HEADWATER STREAM SALAMANDER RESPONSES TO EXPERIMENTAL GRADIENTS OF NUTRIENT ENRICHMENT

by

PHILLIP MARTIN BUMPERS

(Under the Direction of Amy D. Rosemond)

ABSTRACT

Our current understanding of responses of predators to nutrient enrichment of nitrogen (N) and phosphorus (P) is limited. Here, I investigated responses of larval salamanders (*Desmognathus quadramaculatus, Eurycea wilderae*), vertebrate predators in forested headwater streams, to an experimental gradient of N and P enrichment, for two years. Salamander growth rates were stimulated by added nutrients and were positively related to P, but not N concentration. *D. quadramaculatus* increased consumption of prey biomass during enrichment, which was surprisingly driven by an increased proportion of biofilm consuming macroinvertebrates. No change was detected in *E. wilderae* prey biomass or composition. Investigation of threshold elemental ratios indicated that larval salamanders are potentially highly-P limited, but may also be limited by food quantity. Results of this study indicate that P enrichment can propagate through the food web in detritus-based streams and that unexpected food web pathways can emerge as a result of nutrient enrichment.

INDEX WORDS: Salamanders, nutrient enrichment, phosphorus, *Desmognathus quadramaculatus,* bottom-up, top-predator, *Eurycea wilderae,* stoichiometry, growth response, diet, TER

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PHILLIP MARTIN BUMPERS

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PHILLIP MARTIN BUMPERS

Major Professor:	Amy D. Rosemond
Committee:	Jonathan P. Benstead
	Mary Freeman
	John C. Maerz

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
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DEDICATION

To my dad, who taught me at an early age to love the natural world and made sure I intimately knew the streams of the Arkansas Ozarks. And to my grandfathers, who both instilled in me their work ethics and appreciation of nature. Their influences have and will continue to shape me.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Human activities (e.g., burning of fossil fuels, fertilizer application, sewage effluent) have greatly increased the availability of limiting nutrients (primarily nitrogen (N) and phosphorus (P)) in aquatic systems worldwide (Vitousek et al. 1997; Carpenter et al. 1998; Smith and Schindler 2009). Because N and/or P concentration generally limits primary productivity, algal blooms often occur in autotrophic systems, which can lead to dead zones (Smith and Schindler 2009). As a result, regulatory government agencies called upon aquatic resource managers to develop nutrient criteria to help restore and maintain ecological integrity and social benefits of streams (US EPA 1998). Despite this call, the most common impairment to streams and rivers in the U.S. is enrichment of N and P (US EPA 2013). Progress on the development of criteria has been slow, partly due to the complex responses that often result from nutrient enrichment.

The effects of nutrient enrichment on detritus-based systems are less understood than that of algal-based streams, but recent research has revealed profound effects are possible (Cross et al. 2006; Johnson et al. 2006; Benstead et al. 2009). For example, nutrient enrichment reduces detrital standing stocks yet can increase the quality (C:N:P), which has implications for consumer-resource imbalances (Cross et al. 2003; Suberkropp et al. 2010). Most of the research in detritus systems, however, has been conducted in single streams, testing only one concentration and ratio of N and P. Yet, we know that different land uses can produce runoff

with differing concentrations and ratios of N and P (Downing and McCauley 1992), which may create different responses in stream ecosystems, further complicating the development of numerical criteria. Consequently, in order to improve our predictive ability and create effective policy, we must better understand how N and P control ecosystem function and affect food web dynamics.

Developing effective nutrient policy will require understanding the mechanisms by which N and P enrichment affects all levels of the food web (Dodds 2007; Jarvie et al. 2013). The degree to which nutrient enrichment can propagate up food webs and affect higher-level consumers is poorly understood. In both algal and detritus-based streams, top consumers have been shown to respond to nutrient enrichment (Peterson et al. 1993; Johnson et al. 2006). However, other studies have shown that nutrient enrichment has weak indirect effects on top consumers (Borer et al. 2006), indicating the necessity to better understand the roles that N and P play in regulating predator responses, and the conditions by which nutrient enrichment may propagate up food webs.

Larval salamanders are vertebrate predators in many headwater streams in the eastern U.S (Hall et al. 2000; Davic and Welsh 2004). In high gradient streams in which fish don't persist, larval salamanders are the only vertebrate predators in stream systems. Salamanders play critical roles in shaping stream structure and function, regulating macroinvertebrate abundance and community structure (Keitzer and Goforth 2013a), as well as, contributing to nutrient cycling and retention in headwater streams (Milanovich et al. in Milanovich 2010; Keitzer and Goforth 2013b). Moreover, salamanders have a bi-phasic lifecycle, spending time as larvae in streams and the rest of their lives as adults in the terrestrial-aquatic interface, becoming important components of terrestrial and riparian ecosystems (Davic and Welsh 2004). Therefore,

assessing the effects that enrichment has on larval salamanders will aid in understanding how stream (and riparian) ecosystem structure and function may be altered.

