pretreatment period (DIN: 31.0 ± 3.4 µg L^{-1}, SRP: 8.0 ± 1.3µg L^{-1}). Previous results from the instream sampling showed that nutrient enrichment significantly

increased macroinvertebrate biomass and production in the treatment stream relative to the reference stream (Cross et al. 2006, Davis et al. *In review-a*).

Isotopic enrichment— To quantify the effect of nutrient enrichment on resource subsidies and N flow from the treatment stream, we applied an isotopic tracer to both streams. We continuously added 99% ^{15}N-labeled NH_4Cl into the reference and treatment stream to achieve 2500‰ enrichment without additionally affecting nutrient concentrations. The solution was added for 44-days using a battery powered peristaltic pump. The amount of solute released was adjusted daily according to stream discharge, leading to an isotopic release that was proportional to flow and nutrient concentrations (C54: 5.53 g ^{15}N as $^{15}NH_4Cl$, C53: 0.39 g ^{15}N as $^{15}NH_4Cl$).

Emergence sampling— Aquatic emergence was sampled during the typical period of peak emergence from these stream ecosystems (April to June 2005) (J. B. Wallace pers. comm.). On a weekly basis, we affixed 0.25 m^2 emergence traps (0.5 mm mesh) to the stream substrate to collect emerging adult insects that were analyzed for total biomass, abundance, and isotopic composition. To quantify the background isotopic concentration, we also collected emergence samples one week prior to the initiation of the isotopic drip. Traps were deployed at sunrise for 48h at ca. 10m intervals down stream of the isotopic enrichment (10, 20, 30, 40, and 48m). Emergence was collected from the traps every 24 hours and immediately frozen. Most adults were enumerated and identified to family level, but Diptera were only identified to order level. Samples were dried at 60°C and weighed. Because of limited spatial and temporal sampling resolution at the family level, we had to combine individuals from the same order for isotopic analysis. For each sampling date and location, multiple individuals from the same order were composited, ground, and analyzed for isotopic composition using a mass spectrophotometer (Finnigan Delta Plus).

Spider sampling— We used separate methods to quantify the abundance and biomass of ground spiders and arboreal spiders. To collect ground spiders, five transects of pitfall traps were deployed weekly for 48h at ca. 10m intervals down stream of the point of isotopic enrichment. At each of these transects, a pitfall trap (diameter: 11 cm) was deployed at 0m (streamside), 10m, and 25m away from the stream's edge. This grid pattern allowed us to determine the lateral extent to which the positive effects of aquatic emergence were reaching into the upland habitat. Traps were deployed for 48h shortly after sunrise. All individuals were removed, rinsed, and frozen for later analyses. To calculate background isotopic signatures, we also sampled ground spiders one week prior to the initiation of the isotopic drip.

We sampled arboreal spiders via timed-beat sampling at each of the pitfall sampling locations. However, as arboreal spiders are less mobile than ground spiders and may experience localized population depression from excessive sampling (F. Coyle pers. comm.), we only sampled them every two weeks (week 2, 4, and 6 of the isotopic drip). To calculate background isotopic signatures, we also sampled arboreal spiders one week prior to the initiation of the isotopic drip. Shortly after sunrise on a given sampling date, we selected a random direction at each sampling point and spread a 1 m^2 white canvas below the vegetation. We then beat the vegetation for 5-min and collected all individuals that landed on the canvas. Individuals were frozen for later analyses. To increase our sample size for isotopic analysis, we also systematically collected any spider encountered at each sampling location on the final day of isotopic enrichment (d44). The spiders collected during the pretreatment period and on d44 were not used to estimate spider abundance or biomass, but were used for isotopic analyses.

All spiders were enumerated and identified to family level according to Ubick et al. (2005). We then dried them at 60°C and weighed them. Isotopic analyses were limited to those

taxa that possessed sufficient temporal sampling resolution to calculate isotopic enrichment curves. For arboreal spiders, we analyzed the families Tetragnathidae, Araneidae, Anyphaenidae, and Linyphiidae (ca. 70% of the total arboreal spider biomass). For ground spiders, we analyzed the families Amaurobiidae, Gnaphosidae, and Lycosidae (ca. 70% of the total ground spider biomass). Sample sizes of other taxa were not large enough to adequately determine isotopic composition. For these seven families, we composited multiple individuals from the same family that were collected at the same sample location and date (range: 1 - 14 individuals, ca. 40% of the samples had > 1 individual). Composite samples were ground and analyzed using a mass spectrophotometer (Finnigan Delta Plus).

Estimation of Trophic Transfer of ^{15}N— Isotopic equilibria were not achieved in either stream, such that spider ^{15}N signatures were continuing to increase after 44d of isotopic enrichment. Thus, we could not use a two-source mixing model because it assumes that the isotopic signature is at steady state (e.g., Phillips and Gregg 2003). We used the following dynamic mixing model adapted from Hall et al. (1998) to quantify the mass of spider N derived from aquatic emergence N.

$$\delta_{t+1,i,j} = \frac{(\delta_{SP,i,j} * M_{SP}) + (Ing_{SP} * \delta_{AQ_i} * \%Aq) + (Ing_{SP} * \delta_{TERR} * (1 - \%Aq))}{(M_{SP} + Ing_{SP})} \qquad (1)$$

where

$\delta_{t+1,\,i,\,j}$ = the background corrected isotopic signature of spiders for time period $t+1$ (in days) at sampling location i meters down stream of isotopic enrichment (10, 20, 30, 40, 48) and j meters laterally from streamside (0, 10, 25)

$\delta_{SP,\,i,\,j}$ = the background corrected isotopic signature of spiders for time period *t* (in days) at sampling location *i* meters down stream of isotopic enrichment and *j* meters from streamside

M_{SP} = the mass of spider N

Ing_{SP} = the mass-specific N ingestion rate of spiders

$\delta_{AQ,\,i}$ = the background corrected isotopic signature of aquatic emergence at sampling location *i* meters down stream of isotopic enrichment, which was a biomass-weighted average δ^{15}N of emerging adults

%Aq = the proportion of spider N originating from aquatic emergence, and

δ_{TERR} = the background corrected isotopic signature of terrestrial prey.

The isotope values used in the model were background corrected; thus, the calculations represent only changes in the ^{15}N tracer. The mass-specific N ingestion rate of spiders was calculated by multiplying the mass-specific prey ingestion rate by known spider and prey body N content (this study) and spider assimilation efficiency. We used a previously published literature value of 0.03 mg prey / mg spider / day for mass-specific spider ingestion rates (Moulder and Reichle 1972, Tanaka 1991). Based on Moulder and Reichle (1972), spider assimilation efficiency was set at 0.90.

Because we only sampled the isotopic signature of aquatic emergence weekly, but we fit the model on a daily basis, we interpolated aquatic emergence isotopic signatures on a one-day time step. We used these interpolated data in our dynamic mixing model based on equation (1). We fit our dynamic mixing model to the actual isotopic signature of ground spiders and arboreal spiders. To find the best model approximation ($\delta_{t+1,\,i,\,j}$) of the actual isotopic signature, we manually varied the proportion of spider N originating from aquatic emergence (*%Aq*). To assess which *%Aq* value gave us the best approximation of the actual spider isotopic signature,

we used sum of squares to compare the actual isotopic signature with our modeled isotopic signature ($\delta_{t+1, i, j}$). The *%Aq* from the model with the lowest sum of squares was considered the best estimate of the spiders' reliance on aquatic emergence. This process was repeated for each spider family at each sampling location where we had sufficient data to adequately fit the model.

Statistical Analyses— To assess the effects of nutrient enrichment on the abundance and biomass of aquatic emergence we used a one-way repeated measures ANOVA with nutrients as the main effect. Spider biomass and abundance responses were analyzed with a two-way repeated measures ANOVA with the main effects of nutrients (reference vs. treatment stream) and lateral distance from streamside (0m, 10m, and 20m). Including an interaction between nutrients and distance (nutrients x distance) assessed whether the positive effects of increased emergence extended further into the upland habitats along the treatment stream relative to the reference stream. Emergence body N content was analyzed with a one-way ANOVA. Spider body N content was analyzed with a two-way ANOVA with the main effects set to nutrients and lateral distance. We also included an interaction term (nutrients x distance). We used the appropriate transformations when necessary. Because of limited samples sizes, we could not statistically analyze the results from our dynamic mixing model. However, we fit separate models for each downstream sampling location (10, 20, 30, 40, 48m) at a given lateral distance (0, 10, 25m). Using this spatial replication, we could calculate the mean aquatic emergence reliance for a specific lateral distance. We graphically compared these means to qualitatively assess the effects of nutrient enrichment on spider diet.

Use of nutrients in our model violates assumptions of sample independence in ANOVA as our nutrient treatment was pseudoreplicated (sensu Hurlbert 1984). However, it would not have been possible to conduct the combined long-term nutrient enrichment and stable isotope

study on a greater number of streams. Therefore, we suggest increased caution in interpreting the nutrient treatment effect and we provide substantiating additional data where possible.

Results

Effects of nutrients on aquatic emergence— Nutrient enrichment had a significant positive effect on aquatic emergence biomass, but not abundance (Fig. 5.1A and 5.1B). By dividing total biomass by total abundance for each emergence trap on each sampling date, we were able to calculate a rough indicator of a community-level average body size for the emerging adults. In general, nutrient enrichment increased the average individual body size of adults emerging during the entire sampling period (Fig. 5.2). Because we did not directly measure the individual body size of each adult, we could not construct body size spectra for emerging adults or statistically analyze the effects of nutrient enrichment on this community-level metric.

Although nutrient enrichment increased aquatic emergence biomass, this positive effect was not homogenous across taxonomic groups (Table 5.1, Fig. 5.3A – 5.3D). Because of low temporal sampling resolution at the family level, we combined aquatic invertebrate families at the order level to assess whether the effects of enrichment varied between orders. Nutrient enrichment only increased the biomass and abundance of Trichoptera relative to the reference stream. The biomass and abundance of Diptera, Ephemeroptera, and Plecoptera did not increase with nutrient enrichment (Table 5.1, Fig. 5.3A – 5.3D). Insufficient numbers of Odonata were collected for statistical analyses.

Effects of nutrients on arboreal spider abundance and biomass— Combined arboreal spider biomass did not differ between the two streams, but abundance was slightly lower along

the treatment stream (Table 5.2, Fig. 5.4A – 5.4B). Neither their biomass nor abundance differed with proximity to the stream margin (non-significant distance effect) (Table 5.2, Fig. 5.4B).

We found similar trends when arboreal spiders were analyzed at the family level. The biomass and abundance of Tetragnathidae, a family of orb-weavers known to specialize on aquatic emergence, was not significantly different between streams (Table 5.2). The abundance and biomass of Araneidae (vertical orb-weavers) also did not vary between streams (Table 5.2). However, Linyphiidae (sheet-web spiders) biomass and abundance was lower along the treatment stream (Table 5.2). Anyphaenidae biomass and abundance did not differ between streams (Table 5.2). While Araneidae abundance was significantly lower at the stream margin, the biomass and abundance of the other three spider families did not differ between the stream margin and upland habitat (Table 5.2). Thus, despite the lower Linyphiidae biomass along the treatment stream, these results indicate that nutrient enrichment did not have a significant effect on the abundance and biomass of most arboreal spider families.

Effects of nutrients on ground spider abundance and biomass— The biomass and abundance of the combined ground spider community did not differ between the treatment and reference streams (Table 5.3, Fig. 5.4C – 5.4D). However, ground spider biomass and abundance was dramatically lower at the stream margin relative to upland habitat (Table 5.3, Fig. 5.4C – 5.4D). Although the biomass and abundance of the combined ground spider community did not vary between streams, the responses of individual ground spider families differed in regard to nutrient enrichment (Table 5.3). Amaurobiidae biomass, but not abundance, was higher along the treatment stream (Table 5.3). Lycosidae (wolf spiders) biomass and abundance did not differ between the treatment and reference stream (Table 5.3). Gnaphosidae abundance and biomass was lower along the treatment stream relative to the reference stream (Table 5.3).

The biomass and abundance of these three ground spider families were also consistently lower at the stream margin relative to the upland habitat (Table 5.3). Thus, although the biomass and abundance of the combined ground spider community was similar along both streams (Fig. 5.4C – 5.4D), the responses of individual families did vary (Amaurobiidae: treatment > reference, Lycosidae: no difference, Gnaphosidae: reference > treatment) (Table 5.3).

Effects of nutrients on arboreal spider aquatic diet— Based on our dynamic mixing model (Equation 1), we found that three out of the four arboreal spider families along the treatment stream obtained a lower proportion of their N from aquatic emergence relative to the reference stream (Fig. 5.5A – 5.5D). Tetragnathidae along the margin of the reference stream obtained ca. 100% of their N from aquatic emergence (range: all five models returned values of 100%) (Fig. 5.5A), but individuals along the treatment stream only obtained ca. 50% of their N from emergence (range: 15 - 95%) (Fig. 5.5A). Similar patterns for Araneidae were observed (Fig. 5.5B). Araneidae along the margin of the reference stream obtained ca. 75% of their N from aquatic emergence (range: 50 – 100%), but individuals directly along the treatment stream relied less on aquatic emergence (ca. 52%, range: 5 – 100%). Linyphiidae was the only family that increased their utilization of aquatic emergence along the treatment stream (Fig. 5.5C). Under reference conditions, streamside Linyphiidae obtained ca. 12% of their N from emergence (range: 0 – 35%), but this reliance increased to 79% (range: 35 – 100%) along the treatment stream. Anyphaenidae along the side of the reference stream relied heavily on aquatic emergence (ca. 90%, range: 80 – 100%), but this reliance declined along the treatment stream (50%, range: not determined [ND]) (Fig. 5.5D). Along the reference stream, the utilization of aquatic emergence increased slightly for Araneidae in the upland habitat relative to the stream margin (Fig. 5.5B). However, the aquatic emergence reliance of other arboreal spiders declined

slightly in the upland habitats relative to the stream margin (Fig. 5.5A – 5.5D). In sixty percent of the reference Tetragnathidae and Araneidae calculations and one calculation for a treatment Araneidae, the mixing model returned values greater than 100% (i.e., they were more enriched than could be explained by the measured isotopic values of emergence samples). We interpreted these results as the spiders obtaining 100% of their N from aquatic sources (average of those exceeding 100%: 180% max: 500%). Overall, our results indicate that arboreal spiders relied heavily on aquatic emergence, but that this reliance decreased along the treatment stream.

Effects of nutrients on ground spider aquatic diet— Results from our dynamic mixing model show that ground spiders along both the reference and treatment streams largely do not rely on aquatic emergence (Fig. 5.6A – 5.6C). Amaurobiidae at the reference stream margin obtained ca. 10% of their N from emergence (range: ND), but along the treatment stream they obtained no N from aquatic emergence (range: both replicates returned values of 0%). Because of insufficient sample sizes at the stream margin, we could not assess the reliance of streamside Lycosidae on aquatic emergence (Fig. 5.6B). However, individuals 10m from the reference stream obtained 15% of their N from emergence (range: 0 – 45%). Lycosidae along the treatment stream margin and 10m away relied less on aquatic emergence because spiders derived only 0.1% (range: ND) and 0.05% (range: 0 – 0.1%) of their N from emergence, respectively (Fig. 5.6B). Gnaphosidae at the margins of the reference and treatment streams showed no reliance upon aquatic emergence (0%, range: ND) (Fig. 5.6C). The reliance of ground spiders on aquatic emergence was less consistent based on lateral distance, but on average, did not reach above 20% (e.g., Amaurobiidae, Fig. 5.6A). These results indicate that ground spiders along either the reference and treatment streams obtained very little, if any, N from aquatic emergence (Fig. 5.6A – 5.6C).

Effects of nutrients on consumer body nitrogen content— Body N content of aquatic emergence (ANOVA, $F_1 = 0.55$) and arboreal spiders (ANOVA, $F_{1, 262} = 3.60$) did not differ between the treatment and reference streams (Fig. 5.7A – 5.7B). However, we found a significant distance effect on arboreal spider N content (ANOVA, $F_{2, 262} = 4.10$), such that spiders farther from the stream had a slightly lower N content than those living streamside (Fig. 5.7B). This difference of less than 1% was probably not biologically significant. We found a significant nutrient effect (ANOVA, $F_{1, 168} = 33.82$), but not a distance effect (ANOVA, $F_{2, 168} = 1.73$), on ground spider body N content (Fig. 5.7C). Ground spider N content was only ca. 1% lower along the treatment stream, suggesting that this difference was biologically irrelevant.

Discussion

Effects of increased emergence on spider biomass— Despite nutrient enrichment more than doubling aquatic emergence biomass, our results indicate that this increased subsidy was largely not utilized by riparian spiders. Nutrient enrichment stimulated aquatic emergence biomass through increases in average size rather than abundance, and this increased emergence did not lead to increases in the biomass of arboreal and ground spiders. The abundance of arboreal spiders actually decreased slightly along the treatment stream, but this was primarily due to associated declines 10m from the stream margin. Thus, five years of continuous nutrient enrichment increased benthic secondary production (Davis et al. *In review-a*) and aquatic emergence biomass (this study), but this stimulation of stream productivity did not transfer to riparian spiders nor stimulate spider populations.

Our results contrast sharply with earlier studies that showed a positive relationship between the magnitude of aquatic subsidies and their effects on subsidized consumers (Sanzone

et al. 2003, Marczak et al. 2007). When predator biomass is largely based on a prey subsidy, an increase in that subsidy should stimulate predator biomass (Polis et al. 1997, Sanzone et al. 2003) and, similarly, decreased aquatic emergence can lead to declines in terrestrial spider populations (Kato et al. 2003, Marczak and Richardson 2007). We initially predicted that if nutrient enrichment stimulated aquatic emergence biomass, it would increase resource subsidies and stimulate spider biomass and abundance. Although nutrient enrichment increased aquatic emergence biomass, we found no evidence of positive effects on spider populations.

Importance of aquatic emergence— Despite the non-significant increase in total ground or arboreal spider biomass, results from our isotopic enrichment suggested that several arboreal spider families relied heavily on aquatic emergence for their N demand. For example, Tetragnathidae is a spider family that has been previously shown to rely heavily on aquatic emergence (Sanzone et al. 2003, Kato et al. 2004) and has exhibited reduced abundance when aquatic emergence declines (Kato et al. 2003, Marczak and Richardson 2007). Our isotopic results from the reference stream largely support this previous evidence suggesting that Tetragnathidae are aquatic emergence specialists. Araneidae is another arboreal spider family that has largely been thought to rely less on aquatic emergence (Kato et al. 2003, Kato et al. 2004), but there has been limited evidence indicating the importance of aquatic emergence in maintaining their populations (Marczak and Richardson 2007). As Araneidae at the reference stream margin showed substantial reliance on aquatic insect emergence (range: 50 – 100%), our results suggest that aquatic emergence may be more important than previously thought for maintaining this spider family's production.

Results of our ground spiders' estimated reliance on aquatic emergence contrast with findings from Sycamore Creek, AZ, a desert stream whose emergence and aquatic-terrestrial

linkages have been well-studied (e.g., Jackson and Fisher 1986, Sanzone et al. 2003). In our study, isotopic data showed that ground spiders found directly adjacent to our stream margins mostly did not rely on aquatic emergence, which is further supported by our low ground spider abundance and biomass along the stream margin compared to the upland habitat. However, along Sycamore Creek, ground spiders obtained up to ca. 25% of their N from aquatic emergence. Although two families from our study did exhibit the capacity to rely on aquatic emergence at a level similar to Sycamore Creek, the majority of ground spiders along our forested streams obtained a nominal amount of N from aquatic emergence. The relatively greater importance of aquatic emergence to desert ground spider diets indicates that these ground spiders may be more reliant upon aquatic production because of comparatively low terrestrial productivity that does not adequately meet their dietary requirements. It further suggests that the resource demand of spiders in the forest communities surrounding our headwater streams are readily met by terrestrial prey production. Our finding that Coweeta spiders were less reliant on aquatic emergence compared to Sycamore Creek spiders adds to the growing evidence showing that the productivity of the recipient food web can influence the importance of resource subsidies to subsidized consumers (Polis et al. 1997, Nakano and Murakami 2001).

Why did increased emergence not result in increased utilization of emergence or increased biomass of aquatic prey specialists?— Our most interesting and counterintuitive finding was that increased nutrient enrichment did not result in increased dependence of spiders on stream subsidies and in fact, dependence on aquatic subsidies was generally reduced along the treatment stream. However, these contradictory results are likely explained by the increased dominance of large-bodied adults and Trichoptera, which may have been less readily eaten by spiders and reduced the reliance of spiders on emergence. Specifically, the positive effect of

nutrient enrichment on aquatic emergence biomass, but not abundance, suggests that enrichment may have increased the body size of emerging adults and the dominance of large-bodied individuals. A concurrent study also found that nutrient enrichment increased the maximum body size of a dominant stream consumer and increased the relative dominance of large-bodied macroinvertebrate larvae (Davis et al. *In review-b*). These potential shifts in the size structure of the emerging adults may have reduced prey availability for spiders. Although spiders can eat larger-bodied prey (Kato et al. 2003, Kato et al. 2004), other studies have shown that spider diet can be largely dominated by smaller-bodied prey such as Chironomidae and other small Diptera (Nentwig and Wissel 1986, Tanaka 1991, Williams et al. 1995, Henschel et al. 2001). Despite larger prey being captured in spider webs, the difficulties associated with handling larger prey can sometimes increase the rate that spiders reject these prey (Nentwig and Wissel 1986) or increase prey handling time (Olive 1980). Overall, spiders may more readily accept prey that are ca. 50 – 80% of the spider's size, although some larger spiders will consume prey much larger than themselves (Nentwig and Wissel 1986). If spiders in our study also prefer these smaller-bodied prey, then nutrient enrichment may have reduced spiders' reliance on aquatic emergence because of increases in emergence body size.

The increased dominance of Trichoptera may have further reduced this reliance. Although there is limited evidence that spiders can eat Trichoptera (Kato et al. 2004), other evidence suggests that Trichoptera may not comprise a significant proportion of spider diet. Kato et al. (2004) estimated that ca. 40% of spider diet originated from aquatic predators, such as Trichoptera, Plecoptera, Tanypodinae Chironomidae, and Megaloptera. However, because they grouped these taxa together, it is unclear how many Trichoptera they actually consumed. Circumstantial evidence suggests that Trichoptera may not be easily eaten. For instance, because

of their wing scales and increased mobility, Lepidoptera, a close taxonomic relative of Trichoptera, can have a high escape rate from spider webs (Olive 1980). Because Trichoptera have wing hairs that may function the same way as Lepidoptera wing scales, it is possible that spider webs may be less efficient at retaining these prey as well. In fact, spider webs of Tetragnathidae along another stream corridor disproportionately captured Chironomidae and Ephemeroptera adults, despite the high availability of Trichoptera and Plecoptera emergence (Williams et al. 1995). Thus, the greater body size of emerging adults and the increased dominance of Trichoptera may have reduced the reliance of spiders on aquatic emergence.

According to this conceptual framework, if spiders did not eat this increased emergence biomass because of shifts in its composition, these shifts can subsequently explain why increased aquatic emergence did not stimulate the biomass of spiders that typically rely on aquatic emergence (e.g., Tetragnathidae). If spiders primarily consume smaller-bodied Diptera that did not increase with enrichment, then this likely reduced the positive effects of enrichment on spider biomass. Conversely, in Sycamore Creek, where there was a positive relationship between emergence and spider biomass (Sanzone et al. 2003), aquatic emergence is dominated by Chironomidae and Ephemeroptera (primarily *Baetis* spp.) (Jackson and Fisher 1986). Because these taxa are substantially smaller than most of the Trichoptera dominating our stream's emergence, smaller-bodied adults from Sycamore Creek may have been more readily eaten by spiders and helped maintain the positive relationship between spider and emergence biomass. Thus, the net effect of aquatic subsidies on terrestrial predators may not simply be a function of the magnitude of the subsidy, but may also be influenced by a subsidy's community structure because it alters a predator's ability to benefit from it.

Other factors that may affect the dynamic mixing model— Despite this indirect evidence for the reduced importance of aquatic emergence to spiders along the treatment stream, we cannot completely rule out that sampling error may have led to this decline. Because emerging adults are thought to fly upstream during oviposition (MacNeale et al. 2005), the emergence and isotopic signature actually available to a spider at a given sampling location may differ from what actually emerges at that location. For this reason, we attempted to validate the isotopic signature of the aquatic emergence available at a sampling location through additional sampling; however, these methods (e.g., light traps, sticky traps, and sweep netting) proved ineffective for adequately sampling aquatic emergence and their isotopic signatures. The mixing model therefore had to make the assumption that a spider at a given location downstream of the isotopic enrichment was consuming emergence originating from that same downstream distance. If this assumption was violated because emergence was flying upstream, then spiders may be eating aquatic emergence that originated down stream of the spider sampling location. This would have overestimated the isotopic signature of the consumed aquatic emergence and underestimated the spider's reliance on aquatic emergence. Because these watersheds did not differ in their physical or chemical attributes (e.g., discharge, slope, watershed area, elevation, or temperature) (see Lugthart and Wallace 1992), we do not believe that upstream flight differed between the streams. Therefore, any upstream correction would have been applied to both streams and led to similar interstream differences in aquatic emergence reliance. Additional error may have also been introduced into our mixing models because we did not directly measure spider ingestion rates or assimilation efficiencies. Instead we had to use literature values that may have further reduced the accuracy of our mixing model output. However, any change in our assumptions of prey

ingestion rates or assimilation rates would have similarly been applied to both streams, which would have led to similar interstream trends.

Because we applied the same model assumptions and used identical sampling methods in both streams, our interstream differences (i.e., less reliance on aquatic emergence along the treatment stream) are likely robust to these potential violations. Our results indicating that spiders along the treatment stream rely less on aquatic emergence is also indirectly supported by the lack of a significant increase in spider biomass along the treatment stream. If spiders were relying equally on aquatic emergence from both streams, we would have expected increased spider biomass along the treatment stream because of greater resource availability. Because we found no evidence of an increase, it further supports our isotopic evidence that those spiders specializing on aquatic insect emergence were likely not eating this nutrient-enhanced insect emergence.

Effects of nutrient enrichment on spider N content— Despite their reliance upon animal material relatively high in N content, predators can still be N limited because of their relatively greater body N content compared to the N content of their prey (Fagan and Denno 2004, Mayntz et al. 2005). Therefore, we initially predicted that nutrient enrichment would increase the body N content of aquatic emergence, subsequently increasing the N content of spiders and stimulating spider populations along the treatment stream. We did not find a biologically relevant effect of nutrient enrichment on spider body N content or an increased biomass of riparian spiders. This non-significant response of spider N content was likely due to the similar lack of an aquatic emergence N content response. An earlier study from our study streams also showed no effect of enrichment on the body N content of aquatic larvae in these stream food webs (Cross et al. 2003). Thus, our study further indicates that nutrient enrichment may not alter

the body N content of adult stream consumers or the riparian predators that eat them, possibly due to these consumers being relatively homeostatic in terms of body N content (Sterner and Elser 2002).

Fate of aquatic emergence— Nutrient enrichment increased aquatic emergence biomass and decreased the reliance of spiders on aquatic emergence, which suggests that a substantial proportion of this stimulated aquatic emergence was not consumed by riparian spiders and was destined for other fates. Despite a reduction in the consumption of aquatic emergence by riparian spiders along the treatment stream, this emergence may have still benefited other components of the riparian predator community. For example, because aquatic emergence can represent a significant subsidy for bats and avian insectivores (Kurta and Whitaker 1998, Nakano and Murakami 2001, Fukui et al. 2006), the emergence not consumed by spiders may have benefited other such predators. Unconsumed adult emergence also likely returned to the stream ecosystem during oviposition (e.g., Werneke and Zwick 1992); thus, these returning adults may have stimulated instream food web pathways as ovipositing adults died and were consumed by instream detritivores and predators.

In summary, long-term nutrient enrichment more than doubled aquatic emergence biomass, but did not stimulate the biomass or abundance of riparian spider taxa known to rely heavily on aquatic emergence (e.g., Tetragnathidae). In fact, we found that the reliance of spiders on aquatic emergence declined with nutrient enrichment, potentially due to the increased dominance of large-bodied adults and Trichoptera that may not have been eaten by riparian spiders. This suggests that the ability of a subsidy to stimulate predator production may not simply be the gross increase in the magnitude of that subsidy (e.g., Marczak et al. 2007), but may also be determined by its relative composition. As the positive effects of nutrient enrichment are

attenuated by trophic distance (Brett and Goldman 1997), our results indicate that the positive effects of nutrient enrichment on terrestrial spiders may have been similarly attenuated by a trophic transfer extending across an ecosystem boundary. Thus, when environmental change increases the magnitude of an aquatic subsidy, it may not always stimulate terrestrial predator populations. Shifts in the subsidy's structure (e.g., body size or community composition) may reduce a predator's ability to take advantage of subsidy increases.

Acknowledgments

We greatly appreciate the help of S. Dye, C. Anderson, C. Tant, and N. Taylor for their field assistance during the isotopic enrichment. J. B. Wallace, R. Hall, M. Bradford, P. Mulholland, F. Coyle, and A. Fisk gave helpful advice during the initial experimental design. T. Maddox at the University of Georgia Analytical Chemistry Lab provided invaluable assistance in the storage, processing, and analyses of isotopic samples. R. Hall provided expert guidance in the development of the dynamic mixing model. Logistical support was provided by the staff at the Coweeta Hydrologic Laboratory, but most especially by B. Kloeppel, J. Deal, and C. Harper. K. Catlyn at Western Carolina University graciously verified spider identifications. We would also like to acknowledge the Highlands Biological Station for providing research space during the verification of spider samples. S. Dye, A. Mehring, and C. Tant commented on an earlier draft of this manuscript. J. Davis was supported by a NSF Graduate Research Fellowship, a University of Georgia Presidential Fellowship, and a North American Benthological Society Presidential Award.

Table 5.1: P-value results from the repeated measures one-way ANOVA testing the main effects of nutrient enrichment on the abundance and biomass of aquatic emergence orders. Odonata were not analyzed because of insufficient sample sizes.

Combined Emergence	Biomass		Abundance	
Nutrients	$F_{1,8}$ = 16.55	0.0036	$F_{1,8}$ = 0.76	NS
Time	$F_{6,48}$ = 2.58	0.0300	$F_{6,48}$ = 3.34	0.0079
Nutrients*Time	$F_{6,48}$ = 2.03	NS	$F_{6,48}$ = 1.68	NS
Trichoptera	**Biomass**		**Abundance**	
Nutrients	$F_{1,8}$ = 8.95	0.0173	$F_{1,8}$ = 6.37	0.0356
Time	$F_{6,48}$ = 2.71	0.0238	$F_{6,48}$ = 1.11	NS
Nutrients*Time	$F_{6,48}$ = 1.81	NS	$F_{6,48}$ = 3.01	0.0141
Diptera	**Biomass**		**Abundance**	
Nutrients	$F_{1,8}$ = 0.66	NS	$F_{1,8}$ = 0.36	NS
Time	$F_{6,48}$ = 2.77	0.0216	$F_{6,48}$ = 4.40	0.0013
Nutrients*Time	$F_{6,48}$ = 0.62	NS	$F_{6,48}$ = 1.95	NS
Ephemeroptera	**Biomass**		**Abundance**	
Nutrients	$F_{1,8}$ = 0.50	NS	$F_{1,8}$ = 2.63	NS
Time	$F_{6,48}$ = 1.74	NS	$F_{6,48}$ = 1.30	NS
Nutrients*Time	$F_{6,48}$ = 0.62	NS	$F_{6,48}$ = 0.44	NS
Plecoptera	**Biomass**		**Abundance**	
Nutrients	$F_{1,8}$ = 0.01	NS	$F_{1,8}$ = 0.32	NS
Time	$F_{6,48}$ = 2.04	NS	$F_{6,48}$ = 3.92	0.0029
Nutrients*Time	$F_{6,48}$ = 2.11	NS	$F_{6,48}$ = 2.75	0.0224

Table 5.2: P-value results from the repeated measures two-way ANOVA testing the effects of nutrients and distance from the stream margin on arboreal spider biomass and abundance.

Combined Arboreal Spiders	Biomass		Abundance	
Nutrients	$F_{1, 24} = 2.15$	NS	$F_{1, 24} = 8.06$	0.0091
Distance	$F_{2, 24} = 0.62$	NS	$F_{2, 24} = 0.14$	NS
Nutrients*Distance	$F_{2, 24} = 1.69$	NS	$F_{2, 24} = 2.49$	NS
Time	$F_{2, 48} = 2.35$	NS	$F_{2, 48} = 0.25$	NS
Time*Nutrients	$F_{2, 48} = 0.41$	NS	$F_{2, 48} = 2.33$	NS
Time*Distance	$F_{4, 48} = 0.73$	NS	$F_{4, 48} = 2.51$	NS
Tetragnathidae	**Biomass**		**Abundance**	
Nutrients	$F_{1, 24} = 0.31$	NS	$F_{1, 24} = 0.01$	NS
Distance	$F_{2, 24} = 0.38$	NS	$F_{2, 24} = 0.09$	NS
Nutrients*Distance	$F_{2, 24} = 0.39$	NS	$F_{2, 24} = 0.11$	NS
Time	$F_{2, 48} = 4.96$	0.0110	$F_{2, 48} = 4.61$	0.0147
Time*Nutrients	$F_{2, 48} = 0.55$	NS	$F_{2, 48} = 1.31$	NS
Time*Distance	$F_{4, 48} = 1.31$	NS	$F_{4, 48} = 1.08$	NS
Araneidae	**Biomass**		**Abundance**	
Nutrients	$F_{1, 24} = 0.03$	NS	$F_{1, 24} = 3.00$	NS
Distance	$F_{2, 24} = 1.43$	NS	$F_{2, 24} = 4.53$	0.0214
Nutrients*Distance	$F_{2, 24} = 1.25$	NS	$F_{2, 24} = 3.16$	NS
Time	$F_{2, 48} = 0.33$	NS	$F_{2, 48} = 0.03$	NS
Time*Nutrients	$F_{2, 48} = 0.64$	NS	$F_{2, 48} = 1.55$	NS
Time*Distance	$F_{4, 48} = 0.56$	NS	$F_{4, 48} = 0.06$	NS
Linyphiidae	**Biomass**		**Abundance**	
Nutrients	$F_{1, 24} = 4.64$	0.0415	$F_{1, 24} = 15.42$	0.0006
Distance	$F_{2, 24} = 0.20$	NS	$F_{2, 24} = 0.50$	NS
Nutrients*Distance	$F_{2, 24} = 0.48$	NS	$F_{2, 24} = 0.23$	NS
Time	$F_{2, 48} = 0.98$	NS	$F_{2, 48} = 2.35$	NS
Time*Nutrients	$F_{2, 48} = 1.28$	NS	$F_{2, 48} = 1.60$	NS
Time*Distance	$F_{4, 48} = 0.67$	NS	$F_{4, 48} = 0.40$	NS
Anyphaenidae	**Biomass**		**Abundance**	
Nutrients	$F_{1, 24} = 3.23$	NS	$F_{1, 24} = 2.22$	NS
Distance	$F_{2, 24} = 2.39$	NS	$F_{2, 24} = 2.58$	NS
Nutrients*Distance	$F_{2, 24} = 0.21$	NS	$F_{2, 24} = 0.25$	NS
Time	$F_{2, 48} = 0.40$	NS	$F_{2, 48} = 3.03$	NS
Time*Nutrients	$F_{2, 48} = 0.00$	NS	$F_{2, 48} = 0.08$	NS
Time*Distance	$F_{4, 48} = 6.65$	0.0002	$F_{4, 48} = 8.20$	<0.001

Table 5.3: P-value results from the repeated measures two-way ANOVA testing the main effects of nutrient enrichment and distance from the stream margin on ground spider biomass and abundance.

Combined Ground Spiders	Biomass		Abundance	
Nutrients	$F_{1, 24} = 0.33$	NS	$F_{1, 24} = 2.27$	NS
Distance	$F_{2, 24} = 45.21$	<0.0001	$F_{2, 24} = 60.00$	<0.0001
Nutrients*Distance	$F_{2, 24} = 1.09$	NS	$F_{2, 24} = 3.39$	NS
Time	$F_{6, 144} = 9.02$	<0.0001	$F_{6, 144} = 8.69$	<0.0001
Time*Nutrients	$F_{6, 144} = 1.34$	NS	$F_{6, 144} = 0.77$	NS
Time*Distance	$F_{12, 144} = 3.18$	0.0005	$F_{12, 144} = 2.11$	0.0197

Amaurobiidae	Biomass		Abundance	
Nutrients	$F_{1, 24} = 4.89$	0.0368	$F_{1, 24} = 3.91$	NS
Distance	$F_{2, 24} = 9.74$	0.0008	$F_{2, 24} = 4.48$	0.0220
Nutrients*Distance	$F_{2, 24} = 3.90$	0.0340	$F_{2, 24} = 0.70$	NS
Time	$F_{6, 144} = 4.59$	0.0003	$F_{6, 144} = 2.26$	0.0407
Time*Nutrients	$F_{6, 144} = 0.68$	NS	$F_{6, 144} = 1.55$	NS
Time*Distance	$F_{12, 144} = 3.70$	<0.001	$F_{12, 144} = 1.96$	0.0319

Lycosidae	Biomass		Abundance	
Nutrients	$F_{1, 24} = 0.20$	NS	$F_{1, 24} = 2.50$	NS
Distance	$F_{2, 24} = 9.08$	0.0012	$F_{2, 24} = 11.94$	0.0003
Nutrients*Distance	$F_{2, 24} = 1.37$	NS	$F_{2, 24} = 3.28$	NS
Time	$F_{6, 144} = 7.21$	<0.001	$F_{6, 144} = 8.14$	<0.001
Time*Nutrients	$F_{6, 144} = 2.00$	NS	$F_{6, 144} = 3.31$	0.0044
Time*Distance	$F_{12, 144} = 2.73$	0.0024	$F_{12, 144} = 3.30$	0.0003

Gnaphosidae	Biomass		Abundance	
Nutrients	$F_{1, 24} = 4.89$	0.0242	$F_{1, 24} = 4.80$	0.0384
Distance	$F_{2, 24} = 9.74$	0.0012	$F_{2, 24} = 8.94$	0.0013
Nutrients*Distance	$F_{2, 24} = 3.90$	NS	$F_{2, 24} = 0.43$	NS
Time	$F_{6, 144} = 4.59$	<0.001	$F_{6, 144} = 11.58$	<0.001
Time*Nutrients	$F_{6, 144} = 0.68$	NS	$F_{6, 144} = 1.93$	NS
Time*Distance	$F_{12, 144} = 3.70$	0.0297	$F_{12, 144} = 1.84$	0.0468

Fig. 5.1: Aquatic insect emergence biomass (**A**) and abundance (**B**) originating from the reference and treatment streams during the one week pre-isotopic enrichment period and 44d isotopic enrichment (mean ± SE). Emergence traps (0.25 m^2) were deployed on a weekly basis for 48h in both streams.

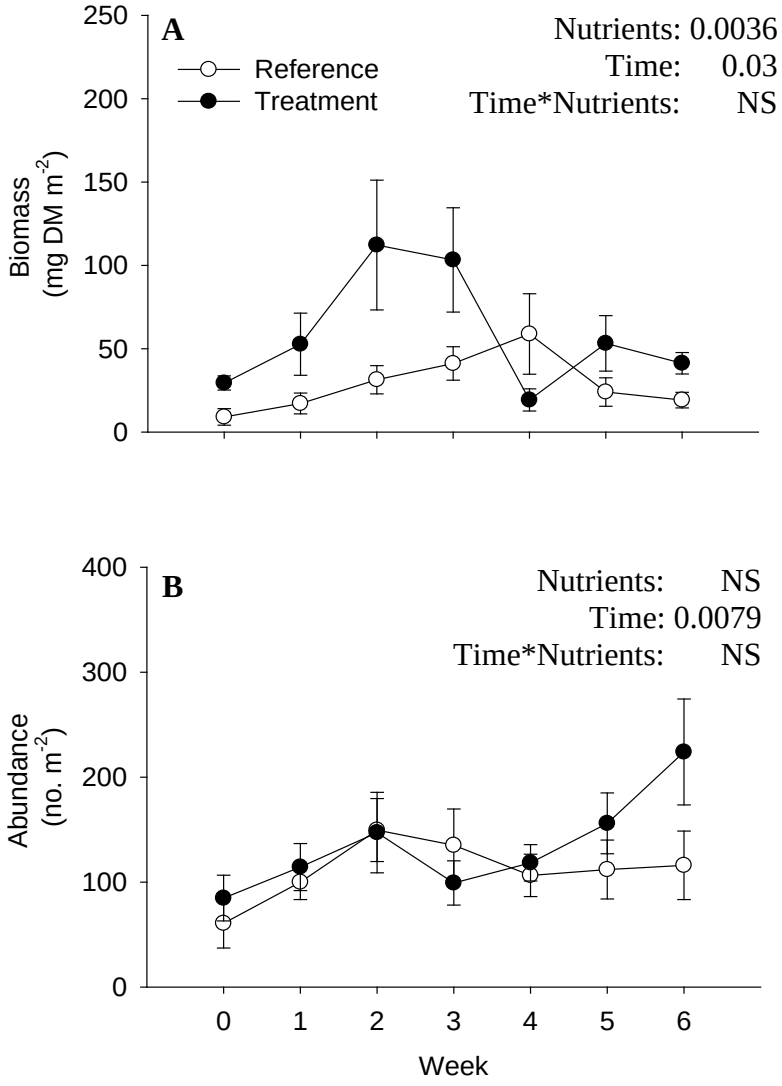

A
Reference
Treatment
Nutrients: 0.0036
Time: 0.03
Time*Nutrients: NS
Biomass (mg DM m-2)
250
200
150
100
50
0
B
Nutrients: NS
Time: 0.0079
Time*Nutrients: NS
Abundance (no. m-2)
400
300
200
100
0
0
1
2
3
4
5
6
Week

Fig. 5.2: Average body size of adult insects emerging across all sampling dates (mean ± SE). Calculated for each sampling date by dividing total biomass per emergence trap divided by total abundance per emergence trap.

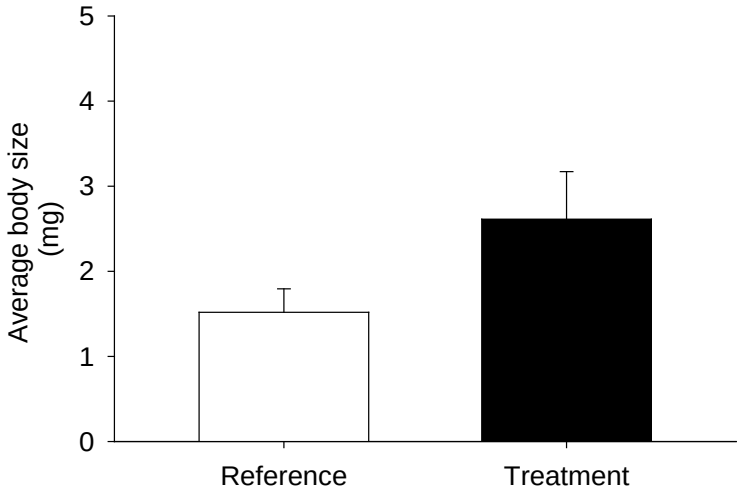

Average body size (mg)
5
4
3
2
1
0
Reference
Treatment

Fig. 5.3: Aquatic emergence biomass (mean ± SE) categorized by insect order: Trichoptera (**A**), Diptera (**B**), Ephemeroptera (**C**), and Plecoptera (**D**). Odonata are not shown because of small sample sizes. Note the different scale used for Diptera compared to other three orders.

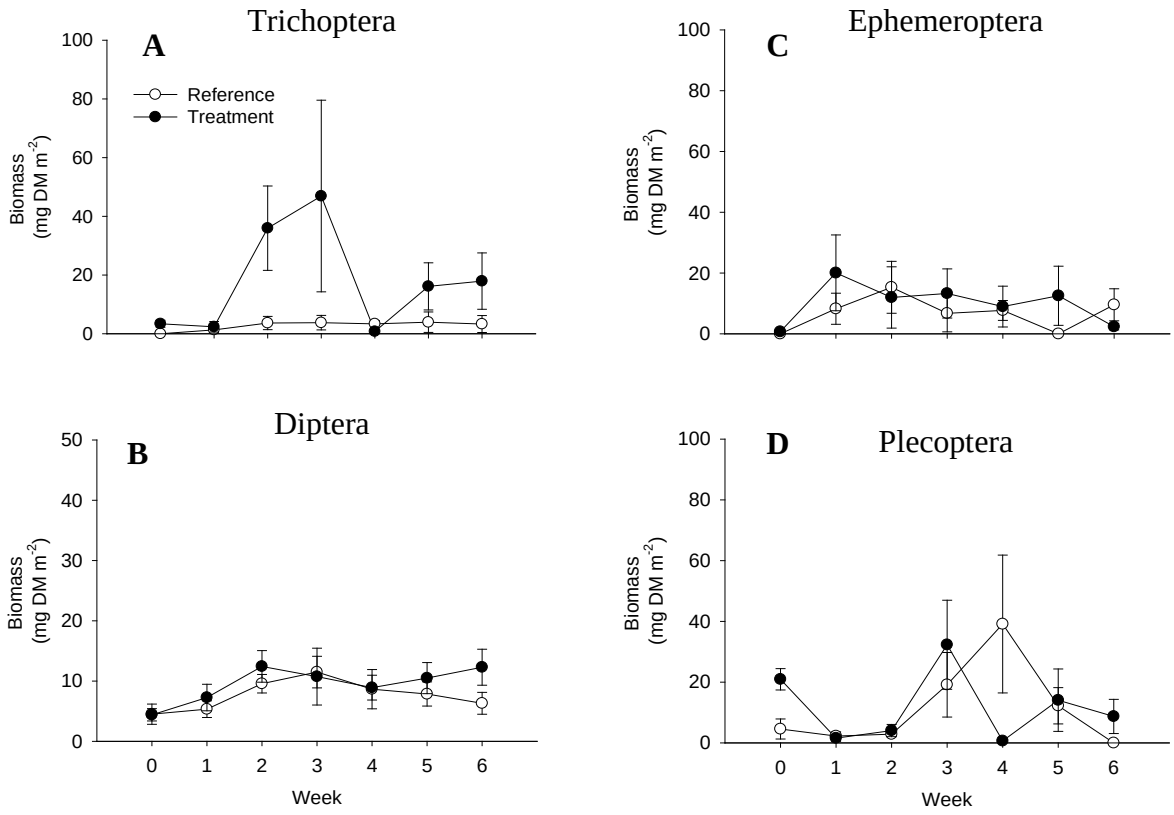

Trichoptera
A
Reference
Treatment
Biomass (mg DM m-2)
Ephemeroptera
C
Diptera
B
Plecoptera
D
Week

Fig. 5.4: The biomass and abundance of combined arboreal (**A** and **B**) and combined ground spiders (**C** and **D**) sampled along both the reference and treatment streams (mean ± SE). Ground spiders were sampled weekly with pitfall traps placed along five transects spaced at 10m intervals downstream of the isotopic enrichment. Each transect sampled at three locations: 0m, 10m, and 25m from the stream margin. Traps were deployed for 48h. Arboreal spiders were sampled semi-weekly at the same locations using timed (5 min) beat sampling of vegetation. NOTE: For simplicity, data are presented as averages over the entire sampling period and do not explicitly incorporate temporal changes. However, actual statistical analyses incorporated time (i.e., repeated measures two-way ANOVA).