Project Overview

The studies in this thesis were part of a larger collaborative project between three universities (University of Georgia, University of Alabama, and Coastal Carolina University) with the overarching objective to determine the responses of heterotrophic streams to a gradient of dissolved N:P ratio and N and P concentration. Two previous nutrient enrichment experiments of a detritus-based stream (at N:P=16:1) showed that detrital quality increases via microbially mediated increases in nutrient content (Cross et al. 2003; Gulis and Suberkropp 2003), which also stimulated breakdown of detrital carbon (Suberkropp et al. 2010). The quality effects on detritus lead to increased production of macroinvertebrates (Cross et al. 2006), which presumably was responsible for the stimulated growth of a larval salamander species (Johnson et al. 2006). These responses led us to question if and how streams differed in their response to differences in the delivery (ratio and concentration) of nutrients, given that landuses can vary in their delivery of nutrients to streams (Dodds and Oakes 2004). Specifically, this thesis focuses on the response of larval salamanders to gradients in N:P ratio and concentration. Further, we aimed to determine the degree to which larval salamander growth is nutrient limited, and whether, enrichment alleviates the stoichiometric constraints they face.

To address our questions we experimentally enriched five headwater streams at the Coweeta Hydrologic Laboratory, a Long-Term Ecological Research site, in Macon County, NC, USA. Coweeta is a 2185-ha heavily forested basin comprised of mixed hardwoods and a dense understory of *Rhododendron maximum* shades the majority of streams. Five streams were

selected in the 559-ha Dryman's fork watershed as study sites and were similar physically and chemically before enrichment. These streams were also advantageous study sites because they were very close in proximity (< 0.5 km apart). Beginning in July 2011 the five streams were continuously enriched along 70-meter treatment reaches for two years (ending July 2013), each receiving different concentrations of N and P such that a gradient of N:P ratios was created. This design allowed us to test the relative importance of N and P in creating responses of stream ecosystem structure and function. To test the objectives of this thesis, we employed a number of methods to assess the effects of enrichment on larval salamanders.

Overview of Thesis Chapters

The overarching goal of this research was to quantify the effects of nutrient enrichment on larval salamanders and mechanistically assess drivers behind observed responses. Our goals were three fold: 1) To measure the growth responses of salamanders to enrichment and determine which nutrient (N or P) was most important in elucidating those responses, 2) to determine potential pathways affecting observed growth responses by quantifying changes in salamander diets, and 3) to develop models predicting the elemental limitations salamanders may face as a predictive tool and to further understand observed growth responses.

Chapter 2 of this thesis addresses the first objective by measuring larval growth rates of two species of larval salamanders in enriched and reference conditions. Results from a previous enrichment experiment indicated that larval salamanders responded positively in terms of growth to nutrient enrichment (Johnson et al. 2006). This chapter aimed to determine if this was a consistently obtainable result and if N or P better explained increased growth rates. We found that both species of larval salamanders had increased growth rates in response to enrichment that

were positively related to the amount of phosphorus added to the streams. Results of this chapter indicate that salamanders respond to relaxation of ecosystem-level P limitation and that enrichment of P will likely propagate further through food webs than enrichment of N alone.

The impetus behind chapter 3 was to understand mechanisms by which increased growth rates might occur. We hypothesized that salamanders could increase growth rates via three potential pathways: 1) Increased consumption of prey (i.e., reduced food limitation), 2) reduced elemental imbalances via changes in prey composition or 3) reduced elemental imbalances via changes in individual prey nutrient content. I sampled gut contents of the two study species in un-enriched and enriched conditions. Results indicated that one species exhibited increased consumption of prey and shifts in diet composition. Unexpectedly, increased consumption of prey was largely driven by taxa that utilize biofilm carbon, rather than detrital carbon. The other salamander species studied showed no detectable pattern that would clearly indicate a mechanism for increased growth rates.

Elemental limitations that larval salamanders may face within a stoichiometric/bioenergetics framework were addressed in chapter 4. Threshold elemental ratios (TER; Frost et al. 2006) were determined for both species to predict the severity of elemental limitation they may face (in regards to diet stoichiometry). This approach was used to help us further understand the mechanisms by which salamanders could respond to nutrient enrichment by modeling the elemental imbalance (in terms of P) between their $TER_{C:P}$ and the nutrient content (C:P) of their prey. This allowed us to determine whether salamanders should respond to changes in nutrient content of their prey or whether increased consumption (energy limitation) would be the most likely predicted mechanism for increased growth rates in response to nutrient enrichment.

In conclusion, this thesis highlights the degree to which enrichment of N and P can propagate through detritus-based food webs. Moreover, it reveals the mechanisms by which responses of predators can depend on life-history traits or occur through unexpected pathways. Responses of larval salamanders observed in this thesis work indicate that nutrient enrichment has the potential to alter functional roles of salamanders (e.g., top-down control of macroinvertebrates), which could add to other nutrient effects on headwater stream ecosystems.

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CHAPTER 2

GROWTH OF VERTEBRATE PREDATORS RESPONDS TO PHOSPHORUS ENRICHMENT MORE THAN NITROGEN ENRICHMENT IN DETRITUS-BASED HEADWATER STREAMS[1]

[1]Bumpers, P.M., J.C. Maerz, A.D. Rosemond, and J.P Benstead. To be submitted to *Ecology Letters*

Abstract

Nutrient-driven perturbations to the base of food webs are predicted to attenuate with trophic distance, so it is unclear whether top consumers will generally respond to anthropogenic nutrient inputs. We used a 2-year, whole-stream enrichment of 5 streams with nitrogen (N) and phosphorus (P) to generate different N:P ratios to examine the effects of N and P concentrations on the growth rates and average cohort size of larval salamanders. Growth rates and average cohort size increased (up to 40% and 60% respectively) with P concentration and was negatively correlated with N:P ratio. Nitrogen concentration was not significantly correlated with growth of salamander larvae. The results of this study suggest that larval salamander growth responds to the relaxation of ecosystem-level P-limitation and that moderate P enrichment can have a relatively large effect on a top predator, despite theoretical dampening of effect size with trophic level.