Arboreal spiders

Ground spiders

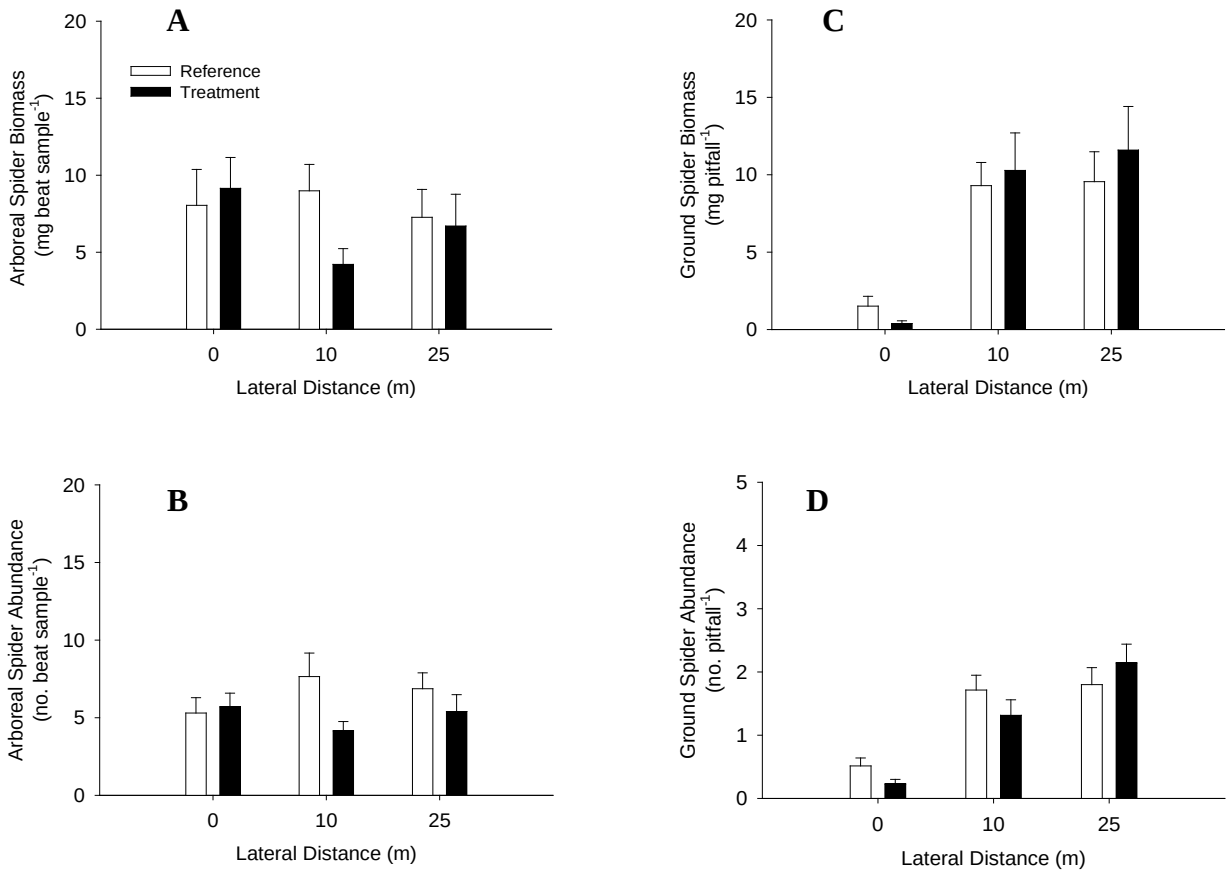


Fig. 5.5: Proportion of arboreal spider N derived from aquatic emergence (mean ± SE) for Tetragnathidae (**A**), Araneidae (**B**), Linyphiidae (**C**), and Anyphaenidae (**D**). Proportions based on the best fitting results from our dynamic mixing model (Equation 1). Numbers above each bar represent the number of composite samples analyzed during the isotopic enrichment that were used to construct the dynamic mixing model. Due to insufficient sample sizes, we did not calculate some proportions (represented by ND [not determined]). Sampling locations as in Fig. 5.2.

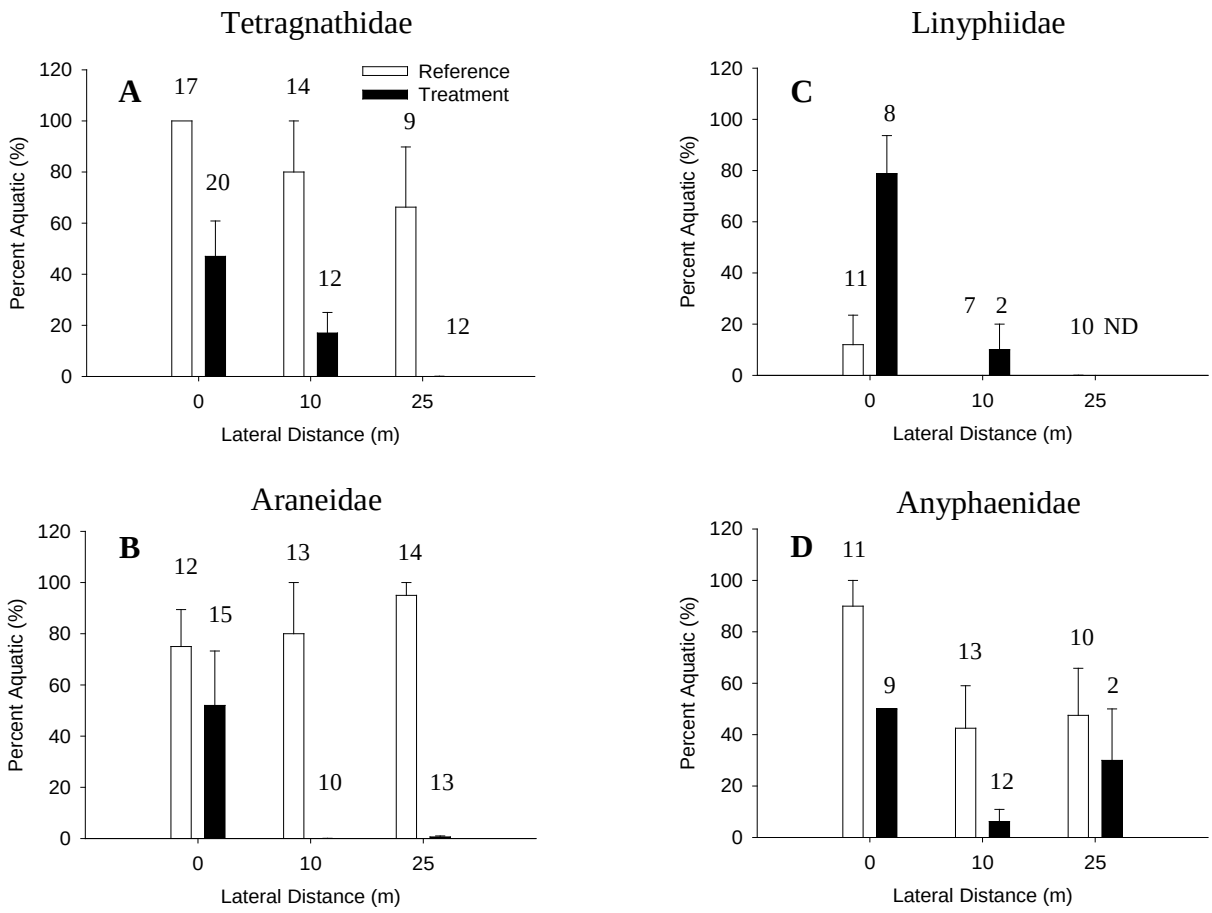
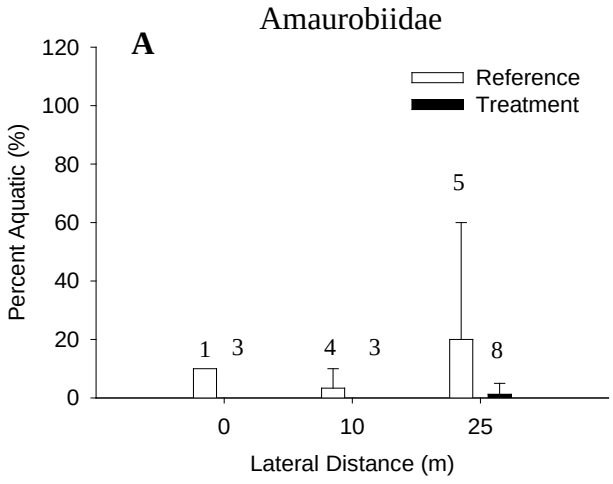
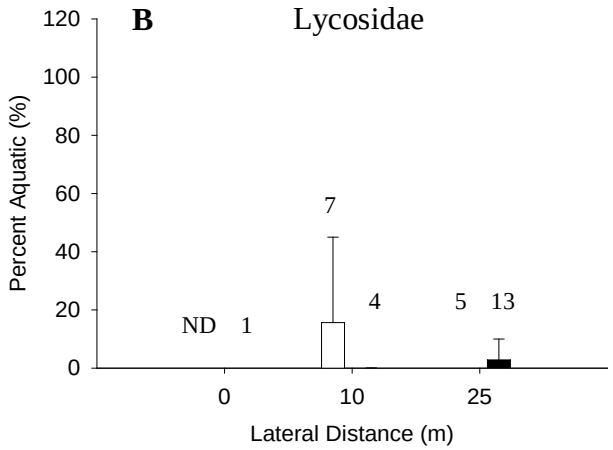

Tetragnathidae
A
Reference
Treatment
17
20
14
12
9
12
Linyphiidae
C
11
8
7
2
10
ND
Araneidae
B
12
15
13
10
14
13
Anyphaenidae
D
11
9
13
12
10
2
Percent Aquatic (%)
Lateral Distance (m)
0
10
25
0
20
40
60
80
100
120

Fig. 5.6: Proportion of ground spider N originating from aquatic emergence (mean ± SE) for Amaurobiidae (**A**), Lycosidae (**B**), and Gnaphosidae (**C**). Proportions based on the best fitting results from our dynamic mixing model (Equation 1). Numbers above each bar represent the number of composite samples analyzed during the isotopic enrichment that were used to construct the dynamic mixing model. Due to insufficient sample sizes, we could not calculate some proportions (represented by ND). Sampling locations as in Fig. 5.2.


Amaurobiidae
A
Reference
Treatment
Percent Aquatic (%)
120
100
80
60
40
20
0
1
3
4
3
5
8
0
10
25
Lateral Distance (m)


B
Lycosidae
Percent Aquatic (%)
120
100
80
60
40
20
0
ND
1
7
4
5
13
0
10
25
Lateral Distance (m)

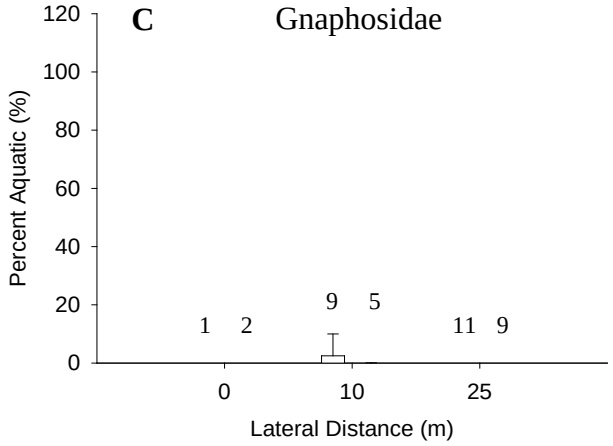
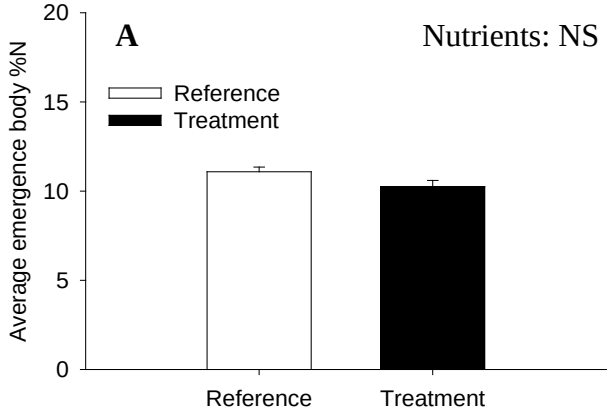
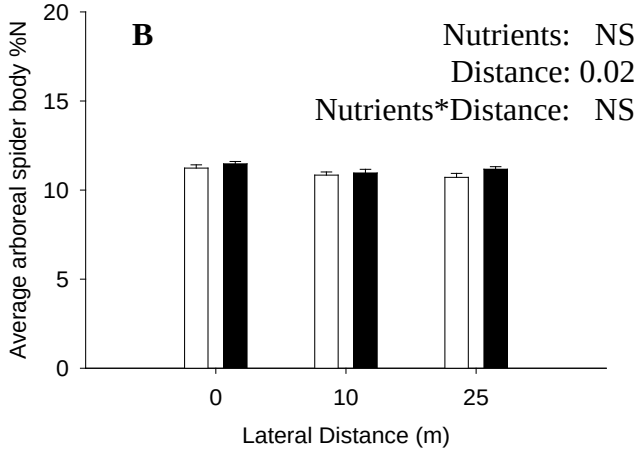
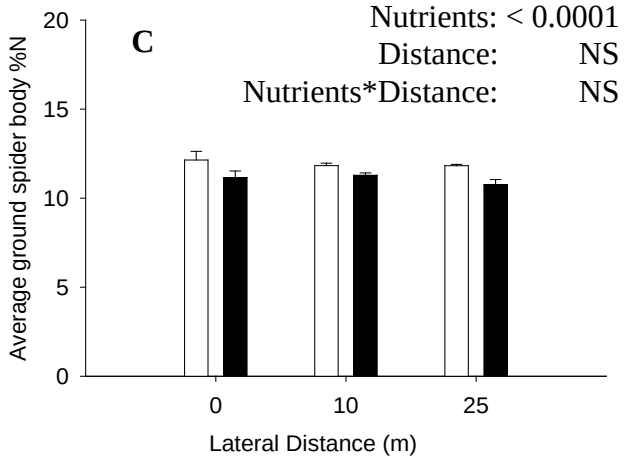

C
Gnaphosidae
Percent Aquatic (%)
120
100
80
60
40
20
0
1
2
9
5
11
9
0
10
25
Lateral Distance (m)

Fig. 5.7: Body N content (mean ± SE) for aquatic emergence (**A**), arboreal spiders (**B**), and ground spiders (**B**). Bars represent the average of all individuals collected at a given lateral distance from the stream margin through the entirety of the sampling period. Emergence body N content was analyzed with a one-way ANOVA, but arboreal and ground spider body N contents were analyzed with a two-way ANOVA.


A
Nutrients: NS
Reference
Treatment
Average emergence body %N
20
15
10
5
0
Reference
Treatment


B
Nutrients: NS
Distance: 0.02
Nutrients*Distance: NS
Average arboreal spider body %N
20
15
10
5
0
0
10
25
Lateral Distance (m)


C
Nutrients: < 0.0001
Distance: NS
Nutrients*Distance: NS
Average ground spider body %N
20
15
10
5
0
0
10
25
Lateral Distance (m)

CHAPTER 6

GENERAL CONCLUSIONS

Effects of nutrient enrichment on detrital resource dynamics— Because of human activities that have increased the mobilization of nitrogen (N) and phosphorus (P), nutrient enrichment has become one of the greatest threats to the functioning and stability of aquatic ecosystems (Vitousek et al. 1997, Smith et al. 1999, Bennett et al. 2001, Smith and Schindler 2009). However, despite nutrient enrichment threatening both algal-based and detritus-based food webs, few studies have quantified these effects on detrital-pathways, a dominant food web pathway in aquatic ecosystems (Mulholland et al. 2001, Moore et al. 2004). This underrepresentation of a widely-distributed ecosystem type has subsequently hindered the development of a comprehensive paradigm describing how aquatic ecosystems respond to excess nutrients (Dodds 2007). Therefore, my dissertation was a component of a larger-scale study that examined the nutrient response of stream consumers and associated shifts in carbon and nutrient dynamics within a detritus-based headwater stream.

To more adequately predict how increases in nutrient loading may alter consumer dynamics in detritus-based food webs, we first need to understand their net effects on detrital resource quantity and quality. However, this understanding at the stream-scale is still limited because of few large-scale nutrient enrichments (e.g., Cross et al. 2006, Benstead et al. 2009) and the previous focus on algal-based food webs that has largely shaped our understanding of nutrient effects (e.g., Peterson et al. 1993, Slavik et al. 2004). Within algal-based food webs, nutrient enrichment can simultaneously increase the quality and quantity of basal resources

because it can increase carbon fixation associated with greater *in situ* primary production (Schindler 1974, Peterson et al. 1993, Schindler et al. 2008, Smith and Schindler 2009). Accordingly, nutrient enrichment is largely believed to stimulate primary consumer and predator production within aquatic food webs. However, within detritus-based food webs nutrient enrichment can increase resource quality (Stelzer et al. 2003, Greenwood et al. 2007), but decrease resource quantity because of increased microbial production that can accelerate detrital processing rates (Greenwood et al. 2007, Benstead et al. 2009). Due to these divergent nutrient responses of resource quantity in algal-based vs. detritus-based food webs, nutrient enrichment may have dramatically different effects on consumers and ecosystem processes in these two ecosystems types. Therefore, because of the potential decline in detrital resource quantity that may increase carbon limitation for consumers, we initially predicted that the positive nutrient response of macroinvertebrate production would be attenuated during the fourth and fifth year of enrichment (this study) compared to shorter-term responses (years one and two: Cross 2004, Cross et al. 2006).

Results from our longer-term nutrient enrichment did not support these predictions. In agreement with results from our two-year enrichment, five years of enrichment continued to stimulate microbial production and increase detrital-quality (C. Tant unpubl. data, Suberkropp et al. *In press*). We also observed significant increases in carbon loss because of increased detrital-processing rates and microbial respiration due to nutrient enrichment (Gulis et al. 2004, C. Tant unpubl. data). This ultimately decreased organic matter standing crop (Gulis et al. 2004, Suberkropp et al. *In press*) and increased carbon export to downstream habitats (Benstead et al. 2009, A. Rosemond unpubl. data). Thus, rather than nutrient enrichment increasing carbon fixation and accrual as in algal-based food webs (Schindler 1974, Peterson et al. 1993), nutrient

enrichment of this detritus-based headwater stream decreased carbon storage and increased carbon loss via microbial respiration and downstream export of fine particulate organic matter (Benstead et al. 2009, A. Rosemond unpubl. data). Because downstream habitats are linked to upstream food webs via resource and macroinvertebrate transport (Vannote et al. 1980), this greater downstream export suggests the potential for these shifts in upstream carbon dynamics to alter the structure and function of downstream food webs.

Although nutrient enrichment dramatically increased carbon loss within the treatment stream (Benstead et al. 2009, Suberkropp et al. *In press*), enrichment still continued to stimulate the production of macroinvertebrate consumers in these detritus-based headwater streams (Chapter 2). This suggests that despite the divergent responses of resource quantity within algal-based (positive) and detritus-based (negative) food webs, nutrient enrichment had similar positive effects on total macroinvertebrate production in these two ecosystem types (this study, Slavik et al. 2004, Cross et al. 2006), at least over our experimental period. Thus, the potential negative effects of reductions in resource quantity on macroinvertebrate production were largely outweighed by the positive effects of resource quality.

It should also be noted that these divergent results likely did not result from differences in the lengths of the experimental enrichments. For instance, the experimental period of our study exceeded the only other long-term experimental enrichment of a stream ecosystem, the autotrophic Kuparuk River (Slavik et al. 2004). While the Kuparuk enrichment ran for 16 years (1983 – 1998), the total number of enrichment days was ca. 730 because this arctic stream was only enriched during summer months (Slavik et al. 2004). Conversely, our forested headwater stream was continuously enriched for ca. 1877 days (2.5x longer than the experimental enrichment of the Kuparuk River). This five-year enrichment would have likely allowed many

of the stream taxa to reach new population levels, because ca. 90% of the stream taxa in these headwater streams have life-cycles of one year or less, and only two taxa have larval periods longer than two years (*Anchytarsus* [1095 days], *Cordulegaster* [1140 days]) (Appendix A, Wallace et al. 1999). Thus, by the fourth and fifth year of enrichment, 90% of taxa would have produced > 4 generations under nutrient-enriched conditions. This suggests that the consumer responses observed during the five-year enrichment were likely not temporary.

Summary of dissertation objectives— Within the context of the long-term nutrient enrichment study, the main objective of my dissertation study was to improve our understanding of how nutrient enrichment altered consumer dynamics in a detritus-based headwater stream. Chapter 2 examined whether five years of continuous enrichment would continue to stimulate both primary consumer and predator production. Because previous enrichment studies that decoupled predator-prey relationships were conducted at limited spatial and temporal scales (Bohannan and Lenski 1999, Stevens and Steiner 2006), these decouplings may have resulted from simplified food webs using few taxa. Therefore, my second chapter (Chapter 2) attempted to validate these small-scale results at larger scales. Because the effects of nutrient enrichment on individual stream taxa can vary (e.g., Peterson et al. 1993, Cross 2004), my third chapter (Chapter 3) attempted to improve our ability to predict these taxon-specific responses to nutrients. Specifically, it examined the role of consumer body size in mediating consumer response to nutrient enrichment. Macroinvertebrate consumers can also be important drivers of many ecosystem processes in headwater streams (Wallace et al. 1991); thus, I quantified the role of a dominant stream consumer in elemental transformations at the stream-level (Chapter 4). Because aquatic emergence can represent an important subsidy of terrestrial predators (Jackson

and Fisher 1986), my final chapter (Chapter 5) assessed whether enrichment increased aquatic emergence biomass and subsequently increased the biomass and abundance of terrestrial spiders.

Chapter 2 Summary— The objective of Chapter 2 was to assess whether nutrient enrichment of a detritus-based headwater stream would continue to stimulate primary consumer and predator production, or whether shifts in food web structure might alter these positive bottom-up effects on higher trophic levels. Results from the first two years of enrichment increased the production and biomass of primary consumers and predators in the treatment stream relative to a reference stream (Cross 2004, Cross et al. 2006). However, continued nutrient enrichment (years 4 and 5) unexpectedly increased the dominance of predator-resistant primary consumers that ultimately decoupled primary consumer and predator production. This truncated resource flows to higher trophic levels and reduced food web efficiency. These results contrasted sharply with the first two years of our enrichment (Cross 2004, Cross et al. 2006) and another long-term experimental manipulation of an arctic stream that continued to stimulate predators and primary consumers even after 16 years of seasonal enrichment (Deegan and Peterson 1992, Slavik et al. 2004). As our study stream differed greatly in food web structure from the arctic stream, this chapter indicated the importance of food web structure in regulating the effects of nutrient enrichment on stream food webs. Specifically, it showed that the net effect of nutrients may be largely determined by prey body size distributions and the prevalence of predator-resistant prey, a relationship that has only been previously shown theoretically or at small-spatial scales (Abrams 1993, Bohannan and Lenski 1999, Chase 1999, Stevens and Steiner 2006). As humans are intentionally and unintentionally enriching aquatic ecosystems to stimulate predator production (Slaney et al. 2003, Compton et al. 2006), results from my second

chapter suggest that these practices may not always have the intended effects on ecosystem productivity.

Chapter 3 Summary— Although nutrient enrichment increased overall consumer production, the effects of nutrient enrichment on individual taxa and functional feeding groups varied (Chapter 2, Cross et al. 2005b, Cross et al. 2006). Thus, the goal of Chapter 3 was to better understand the importance of species-specific traits in determining a consumer's response to nutrient enrichment. Specifically, by grouping stream primary consumers and predators into body size classes and assessing their nutrient enrichment response, I tested whether consumers of similar body size responded similarly to nutrient enrichment. During the first two years of enrichment, all consumers regardless of body size increased with nutrient enrichment. However, because larger prey obtained predator-size refugia that likely decreased their predation risk, their biomass and abundance continued to increase during years four and five of enrichment. Conversely, the abundance and biomass of small-bodied primary consumers returned to pretreatment levels in years four and five. This consumer body size response varied with trophic level because large-bodied predators did not increase during this same time period. Because these observed increases in large-bodied primary consumers occurred despite substantial declines in resource quantity (Suberkropp et al. *In press*), our results suggest that the positive effects of resource quality outweighed the potential negative effects of resource quantity on consumer body size (e.g., Boersma and Kreutzer 2002). A similar positive nutrient response of large-bodied primary consumers was observed in an algal-based stream food web (Bourassa and Morin 1995), indicating similar consumer body size responses can occur despite differences in resource base (algal vs. detrital).

Chapter 4 Summary— The objective of Chapter 4 was to assess the functional role of a dominant stream consumer, *Pycnopsyche* spp., in driving some of the changes in ecosystem processes observed within our larger-scale nutrient enrichment experiment. Previous results from this experimental study indicated that nutrient enrichment increased leaf breakdown rates and carbon export (C. Tant unpubl. data, Greenwood 2004, Greenwood et al. 2007, Benstead et al. 2009). Because stream consumers can be important drivers of these processes (Cuffney et al. 1990, Wallace et al. 1991, Cross et al. 2005c), we used laboratory-based assimilation experiments and field-based excretion experiments to assess the effect of nutrient enrichment on *Pycnopsyche*'s N, P, and C assimilation and excretion. Enrichment significantly increased the rate at which *Pycnopsyche* assimilated N and P, but not C. Nutrient enrichment also increased *Pycnopsyche*'s rate of N and P excretion. Surprisingly, despite significant increases in stream N and P concentrations due to nutrient enrichment, *Pycnopsyche* assimilated relatively greater proportions of P compared to N, which suggested that they were differentially sequestering P. We found similar trends when these results were coupled with our known *Pycnopsyche* standing biomass and production values (Chapter 2); thus, *Pycnopsyche* affected elemental transformations at the stream-level via simultaneous increases in standing stocks, secondary production, and assimilation rates. These results were largely in agreement with previous results from our ecosystem-level manipulation (Cross et al. 2003, Rosemond et al. 2008) and further indicates that consumers in these headwater streams are principally limited by P availability and secondarily by N availability.

Chapter 5 Summary— Stream food webs are linked to the surrounding riparian food web via aquatic insect emergence that subsidize terrestrial predators (Jackson and Fisher 1986, Baxter et al. 2005). Thus, my final chapter (Chapter 5) assessed whether the observed doubling of

secondary production in the treatment stream (see Chapter 2) would similarly stimulate aquatic emergence subsidies and increase spider biomass and abundance. Despite nutrient enrichment more than doubling aquatic emergence biomass, this increased emergence mostly did not stimulate the biomass or abundance of terrestrial spiders along the treatment stream. Under reference conditions, arboreal spiders relied heavily on aquatic emergence for meeting their N demand. However, nutrient enrichment decreased this reliance in three of the four arboreal spider families, likely because of shifts in the composition of the emergence community. Enrichment dramatically increased the average body size of emerging adults and the relative dominance of Trichoptera, two groups of prey that may not be readily eaten by spiders (Nentwig and Wissel 1986, Tanaka 1991, Williams et al. 1995, Henschel et al. 2001). Thus, when environmental change stimulates the production of an aquatic subsidy, it may not always benefit those predators that specialize on that subsidy. It also suggests that the net effect of resource subsidies on consumers is not simply a function of the magnitude of the subsidy (e.g., Marczak et al. 2007), but may also depend on the subsidy's composition that can alter a consumer's ability to benefit from it.

General conclusions— The observed decoupling of predator and primary consumer production associated with the longer-term enrichment contrasted sharply with results from the first two years of the nutrient enrichment (Cross et al. 2006) and another long-term enrichment (Deegan and Peterson 1992, Peterson et al. 1993, Slavik et al. 2004). Because these earlier enrichments largely maintained a positive relationship between primary consumer and predator responses, these dissimilar results accentuate the difficulties in predicting stream responses to nutrient enrichment from this limited number of long-term experiments. Specifically, if the experimental enrichment had ceased after only two years of enrichment, we would have come to

similar conclusions as earlier experimental manipulations (i.e., nutrients stimulate primary consumer and predator production). However, after 4+ years of continuous enrichment, we came to a dramatically different conclusion; nutrient enrichment does not stimulate predator production despite dramatic increases in primary consumer production. These divergent empirical results clearly demonstrate the continued importance of multi-year studies in helping to predict the long-term effects of nutrients on aquatic ecosystems. Moreover, it suggests the need for a re-evaluation of the common assumption that nutrients simulate predator and ecosystem productivity.

Our results also differed from empirical evidence indicating a positive relationship between aquatic emergence and spider biomass (Sanzone et al. 2003, Marczak et al. 2007). Despite nutrient enrichment substantially increasing aquatic insect emergence biomass, it did not stimulate terrestrial spider biomass. Shifts in the size and community structure of the aquatic emergence likely contributed to this non-significant spider response. Thus, my findings suggest that shifts in the community structure associated with nutrient enrichment may not only attenuate the positive effects of nutrients on instream predators, but may similarly attenuate the positive cross-boundary effects of aquatic emergence on riparian predators.

Because a shift in consumer body size was a prominent mechanism likely driving the results in three out of the four data chapters presented here, it emphasizes the importance of species-specific traits, such as consumer body size, in mediating the response of aquatic food webs to nutrient enrichment. Specifically, consumers are important drivers of many ecosystem-level processes in stream food webs (Wallace and Webster 1996, Wallace and Hutchens 2000); thus, it logically follows that the ecosystem-level effects of nutrients on streams should be similarly propagated through changes in consumer structure and functional traits. This suggests

that our observed effects of nutrient enrichment on stream function may have been dramatically different in another aquatic food web that possessed a totally different suite of consumer taxa or alternatively, nutrient enrichment may evoke the same changes in food web structure in other ecosystems as we observed here. Therefore, to more generally predict the ecosystem-level responses of aquatic ecosystem, we need to better understand how consumer body size and other species-specific traits help determine a consumer's response to nutrient enrichment. By testing for nutrient enrichment effects in a diversity of ecosystems that differ in structure, we will be able to better assess what factors determine how aquatic ecosystems respond to nutrient enrichment. Only through these studies will we be able to ultimately develop a general paradigm to predict how nutrients alter the structure and function of aquatic ecosystems.

REFERENCES

Abell, R. A., D. M. Olson, E. Dinerstein, P. T. Hurley, J. T. Diggs, W. Eichbaum, S. Walters, W. Wettengel, T. Allnutt, C. J. Loucks, and P. Hedao. 2000. Freshwater ecoregions of North America: a conservation assessment. Island Press, Washington, D.C.

Abrams, P. A. 1993. Effect of increased productivity on the abundances of trophic levels. American Naturalist 141:351-371.

APHA. 1998. Standard methods for the examination of water and wastewater. 20th edition. American Public Health Association, Washington, D.C., USA.

Ballinger, A., and P. S. Lake. 2006. Energy and nutrient fluxes from rivers and streams into terrestrial food webs. Marine and Freshwater Research 57:15-28.

Baxter, C. V., K. D. Fausch, M. Murakami, and P. L. Chapman. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. Ecology 85:2656-2663.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zone. Freshwater Biology 50:201-220.

Beaty, S. R., K. Fortino, and A. E. Hershey. 2006. Distribution and growth of benthic macroinvertebrates among different patch types of the littoral zones of two arctic lakes. Freshwater Biology 51:2347-2361.

Benke, A., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the

southeastern United States. Journal of the North American Benthological Society 18:308-343.

Benke, A., and J. B. Wallace. 1997. Trophic basis of production among riverine caddisflies: implications for food web analysis. Ecology 78:1132-1145.

Benke, A. C. 1979. Modification of the Hynes method for estimating secondary production with particular significance for multivoltine populations. Limnology and Oceanography 24:168-171.

Benke, A. C. 1993. Concepts and patterns of invertebrate production in running waters. Verh. Internat. Verein. Limnol. 25:15-38.

Bennett, E. M., S. R. Carpenter, and N. F. Caraco. 2001. Human impacts on erodable phosphorus and eutrophication: a global perspective. Bioscience 51:227-234.

Benstead, J. P., L. A. Deegan, B. J. Peterson, A. D. Huryn, W. B. Bowden, K. Suberkropp, K. M. Buzby, A. C. Green, and J. A. Vacca. 2005. Responses of a beaded Arctic stream to short-term N and P fertilisation. Freshwater Biology 50:277-290.

Benstead, J. P., A. D. Rosemond, W. F. Cross, J. B. Wallace, S. L. Eggert, K. Suberkropp, V. Gulis, J. Greenwood, and C. J. Tant. 2009. Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. Ecology 90:2556-2566.

Boersma, M., and C. Kreutzer. 2002. Life at the edge: is food quality really of minor importance at low quantities? Ecology 83:2552-2561.

Bohannan, B. J. M., and R. E. Lenski. 1999. Effect of prey heterogeneity on the response of a model food chain to resource enrichment. American Naturalist 153:73-82.

Bourassa, N., and A. Morin. 1995. Relationships between size structure of invertebrate assemblages and trophy and substrate composition in streams. Journal of the North American Benthological Society 14:393-403.

Bremigan, M. T., and R. A. Stein. 1994. Gape-dependent larval foraging and zooplankton size: implications for fish recruitment across systems. Canadian Journal of Fisheries and Aquatic Sciences 51:913-922.

Brett, M. T., and C. R. Goldman. 1997. Consumer versus resource control in freshwater pelagic food webs. Science 275:384-386.

Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150:28-35.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771-1789.

Burdon, F. J., and J. S. Harding. 2008. The linkage between riparian predators and aquatic insects across a stream-resource spectrum. Freshwater Biology 53:330-346.

Carpenter, S. R. 1996. Microcosm experiments have limited relevance for community and ecosystem ecology. Ecology 77:677-680.

Carpenter, S. R., T. M. Frost, D. Heisey, and T. K. Kratz. 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. Ecology 70:1142-1152.

Cebrian, J. 1999. Patterns in the fate of production in plant communities. American Naturalist 154:449-468.

Chase, J. M. 1999. Food web effects of prey size refugia: variable interactions and alternative stable equilibria. American Naturalist 154:559-570.

Cole, J. J., S. R. Carpenter, M. L. Pace, M. C. V. d. Bogert, J. L. Kitchell, and J. R. Hodgson. 2006. Differential support of lake food webs by three types of terrestrial carbon. Ecology Letters 9:558-568.

Compton, J. E., C. P. Anderson, D. L. Phillips, J. R. Brooks, M. G. Johnson, M. R. Church, W. E. Hogsett, M. A. Cairns, P. T. Rygiewicz, B. C. McComb, and C. D. Shaff. 2006. Ecological and water quality consequences of nutrient addition for salmon restoration in the Pacific Northwest. Frontiers in Ecology and the Environment 4:18-26.

Creed, R. P., R. P. Cherry, J. R. Pflaum, and C. J. Wood. 2009. Dominant species can produce a negative relationship between species diversity and ecosystem function. Oikos 118:723-732.

Cross, W. F. 2004. Nutrient enrichment of a detritus-based stream ecosystem: effects on invertebrate community structure and function. Dissertation. University of Georgia, Athens, GA, USA.

Cross, W. F., J. P. Benstead, P. C. Frost, and S. A. Thomas. 2005a. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50:1895-1912.

Cross, W. F., J. P. Benstead, A. D. Rosemond, and J. B. Wallace. 2003. Consumer-resource stoichiometry in detritus-based streams. Ecology Letters 6:721-732.

Cross, W. F., B. R. Johnson, J. B. Wallace, and A. D. Rosemond. 2005b. Contrasting response of stream detritivores to long-term nutrient enrichment. Limnology and Oceanography 50:1730-1739.

Cross, W. F., A. D. Rosemond, J. P. Benstead, S. L. Eggert, and J. B. Wallace. 2005c. Differential effects of consumers on C, N, and P dynamics: insights from long-term

research. Pages 235-247 *in* P. C. de Ruiter, V. Wolters, and J. C. Moore, editors. Dynamic food webs: multispecies assemblages, ecosystem development, and environmental change Academic Press, Boston, MA.

Cross, W. F., J. B. Wallace, and A. D. Rosemond. 2007. Nutrient enrichment reduces constraints on material flows in a detritus-based food web. Ecology 88:2563-2575.

Cross, W. F., J. B. Wallace, A. D. Rosemond, and S. L. Eggert. 2006. Whole-system nutrient enrichment increases secondary production in a detrital-based ecosystem. Ecology 87:1556-1565.

Crowl, T. A., and A. P. Covich. 1990. Predator induced life-history shifts in a freshwater snail. Science 247:949-951.

Cuffney, T. F., J. B. Wallace, and G. J. Lugthart. 1990. Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. Freshwater Biology 23:281-299.

Davic, R. D. 1991. Ontogenetic shift in diet of *Desmognathus quadramaculatus*. Journal of Herpetology 25:108-111.

Davis, J. M., A. D. Rosemond, S. L. Eggert, W. F. Cross, and J. B. Wallace. *In review-a*. Long-term nutrient enrichment decouples predator and prey production.

Davis, J. M., A. D. Rosemond, S. L. Eggert, W. F. Cross, and J. B. Wallace. *In review-b*. Nutrient enrichment differentially affects body sizes of primary consumers and predators in a detritus-based ecosystem.

Deegan, L. A., and B. J. Peterson. 1992. Whole-river fertilization stimulates fish production in an arctic tundra river. Canadian Journal of Fisheries and Aquatic Sciences 49:1890-1901.

Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321:926-929.

Dodd, C. K., Jr. 1997. Imperiled amphibians: a historical perspective. Pages 165-200 *in* G. W. Benz and D. E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Special Publication 1, Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, GA.

Dodds, W. K. 2007. Trophic state, eutrophication and nutrient criteria in streams. Trends in Ecology & Evolution 22:669-676.

Eggert, S. L., and J. B. Wallace. 2003. Reduced detrital resources limit *Pycnopsyche gentilis* (Trichoptera: Limnephilidae) production and growth. Journal of the North American Benthological Society 22:388-400.

Eggert, S. L., and J. B. Wallace. 2007. Wood biofilm as a food resource for stream detritivores. Limnology and Oceanography 52:1239-1245.

Elser, J. J., D. R. Dobberfuhl, N. A. MacKay, and J. H. Schampel. 1996. Organism size, life history, and N:P stoichiometry. Bioscience 46:674-684.

Emmerson, M., and D. Raffaelli. 2004. Predator-prey body size, interaction strength and the stability of a real food web. Journal of Animal Ecology 73:399-409.

Evans-White, M. A., and G. A. Lamberti. 2006. Stoichiometry of consumer-driven nutrient recycling across nutrient regimes in streams. Ecology Letters 9:1186-1197.

Fagan, W. F., and R. F. Denno. 2004. Stoichiometry of actual vs. potential predator-prey interactions: insights into nitrogen limitation for arthropod predators. Ecology Letters 7:876-883.

Finlay, K., B. E. Beisner, A. Patoine, and B. Pinel-Alloul. 2007. Regional ecosystem variability drives the relative importance of bottom-up and top-down factors for zooplankton size spectra. Canadian Journal of Fisheries and Aquatic Sciences 64:516-529.

Frost, P. C., J. P. Benstead, W. F. Cross, H. Hillebrand, J. H. Larson, M. A. Xenopoulos, and T. Yoshida. 2006. Threshold elemental ratios of carbon and phosphorus in aquatic consumers. Ecology Letters 9:774-779.

Fukui, D., M. Murakami, S. Nakano, and T. Aoi. 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. Journal of Animal Ecology 75:1252-1258.

Golden, H. E., and L. A. Deegan. 1998. The trophic interactions of young Arctic grayling (*Thymallus arcticus*) in an Arctic tundra stream. Freshwater Biology 39:637-648.

Golladay, S. W., J. R. Webster, and E. F. Benfield. 1983. Factors affecting food utilization by a leaf shredding aquatic insect: leaf species and conditioning time. Holarctic Ecology 6:157-162.

Gonzalez, J. M., and M. A. S. Graca. 2003. Conversion of leaf litter to secondary production by a shredding caddisfly. Freshwater Biology 48:1578-1592.

Greenwood, J. 2004. The response of detrital and autotrophic resources to long-term nutrient enrichment in a detritus-based headwater stream. Dissertation. University of Georgia, Athens, GA, USA.

Greenwood, J. L., A. D. Rosemond, J. B. Wallace, W. F. Cross, and H. S. Weyers. 2007. Nutrients stimulate leaf breakdown rates and detritivore biomass: bottom-up effects via heterotrophic pathways. Oecologia 151:637-649.

Gruner, D. S., J. E. Smith, E. W. Seabloom, S. A. Sandin, J. T. Ngai, H. Hillebrand, W. S. Harpole, J. J. Elser, E. E. Cleland, M. E. S. Bracken, E. T. Borer, and B. M. Bolker. 2008.

A cross-system synthesis of consumer and nutrient resource control on producer biomass. Ecology Letters 11:740-755.

Gulis, V., A. D. Rosemond, K. Suberkropp, H. S. Weyers, and J. P. Benstead. 2004. Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams. Freshwater Biology 49:1437-1447.

Gulis, V., and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology 48:123-134.

Hall, R. O., B. J. Koch, M. C. Marshall, B. W. Taylor, and L. M. Tronstad. 2007. How body size mediates the role of animals in nutrient cycling in aquatic ecosystems. Pages 286-305 *in* A. Hildrew, D. Raffaelli, and R. Edmonds-Brown, editors. Body size: the structure and function of aquatic ecosystems. Cambridge University Press, Cambridge, UK.

Hall, R. O., B. J. Peterson, and J. L. Meyer. 1998. Testing a nitrogen-cycling model of a forest stream by using a nitrogen-15 tracer addition. Ecosystems 1:283-298.

Hall, R. O., J. L. Tank, and M. F. Dybdahl. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. Frontiers in Ecology and the Environment 1:407-411.

Hall, R. O., J. B. Wallace, and S. L. Eggert. 2000. Organic matter flow in stream food webs with reduced detrital resource base. Ecology 81:3445-3463.

Henschel, J. R., D. Mahsberg, and H. Stumpf. 2001. Allochthonous aquatic insects increase predation and decrease herbivory in river shore food webs. Oikos 93:429-438.

Herbst, G. N. 1980. Effects of burial on food value and consumption of leaf detritus by aquatic invertebrates in a lowland forest stream. Oikos 35:411-424.

Herbst, G. N. 1982. Effects of leaf type on the consumption rates of aquatic detritivores. Hydrobiologia 89:77-87.

Hessen, D. O., G. I. Agren, T. R. Anderson, J. J. Elser, and P. C. de Ruiter. 2004. Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 85:1179-1192.

Hurlbert, S. H. 1984. Pseudreplication and the design of ecological field experiments. Ecological Monographs 54:187-211.

Huryn, A. D., and A. C. Benke. 2007. Relationship between biomass turnover and body size for stream communities. Pages 55-76 *in* A. Hildrew, D. Raffaelli, and R. Edmonds-Brown, editors. Body size: the structure and function of aquatic ecosystems. Cambridge University Press.

Huryn, A. D., and J. B. Wallace. 1988. Community structure of Trichoptera in a mountain stream: spatial patterns of production and functional organization. Freshwater Biology 20:141-155.

Hutchens, J. J., Jr., E. F. Benfield, and J. R. Webster. 1997. Diet and growth of a leaf-shredding caddisfly in southern Appalachian streams of contrasting disturbance history. Hydrobiologia 346:193-201.

Hynes, H. B. N. 1975. The stream and its valley. Verh. Internat. Verein. Limnol. 19:1-15.

Isphording, W. C., and J. F. Fitzpatrick, Jr. 1992. Geologic and evolutionary history of drainage systems in the southeastern United States. Pages 19-56 *in* C. T. Hackney, S. M. Adams, and W. H. Martin, editors. Biodiversity of the southeastern united states: aquatic communities. John Wiley and Sons, Inc., New York, NY.

Jackson, J. K., and S. G. Fisher. 1986. Secondary production, emergence, and export of aquatic insects of a sonoran desert stream. Ecology 67:629-638.

Johnson, B. R., and J. B. Wallace. 2005. Bottom-up limitation of a stream salamander in a detritus-based food web. Canadian Journal of Fisheries and Aquatic Sciences 62:301-311.