Introduction

Effective ecosystem management requires understanding how perturbations affect ecosystem structure and function (Palmer & Febria 2012), including how they propagate through food webs. Excessive nutrient inputs are a major source of impairment to streams and rivers in the U.S. (Carpenter *et al.* 1998; USEPA 2013). Nutrient enrichment has negative consequences for most aquatic ecosystems, and its effects on autotrophic pathways (e.g., algal biomass) are well known (Smith & Schindler 2009). Increased nutrient availability has been shown to propagate through algal-based food webs and affect top predators such as arctic grayling (Slavik *et al.* 2004). However, some studies indicate that enrichment may not create significant growth and production responses at top trophic levels (Borer *et al*. 2006).

Bottom-up effects of nutrient enrichment may attenuate quickly, often at adjacent trophic levels, relative to top down effects (Brett & Goldman 1997; Borer *et al.* 2006). Reduced resource diversity and palatability, as well as increased dominance of predator-resistant prey, likely contributes to such attenuation of bottom-up forces (Brett & Goldman 1997; Borer *et al.* 2006; Davis *et al.* 2010). Nonetheless, many secondary and tertiary consumers are resource-limited and some show responses to changes in the quality of basal resources (Malzahn *et al.* 2007). Determining the conditions that promote bottom-up propagation of nutrient enrichment is important for identifying systems that may be most sensitive to anthropogenic alterations of resource availability.

Organismal growth, reproduction, and maintenance are often limited by a scarcity of energy and/or elements in the environment (Frost *et al.* 2005). The balance of elements is of particular importance to animals, as they often face unbalanced diets with regards to nutrients, particularly carbon (C), nitrogen (N), and phosphorus (P) (Sterner & Elser 2002), as well as

specific dietary requirements (e.g., fatty acids, lipids; Brett & Muller-Navarra 1997; Wilder *et al.* 2013). Changes in nutrient availability, as occurs with anthropogenic nutrient enrichment, can alter the imbalances consumers face (Cross *et al.* 2007). These effects are predicted to be much greater for primary consumers due to the variation and plasticity in nutrient content of basal resources; there is much less variation in nutrient content of primary consumers (Sterner and Elser 2002). However, some variation in nutrient stoichiometry of primary consumers exists (Frost *et al.* 2006) and can be exploited by predators. Thus, growth of top predators due to nutrient enrichment may be a function of changes in the quantity and/or quality of basal resources that lead to increased prey production and/or altered prey quality.

The relative importance of N and P on growth of top predators in natural ecosystems is poorly studied. Consumers with high nitrogen demand (i.e., high body N:P) may often be limited by N over P; however, consumers with high body P demands, such as fast-growing species and vertebrates, may be more prone to P limitation (Sterner and Elser 2002). The propagation of bottom-up effects on higher-level consumers may depend on nutrient availability and the degree to which primary consumer production is limited by the same factors that limit the production of higher-level consumers.

Recent research in detritus-based streams found that nutrient enrichment at a 16:1 N:P ratio increased production via reduced stoichiometric constraints of primary consumers and increased growth rates of a top vertebrate predator (Cross *et al.* 2006, 2007; Johnson *et al.* 2006). Detrital-based systems offer an opportunity to examine the effects of basal resource quality on food webs, even when the quantity of basal carbon decreases due to enrichment (Suberkropp *et al.* 2010). Anthropogenic effects on nutrient supplies often disproportionately alter nutrient ratios

(Downing & McCauley 1992), but it is not known whether different concentrations of N versus P affect the propagation of eutrophication to high-level consumers in heterotrophic systems.

In our system, we predicted that top predators would more likely be P-limited than N-limited. Although prey production was related to reduced imbalances in both C:N and C:P of basal resources in a previous study (Cross *et al.* 2007), larval salamanders are imbalanced in N:P (lower) relative to prey due to moderate skeletal P demand (Milanovich *et al*, in Milanovich 2010). However, increased prey quantity, whether N or P driven, could also contribute to increased salamander growth. Thus our results on the relative importance of N or P in these systems would illuminate which element is of primary importance to prey populations as well as to predators (via either quantity or quality of prey) if propagating effects were observed. Here, we used a 2-year enrichment of five forested headwater streams to measure the response of the two most common larval salamander species to N and P enrichment. We show that larval salamander growth responds to the relaxation of ecosystem-level P limitation and suggest that N enrichment of headwater systems may not propagate to higher trophic levels without additional inputs of P.