Kato, C., T. Iwata, S. Nakano, and D. Kishi. 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. Oikos 103:113-120.

Kato, C., T. Iwata, and E. Wada. 2004. Prey use by web-building spiders: stable isotope analyses of trophic flow at a forest-stream ecotone. Ecological Research 19:633-643.

Kawaguchi, Y., T. Taniguchi, and S. Nakano. 2003. Terrestrial invertebrate inputs determine the local abundance of stream fishes in a forested stream. Ecology 84:701-708.

Kurta, A., and J. O. Whitaker. 1998. Diet of the endangered Indiana bat (*Myotis sodalis*) on the northern edge of its range. American Midland Naturalist 140:280-286.

Lugthart, G. J., and J. B. Wallace. 1992. Effects of disturbance on benthic functional structure and production in mountain streams. Journal of the North American Benthological Society 11:138-164.

Lurling, M., and E. Van Donk. 1997. Life history consequences for *Daphnia pulex* feeding on nutrient-limited phytoplankton. Freshwater Biology 38:693-709.

MacNeale, K. H., B. L. Peckarsksy, and G. E. Likens. 2005. Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. Freshwater Biology 50:1117-1130.

Marczak, L. B., and J. S. Richardson. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. Journal of Animal Ecology 76:687-694.

Marczak, L. B., R. M. Thompson, and J. S. Richardson. 2007. Meta-analysis: trophic level, habitat, and productivity shape the food web effects of resource subsidies. Ecology 88:140-148.

Mayntz, D., D. Raubenheimer, M. Salomon, S. Toft, and S. J. Simpson. 2005. Nutrient-specific foraging in invertebrate predators. Science 307:111-112.

Merritt, R. W., and K. W. Cummins, editors. 1996. An introduction to the aquatic insects of North America. 3rd edition. Kendall Hunt, Dubuque.

Meyer, J. L., and J. O'Hop. 1983. Leaf-shredding insects as a source of dissolved organic carbon in headwater streams. American Midland Naturalist 109:175-183.

Meyer, J. L., D. L. Strayer, J. B. Wallace, S. L. Eggert, G. S. Helfman, and N. E. Leonard. 2007. The contribution of headwater streams to biodiversity in river networks. Journal of the American Water Resources Association 43:86-103.

Meyer, J. L., and J. B. Wallace. 2001. Lost linkages and lotic ecology: rediscovering small streams. Pages 295-317 *in* M. C. Press, N. J. Huntly, and S. Levin, editors. Ecology: achievement and challenge. Blackwell Science, Oxford.

Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. d. Ruiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni, and D. H. Wall. 2004. Detritus, trophic dynamics, and biodiversity. Ecology Letters 7.

Morse, J. C., B. P. Stark, W. P. McCafferty, and K. J. Tennessen. 1997. Southern Appalachian and other southeastern streams at risk: implications for mayflies, dragonflies and damselflies, stoneflies, and caddisflies. Pages 17-42 *in* G. W. Benz and D. E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Southeast Aquatic Research Institute Special Publication 1, Lenz Design and Communications, Decatur, GA.

Moulder, B. C., and D. E. Reichle. 1972. Significance of spider predation in energy dynamics of forest floor arthropod communities. Ecological Monographs 42:473-498.

Mulholland, P. J., C. S. Fellows, J. L. Tank, N. B. Grimm, J. R. Webster, S. K. Hamilton, E. Marti, L. Ashkenas, W. B. Bowden, W. K. Dodds, W. H. McDowell, M. J. Paul, and B. J. Peterson. 2001. Inter-biome comparison of factors controlling stream metabolism. Freshwater Biology 46:1503-1517.

Naeem, S., and J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. Ecology Letters 6:567-579.

Nakano, S., H. Miyasaka, and N. Kuhara. 1999. Terrestrial-aquatic linkages: riparian arthropod inputs alter trophic cascades in a stream food web. Ecology 80:2435-2441.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamics interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences of the United States of America 98:166-170.

Nentwig, W., and C. Wissel. 1986. A comparison of prey lengths among spiders. Oecologia 68:595-600.

Oksanen, L., S. D. Fretwell, J. Arruda, and P. Niemela. 1981. Exploitation ecosystems in gradients of primary productivity. American Naturalist 118:240-261.

Olive, C. W. 1980. Foraging specialization in orb-weaving spiders. Ecology 61:1133-1144.

Osenberg, C. W., R. J. Schmitt, S. J. Holbrook, K. E. Abusaba, and A. R. Flegal. 1994. Detection of environmental impacts: natural variability, effect size, and power analysis. Ecological Applications 4:16-30.

Otto, C. 1974. Growth and energetics in a larval population of *Potamophylax cingulatus* (Steph) (Trichoptera) in a south Swedish stream. Journal of Animal Ecology 43:339-361.

Pace, M. L., J. J. Cole, S. R. Carpenter, J. F. Kitchell, J. R. Hodgson, M. C. V. d. Bogert, D. L. Bade, E. S. Kritzberg, and D. Bastviken. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427:240-243.

Pandian, T. J., and M. P. Marian. 1986. An indirect procedure for the estimation of assimilation efficiency of aquatic insects. Freshwater Biology 16:93-98.

Peacor, S. D., and E. E. Werner. 2000. Predator effects on an assemblage of consumers through induced changes in consumer foraging behavior. Ecology 81:1998-2010.

Peckarsky, B. L., B. W. Taylor, A. R. McIntosh, M. A. McPeek, and D. A. Lytle. 2001. Variation in mayfly size at metamorphosis as a development response to risk of predation. Ecology 82:740-757.

Peterson, B. J., L. Deegan, J. Helfrich, J. E. Hobbie, M. Hullar, B. Moller, T. E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M. A. Lock, D. M. Fiebig, V. McKinley, M. C. Miller, J. R. Vestal, R. Ventullo, and G. Volk. 1993. Biological responses of a tundra river to fertilization. Ecology 74:653-672.

Peterson, B. J., J. E. Hobbie, A. E. Hershey, M. A. Lock, T. E. Ford, J. R. Vestal, V. L. McKinley, M. A. J. Hullar, M. C. Miller, R. M. Ventullo, and G. S. Volk. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science 229:1383-1386.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowden, H. M.Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86-90.

Phillips, D. L., and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261-269.

Poff, N. L., M. A. Palmer, P. A. Angermeier, R. L. Vadas, Jr., C. K. Hakenkamp, A. Bely, P. Arensburger, and A. P. Martinm. 1993. Size structure of the metazoan community in a Piedmont Stream. Oecologia 95:202-209.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics 28:289-316.

Post, D. M., M. L. Pace, and N. G. Hairston. 2000. Ecosystem size determines food-chain length in lakes. Nature 405:1047-1049.

Power, M. E., J. C. Marks, and M. S. Parker. 1992. Variation in the vulnerability of prey to different predators - community-level consequences. Ecology 73:2218-2223.

Power, M. E., M. S. Parker, and W. E. Dietrich. 2008. Seasonal reassembly of a river food web: floods, droughts, and impacts of fish. Ecological Monographs 78:263-282.

Power, M. E., M. S. Parker, and J. T. Wootton. 1996. Disturbance and food chain length in rivers. Pages 286-297 *in* G. A. Polis and K. O. Winemiller, editors. Food webs: integration of patterns and dynamics. Chapman and Hall, New York, New York.

Power, M. E., W. E. Rainey, M. S. Parker, J. L. Sabo, A. Smyth, S. Khandwala, J. C. Finlay, F. C. McNeely, K. Marsee, and C. Anderson. 2004. River-to-watershed subsidies in an old-growth conifer forest. Pages 217-240 *in* G. A. Polis, M. E. Power, and G. R. Huxel, editors. Food Webs at the Landscape Level. University of Chicago, Chicago.

Reynolds, S. E. 1990. Feeding in caterpillars: maximizing or optimizing food acquisition? Pages 106-118 *in* J. Mellinger, editor. Animal Nutrition and Transport Processes: 1. Nutrition in Wild and Domestic Animals. Karger, New York, NY.

Romaniszyn, E. D., J. J. Hutchens, and J. B. Wallace. 2007. Aquatic and terrestrial invertebrate drift in southern Appalachian Mountain streams: implications for trout food resources. Freshwater Biology 52:1-11.

Romanovsky, Y. E., and I. Y. Feniova. 1985. Competition among cladocera: effect of different levels of food supply. Oikos 44:243-252.

Rosemond, A. D., W. F. Cross, J. L. Greenwood, V. Gulis, S. L. Eggert, K. Suberkropp, J. B. Wallace, and S. E. Dye. 2008. Nitrogen versus phosphorus demand in a detritus-based headwater stream: what drives microbial to ecosystem response? Verh. Internat. Verein. Limnol. 30:651-655.

Rosemond, A. D., C. M. Pringle, A. Ramirez, and M. J. Paul. 2001. A test of top-down and bottom-up control in a detritus-based food web. Ecology 82:2279-2293.

Rosemond, A. D., C. M. Pringle, A. Ramirez, M. J. Paul, and J. L. Meyer. 2002. Landscape variation in phophorus concentration and effects on detritus-based tropical streams. Limnology and Oceanography 47:278-289.

Rosemond, A. D., C. M. Swan, J. S. Kominoski, and S. E. Dye. *In press*. Non-additive effects of litter mixing are suppressed in a nutrient-enriched stream. Oikos.

Rosenzweig, M. L. 1971. Paradox of enrichment: destabilization of exploitation ecosystems in ecological times. Science 171:385-387.

Sabo, J. L., and M. E. Power. 2002a. Numerical response of lizards to aquatic insects and shore-term consequences for terrestrial prey. Ecology 83:3023-3036.

Sabo, J. L., and M. E. Power. 2002b. River-watershed exchange: effects of riverine subsidies on riparian lizards and their terrestrial prey. Ecology 83:1860-1869.

Sanzone, D. M., J. L. Meyer, E. Marti, E. P. Gardiner, J. L. Tank, and N. B. Grimm. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. Oecologia 134:238-250.

Schindler, D. W. 1974. Eutrophication and recovery in experimental lakes: implications for lake management. Science 184:897-899.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science 195:260-262.

Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng, and S. E. M. Kasian. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences of the United States of America 105:11254-11258.

Short, R. A., and P. E. Maslin. 1977. Processing of leaf litter by a stream detritivore: effect on nutrient availability to collectors. Ecology 58:935-938.

Singer, G. A., and T. J. Battin. 2007. Anthropogenic subsidies alter stream consumer-resource stoichiometry, biodiversity, and food chains. Ecological Applications 17:376-389.

Slaney, P. A., B. R. Ward, and J. C. Wightman. 2003. Experimental nutrient addition to the Keogh River and application to the Salmon River in coastal British Columbia. Pages 111-126 *in* J. Stockner, editor. Nutrients in Salmonid Ecosystems: Sustaining Production and Biodiversity. American Fisheries Society, Bethesda, MD.

Slavik, J., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey, and J. E. Hobbie. 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. Ecology 85:939-954.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends in Ecology & Evolution 24:201-207.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100:179-196.

Smock, L. A., G. M. Metzler, and J. E. Gladden. 1989. Role of debris dams in the structure and functioning of low-gradient headwater streams. Ecology 70:1989.

Sprules, W. G., and M. Munawar. 1986. Plankton size spectra in relation to ecosystem productivity, size, and perturbation. Canadian Journal of Fisheries and Aquatic Sciences 43:1789-1794.

Stelzer, R. S., J. Heffernan, and G. E. Likens. 2003. The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. Freshwater Biology 48:1925-1937.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, NJ.

Stevens, M. H. H., and C. E. Steiner. 2006. Effects of predation and nutrient enrichment on a food web with edible and inedible prey. Freshwater Biology 51:666-671.

Suberkropp, K., V. Gulis, A. D. Rosemond, and J. P. Benstead. *In press*. Nutrient enrichment shifts microbial and detrital pathways in a headwater stream ecosystem.

Tanaka, K. 1991. Food consumption and diet composition of the web-building spider *Agelena limbata* in two habitats. Oecologia 86:8-15.

Tessier, A. J., L. L. Henry, C. E. Goulden, and M. W. Durand. 1983. Starvation in *Daphnia*: energy reserves and reproductive allocation. Limnology and Oceanography 28:667-676.

Ubick, D., P. Paquin, P. E. Cushing, and V. Roth, editors. 2005. Spiders of North America: an identification manual. American Arachnological Society.

Vanni, M. J. 1986. Competition in zooplankton communities: suppression of small species by *Daphnia Pulex*. Limnology and Oceanography 31:1039-1056.

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual Review of Ecology and Systematics 33:341-370.

Vanni, M. J., A. M. Bowling, E. M. Dickman, R. S. Hale, K. A. Higgins, M. J. Horgan, L. B. Knoll, W. H. Renwick, and R. A. Stein. 2006. Nutrient cycling by fish supports relatively more primary production as lake productivity increases. Ecology 87:1696-1709.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7:737-750.

Wallace, J. B., T. F. Cuffney, J. R. Webster, G. J. Lugthart, K. Chung, and B. S. Goldowitz. 1991. Export of fine organic particles from headwater streams: effects of season, extreme discharges, and invertebrate manipulations. Limnology and Oceanography 36:670-682.

Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102-104.

Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1999. Effects of resource limitation on a detrital-based ecosystem. Ecological Monographs 69:409-442.

Wallace, J. B., and J. J. Hutchens. 2000. Effects of invertebrates in lotic ecosystem processes. Pages 73-96 *in* D. C. Coleman and P. F. Hendrix, editors. Invertebrates as webmasters in ecosystems. CAB International, New York, NY.

Wallace, J. B., and J. R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. Annual Review of Entomology 41.

Warren, M. L., Jr., P. L. Angermeier, B. M. Burr, and W. R. Haag. 1997. Decline of a diverse fish fauna: patterns of imperilment and protection in the southeastern United States. Pages 105-164 *in* G. W. Benz and D. E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Special Publication 1, Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, GA.

Waters, T. F. 1969. Subsampler for dividing large samples of stream invertebrate drift. Limnology and Oceanography 14:813-815.

Werneke, U., and P. Zwick. 1992. Mortality of the terrestrial adult and aquatic nymphal life stages of *Baetis vernus* and *Baetis rhodani* in the Breitenbach, Germany (Insecta: Ephemeroptera). Freshwater Biology 28:249-255.

Whiles, M. R., A. D. Huryn, B. W. Taylor, and J. D. Reeve. 2009. Influence of handling stress and fasting on estimates of ammonium excretion by tadpoles and fish: recommendations for designing excretion experiments. Limnology and Oceanography-Methods 7:1-7.

Williams, D. D., L. G. Ambrose, and L. N. Browning. 1995. Trophic dynamics of two sympatric species of riparian spider (Araneae, Tetragnathidae). Canadian Journal of Zoology-Revue Canadienne De Zoologie 73:1545-1553.

Wipfli, M. S., and D. P. Gregovich. 2002. Export of invertebrates and detritus from fishless headwater streams in southeastern Alaska: implications for downstream salmonid production. Freshwater Biology 47:957-969.

Witman, J. D., J. C. Ellis, and W. B. Anderson. 2004. The influence of physical processes, organisms, and permeability on cross-ecosystem fluxes. Pages 335-358 *in* G. A. Polis, M. E. Power, and G. R. Huxel, editors. Food Webs: at the landscape level. University of Chicago Press, Chicago, IL.

Woodward, G., and P. Warren. 2007. Body size and predatory interactions in freshwaters: scaling from individuals to communities. Pages 98-117 *in* A. G. Hildrew, D. G. Raffaelli, and R. Edmonds-Brown, editors. Body size: the structure and function of aquatic ecosystems. Cambridge University Press, Cambridge, UK.

Wootton, J. T., M. S. Parker, and M. E. Power. 1996. Effects of disturbance on river food webs. Science 273.

APPENDIX A

Mean annual abundance (A, no m^{-2}), biomass (B, mg AFDM m^{-2}), and production (P, mg AFDM m^{-2} yr^{-1}) of taxa in each functional feeding group (as defined by Wallace et al. 1999). Data grouped by mixed substrate and bedrock outcrop habitats in the reference stream (C53) and the treatment (C54) streams. Insect orders are as follows: E = Ephemeroptera, P = Plecoptera, T = Trichoptera, C = Coleoptera, O = Odonata, D = Diptera, L = Lepidoptera, NI = non-insect. CPI = cohort production interval in days or, where indicated, the assumed annual P/B that was used for production calculations. The symbol '‡' indicates that the instantaneous growth rate method was used to calculate secondary production (see Cross et al. 2005 for growth rate methods).
We divided the study into three time periods: pretreatment (PRE 1 and PRE 2; July 1998 – August 2000; 22 months), short-term response (ENR 1 and ENR 2; September 2000 – August 2002; 26 months), and long-term response (ENR 4 and ENR 5; September 2003 – August 2005; 24 months). The third year of enrichment (ENR 3; September 2002 – August 2003) was not included in the analyses. Data from the pretreatment and short-term enrichment period have been previously reported (Cross 2004, Cross et al. 2006).

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Scrapers										
Epeorus sp.	340	E	C53	Pre 1	1	7	13	6	1	8
				Pre 2	34	2	6	16	8	29
				Enr 1	0	0	0	14	5	41
				Enr 2	0	0	0	4	1	8
				Enr 4	2	<1	2	28	26	94
				Enr 5	0	0	0	4	6	16
			C54	Pre 1	0	0	0	2	<1	6
				Pre 2	4	<1	1	11	11	23
				Enr 1	4	16	19	18	53	146
				Enr 2	0	0	0	32	30	127
				Enr 4	0	0	0	26	27	87
				Enr 5	4	<1	<1	12	15	41

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Baetis sp.	120	E	C53	Pre 1	0	0	0	22	0	4
				Pre 2	0	0	0	132	2	36
				Enr 1	0	0	0	66	1	16
				Enr 2	17	<1	2	94	5	70
				Enr 4	0	0	0	246	5	104
				Enr 5	33	<1	6	141	5	48
			C54	Pre 1	0	0	0	36	1	20
				Pre 2	9	<1	1	41	1	18
				Enr 1	0	0	0	23	<1	6
				Enr 2	0	0	0	77	2	25
				Enr 4	0	0	0	174	10	118
				Enr 5	0	0	0	117	3	49
Hydroptila sp.	365	T	C53	Pre 1	0	0	0	26	4	9
				Pre 2	33	6	6	48	8	16
				Enr 1	4	1	2	58	10	19
				Enr 2	33	6	7	86	14	16
				Enr 4	0	0	0	3	<1	0
				Enr 5	0	0	0	9	2	3
			C54	Pre 1	0	0	0	56	9	15
				Pre 2	1	<1	<1	123	21	30
				Enr 1	0	0	0	58	10	20
				Enr 2	0	0	0	10	2	1
				Enr 4	0	0	0	0	0	0
				Enr 5	0	0	0	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Neophylax sp.	213	T	C53	Pre 1	0	0	0	13	0	2
				Pre 2	1	0	0	9	0	1
				Enr 1	1	<1	1	11	<1	1
				Enr 2	0	0	0	40	<1	4
				Enr 4	<1	<1	<1	11	<1	7
				Enr 5	0	0	0	14	<1	2
			C54	Pre 1	0	0	0	23	<1	3
				Pre 2	1	<1	2	12	2	10
				Enr 1	2	<1	2	43	1	12
				Enr 2	1	<1	<1	171	3	28
				Enr 4	4	2	11	596	62	459
				Enr 5	40	3	28	142	11	80
Ectopria sp.	365	C	C53	Pre 1	7	2	12	26	5	25
				Pre 2	10	1	7	90	22	94
				Enr 1	6	1	3	61	16	54
				Enr 2	7	3	8	32	15	34
				Enr 4	3	2	3	23	13	40
				Enr 5	0	0	0	50	9	25
			C54	Pre 1	2	<1	2	10	2	8
				Pre 2	5	2	4	6	8	14
				Enr 1	11	<1	3	60	15	38
				Enr 2	0	0	0	15	3	8
				Enr 4	8	6	27	18	8	28
				Enr 5	18	<1	4	30	5	17

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Elmidae	365	C	C53	Pre 1	0	0	0	20	1	3
				Pre 2	33	0	1	0	0	0
				Enr 1	0	0	0	0	0	0
				Enr 2	2	<1	<1	1	<1	<1
				Enr 4	0	0	0	0	0	0
				Enr 5	2	<1	2	0	0	0
			C54	Pre 1	0	0	0	6	<1	<1
				Pre 2	9	<1	1	7	1	1
				Enr 1	0	0	0	7	<1	1
				Enr 2	11	<1	1	3	<1	<1
				Enr 4	0	0	0	4	1	2
				Enr 5	<1	<1	<1	10	2	5
Total Scrapers			C53	Pre 1	8	9	25	112	10	50
				Pre 2	112	9	19	294	39	175
				Enr 1	10	1	6	209	31	131
				Enr 2	60	8	16	257	36	131
				Enr 4	5	2	6	312	45	244
				Enr 5	35	1	8	218	21	94
			C54	Pre 1	2	0	2	133	13	52
				Pre 2	29	2	9	200	44	96
				Enr 1	17	17	24	211	79	222
				Enr 2	12	1	1	308	40	190
				Enr 4	12	7	37	818	109	694
				Enr 5	63	3	31	312	34	191

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Shredders										
Leuctra spp.	340	P	C53	Pre 1	918	49	180	30	1	3
				Pre 2	1539	73	392	49	2	9
				Enr 1	1271	41	304	119	2	17
				Enr 2	1454	55	382	395	11	106
				Enr 4	2536	66	504	27	1	8
				Enr 5	2319	64	508	46	<1	6
			C54	Pre 1	297	17	99	116	4	26
				Pre 2	750	31	120	78	5	28
				Enr 1	3847	145	1145	83	5	29
				Enr 2	1056	55	349	43	1	12
				Enr 4	386	13	88	89	1	15
				Enr 5	287	18	82	132	4	24
Tallaperla spp.	540	P	C53	Pre 1	785	92	218	306	45	143
				Pre 2	1574	127	334	251	54	160
				Enr 1	573	161	442	465	96	275
				Enr 2	613	173	360	622	112	333
				Enr 4	621	161	651	357	110	397
				Enr 5	393	79	335	329	98	434
			C54	Pre 1	239	51	81	292	37	90
				Pre 2	511	107	222	295	50	135
				Enr 1	681	171	335	258	35	91
				Enr 2	385	113	227	191	32	80
				Enr 4	188	124	487	205	122	458
				Enr 5	202	76	344	177	78	252

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Lepidostoma spp.	246	T	C53	Pre 1	196	19	144	19	<1	4
				Pre 2	592	46	387	6	<1	2
				Enr 1	607	39	381	13	1	7
				Enr 2	615	76	667	31	1	14
				Enr 4	1157	81	727	29	1	12
				Enr 5	439	42	301	1	<1	<1
			C54	Pre 1	171	6	54	26	<1	4
				Pre 2	396	74	533	9	<1	2
				Enr 1	365	109	725	16	<1	3
				Enr 2	402	83	765	65	2	25
				Enr 4	26	8	53	7	<1	5
				Enr 5	25	9	43	0	0	0
Pycnopsyche spp.	275	T	C53	Pre 1	323	63	895	47	6	94
				Pre 2	92	34	367	4	1	15
				Enr 1	318	95	706	15	<1	88
				Enr 2	263	88	680	72	1	61
				Enr 4	592	295	2349	215	13	302
				Enr 5	107	271	1458	0	0	0
			C54	Pre 1	520	137	1349	60	2	76
				Pre 2	213	192	1356	6	0	5
				Enr 1	775	557	3497	10	0.2	115
				Enr 2	567	1152	8482	44	4	111
				Enr 4	491	2053	14183	45	45	236
				Enr 5	431	3126	18390	19	10	52