Methods

Study Site

We conducted our study in five adjacent, fully forested headwater streams at the Coweeta Hydrologic Laboratory, a Long Term Ecological Research site in Macon County, North Carolina, U.S.A. Coweeta is a heavily forested 2185-ha basin in the Blue Ridge physiographic province of the southern Appalachian Mountains (see Swank & Crossley 1988). Forests surrounding our study streams were dominated by oak, maple, and poplar, with a dense

understory of *Rhododendron maximum* shading the streams year-round. The five streams used in this study were located in the 559-ha Dryman's Fork watershed. Streams were similar in terms of background chemical and physical characteristics (i.e., pH, gradient, temperature, elevation, discharge). Ambient soluble reactive phosphorus (SRP) concentrations were very low (mean across 5 streams; 2.8 μg/L; range 2.5-3.1 μg/L). Ambient dissolved inorganic nitrogen (DIN) concentrations were more variable but still relatively low (NO_3-N mean, 74 μg/L; range 10.4-179 μg/L; NH_4-N mean, 8 μg/L; range 6.6-8.9), which resulted in a mean ambient dissolved N:P range of 15:1-138:1.

Experimental enrichment

We performed a 2-year (July 2011-July 2013; YR1, YR2) nutrient addition experiment and conducted pre-treatment sampling for one year (June 2010-June 2011, PRE) prior to enrichment. Beginning in July 2011, nitrogen (21% N liquid NH_4NO_3) and phosphorus (as food grade phosphoric acid, 85% H_3PO_4, Colonial Chemical Solutions Inc., Savannah, GA, U.S.A) were continuously added to the experimental streams. Each of the five streams received different added concentrations of N and P to create a gradient in stream water N:P (Tables 2.1, Appendix 2.1 Table S1; hereafter streams will be referred to by their target N:P ratio). Using a solar-powered discharge-proportional injection system, nutrients were mixed with ambient stream water in an irrigation line that ran the length of each 70-m experimental reach and dripped through valves approximately every 5 m to ensure a well-mixed nutrient treatment (see Appendix 2.1 Fig. S1 for a depiction of treatment apparatus). Water samples were taken every 2 weeks at three points along the 70-m reaches to confirm consistent downstream nutrient concentrations.

Focal species

Eurycea wilderae Dunn (*Ew*) and *Desmognathus quadramaculatus* Holbrook (*Dq*) dominate abundance and biomass, respectively, of salamander assemblages in our study streams (Milanovich *et al*. in review, Peterman *et al*. 2008). *Eurycea wilderae* has a larval period of approximately 12 months (mo), metamorphosing at 18-24 mm snout-vent length (SVL) (Bruce 1988). In contrast, *Dq* has a larval stage of 36-48 mo and metamorphoses at approximately 40-45 mm SVL (Bruce *et al.* 2002). Both salamanders are secondary to tertiary consumers, with *Dq* having a greater ability to consume larger predatory macroinvertebrates. Larvae are moderately P-rich due to their heavy investment in bone formation (~1.5-3.5% body P; Bumpers et al. *unpublished*).

Salamander sampling

Salamander growth rates and cohort size were determined using three separate sampling methods. We assessed growth rates of *Dq* using two methods: an enclosure study and capture-mark-recapture (CMR) of free-roaming individuals. For the enclosure study, plastic-framed mesh enclosures (~0.52 m^2) were placed in streams and lined with cobble and detritus from the nearby streambed (Appendix 2.1 Fig. S1). Mesh size (~1.5-mm) was such that larvae could not escape but water and invertebrates in the size classes consumed by salamanders passed freely. Seven larval young-of–the-year *Dq* were hand-captured, measured to the nearest mm (SVL), weighed, uniquely marked with a visual implant elastomer tag (VIE, Northwest Marine Technologies Inc., Shaw Island, WA, U.S.A), and released in each enclosure. Two enclosures were placed in each of the five treatment streams (n = 10 treatment enclosures, 7 larvae each). Approximately three months later, larvae were measured and weighed again to obtain growth rates. This experiment

was repeated three times (Spring 2012, Fall 2012, Spring 2013). Two control enclosures were placed upstream of the nutrient release point in stream 32:1 for Spring 2012 and Fall 2012 (n = 2 control enclosures). In Spring 2013, two control enclosures were placed above the nutrient release points in streams 32:1 and 8:1 (n = 4 control enclosures).

Growth of free-roaming individuals was determined using a robust capture-mark-recapture (RCMR) beginning in July 2011 and ending July 2013. Salamanders were non-destructively sampled using litter bags deployed in the streams (Nowakowski & Maerz 2009; Cecala *et al.* in review). Two bags (40 × 20 cm made with 2.25-cm^2 plastic netting) were filled with leaf litter from the riparian area and placed approximately every 5 m in the wetted portion of the 70-m treatment reaches with a large rock on top to prevent dislodgement. Litter bags were deployed at least 48 hours prior to sampling. We sampled salamanders each month (n = 11 primary occasions) during the active growing season (generally March – October) on three consecutive days (n = 31 secondary occasions). To collect salamanders from litter bags, we quickly lifted the bag into a fine mesh dipnet and transferred it to a bucket of water. We agitated the bag in the bucket and then replaced it in the stream. Contents of the bucket were filtered through the fine mesh dipnet, and any captured individuals were weighed, measured (SVL), and uniquely tagged with a VIE tag. We released salamanders on the upstream side of the bags to prevent downstream drift. Salamanders moved freely in and out of the litter bags during and between secondary and primary occasions. Growth rates for *Dq* were estimated from changes in SVL between initial capture and subsequent recaptures. To estimate changes in mass, SVL was converted to dry mass (DM) using a length-mass regression derived for *Dq* in Coweeta streams (Milanovich & Maerz *unpublished data*):

$$M = 0.0014\ L^{3.26}\ (P<0.001,\ R^2=0.84,\ \text{n=79})$$

where *M* is larval mass (mg DM) and *L* is the snout-vent length (mm). Individual daily growth rate (*g*) was then calculated using the following equation (Johnson *et al.* 2006):

$$g = (\ln (W_{fn}) - \ln (W_{in}))/t$$

Where W_{in} was the initial DM and W_{fn} is the final DM of a salamander, and t=time interval in days.

Growth rate was inversely related to initial size of an individual, with smaller individuals typically having higher growth rates than larger individuals (enclosure larvae $F_{1, 192} = 167.7$, $P < 0.001$, $R^2 = 0.46$, $n = 194$; CMR larvae $F_{1, 96} = 50.84$, $P < 0.001$, $R^2 = 0.35$, $n = 98$). We standardized growth rates with respect to initial SVL (SVL_i) by regressing the relationship of SVL_i against growth rate for the enclosure larvae (both control and treatment individuals together) and individuals from the CMR component of the study. The following equations were then used to determine predicted growth rates (PG) for a given SVL for enclosure and RCMR individuals respectively:

$$PG = -0.0142*\ln (SVL_i) + 0.0474$$

$$PG = -0.0049*\ln (SVL_i) + 0.0189$$

The SVL_i adjusted growth rate was calculated by subtracting the predicted growth rate (PG) from the observed growth rate (g), with positive values indicating a growth rate higher than predicted by initial size.

Eurycea wilderae were too small to mark during RCMR, therefore, we used changes in mean individual size among primary occasions to estimate growth. Unlike *Dq*, *Ew* was present in streams as single annual cohorts that metamorphose after 12-14 months, so cohort measures of growth are reasonable for this species. Average body size for each stream was determined one year prior to enrichment (PRE) and during both years of enrichment (YR1, YR2).

Statistical Analyses

We used linear regression and an information-theoretic approach to evaluate the effects of added and measured nutrient concentrations and ratios (Table 2.1, Appendix 2.1), and season on *Dq* stream-averaged adjusted growth rates for each of the five streams. Akaike's Information Criterion corrected for small sample size (AICc) was used to determine the most parsimonious model for growth (Burnham & Anderson 2002). Nutrient ratios were log-transformed to meet assumptions of a linear model. For enclosure growth, we included all three enclosure time periods (Spring 2012, Fall 2012, Spring 2013) together in our models. Capture-mark-recapture data were analyzed using linear regression on the average stream SVL_i adjusted growth rate for the 2-year treatment period. To determine if treatment had an effect on *Ew*, SVL was averaged for each stream just after hatching (July or September) and just before metamorphosing (May, hereafter "metamorph") to approximate changes in average body size. Averages were then compared using linear regression and ANOVA. Pre-treatment metamorph SVL was assessed using ANOVA on all individuals captured to determine if there were pre-existing differences among streams. All statistical analyses were performed in R statistical software version 3.0.2 (R Core Development Team 2013).

Results

Effectiveness of enrichment

Concentrations and ratios of the dosing treatment successfully created a gradient of both concentration and ratio (Appendix 2.1 Table S1). Measured concentrations and ratios varied by stream and year, but were generally reflective of targets (Table 2.1). Measured stream nutrient concentrations were elevated 2.5-31× and 3-10× above background for SRP and DIN,

respectively (Table 2.1). Measured stream water N:P ratios were always higher than added ratios, indicating preferential uptake of P, even in the lowest N:P stream. Throughout the study, water temperature ranged from 1°-19.5° C (annual mean ~10.5° C) and did not differ among streams. Annual average discharge was 11.2 L/s ± 2.2 SE, 5.8 L/s ± 1.6 SE, and 6.9 L/s ± 1.4 SE for PRE, YR1 and YR2, respectively across all streams. Annual average discharge among streams also varied each year ranging from 5.1-16.8, 2.69-12.2, and 4.9-12.5 for PRE, YR1, and YR2, respectively. PRE annual average discharge was higher than YR1 and YR2 in all streams except YR2 stream 2:1.

Dq growth in enclosures

Growth was calculated on 196 larval *Dq* from the three enclosure studies (Appendix 2.1 Table S2). We recovered 75 (89%), 50 (60%), and 71 (72%) larvae for Spring 2012, Fall 2012, and Spring 2013, respectively (Table S2). The top AIC model included added P concentration and season (Fig. 2.1A, Appendix 2.1 Table S3; $F_{2, 16} = 14.38$, $P < 0.001$ Adj. $R^2 = 0.61$). Six of the 7 models in the 95% confidence set included both measured and added P or N:P ratio and all included season as a variable (Table S3). Growth rates were negatively correlated with N:P ratio, with the 3 mo-average measured N:P explaining the data the best with season as a variable (Fig. 2.1C, Table S3; $F_{2, 16} = 13.34$, $P < 0.001$, Adj. $R^2 = 0.58$). Nitrogen concentration was not an important predictor in enclosure residual growth (Fig. 2.1B). Average residual growth rates in the enrichment enclosures were always higher compared to the control enclosures within a respective time period (Fig. 2.1). Among all streams, growth was higher in both spring seasons compared to those measured in the fall, but differences in growth among treatments were similar

across seasons but most pronounced in the fall (Fig. 2.1). Growth in the highest P treatment (2:1) represented an ~ 30% and 40% increase over controls in spring and fall, respectively.