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Fattigia pele	664	T	C53	Pre 1	112	158	233	0	0	0
				Pre 2	64	74	194	0	0	0
				Enr 1	95	133	306	0	0	0
				Enr 2	143	135	323	0	0	0
				Enr 4	269	273	623	0	0	0
				Enr 5	303	209	510	0	0	0
			C54	Pre 1	167	55	152	0	0	0
				Pre 2	105	111	263	0	0	0
				Enr 1	264	149	435	0	0	0
				Enr 2	136	116	336	0	0	0
				Enr 4	171	234	632	0	0	0
				Enr 5	106	253	645	1	<1	<1
Psilotreta sp.	335	T	C53	Pre 1	35	10	33	0	0	0
				Pre 2	15	7	26	0	0	0
				Enr 1	3	9	19	0	0	0
				Enr 2	5	5	20	0	0	0
				Enr 4	18	10	51	0	0	0
				Enr 5	3	9	31	0	0	0
			C54	Pre 1	2	1	3	1	<1	<1
				Pre 2	10	11	55	0	0	0
				Enr 1	16	15	77	0	0	0
				Enr 2	15	41	100	0	0	0
				Enr 4	4	27	107	0	0	0
				Enr 5	10	49	220	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Molophilus sp.	365	D	C53	Pre 1	555	64	318	1	<1	1
				Pre 2	446	60	307	0	0	0
				Enr 1	189	33	179	1	1	4
				Enr 2	207	39	237	0	0	0
				Enr 4	192	34	153	1	<1	2
				Enr 5	255	59	324	0	0	0
			C54	Pre 1	116	23	86	0	0	0
				Pre 2	199	55	223	10	<1	1
				Enr 1	327	97	455	0	0	0
				Enr 2	156	70	325	6	0	5
				Enr 4	324	179	690	0	0	0
				Enr 5	359	107	538	5	<1	<1
Tipula sp.	310	D	C53	Pre 1	31	220	961	1	7	48
				Pre 2	45	190	1018	5	10	81
				Enr 1	55	230	1406	15	47	323
				Enr 2	67	261	1583	18	38	98
				Enr 4	48	404	1663	0	0	0
				Enr 5	42	344	1346	6	8	95
			C54	Pre 1	44	134	721	3	14	109
				Pre 2	39	98	512	5	11	83
				Enr 1	63	233	1382	1	4	16
				Enr 2	75	328	1818	4	8	77
				Enr 4	39	635	2820	6	2	18
				Enr 5	36	275	1134	2	10	14

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Lipsothrix sp.	*5	D	C53	Pre 1	1	2	8	0	0	0
				Pre 2	4	6	28	0	0	0
				Enr 1	1	<1	1	0	0	0
				Enr 2	0	0	0	0	0	0
				Enr 4	10	17	86	0	0	0
				Enr 5	10	32	161	0	0	0
			C54	Pre 1	1	3	14	0	0	0
				Pre 2	0	0	0	0	0	0
				Enr 1	4	15	74	0	0	0
				Enr 2	0	0	0	0	0	0
				Enr 4	0	0	0	0	0	0
				Enr 5	<1	5	25	0	0	0
Limonia sp.	340	D	C53	Pre 1	2	1	3	0	0	0
				Pre 2	1	<1	1	0	0	0
				Enr 1	12	6	25	0	0	0
				Enr 2	9	6	24	0	0	0
				Enr 4	55	10	51	0	0	0
				Enr 5	11	3	13	0	0	0
			C54	Pre 1	9	<1	2	0	0	0
				Pre 2	0	0	0	0	0	0
				Enr 1	7	3	10	0	0	0
				Enr 2	6	6	19	10	<1	2
				Enr 4	<1	<1	<1	0	0	0
				Enr 5	<1	<1	1	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Anchytarsus sp.	1095	C	C53	Pre 1	7	3	5	2	1	2
				Pre 2	23	7	13	0	0	0
				Enr 1	4	5	1	0	0	0
				Enr 2	13	10	14	0	0	0
				Enr 4	13	15	17	0	0	0
				Enr 5	13	15	35	0	0	0
			C54	Pre 1	6	1	4	0	0	0
				Pre 2	5	8	10	0	0	0
				Enr 1	57	10	1	0	0	0
				Enr 2	8	6	8	0	0	0
				Enr 4	1	<1	<1	0	0	0
				Enr 5	8	4	5	0	0	0
Cambarus bartoni	*5	NI	C53	Pre 1	4	349	203	0	0	0
				Pre 2	8	715	415	0	0	0
				Enr 1	6	118	68	0	0	0
				Enr 2	3	119	69	0	0	0
				Enr 4	9	1160	673	0	0	0
				Enr 5	3	627	364	0	0	0
			C54	Pre 1	0	0	0	0	0	0
				Pre 2	2	249	144	0	0	0
				Enr 1	0	0	0	0	0	0
				Enr 2	0	0	0	0	0	0
				Enr 4	4	868	497	0	0	0
				Enr 5	1	3	2	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Total Shredders			C53	Pre 1	2975	681	3198	406	59	407
				Pre 2	4393	623	3434	315	67	376
				Enr 1	3127	751	3327	628	147	438
				Enr 2	3387	849	4768	1139	164	1024
				Enr 4	5520	2527	7547	629	126	721
				Enr 5	3897	1756	5386	382	107	535
			C54	Pre 1	1572	428	2636	499	59	395
				Pre 2	2229	686	3531	403	67	395
				Enr 1	6403	1504	7802	368	44	163
				Enr 2	2805	1970	12805	362	47	410
				Enr 4	1635	4120	19557	352	171	732
				Enr 5	1466	3924	21429	336	102	343
C-gatherers										
Paraleptophlebia sp.	340	E	C53	Pre 1	210	6	38	18	4	19
				Pre 2	265	15	109	11	1	8
				Enr 1	59	1	7	9	<1	1
				Enr 2	76	7	27	0	0	0
				Enr 4	243	20	84	2	<1	<1
				Enr 5	71	3	25	0	0	0
			C54	Pre 1	89	4	26	53	3	17
				Pre 2	140	12	65	86	3	27
				Enr 1	148	9	56	1	0	1
				Enr 2	142	13	56	29	1	4
				Enr 4	94	27	145	0	0	0
				Enr 5	121	23	144	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Serratella sp.	330	E	C53	Pre 1	0	0	0	165	30	174
				Pre 2	129	4	51	99	24	154
				Enr 1	33	<1	9	156	28	218
				Enr 2	4	0	1	242	32	299
				Enr 4	35	<1	8	269	123	615
				Enr 5	0	0	0	264	42	240
			C54	Pre 1	27	1	42	400	33	189
				Pre 2	61	1	10	383	93	541
				Enr 1	31	4	19	623	221	1216
				Enr 2	52	9	57	459	89	596
				Enr 4	18	2	9	211	169	751
				Enr 5	8	6	25	297	130	657
Stenonema sp.	340	E	C53	Pre 1	86	14	71	26	0	8
				Pre 2	129	21	120	1	<1	<1
				Enr 1	2	<1	3	44	11	43
				Enr 2	41	18	67	0	0	0
				Enr 4	46	33	163	2	<1	2
				Enr 5	35	36	137	0	0	0
			C54	Pre 1	17	4	25	1	<1	1
				Pre 2	9	8	37	5	1	6
				Enr 1	18	23	84	0	0	0
				Enr 2	82	53	234	6	1	4
				Enr 4	22	50	205	0	0	0
				Enr 5	25	68	273	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Amphinemura sp.	300	P	C53	Pre 1	107	1	15	441	14	99
				Pre 2	211	10	66	297	25	136
				Enr 1	49	2	16	339	12	110
				Enr 2	188	5	79	479	25	283
				Enr 4	211	16	108	476	39	255
				Enr 5	53	2	12	739	24	206
			C54	Pre 1	45	1	10	746	27	169
				Pre 2	131	8	22	959	33	254
				Enr 1	327	26	139	1298	52	474
				Enr 2	918	97	975	1101	44	488
				Enr 4	42	17	73	336	22	228
				Enr 5	41	7	44	867	37	226
Soyedina sp.	300	P	C53	Pre 1	4	0	0	0	0	0
				Pre 2	0	0	0	0	0	0
				Enr 1	5	1	7	2	<1	3
				Enr 2	48	8	51	2	1	5
				Enr 4	162	7	37	16	<1	4
				Enr 5	3	<1	3	0	0	0
			C54	Pre 1	0	0	0	1	<1	0
				Pre 2	0	0	0	0	0	0
				Enr 1	42	3	15	0	0	0
				Enr 2	53	11	54	6	1	3
				Enr 4	23	<1	1	0	0	0
				Enr 5	3	2	7	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Lype diversa	332	T	C53	Pre 1	64	4	19	0	0	0
				Pre 2	258	12	71	1	<1	0
				Enr 1	58	7	48	4	<1	<1
				Enr 2	48	5	38	3	0	1
				Enr 4	412	32	194	0	0	0
				Enr 5	297	27	165	0	0	0
			C54	Pre 1	22	5	19	0	0	0
				Pre 2	41	4	24	0	0	0
				Enr 1	34	15	66	1	<1	1
				Enr 2	113	31	163	1	<1	2
				Enr 4	120	136	755	0	0	0
				Enr 5	183	90	542	0	0	0
Chironomidae (non-Tanypodinae)	‡	D	C53	Pre 1	42495	109	1005	9164	18	293
				Pre 2	50931	134	1704	7997	18	322
				Enr 1	32079	83	1261	7422	19	333
				Enr 2	32143	98	1279	12751	35	582
				Enr 4	44882	163	1582	7845	30	377
				Enr 5	37028	120	1236	7047	15	229
			C54	Pre 1	19252	80	803	12393	30	480
				Pre 2	30658	194	1457	18214	43	854
				Enr 1	58493	204	3968	15240	44	1097
				Enr 2	41919	180	2817	11389	28	764
				Enr 4	13420	164	1065	6928	34	938
				Enr 5	19203	218	1818	16020	50	824

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Leptotarus sp.	365	D	C53	Pre 1	13	107	536	0	0	0
				Pre 2	8	104	545	0	0	0
				Enr 1	2	162	1095	0	0	0
				Enr 2	0	0	0	0	0	0
				Enr 4	5	74	200	0	0	0
				Enr 5	9	21	76	0	0	0
			C54	Pre 1	2	28	95	0	0	0
				Pre 2	1	48	435	0	0	0
				Enr 1	6	40	205	0	0	0
				Enr 2	11	71	260	0	0	0
				Enr 4	0	0	0	0	0	0
				Enr 5	2	71	202	0	0	0
Nymphomyiidae	*5	D	C53	Pre 1	0	0	0	7	0	0
				Pre 2	0	0	0	4	<1	<1
				Enr 1	0	0	0	1	0	0
				Enr 2	0	0	0	6	<1	<1
				Enr 4	5	<1	<1	10	<1	<1
				Enr 5	25	<1	<1	21	<1	<1
			C54	Pre 1	3	<1	<1	102	1	3
				Pre 2	8	<1	<1	91	1	3
				Enr 1	6	<1	<1	90	1	3
				Enr 2	0	0	0	30	<1	1
				Enr 4	1	<1	<1	9	<1	<1
				Enr 5	<1	<1	<1	5	<1	<1

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Ormosia sp.	*5	D	C53	Pre 1	4	1	3	0	0	0
				Pre 2	38	5	25	0	0	0
				Enr 1	3	3	14	0	0	0
				Enr 2	2	2	11	0	0	0
				Enr 4	2	<1	2	0	0	0
				Enr 5	3	<1	5	0	0	0
			C54	Pre 1	4	0	0	0	0	0
				Pre 2	1	0	1	0	0	0
				Enr 1	20	5	24	0	0	0
				Enr 2	3	3	13	0	0	0
				Enr 4	3	9	44	0	0	0
				Enr 5	8	6	32	0	0	0
Sciaridae	365	D	C53	Pre 1	547	11	56	3	<1	<1
				Pre 2	410	9	64	0	0	0
				Enr 1	259	8	43	0	0	0
				Enr 2	129	7	25	0	0	0
				Enr 4	185	7	34	1	<1	<1
				Enr 5	115	6	23	0	0	0
			C54	Pre 1	246	6	24	2	<1	<1
				Pre 2	302	17	71	1	<1	<1
				Enr 1	1355	33	187	1	<1	<1
				Enr 2	75	4	15	0	0	0
				Enr 4	38	3	8	0	0	0
				Enr 5	65	5	15	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Copepoda	*18	NI	C53	Pre 1	22990	23	414	659	1	13
				Pre 2	33732	34	607	390	<1	7
				Enr 1	16441	17	297	1652	2	31
				Enr 2	20665	21	372	2850	3	52
				Enr 4	35762	36	644	347	<1	6
				Enr 5	24609	25	444	385	<1	7
			C54	Pre 1	12213	12	220	199	<1	4
				Pre 2	30563	31	551	287	<1	5
				Enr 1	59365	59	1069	1050	1	18
				Enr 2	43695	44	786	420	<1	7
				Enr 4	12332	12	221	2406	2	29
				Enr 5	14325	14	258	3397	3	61
Nematoda	*5	NI	C53	Pre 1	14752	12	58	162	0	1
				Pre 2	13589	12	62	260	<1	2
				Enr 1	11117	12	62	266	<1	2
				Enr 2	10612	11	54	385	<1	2
				Enr 4	12502	11	55	253	<1	1
				Enr 5	5521	4	22	105	<1	<1
			C54	Pre 1	2053	2	9	36	<1	<1
				Pre 2	13744	15	73	169	<1	1
				Enr 1	26327	29	144	223	<1	2
				Enr 2	20510	21	106	529	1	4
				Enr 4	7851	11	56	699	1	3
				Enr 5	8976	13	66	648	<1	4

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Oligochaeta	*5	NI	C53	Pre 1	6519	80	398	350	1	5
				Pre 2	7020	102	509	119	<1	1
				Enr 1	4905	96	482	225	1	3
				Enr 2	4239	88	439	461	1	3
				Enr 4	5049	201	1006	82	<1	1
				Enr 5	5441	124	619	217	2	11
			C54	Pre 1	1805	104	519	43	<1	1
				Pre 2	6201	153	767	192	1	7
				Enr 1	15636	201	1004	111	<1	2
				Enr 2	9849	235	1174	203	1	6
				Enr 4	940	297	1483	0	0	0
				Enr 5	1959	189	945	304	1	6
Total C-gatherers			C53	Pre 1	87778	261	3572	10993	68	704
				Pre 2	106711	357	3389	9180	69	629
				Enr 1	65011	230	2248	10119	74	743
				Enr 2	68194	269	2442	17180	97	1227
				Enr 4	99503	600	4116	9303	193	1262
				Enr 5	73209	369	2767	8779	83	693
			C54	Pre 1	35777	220	1695	13976	94	863
				Pre 2	81858	442	3077	20388	175	1698
				Enr 1	161802	611	6775	18638	319	2813
				Enr 2	117410	700	6451	14173	165	1879
				Enr 4	34902	728	4066	10590	228	1950
				Enr 5	44920	713	4368	21538	223	1778

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
C-filterers										
Diplectrona modesta	332	T	C53	Pre 1	297	10	58	36	5	36
				Pre 2	430	21	187	7	3	23
				Enr 1	183	12	82	25	2	24
				Enr 2	232	15	106	10	3	20
				Enr 4	157	54	329	2	1	7
				Enr 5	49	19	71	0	0	0
			C54	Pre 1	128	18	108	62	10	48
				Pre 2	368	62	353	17	2	22
				Enr 1	517	59	320	1	1	2
				Enr 2	350	107	534	20	5	42
				Enr 4	471	331	2016	160	30	87
				Enr 5	212	139	704	68	18	93
Diplectrona metaqui	332	T	C53	Pre 1	4	1	5	1	1	1
				Pre 2	0	0	0	0	0	0
				Enr 1	1	3	8	0	0	0
				Enr 2	2	3	8	10	5	28
				Enr 4	12	14	55	0	0	0
				Enr 5	2	2	5	0	0	0
			C54	Pre 1	0	0	0	0	0	0
				Pre 2	0	0	0	0	0	0
				Enr 1	1	1	5	0	0	0
				Enr 2	0	0	0	0	0	0
				Enr 4	0	0	0	0	0	0
				Enr 5	0	0	0	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Parapsyche cardis	332	T	C53	Pre 1	68	1	20	904	135	871
				Pre 2	1	1	2	1217	157	1343
				Enr 1	1	<1	1	388	106	742
				Enr 2	1	<1	1	414	88	694
				Enr 4	2	<1	3	740	219	1619
				Enr 5	3	1	7	693	135	776
			C54	Pre 1	2	9	17	652	126	1000
				Pre 2	0	0	0	467	131	909
				Enr 1	0	0	0	404	112	759
				Enr 2	18	2	23	665	199	1422
				Enr 4	1	12	29	190	238	1497
				Enr 5	<1	<1	1	344	473	2239
Wormaldia sp. (summer cohort)	130	T	C53	Pre 1	1	<1	3	6	1	12
				Pre 2	104	2	28	15	2	22
				Enr 1	31	5	42	7	2	22
				Enr 2	17	3	58	25	2	48
				Enr 4	10	1	12	10	1	15
				Enr 5	11	3	27	32	5	64
			C54	Pre 1	27	3	35	74	15	105
				Pre 2	15	4	29	14	3	24
				Enr 1	28	10	106	88	9	113
				Enr 2	16	3	41	23	1	28
				Enr 4	2	2	5	61	6	34
				Enr 5	10	5	52	31	<1	7

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Wormaldia sp. (winter cohort)	236	T	C53	Pre 1	3	0	1	7	1	3
				Pre 2	37	3	28	21	2	11
				Enr 1	19	1	13	13	2	11
				Enr 2	80	3	47	55	5	61
				Enr 4	14	2	15	8	<1	6
				Enr 5	51	4	28	18	2	16
			C54	Pre 1	5	<1	2	36	5	31
				Pre 2	33	4	20	20	2	11
				Enr 1	106	14	94	92	5	46
				Enr 2	90	12	30	93	6	77
				Enr 4	9	8	76	2	<1	51
				Enr 5	16	6	53	14	3	29
Dolophilodes distinctus	269	T	C53	Pre 1	1	1	1	0	0	0
				Pre 2	1	3	6	0	0	0
				Enr 1	0	0	0	0	0	0
				Enr 2	0	0	0	0	0	0
				Enr 4	0	0	0	0	0	0
				Enr 5	<1	1	2	0	0	0
			C54	Pre 1	1	<1	2	1	<1	1
				Pre 2	0	0	0	0	0	0
				Enr 1	0	0	0	0	0	0
				Enr 2	0	0	0	10	6	28
				Enr 4	<1	<1	<1	0	0	0
				Enr 5	<1	<1	1	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Simuliidae	180	D	C53	Pre 1	11	<1	3	250	2	35
				Pre 2	34	<1	3	276	5	57
				Enr 1	8	<1	1	205	3	39
				Enr 2	8	<1	1	62	1	13
				Enr 4	0	0	0	676	12	115
				Enr 5	34	<1	3	505	10	113
			C54	Pre 1	0	0	0	125	2	19
				Pre 2	1	<1	<1	19	1	5
				Enr 1	38	2	16	109	4	46
				Enr 2	2	<1	2	53	3	26
				Enr 4	0	0	0	4	<1	1
				Enr 5	0	0	0	458	9	72
Dixa sp.	365	D	C53	Pre 1	332	1	6	64	0	2
				Pre 2	273	2	12	26	1	2
				Enr 1	107	2	12	208	1	5
				Enr 2	205	3	23	81	1	7
				Enr 4	81	<1	4	45	1	6
				Enr 5	178	2	9	30	<1	1
			C54	Pre 1	42	<1	2	30	<1	1
				Pre 2	50	1	4	17	<1	1
				Enr 1	152	2	13	60	<1	2
				Enr 2	40	1	5	38	<1	3
				Enr 4	22	<1	1	19	<1	3
				Enr 5	57	1	3	19	<1	<1

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Sphaeridae	280	NI	C53	Pre 1	63	4	18	0	0	0
				Pre 2	30	5	23	0	0	0
				Enr 1	23	2	20	0	0	0
				Enr 2	29	7	36	0	0	0
				Enr 4	139	11	54	0	0	0
				Enr 5	21	3	16	0	0	0
			C54	Pre 1	8	1	4	0	0	0
				Pre 2	18	2	14	0	0	0
				Enr 1	148	15	114	0	0	0
				Enr 2	106	9	70	0	0	0
				Enr 4	58	7	42	4	<1	<1
				Enr 5	24	3	9	1	<1	<1
Total C-filterers			C53	Pre 1	778	18	114	1268	145	960
				Pre 2	909	37	289	1562	169	1459
				Enr 1	373	26	177	845	115	842
				Enr 2	573	33	279	657	104	871
				Enr 4	415	84	473	1482	235	1768
				Enr 5	348	35	167	1277	153	970
			C54	Pre 1	212	32	170	979	158	1207
				Pre 2	484	72	420	554	138	972
				Enr 1	989	103	666	754	130	968
				Enr 2	621	134	706	902	220	1626
				Enr 4	562	361	2169	439	276	1674
				Enr 5	322	156	824	935	503	2439

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Invertebrate predators										
Cordulegaster sp.	1140	O	C53	Pre 1	63	99	161	0	0	0
				Pre 2	32	191	339	0	0	0
				Enr 1	86	88	158	0	0	0
				Enr 2	23	46	121	0	0	0
				Enr 4	15	51	164	0	0	0
				Enr 5	7	131	158	0	0	0
			C54	Pre 1	44	153	185	0	0	0
				Pre 2	79	151	370	0	0	0
				Enr 1	81	183	224	0	0	0
				Enr 2	27	218	223	0	0	0
				Enr 4	9	297	267	0	0	0
				Enr 5	3	48	34	0	0	0
Lanthus sp.	660	O	C53	Pre 1	42	115	277	1	0	3
				Pre 2	89	256	670	1	9	14
				Enr 1	62	185	488	0	0	0
				Enr 2	61	164	567	4	47	138
				Enr 4	176	354	903	0	0	0
				Enr 5	46	308	864	2	36	118
			C54	Pre 1	46	73	184	0	0	0
				Pre 2	159	272	674	1	<1	1
				Enr 1	65	191	439	0	0	0
				Enr 2	51	295	686	0	0	0
				Enr 4	57	332	805	0	0	0
				Enr 5	12	174	323	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Sweltsa sp.	630	P	C53	Pre 1	18	1	2	1	<1	<1
				Pre 2	138	2	6	0	0	0
				Enr 1	8	1	4	0	0	0
				Enr 2	4	2	4	0	0	0
				Enr 4	34	2	8	17	<1	1
				Enr 5	4	2	5	0	0	0
			C54	Pre 1	16	1	1	0	0	0
				Pre 2	9	2	3	0	0	0
				Enr 1	2	3	2	0	0	0
				Enr 2	1	1	1	0	0	0
				Enr 4	103	6	19	75	2	4
				Enr 5	89	10	24	5	<1	<1
Beloneuria sp.	660	P	C53	Pre 1	32	33	80	6	10	43
				Pre 2	95	77	114	5	<1	<1
				Enr 1	20	75	190	6	1	6
				Enr 2	51	130	320	38	7	37
				Enr 4	69	213	533	5	10	36
				Enr 5	195	68	204	7	3	16
			C54	Pre 1	20	38	95	51	16	82
				Pre 2	179	182	566	233	13	61
				Enr 1	58	147	428	23	17	56
				Enr 2	29	117	290	16	6	24
				Enr 4	45	37	76	36	67	105
				Enr 5	256	51	162	10	25	48