Growth of free-roaming individuals: Dq

Over the 2-year enrichment, growth rates were calculated on 102 individuals across all five streams (Appendix 2.1 Table S2). Intervals between capture and recaptures varied from 19 days to over 400 days. Patterns of growth among streams were positively correlated with added P and consistent with patterns observed among enclosures; however, the correlation between added P and growth among CMR larvae was not statistically significant ($F_{1, 3} = 3.986$, $P = 0.14$, Adj. $R^2 = 0.43$). Instead, the top AIC model included the 2-year measured stream water P concentration, which was significantly correlated with growth (Figure 2.2A, Appendix 2.1 Table S4; $F_{1, 3} = 21.41$, $P = 0.02$, Adj. $R^2 = 0.84$). No other models were significant.

Size variation of Ew

We captured 482 larval *Ew* across all five streams during the 3-year sampling period in the months of July (September in one case) and May (Appendix 2.1 Table S5). In all three years (PRE, YR1, YR2), there was no difference in average hatchling SVL between streams ($F_{4, 10} = 0.133$, $P = 0.97$). Metamorph SVL was not different across the five streams pre-treatment ($F_{4, 55} = 0.78$, $P = 0.54$), but average metamorph SVL was positively and significantly correlated with added P concentration during enrichment (Fig. 2.2B, $F_{1, 8} = 8.35$, $P = 0.02$, Adj. $R^2 = 0.45$) and was negatively and significantly correlated with added N:P ratio ($F_{1, 8} = 13.43$, $P = 0.006$, Adj. $R^2 = 0.58$) and added N concentration ($F_{1, 8} = 6.827$, $P = 0.039$,Adj. $R^2 = 0.39$). Average SVL across all streams and both years increased by 1.29× compared to PRE (range 1.08-1.45×). Average

SVL in stream 2:1, which had the largest increase, was 1.66× larger in YR2 compared to PRE and 1.22× larger than the average *Ew* size in stream 128:1 over both years.

Discussion

Salamanders are the most abundant predatory vertebrates in fishless headwater streams, and our study demonstrates that larval salamander growth in headwaters is limited by, and responsive to relaxation of, ecosystem-level P limitation. Increased predator growth likely occurred through a combination of relaxation of consumer-food imbalances, increased prey production and a lack of gape limitation in these top predators. Here, largely single nutrient effects propagated up food webs from basal resources to top predators in detritus-based systems.

Our study design allowed for tests of the relative strengths of P versus N limitation across natural, and long-term experimental enrichment conditions, with some limitations. In this study, N was not related to larval salamander growth. However, our P enrichments (from low to high) were crossed with N enrichments (high to low), such that our lowest P treatment (ca 11.3 μg/L SRP) received ca 415 μg/L DIN above background, whereas the highest P treatment (ca 81.3 μg/L SRP) received ca 75 μg/L DIN above background averaged over the study period (Tables 1, S1). Thus, the increase in growth over controls in our lowest P treatment can be partially attributable to increased N; however, the significant relationships between P and growth we observed even at low measured DIN (83 μg/L in the highest P stream, Table 2.1) attest to the overriding importance of P in determining salamander response. The apparent negative relationships between salamander growth and size and both N:P ratio and N concentrations reflected the nature of our experimental design, where N and P were inversely related. In addition, caged and free-roaming *D. quadramaculatus* growth rates were significantly correlated

with different variables of P. Added P concentration explained caged growth rates the best while the 2-year average measured P concentration explained CMR growth rates. We attribute this difference to the times scales over which growth rates in these two studies were calculated. We further note that measured P concentrations also explained caged growth rates relatively well (Appendix 2.1 Table S3). We suggest that prior research demonstrating positive salamander growth in response to N and P enrichment (16:1; Johnson *et al.* 2006) was likely a response to P enrichment, but can also be attributed to some alleviation of both N and P limitation. These results may be important for understanding both natural patterns of variation in larval salamander life histories and salamander population responses to anthropogenic effects on nutrient supply. The ultimate availability of P is largely driven by the parent-material and age of underlying geologic substrate (Vitousek *et al*. 2010). Natural variation in geologic P availability could contribute to the understanding of known variation in geographic patterns of life histories of salamanders (Morrison & Hero 2003). Moreover, at low elevations, streams may receive high amounts of N and P through agricultural and residential runoff; however, in high elevation headwaters, N enrichment occurs through atmospheric N deposition from combustion of fossil fuels without concurrent P enrichment (Vitousek *et al*. 1997). Consequently, our results suggest that N enrichment in high-elevation headwaters may not stimulate stream productivity and associated responses by in-stream consumers to the extent that P enrichment would.

The conditions in our streams that would allow propagation of nutrients to occur were likely mediated by reduced gape limitation, increased production of salamander prey, and/or changes in prey quality. Salamanders would only respond to enrichment through changes in their prey (assuming no direct toxicity). It is likely that salamander prey responded to enrichment in our stream similarly to that of Cross *et al*. (2007). We do not yet fully know to what extent P

enrichment stimulated invertebrate production in this study, but preliminary results indicate that some important prey taxa for *D. quadramaculatus* exhibited increased production in all streams during the first year of enrichment (e.g. *Tallaperla*, *Leuctra* L.M. Demi, *unpublished data*). Preliminary analysis has also revealed that biomass of *Chironomidae*, which are consistently the most important prey resource for larval *E. wilderae* and other closely related congeners (Johnson & Wallace 2005; Barrett *et al.* 2012), increased in 4 of 5 streams during the first year of enrichment (L.M. Demi, *unpublished data*).

Long-term nutrient enrichment has been shown to decouple macroinvertebrate predator-prey production due to gape limitation of macroinvertebrate predators in detritus-based systems (Davis *et al.* 2010). However, due to the relatively large size of most larval salamanders, they are less likely to be gape limited. Therefore, larval salamanders should increase consumption of prey in both number of prey and size of prey consumed in response to enrichment, likely increasing growth rates. Moreover, previous studies in diverse detrital systems have shown that larval salamander growth rates and biomass are correlated with prey biomass (Johnson & Wallace 2005; Huntsman *et al.* 2011). Therefore, increased secondary production of prey in response to nutrient enrichment would likely stimulate salamander growth rates.

Larval salamander growth responses to P availability may also be related to reduced stoichiometric constraints (reduced nutrient limitation). The use of threshold elemental ratios (TER), the point at which a consumer's elemental limitation shifts between one element and another, provides a realistic way to predict how consumers would respond to stoichiometric changes in their prey (Frost *et al.* 2006). Both salamanders in our study have body C:P ratios of ~ 50-60 (P.M. Bumpers *unpublished data*, but see Milanovich et al. in Milanovich 2010). Multiplying body C:P by 2.4 would equate to a $TER_{C:P}$ of ~120-144 (Frost *et al.* 2006). A

$TER_{C:P}$ this low indicates that larval salamanders are likely more limited by P over C when food is abundant because the majority of their prey have C:P ratios higher than this (Cross *et al.* 2003). Shifts in the stoichiometry of important prey or changes in prey community composition that increase the abundance or biomass of higher P-content prey (e.g. low C:P prey) should stimulate growth efficiency of larval salamanders. Experimental enrichment can alter nutrient content of primary consumer macroinvertebrates due to increased nutrient content of organic matter via associated microbes (Cross *et al*. 2003; Suberkropp *et al*. 2010). In addition, recent laboratory experiments show that release from elemental limitation can travel up the food chain to affect larval fish condition (Boersma *et al.* 2008; Dickman *et al.* 2008). Future analyses of TERs and the potential for shifts in prey stoichiometry (either through composition changes or direct changes in individual body stoichiometry) will illuminate the extent to which stoichiometric imbalances contributed to increased growth rates in our study.

Our research indicates that top consumers in detritus-based and living plant-based systems may respond similarly to nutrient enrichment despite fundamental differences in the response of the respective primary basal resources (i.e., decreased detrital standing stocks vs. increased algal standing stocks) and indicate that headwater stream food webs may be altered through nutrient enrichment. Nutrient-driven increases of growth rates and size of larval salamanders may create top-down feedbacks. These top-down effects could have larger consequences cascading through the food web since top-down effects are predicted to propagate through more trophic levels than bottom-up, and create larger indirect effects (Borer *et al.* 2006). Indeed, a recent study in our region demonstrates that larval *E. wilderae* and *D. quadramaculatus* predation affects stream invertebrate composition and abundance (Keitzer & Goforth 2013a). Furthermore, both species play key roles in N and P storage and recycling in

headwater streams (Keitzer & Goforth 2013b; Milanovich *et al*. in Milanovich 2010). Therefore, long-term consequences of increased growth rates of salamanders will likely affect the function of primary consumers (e.g., shredders, collectors) as salamanders may exhibit strong top-down control of them, further altering the flow of carbon, as well as nutrient cycling in headwater streams.

Ours is the first study to separately manipulate N and P availability in multiple natural streams and measure their effects on top predators. Despite bottom-up effects generally having limited propagation to top consumers in many systems, we saw significant and relatively rapid responses in top consumer growth to specific nutrient enrichment, on the order of up to 40% increased growth rate (Dq) and 66% increased size (Ew) at our highest P concentrations. This effect occurred at relatively low to moderate concentrations of P and N relative to those that are observed across landscape gradients in the U.S. and Europe (Woodward *et al*. 2012; USEPA 2013). This study supports other evidence that nutrient perturbations of stream food webs can affect higher trophic levels (Slavik *et al.* 2004), and that P may be more important than N in regulating vertebrate predator responses.

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Table 2.1. Measured dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) concentrations (μg/L) in the five treatment streams. Target is the concentration that was the desired concentration added to the stream. PRE represents stream concentrations in the year prior to enrichment. Year 1 (YR1) and year 2 (YR2) are the measured concentrations in the stream water during treatment. All concentrations are annual means ± 1 SE from bi-weekly water samples.