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Isoperla spp.	300	P	C53	Pre 1	310	8	81	76	7	42
				Pre 2	154	5	57	40	6	50
				Enr 1	147	8	79	36	1	15
				Enr 2	38	3	36	22	4	35
				Enr 4	177	21	163	104	27	178
				Enr 5	39	11	50	55	8	53
			C54	Pre 1	116	8	56	124	17	116
				Pre 2	149	12	36	184	27	125
				Enr 1	489	33	257	149	24	156
				Enr 2	271	51	368	188	26	198
				Enr 4	50	37	172	141	52	246
				Enr 5	118	26	178	178	44	313
Malerikus hastatus	660	P	C53	Pre 1	1	<1	<1	1	1	<1
				Pre 2	0	0	0	0	0	0
				Enr 1	0	0	0	0	0	0
				Enr 2	0	0	0	0	0	0
				Enr 4	0	0	0	0	0	0
				Enr 5	0	0	0	0	0	0
			C54	Pre 1	5	2	7	2	1	2
				Pre 2	1	1	1	10	4	8
				Enr 1	0	0	0	0	0	0
				Enr 2	0	0	0	1	1	<1
				Enr 4	0	0	0	0	0	0
				Enr 5	0	0	0	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Rhyacophila spp.	340	T	C53	Pre 1	158	12	74	20	6	49
				Pre 2	305	17	130	41	4	36
				Enr 1	47	15	103	78	4	64
				Enr 2	87	12	94	41	7	64
				Enr 4	163	36	183	71	30	209
				Enr 5	78	52	192	102	27	195
			C54	Pre 1	82	19	122	127	7	83
				Pre 2	71	36	177	122	29	178
				Enr 1	167	60	388	104	13	112
				Enr 2	176	57	424	180	20	169
				Enr 4	78	100	529	162	53	228
				Enr 5	97	57	320	124	70	299
Pseudogoera singularis	365	T	C53	Pre 1	3	1	3	131	3	29
				Pre 2	2	<1	<1	62	4	20
				Enr 1	17	<1	2	56	1	9
				Enr 2	2	<1	2	67	4	16
				Enr 4	34	<1	1	67	5	27
				Enr 5	2	<1	2	92	2	12
			C54	Pre 1	22	3	12	175	2	17
				Pre 2	14	15	43	168	9	90
				Enr 1	19	14	16	236	28	163
				Enr 2	44	23	132	274	19	101
				Enr 4	19	2	12	197	20	132
				Enr 5	6	3	10	124	10	44

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Ceratopogonidae	365	D	C53	Pre 1	4410	132	865	108	7	32
				Pre 2	7052	233	1272	110	3	16
				Enr 1	4172	144	983	105	3	25
				Enr 2	3704	154	794	89	3	23
				Enr 4	3255	156	856	58	3	18
				Enr 5	2168	99	583	90	2	20
			C54	Pre 1	1651	78	427	106	9	35
				Pre 2	3909	194	1001	73	4	21
				Enr 1	7001	290	1850	51	2	13
				Enr 2	3568	195	1055	46	4	21
				Enr 4	1650	120	608	35	1	9
				Enr 5	3471	150	806	108	9	31
Hexatoma spp.	365	D	C53	Pre 1	865	82	472	0	0	0
				Pre 2	1048	106	522	10	<1	1
				Enr 1	597	108	703	18	2	16
				Enr 2	312	46	334	1	<1	2
				Enr 4	459	96	493	8	6	27
				Enr 5	585	92	487	1	<1	<1
			C54	Pre 1	454	67	364	6	1	7
				Pre 2	823	134	799	9	<1	18
				Enr 1	1087	183	1161	10	1	10
				Enr 2	348	108	666	6	<1	2
				Enr 4	247	179	814	7	2	3
				Enr 5	290	134	652	5	2	5

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
nr. *Pedicia* sp.	340	D	C53	Pre 1	78	15	84	0	0	0
				Pre 2	78	14	92	0	0	0
				Enr 1	6	35	684	0	0	0
				Enr 2	70	30	186	0	0	0
				Enr 4	81	37	214	0	0	0
				Enr 5	85	40	299	0	0	0
			C54	Pre 1	0	0	0	0	0	0
				Pre 2	5	21	62	0	0	0
				Enr 1	14	24	130	0	0	0
				Enr 2	3	3	20	0	0	0
				Enr 4	4	27	57	0	0	0
				Enr 5	16	16	57	1	<1	<1
Pedicia sp.	365	D	C53	Pre 1	8	28	88	0	0	0
				Pre 2	8	38	14	3	<1	1
				Enr 1	10	26	84	0	0	0
				Enr 2	4	30	78	5	1	10
				Enr 4	10	50	188	0	0	0
				Enr 5	2	38	31	0	0	0
			C54	Pre 1	5	1	5	1	<1	<1
				Pre 2	5	52	172	0	0	0
				Enr 1	4	52	80	0	0	0
				Enr 2	6	50	64	0	0	0
				Enr 4	2	2	3	0	0	0
				Enr 5	6	4	9	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Dicranota spp.	310	D	C53	Pre 1	32	17	79	66	1	12
				Pre 2	277	10	86	42	1	11
				Enr 1	85	5	42	22	1	7
				Enr 2	85	4	37	91	2	25
				Enr 4	291	18	140	75	4	26
				Enr 5	134	12	91	112	1	17
			C54	Pre 1	20	3	27	27	1	8
				Pre 2	77	7	36	97	1	21
				Enr 1	216	30	163	78	2	18
				Enr 2	142	37	235	132	4	44
				Enr 4	64	33	163	65	9	32
				Enr 5	86	26	120	96	5	27
Glutops sp.	365	D	C53	Pre 1	5	13	39	0	0	0
				Pre 2	2	11	28	0	0	0
				Enr 1	2	19	32	0	0	0
				Enr 2	4	15	51	0	0	0
				Enr 4	9	39	118	0	0	0
				Enr 5	6	32	83	0	0	0
			C54	Pre 1	17	42	148	0	0	0
				Pre 2	18	56	185	0	0	0
				Enr 1	11	53	155	0	0	0
				Enr 2	20	33	147	0	0	0
				Enr 4	4	70	261	0	0	0
				Enr 5	3	30	75	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Tanypodinae	340	D	C53	Pre 1	3765	4	58	186	1	7
				Pre 2	5435	12	111	160	<1	4
				Enr 1	1585	6	50	170	1	5
				Enr 2	1449	7	51	219	1	8
				Enr 4	1526	6	53	537	2	16
				Enr 5	1263	8	48	70	<1	1
			C54	Pre 1	280	2	15	49	<1	3
				Pre 2	2038	15	97	233	1	6
				Enr 1	3057	18	125	177	1	7
				Enr 2	2477	22	143	45	<1	2
				Enr 4	1347	24	150	397	3	12
				Enr 5	1059	13	124	145	1	6
Empididae	340	D	C53	Pre 1	370	2	23	104	2	12
				Pre 2	93	1	5	108	3	21
				Enr 1	154	2	13	67	2	13
				Enr 2	90	2	12	119	1	9
				Enr 4	227	11	52	85	3	18
				Enr 5	77	4	18	190	3	19
			C54	Pre 1	79	<1	5	108	3	17
				Pre 2	156	2	16	185	5	28
				Enr 1	361	9	51	139	2	13
				Enr 2	141	7	35	75	1	7
				Enr 4	190	31	95	43	4	11
				Enr 5	424	12	59	233	2	6

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Pilaria sp.	365	D	C53	Pre 1	3	0	1	0	0	0
				Pre 2	0	0	0	0	0	0
				Enr 1	1	<1	<1	0	0	0
				Enr 2	0	0	0	0	0	0
				Enr 4	40	3	10	0	0	0
				Enr 5	4	1	5	0	0	0
			C54	Pre 1	0	0	0	0	0	0
				Pre 2	0	0	0	0	0	0
				Enr 1	0	0	0	0	0	0
				Enr 2	1	<1	<1	0	0	0
				Enr 4	3	4	11	0	0	0
				Enr 5	8	7	19	0	0	0
Pseudolimnolphila sp.	365	D	C53	Pre 1	6	4	11	1	1	3
				Pre 2	48	5	37	0	0	0
				Enr 1	89	14	81	2	<1	5
				Enr 2	153	20	102	3	<1	1
				Enr 4	34	13	45	0	0	0
				Enr 5	22	7	24	0	0	0
			C54	Pre 1	55	17	50	1	<1	<1
				Pre 2	109	41	175	0	0	0
				Enr 1	341	82	434	0	0	0
				Enr 2	339	182	676	10	<1	3
				Enr 4	20	29	93	2	<1	<1
				Enr 5	8	9	31	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Rhabdomastix sp.	*5	D	C53	Pre 1	1	0	2	0	0	0
				Pre 2	3	2	9	0	0	0
				Enr 1	1	<1	2	0	0	0
				Enr 2	0	0	0	1	<1	1
				Enr 4	2	2	8	0	0	0
				Enr 5	<1	1	6	0	0	0
			C54	Pre 1	1	<1	1	1	2	9
				Pre 2	3	8	41	0	0	0
				Enr 1	0	0	0	0	0	0
				Enr 2	7	16	82	0	0	0
				Enr 4	0	0	0	0	0	0
				Enr 5	0	0	0	0	0	0
Dolichopodidae	300	D	C53	Pre 1	10	9	33	0	0	0
				Pre 2	46	13	66	0	0	0
				Enr 1	7	6	21	0	0	0
				Enr 2	11	6	21	1	<1	3
				Enr 4	5	2	4	0	0	0
				Enr 5	26	13	68	0	0	0
			C54	Pre 1	28	2	14	0	0	0
				Pre 2	78	25	126	0	0	0
				Enr 1	63	23	83	0	0	0
				Enr 2	12	6	25	0	0	0
				Enr 4	22	4	17	0	0	0
				Enr 5	61	5	24	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Turbellaria	*5	NI	C53	Pre 1	38	0	2	34	0	2
				Pre 2	364	3	15	98	1	6
				Enr 1	49	<1	2	40	1	4
				Enr 2	46	1	4	93	1	7
				Enr 4	123	3	14	21	1	6
				Enr 5	77	1	7	29	<1	2
			C54	Pre 1	61	1	4	16	<1	1
				Pre 2	28	<1	1	20	<1	2
				Enr 1	26	<1	2	12	<1	1
				Enr 2	60	1	5	21	<1	1
				Enr 4	<1	<1	<1	0	0	0
				Enr 5	4	<1	1	0	0	0
Acari	*5	NI	C53	Pre 1	2485	7	33	1726	5	23
				Pre 2	4820	13	64	1727	5	23
				Enr 1	1937	5	26	1130	3	15
				Enr 2	2118	6	28	750	2	10
				Enr 4	1870	5	25	828	2	11
				Enr 5	1578	4	21	601	2	8
			C54	Pre 1	767	2	10	485	1	7
				Pre 2	1446	4	19	693	2	9
				Enr 1	3653	10	49	755	2	10
				Enr 2	1602	4	21	641	2	9
				Enr 4	1089	3	14	960	3	9
				Enr 5	1361	4	18	969	3	13

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Total invertebrate predators			C53	Pre 1	12703	582	2468	2461	44	257
				Pre 2	20088	1009	3635	2406	37	203
				Enr 1	9080	741	3745	1730	20	184
				Enr 2	8312	678	2839	1544	80	386
				Enr 4	8598	1118	4174	1879	93	576
				Enr 5	6398	924	3245	1352	86	462
			C54	Pre 1	3769	512	1732	1280	60	385
				Pre 2	9353	1232	4598	2026	96	567
				Enr 1	16712	1404	6036	1734	91	559
				Enr 2	9325	1426	5297	1636	83	581
				Enr 4	5004	1337	4163	2119	216	791
				Enr 5	7378	777	3045	1998	171	791
Vertebrate Predators (Salamanders)										
Eurycea sp.	365	NI	C53	Pre 1	1	5	9	0	0	0
				Pre 2	2	11	40	0	0	0
				Enr 1	1	2	3	0	0	0
				Enr 2	1	2	8	0	0	0
				Enr 4	1	6	15	0	0	0
				Enr 5	<1	3	12	0	0	0
			C54	Pre 1	2	17	24	0	0	0
				Pre 2	2	18	15	2	6	35
				Enr 1	0	0	0	0	0	0
				Enr 2	1	4	5	0	0	0
				Enr 4	0	0	0	0	0	0
				Enr 5	0	0	0	0	0	0

Appendix A continued:

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Desmognathus spp.	880	NI	C53	Pre 1	2	54	68	1	13	37
				Pre 2	3	68	120	2	47	56
				Enr 1	4	75	84	0	0	0
				Enr 2	2	55	119	0	0	0
				Enr 4	6	146	164	1	18	49
				Enr 5	3	56	110	0	0	0
			C54	Pre 1	1	53	46	2	44	171
				Pre 2	2	62	82	0	0	0
				Enr 1	2	30	38	0	0	0
				Enr 2	2	37	89	1	85	140
				Enr 4	1	15	42	0	0	0
				Enr 5	2	25	13	0	0	0
Total vertebrate predators (Salamanders)			C53	Pre 1	3	59	77	1	13	37
				Pre 2	4	79	160	2	47	56
				Enr 1	4	77	88	0	0	0
				Enr 2	2	57	128	0	0	0
				Enr 4	7	152	179	1	18	49
				Enr 5	3	59	122	0	0	0
			C54	Pre 1	3	70	70	2	44	171
				Pre 2	4	81	97	2	6	35
				Enr 1	2	30	38	0	0	0
				Enr 2	3	41	94	1	85	140
				Enr 4	1	15	42	0	0	0
				Enr 5	2	25	13	0	0	0

Appendix A continued

					Mixed Substrate			Bedrock Outcrops		
Functional group or taxon	CPI	Order	Site	Year	A	B	P	A	B	P
Other rare taxa (Not included in analyses)										
Palaeagapetus sp.	*5	T	C54	Enr 4	0	0	0	0	0	0
				Enr 5	<1	<2	3	0	0	0
Micrasema sp.	*5	T	C54	Enr 4	0	0	0	13	4	20
				Enr 5	0	0	0	20	4	19
Thaumalea sp.	*5	D	C54	Enr 4	5	<1	<1	0	0	0
				Enr 5	0	0	0	2	<1	<1
Pericoma sp.	*5	D	C54	Enr 4	5	<1	<1	0	0	0
				Enr 5	19	<1	1	0	0	0
Pyralidae	*5	L	C54	Enr 4	<1	<1	3	0	0	0
				Enr 5	<1	<1	3	2	18	90

APPENDIX B

Probabilities of change from the randomized intervention analysis (RIA) for abundance and biomass of individual taxa and functional feeding groups in mixed substrate habitat for the reference (C53) and treatment (C54) streams. RIA tests the null hypothesis of no change in abundance or biomass time series between the reference and treatment stream (Carpenter et al. 1989). Probabilities of change based on 1,000 random permutations of interstream differences. We divided the study into three time periods: pretreatment (PRE 1 and PRE 2; July 1998 – August 2000; 22 months), short-term response (ENR 1 and ENR 2; September 2000 – August 2002; 26 months), and long-term response (ENR 4 and ENR 5; September 2003 – August 2005; 24 months). The third year of enrichment (ENR 3; September 2002 – August 2003) was not included in the analyses. Short-term probabilities of change are reported elsewhere (Cross 2004). Insect orders as designated in Appendix A. Bold values indicate significant RIA. Direction of change indicated by '+' (positive) or '-' (negative). Several taxa were not tested because of insufficient sample sizes (represented by '…').

		Long vs. Pre		Long vs. Short	
		P-value	P-value	P-value	P-value
Functional group or taxon	Order	A	B	A	B
Scrapers					
Epeorus sp.	E	…	…	…	…
Baetis sp.	E	…	…	…	…
Hydroptila sp.	T	…	…	…	…
Neophylax sp.	T	…	…	…	…
Ectopria sp.	C	**(+) 0.050**	0.210	0.256	0.156
Elmidae	C	…	…	…	…
Total Scrapers		0.250	**(+) <0.0001**	0.308	**(+) 0.057**

Appendix B continued:

		Long vs. Pre		Long vs. Short	
		P-value	P-value	P-value	P-value
Functional group or taxon	Order	A	B	A	B
Shredders					
Leuctra spp.	P	**(-) 0.002**	0.384	**(-) <0.0001**	**(+) 0.001**
Tallaperla spp.	P	**(+) 0.0487**	0.891	0.086	0.888
Lepidostoma spp.	T	**(-) <0.0001**	**(-) <0.0001**	**(-) 0.005**	**(-) <0.0001**
Pycnopsyche spp.	T	0.811	**(+) <0.0001**	0.414	**(+) 0.026**
Fattigia pele	T	**(-) 0.004**	0.352	**(-) 0.005**	0.956
Psilotreta sp.	T	0.514	0.094	0.0603	0.583
Molophilus sp.	D	**(-) 0.002**	**(+) 0.002**	0.396	0.160
Tipula sp.	D	0.333	0.596	0.116	0.592
Lipsothrix sp.	D	**(-) 0.025**	**(-) 0.054**	**(-) 0.002**	**(-) 0.004**
Limonia sp.	D	**(-) 0.002**	**(-) 0.019**	**(-) 0.014**	0.147
Anchytarsus sp.	C	0.115	**(-) 0.002**	0.383	**(-) 0.036**
Cambarus bartoni	NI	0.519	0.930	0.568	0.471
Total Shredders w/ *Cambarus*		0.098	**(+) 0.011**	**(-) <0.0001**	0.252
Total Shredders w/o *Cambarus*		0.129	**(+) 0.002**	**(-) <0.0001**	0.083
C-gatherers					
Paraleptophlebia sp.	E	0.244	0.091	0.137	0.439
Serratella sp.	E	0.774	**(+) 0.052**	0.626	0.605
Stenonema sp.	E	0.063	0.077	0.544	0.947
Amphinemura sp.	P	0.689	0.425	**(-) 0.001**	**(-) 0.038**
Soyedina sp.	P	0.101	0.171	**(-) 0.033**	0.373
Lype diversa	T	0.507	**(+) 0.005**	**(-) 0.001**	**(+) 0.006**
Chironomidae (non-Tanypodinae)	D	0.562	**(+) 0.014**	**(-) <0.0001**	0.136
Leptotarus sp.	D	0.319	0.395	**(-) <0.0001**	0.918

Appendix B continued:

		Long vs. Pre		Long vs. Short	
		P-value	P-value	P-value	P-value
Functional group or taxon	Order	A	B	A	B
C-gatherers cont'd					
Nymphomyiidae	D	**(-) 0.017**	**(-) 0.015**	**(-) 0.011**	**(-) 0.018**
Ormosia sp.	D	0.567	**(+) 0.014**	0.745	0.199
Sciaridae	D	0.235	0.741	**(-) 0.016**	**(-) 0.008**
Copepoda	NI	0.104	0.095	**(-) <0.0001**	**(-) <0.0001**
Nematoda	NI	**(+) 0.015**	**(+) 0.001**	**(-) <0.0001**	**(-) 0.003**
Oligochaeta	NI	0.471	0.542	**(-) <0.0001**	0.587
Total C-gatherers		0.613	**(+) 0.010**	**(-) <0.0001**	0.251
C-filterers					
Diplectrona modesta	T	**(+) 0.007**	**(+) <0.0001**	0.870	**(+) 0.002**
Diplectrona metaqui	T	…	…	…	…
Parapsyche cardis	T	0.510	0.725	0.361	0.429
Wormaldia sp. (summer cohort)	T	0.501	0.775	0.676	0.743
Wormaldia sp. (winter cohort)	T	0.507	0.175	**(-) 0.039**	0.153
Dolophilodes distinctus	T	…	…	…	…
Simuliidae	D	0.621	0.308	…	**(+) 0.038**
Dixa sp.	D	0.227	0.963	0.674	0.880
Sphaeridae	NI	0.901	0.769	**(-) 0.002**	**(+) 0.060**
Total C-filterers		**(+) 0.010**	**(+) <0.0001**	0.226	**(+) 0.019**
Total primary consumers w/ crayfish		0.596	**(+) 0.003**	**(-) <0.0001**	0.299
Total primary consumers w/o crayfish		0.604	**(+) <0.0001**	**(-) <0.0001**	0.131

Appendix B continued:

		Long vs. Pre		Long vs. Short	
		P-value	P-value	P-value	P-value
Functional group or taxon	Order	A	B	A	B
Invertebrate predators					
Cordulegaster sp.	O	0.894	0.294	0.379	0.461
Lanthus sp.	O	0.080	0.493	**(-) 0.046**	0.217
Sweltsa sp.	P	**(+) 0.004**	**(+) 0.008**	**(+) 0.010**	**(+) 0.034**
Beloneuria sp.	P	0.998	**(-) 0.006**	0.954	**(-) 0.01**
Isoperla spp.	P	0.252	0.195	**(-) 0.002**	0.104
Malerikus hastatus	P	…	…	…	…
Rhyacophila spp.	T	0.244	0.334	**(-) 0.002**	0.738
Pseudogoera singularis	T	0.441	0.140	0.386	**(-) 0.059**
Ceratopognidae	D	**(+) 0.004**	0.128	0.211	**(-) 0.049**
Hexatoma spp.	D	0.700	**(+) 0.057**	**(-) 0.022**	0.872
nr. *Pedicia* sp.	D	0.808	0.397	0.192	0.947
Pedicia sp.	D	0.800	0.477	0.903	0.122
Dicranota spp.	D	0.847	**(+) 0.012**	**(-) 0.015**	0.230
Glutops sp.	D	**(-) <0.0001**	0.505	**(-) <0.0001**	0.791
Tanypodinae	D	**(+) 0.001**	**(+) 0.007**	**(-) 0.002**	0.645
Empididae	D	0.107	**(+) 0.022**	0.730	0.153
Pilaria sp.	D	0.719	0.561	0.472	0.547
Pseudolimnolphila sp.	D	**(-) 0.039**	0.210	**(-) <0.0001**	**(-) 0.004**
Rhabdomastix sp.	D	0.604	0.158	**(-) 0.051**	0.999
Dolichopodidae	D	0.941	0.638	0.946	0.791
Turbellaria	NI	0.872	0.310	0.536	0.071
Acari	NI	**(+) 0.008**	**(+) 0.008**	0.302	0.256
Total invertebrate predators		**(+) 0.001**	0.963	**(-) 0.006**	**(-) 0.001**
Total invertebrate consumers w/ crayfish		0.947	(+) 0.008	**(-) < 0.0001**	0.699
Total invertebrate consumers w/o crayfish		0.944	(+) 0.002	**(-) < 0.0001**	0.457

APPENDIX C

Probabilities of change from the randomized intervention analysis (RIA) for abundance and biomass of individual taxa and functional feeding groups in bedrock outcrop habitat for the reference (C53) and treatment (C54) streams. RIA tests the null hypothesis of no change in abundance or biomass time series between the reference and treatment stream (Carpenter et al. 1989). Probabilities of change based on 1,000 random permutations of interstream differences. We divided the study into three time periods: pretreatment (PRE 1 and PRE 2; July 1998 – August 2000; 22 months), short-term response (ENR 1 and ENR 2; September 2000 – August 2002; 26 months), and long-term response (ENR 4 and ENR 5; September 2003 – August 2005; 24 months). The third year of enrichment (ENR 3; September 2002 – August 2003) was not included in the analyses. Short-term probabilities of change are reported elsewhere (Cross 2004). Insect orders as designated in Appendix A. Bold values indicate significant RIA. Direction of change indicated by '+' (positive) or '-' (negative). Several taxa were not tested because of insufficient sample sizes (represented by '…').

		Long vs. Pre		Long vs. Short	
		P-value	P-value	P-value	P-value
Functional group or taxon	Order	A	B	A	B
Scrapers					
Epeorus sp.	E	**(+) 0.003**	0.167	0.626	0.099
Baetis sp.	E	0.792	0.857	0.586	0.692
Hydroptila sp.	T	0.132	0.126	0.340	0.371
Neophylax sp.	T	**(+) <0.0001**	**(+) <0.0001**	**(+) 0.021**	**(+) <0.0001**
Ectopria sp.	C	0.124	0.233	0.998	0.432
Elmidae	C	…	…	…	…
Total Scrapers		0.101	**(+) 0.032**	0.223	0.614

Appendix C continued:

		Long vs. Pre		Long vs. Short	
		P-value	P-value	P-value	P-value
Functional group or taxon	Order	A	B	A	B
Shredders					
Leuctra spp.	P	0.917	0.453	**(+) 0.010**	0.085
Tallaperla spp.	P	0.424	0.688	**(+) 0.021**	**(+) 0.049**
Lepidostoma spp.	T	0.285	0.546	0.101	0.584
Pycnopsyche spp.	T	0.224	**(+) 0.002**	**(-) 0.045**	**(+) 0.004**
Fattigia pele	T	…	…	…	…
Psilotreta sp.	T	…	…	…	…
Molophilus sp.	D	…	…	…	…
Tipula sp.	D	0.628	0.753	**(+) 0.014**	**(+) 0.009**
Lipsothrix sp.	D	…	…	…	…
Limonia sp.	D	…	…	…	…
Anchytarsus sp.	C	…	…	…	…
Cambarus bartoni	NI	…	…	…	…
Total Shredders w/ *Cambarus*		0.617	0.406	**(+) <0.0001**	**(+) 0.003**
Total Shredders w/o *Cambarus*		0.617	0.406	**(+) <0.0001**	**(+) 0.003**
C-gatherers					
Paraleptophlebia sp.	E	**(-) 0.017**	0.999	0.594	0.472
Serratella sp.	E	**(-) 0.018**	0.773	**(-) 0.019**	0.190
Stenonema sp.	E	…	…	…	…
Amphinemura sp.	P	0.148	0.869	**(-) 0.009**	0.072
Soyedina sp.	P	…	…	…	…
Lype diversa	T	…	…	…	…
Chironomidae (non-Tanypodinae)	D	0.524	0.987	0.838	0.363
Leptotarus sp.	D	…	…	…	…

Appendix C continued:

		Long vs. Pre		Long vs. Short	
		P-value	P-value	P-value	P-value
Functional group or taxon	Order	A	B	A	B
C-gatherers cont'd					
Nymphomyiidae	D	**(-) <0.0001**	**(-) <0.0001**	**(-) 0.002**	**(-) 0.005**
Ormosia sp.	D	…	…	…	…
Sciaridae	D	…	…	…	…
Copepoda	NI	**(+) <0.0001**	**(+) <0.0001**	**(+) <0.0001**	**(+) <0.0001**
Nematoda	NI	**(+) <0.0001**	**(+) <0.0001**	**(+) 0.001**	**(+) 0.002**
Oligochaeta	NI	0.378	0.460	0.277	0.425
Total C-gatherers		0.979	0.845	0.344	0.195
C-filterers					
Diplectrona modesta	T	0.094	**(+) 0.009**	**(+) <0.0001**	**(+) 0.001**
Diplectrona metaqui	T	…	…	…	…
Parapsyche cardis	T	0.296	**(+) 0.031**	0.556	0.078
Wormaldia sp. (summer cohort)	T	0.685	0.346	0.476	0.377
Wormaldia sp. (winter cohort)	T	0.372	0.602	0.303	0.841
Dolophilodes distinctus	T	…	…	…	…
Simuliidae	D	0.459	0.430	0.137	0.078
Dixa sp.	D	0.378	0.098	0.296	0.158
Sphaeridae	NI	…	…	…	…
Total C-filterers		0.315	**(+) 0.017**	0.312	0.060
Total primary consumers		0.953	**(+) 0.012**	0.338	**(+) 0.036**

Appendix C continued:

		Long vs. Pre		Long vs. Short	
		P-value	P-value	P-value	P-value
Functional group or taxon	Order	A	B	A	B
Invertebrate predators					
Cordulegaster sp.	O	…	…	…	…
Lanthus sp.	O	…	…	…	…
Sweltsa sp.	P	…	…	…	…
Beloneuria sp.	P	0.176	**(+) 0.026**	0.211	**(+) 0.018**
Isoperla spp.	P	0.928	0.346	0.333	0.449
Malerikus hastatus	P	…	…	…	…
Rhyacophila spp.	T	0.369	0.447	0.506	0.334
Pseudogoera singularis	T	0.716	0.064	**(-) 0.040**	0.448
Ceratopognidae	D	0.814	0.894	0.43	0.645
Hexatoma spp.	D	0.338	0.879	0.494	0.948
nr. *Pedicia* sp.	D	…	…	…	…
Pedicia sp.	D	…	…	…	…
Dicranota spp.	D	0.712	0.113	**(-) 0.042**	0.309
Glutops sp.	D	…	…	…	…
Tanypodinae	D	0.663	0.286	0.992	0.224
Empididae	D	0.663	0.205	0.740	0.450
Pilaria sp.	D	…	…	…	…
Pseudolimnolphila sp.	D	…	…	…	…
Rhabdomastix sp.	D	…	…	…	…
Dolichopodidae	D	…	…	…	…
Turbellaria	NI	0.873	0.480	0.262	0.355
Acari	NI	**(+) <0.0001**	**(+) <0.0001**	**(+) 0.035**	**(+) 0.041**
Total invertebrate predators		**(+) 0.024**	0.235	0.473	0.141
Total invertebrate consumers		0.883	**(+) 0.008**	0.313	**(+) 0.023**

APPENDIX D

Average biomass and abundance of arboreal spider families collected during 5-minute beat sampling. Values represent averages of 5 replicate samples collected along the reference (C53) and treatment (C54) streams on each sampling date. Distance represents the lateral distance from the stream margin. Arboreal spiders were sampled semi-weekly. Spiders at 25m from the stream margin were not sampled during the pretreatment isotopic enrichment period (PRE).

			Biomass (mg beat sample^{-1})				Abundance (no. beat sample^{-1})			
		Distance	Week				Week			
WS	Spider Family	(m)	PRE	2	4	6	PRE	2	4	6
53	Pisauridae	0	0	0.08	0	0	0	0.2	0	0
		10	0	3.75	1.32	0	0	0.2	0.4	0
		25	ND	0.06	0	0	ND	0.8	0	0
53	Thomisidae	0	0	0	0	0	0	0	0	0
		10	0	1.17	0.17	3.25	0	0.2	0.2	0.6
		25	ND	0	0.43	0	ND	0	0.2	0
53	Anyphaenidae	0	0.6	3.05	1.06	0.69	1.2	6	1.4	0.8
		10	0.92	0.5	2.42	2.96	2	1.2	2.4	3
		25	ND	1.06	0.97	1.53	ND	1.8	1	1.6
53	Uloboridae	0	0	0	0.27	0.02	0	0	0.2	0.2
		10	0	0	0.02	0.04	0	0	0.2	0.4
		25	ND	0	0	0.03	ND	0	0	0.4
53	Tetragnathidae	0	0.54	0.13	3.58	3.01	0.8	0.2	1	0.4
		10	1.13	1.62	1.47	1.61	1	1.2	1.2	0.8
		25	ND	1.07	1.7	3.12	ND	0.8	0.8	0.6

Appendix D continued:

			Biomass (mg beat sample^{-1})				Abundance (no. beat sample^{-1})			
			Week				Week			
WS	Spider Family	Distance (m)	PRE	2	4	6	PRE	2	4	6
53	Hahniidae	0	0	0	0	0	0	0	0	0
		10	0	0.01	0	0	0	0.2	0	0
		25	ND	0	0	0	ND	0	0	0
53	Dicytinidae	0	0	0.15	0	0.18	0	0.2	0	0.2
		10	0	0	0.32	0.18	0	0	0.4	0.2
		25	ND	0	0	0.31	ND	0	0	0.4
53	Theridae	0	0	0.41	0	0.15	0	0.4	0	0.2
		10	0	0	0.23	1.6	0	0	0.2	1
		25	ND	0.32	0.52	1.47	ND	0.2	0.4	0.4
53	Salticidae	0	0	1.14	0	0	0	0.2	0	0
		10	0.3	0	0.82	0.89	0.2	0	0.2	0.2
		25	ND	0.11	0.27	0.77	ND	0.2	0.2	0.4
53	Corinnidae	0	0	0	0	0	0	0	0	0
		10	2.07	0	0	0	0.2	0	0	0
		25	ND	0	0	3.51	ND	0	0	0.2
53	Liocranidae	0	0	0	0.7	0	0	0	0.2	0
		10	0	0	0	0	0	0	0	0
		25	ND	0	0	0.65	ND	0	0	0.2

Appendix D continued:

			Biomass (mg beat sample^{-1})				Abundance (no. beat sample^{-1})			
			Week				Week			
WS	Spider Family	Distance (m)	PRE	2	4	6	PRE	2	4	6
53	Unknown A	0	0	0.12	0.3	0	0	0.2	0.6	0
		10	0	0	0.29	0.12	0	0	0.4	0.2
		25	ND	0.12	0	0	ND	0.2	0	0
53	Araneidae	0	3.21	0.45	4.58	7.41	0.8	1.6	1	0.8
		10	1.71	1.59	1.98	0.7	1	2.4	3.2	2.4
		25	ND	0.54	1.63	0.98	ND	3	1.4	1.6
53	Linyphiidae	0	0	0.21	0.05	0.1	0	1.2	0.6	0.6
		10	0.1	0.43	0.21	0.06	0.4	1.2	1.4	0.2
		25	ND	0.17	0.35	0.12	ND	2	1.4	0.4
54	Pisauridae	0	0	0.59	1.04	0.87	0	0.2	0.8	0.6
		10	0	0	0	0	0	0	0	0
		25	ND	0	0	0.78	ND	0	0	0.2
54	Thomisidae	0	0.07	0	0	0	0.2	0	0	0
		10	0	0	0.23	0	0	0	0.2	0
		25	ND	0.2	0	0.92	ND	0.2	0	0.6
54	Anyphaenidae	0	1.39	2	0.29	0.55	3.2	4.6	0.6	0.6
		10	0.9	0.65	2.11	1.56	2.2	1.2	2.8	1.8
		25	ND	0.2	0.4	0.81	ND	0.4	0.6	0.8

Appendix D continued:

			Biomass (mg beat sample^{-1})				Abundance (no. beat sample^{-1})			
			Week				Week			
WS	Spider Family	Distance (m)	PRE	2	4	6	PRE	2	4	6
54	Uloboridae	0	0	0	0	0	0	0	0	0
		10	0	0	0	0.48	0	0	0	0.6
		25	ND	0	0	0.03	ND	0	0	0.4
54	Tetragnathidae	0	1	0.96	7.56	4.97	0.8	0.4	2.6	0.8
		10	0.61	0.9	2.86	2.59	1	0.2	1	0.6
		25	ND	0.24	1.32	5.26	ND	0.4	0.8	0.6
54	Dicytinidae	0	0	0	0	0	0	0	0	0
		10	0	0	0	0.5	0	0	0	0.4
		25	ND	0	0	0.45	ND	0	0	0.8
54	Theridae	0	0	0	0.24	0	0	0	0.2	0
		10	0	0	0.06	0.43	0	0	0.2	0.2
		25	ND	0	0.46	1.1	ND	0	0.6	0.6
54	Salticidae	0	0.46	0.42	0.32	0	0.4	0.6	0.2	0
		10	0.12	0.76	0	0	0.2	0.4	0	0
		25	ND	1.02	0	0.4	ND	0.4	0	0.2
54	Mysmenidae	0	0	0	0.09	0	0	0	0.2	0
		10	0	0	0	0	0	0	0	0
		25	ND	0	0	0	ND	0	0	0

Appendix D continued:

			Biomass (mg beat sample^{-1})				Abundance (no. beat sample^{-1})			
			Week				Week			
WS	Spider Family	Distance (m)	PRE	2	4	6	PRE	2	4	6
54	Unknown A	0	0.15	0	0	0	0.2	0	0	0
		10	0.08	0	0	0.27	0.2	0	0	0.2
		25	ND	0.06	0	0	ND	0.2	0	0
54	Unknown B	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0
		25	ND	0	0	0.19	ND	0	0	0.2
54	Araneidae	0	4.06	1.47	0.31	7.54	1.6	0.6	1.4	1.2
		10	0.04	0.34	1.19	0.05	0.2	1.2	0.8	0.4
		25	ND	2.42	1.63	1.99	ND	1	2.8	3.6
54	Linyphiidae	0	0	0.01	0.18	0.06	0	0.2	0.4	0.2
		10	0	0.01	0	0.09	0	0.2	0	0.4
		25	ND	0.04	0.18	0	ND	0.4	0.4	0

APPENDIX E

Average biomass and abundance of ground spider families collected weekly with pitfall traps. Values represent average of five replicates collected along the reference (C53) and treatment (C54) streams on each sampling date.

			Biomass (mg pitfall^{-1})							Abundance (no. pitfall^{-1})						
		Distance	Week							Week						
WS	Spider Family	(m)	PRE	1	2	3	4	5	6	PRE	1	2	3	4	5	6
53	Thomisidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	2.67	0.76	0	0	0	0	6.38	0.2	0.2	0	0	0	0	0.4
		25	3.45	0	3.5	0	2.76	0	2.32	0.2	0	0.2	0	0.2	0	0.2
53	Anyphaenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0	0.09	0	0	0	0	0	0	0.2	0	0	0
53	Gnaphosidae	0	0	0	0	1.94	0.78	0	0	0	0	0	0.4	0.2	0	0
		10	0	1.24	3.09	3.27	7.24	0	0.66	0	0.2	0.6	0.8	1.2	0	0.2
		25	0	0	6.5	3.91	4.19	0.62	1.6	0	0	1.2	0.6	1	0.2	0.2
53	Lycosidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	2.28	2.02	3.59	0	12.7	0	0	0.2	0.2	0.4	0	1
		25	4.17	1.3	0.99	7.51	8.5	0	0	0.4	0.2	0.2	0.6	0.6	0	0
53	Tetragnathidae	0	0	0	0.25	0.56	0	0	0	0	0	0.2	0.2	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	Amaurobiidae	0	2.08	0.03	0	1.81	0	0	2.17	0.2	0.2	0	0.2	0	0	0.2
		10	5.12	6.12	0	0	0	0.11	0.31	0.6	0.6	0	0	0	0.2	0.4
		25	2.04	0.21	1.69	0	0.12	0.12	0.07	0.2	0.2	0.2	0	0.4	0.2	0.2

Appendix E continued:

			Biomass (mg pitfall^{-1})							Abundance (no. pitfall^{-1})						
		Distance	Week							Week						
WS	Spider Family	(m)	PRE	1	2	3	4	5	6	PRE	1	2	3	4	5	6
53	Hahniidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0.12	0	0	0	0.16	0	0	0.2	0	0	0	0.2	0	0
53	Dictynidae	0	0	0	0.14	0	0	0	0	0	0	0.2	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0.32	0	0	0	0	0	0	0.2	0	0	0	0
53	Theridiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0.08	0	0	0	0	0	0	0.2	0	0	0	0	0	0
		25	0.09	0	0	0	0	0	0	0.2	0	0	0	0	0	0
53	Salticidae	0	0.39	0	0	0	0	0	0	0.2	0	0	0	0	0	0
		10	3.2	1.48	1.25	0	0	0	0	0.4	0.4	0.4	0	0	0	0
		25	0.05	0	0	0	0	0	0	0.2	0	0	0	0	0	0
53	Atypidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0	0	4.45	0	0	0	0	0	0	0.2	0	0
53	Corinnidae	0	0	0	0	0	0.06	0	0	0	0	0	0	0.2	0	0
		10	0	0	0	0	0.05	0	0	0	0	0	0	0.2	0	0
		25	0	0	0	0	0	0.18	0	0	0	0	0	0	0.4	0

Appendix E continued:

			Biomass (mg pitfall^{-1})							Abundance (no. pitfall^{-1})						
		Distance	Week							Week						
WS	Spider Family	(m)	PRE	1	2	3	4	5	6	PRE	1	2	3	4	5	6
53	Ctenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0	0	1.46	0	0.96	0	0	0	0	0.2	0	0.2
53	Liocranidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0.05	0	0	0.15	0	0.06	0	0.2	0	0	0.2	0	0.2
53	Tengellidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	2.76	0	0	0	0	0	0	0.2	0	0	0	0	0	0
53	Unknown	0	0	0	0.03	0	0	0	0	0	0	0.4	0	0	0	0
		10	0	0	0.01	0	0	0	0	0	0	0.2	0	0	0	0
		25	0	0	0	0	0.03	0	0	0.2	0	0	0	0.2	0	0
53	Linyphiidae	0	0.27	0.05	0.01	0	0	0	0	0.4	0.2	0.2	0	0	0	0
		10	0.19	0	0.01	0.95	0.01	0.15	0.11	1.2	0	0.2	0.4	0.2	0.4	0.4
		25	0.11	0.05	0.06	0.09	0.01	0	0	0.6	0.2	0.4	0.4	0.2	0	0
54	Pisauridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	1.91	0	0	0	0	0	0	0.2	0	0	0
		25	0	17.78	3.73	0	0	0	0	0	0.2	0.4	0	0	0	0

Appendix E continued:

			Biomass (mg pitfall^{-1})							Abundance (no. pitfall^{-1})						
		Distance	Week							Week						
WS	Spider Family	(m)	PRE	1	2	3	4	5	6	PRE	1	2	3	4	5	6
54	Thomisidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	1.6	0	0.63	0	0.73	0	0	0.2	0	0.2	0	0.2	0	0
54	Anyphaenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	4.51	0	0	0	0	0	0	0.2	0	0	0	0	0	0
		25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	Gnaphosidae	0	0	0	0	0	1.08	0	0	0	0	0	0	0.2	0	0
		10	0	0	0.91	0	5.24	0	0.92	0	0	0.2	0	1.2	0	0.2
		25	0	0	1.64	1.45	4.31	0	1.26	0	0	0.2	0.4	1	0	0.4
54	Lycosidae	0	0.22	0	0	0	0	0	0.52	0.2	0	0	0	0	0	0.2
		10	0	1.28	0	0	3.16	0	5.19	0	0.2	0	0	0.4	0	0.6
		25	0	0	2.12	0	17	2.58	10.53	0	0	0.2	0	3	0.6	2
54	Amaurobiidae	0	0.16	0	0.35	0.19	0	0	0.19	0.2	0	0.4	0.2	0	0	0.2
		10	27.14	8.06	5.79	0	3.41	0	0.15	1.6	0.8	0.8	0	0.4	0	0.2
		25	1.23	0	2.05	2.62	2.68	0	4.36	0.2	0	0.8	0.4	0.4	0	0.6
54	Hahniidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0.94	0	0.12	0.12	0	0	0	0.8	0	0.2	0.2	0	0	0
		25	0.45	0.48	0.12	0.48	0	0.14	0.1	0.8	0.6	0.2	0.8	0	0.2	0.2

Appendix E continued:

			Biomass (mg pitfall^{-1})							Abundance (no. pitfall^{-1})						
		Distance	Week							Week						
WS	Spider Family	(m)	PRE	1	2	3	4	5	6	PRE	1	2	3	4	5	6
54	Dictynidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0.95	1.34	0	0	0	0	0	0.2	0.2	0	0	0	0
		25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	Salticidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0.24	0	0.44	0	0.14	0	0	0.2	0	0.2	0	0.2	0	0
		25	0.51	0	0.36	0	0	0	0	0.4	0	0.2	0	0	0	0
54	Antrodiaetidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0	0	0	0.2	0	0	0	0	0	0	0.2	0
54	Corinnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0.02	0	0	0	0	0	0	0.2	0
		25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	Ctenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	2.42	0	3.01	0	0	0	0	0.4	0	0.4
		25	0	0	0	0	2.81	0	0	0	0	0	0	0.4	0	0
54	Cybaeiidae	0	0	0	0.32	0	0	0	0	0	0	0.4	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0	0	0.21	0	0	0	0	0	0	0.2	0	0

Appendix E continued:

			Biomass (mg pitfall^{-1})							Abundance (no. pitfall^{-1})						
		Distance	Week							Week						
WS	Spider Family	(m)	PRE	1	2	3	4	5	6	PRE	1	2	3	4	5	6
54	Liocranidae	0	0	0	0	0	0	0	0.05	0	0	0	0	0	0	0.2
		10	0	0	0	0	0	0	0.07	0	0	0	0	0	0	0.2
		25	0	0	0	0	0.01	0	0.15	0	0	0	0	0.2	0	0.6
54	Tengellidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	1.6	0	0	0	0	0	0	0.2	0	0	0	0
		25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	Araneidae	0	0	0	0	0	0.06	0	0	0	0	0	0	0.2	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0.12	0	0	0	0	0	0	0.2	0	0	0	0
54	Linyphiidae	0	0.04	0.04	0.03	0.07	0.03	0	0	0.2	0.2	0.4	0.2	0.6	0	0
		10	0.14	0.18	0.05	0.04	0.05	0	0.06	0.8	0.8	0.4	0.4	0.6	0	0.2
		25	0.32	0.02	0.05	0.16	0.06	0	0	2	0.6	0.2	0.6	0.4	0	0