		Stream				
		2:1	8:1	16:1	32:1	128:1
DIN	Target	81.3	243.9	365.8	487.7	650.3
	PRE	18.1 ±1.5	111.6 ±17.3	37.4 ±5.7	188.8 ±14.4	56.6 ±7.8
	YR1	100.4 ±9.1	251.2 ±14.3	381.7 ±35.4	510.6 ±37.3	364.3 ±29.2
	YR2	66.3 ±6.2	145.2 ±10.4	277.4 ±32.9	215.6 ±11.5	254.0 ±25.3
SRP	Target	90.0	67.5	50.6	33.8	11.3
	PRE	2.9 ±0.2	2.5 ±0.2	3.0 ±0.5	3.1 ±0.3	2.5 ±0.2
	YR1	42.4 ±3.1	78.4 ±5.3	40.6 ±2.6	30.4 ±2.1	8.2 ±0.6
	YR2	53.3 ±5.2	31.6 ±3.9	30.4 ±3.1	14.2 ±1.1	6.2 ±0.5
N:P	Target	2.0	8.0	16.0	31.9	127.4
	PRE	15.3 ±1.8	95.0 ±16.3	30.0 ±4.5	138.3 ±10.8	48.9 ±7.1
	YR1	8.4 ±1.1	18.6 ±3.5	25.5 ±3.3	54.0 ±7.5	159.6 ±15.1
	YR2	5.6 ±1.5	37.4 ±8.7	24.2 ±3.5	52.9 ±7.9	113.1 ±13.5

Figure Legends

Fig 2.1. SVL_i adjusted growth rates for *Dq* larvae in enclosures correlated with A) added SRP concentration, B) added DIN concentration, and C) added N:P ratio. Regression lines indicate a significant overall effect and are separated by season (Combined Spring=Solid, Fall=Dashed). Residual growth rates are averages per stream per season. Error bars are ± SE and represent variation within a given stream for that season. Note: N:P ratios are displayed on a log scale but the axis is labeled with actual N:P ratio.

Fig 2.2. Growth responses of CMR Dq and Ew. A) Average SVL_i adjusted growth rates for free-roaming *Dq* larvae from CMR. Residual growth rates were positively correlated with measured P concentration, with stream 8:1 having the highest average residual growth. Values are the average from recaptures over the 2 years of enrichment. Error bars represent ± SE. B) Average SVL of *Ew* for YR1 and YR2 of enrichment. Values are the average cohort size of larvae captured during CMR in May of their respective treatment years just before metamorphosis. The grey bar represents the range of SVLs measured in May, pre-treatment, just before metamorphosing. The dotted line is the average pre-treatment SVL across all 5 streams.

Fig 2.1

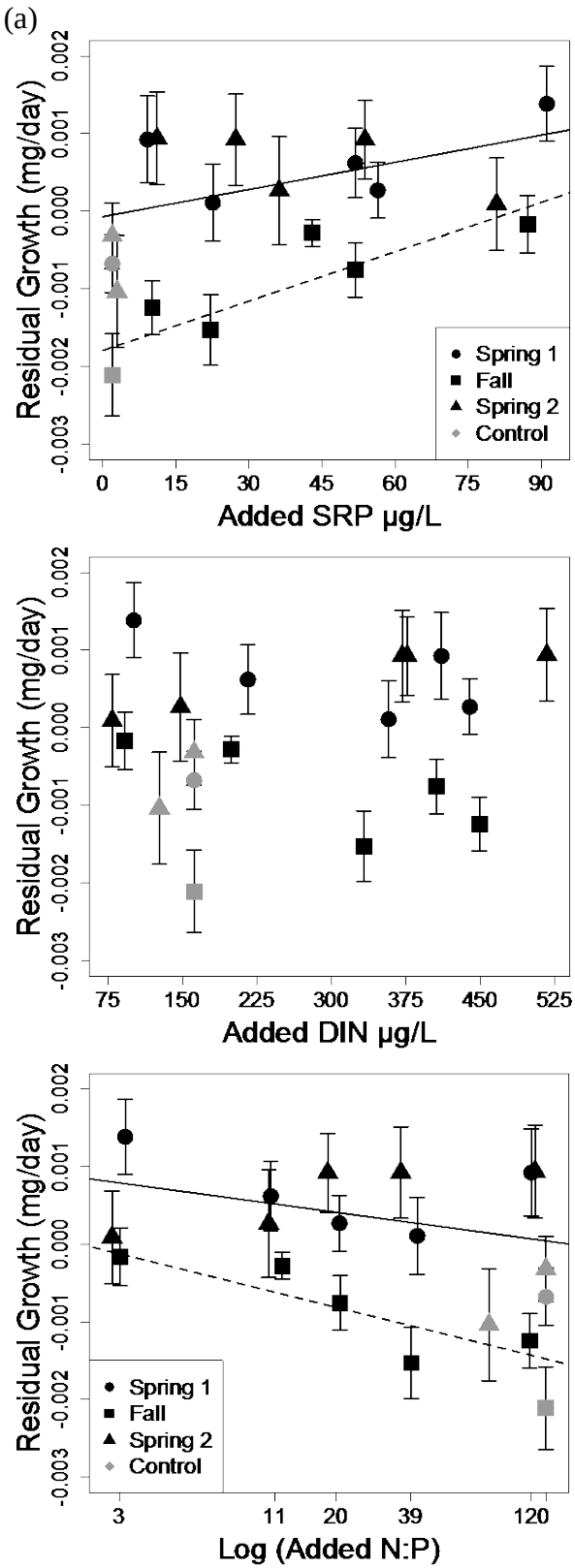
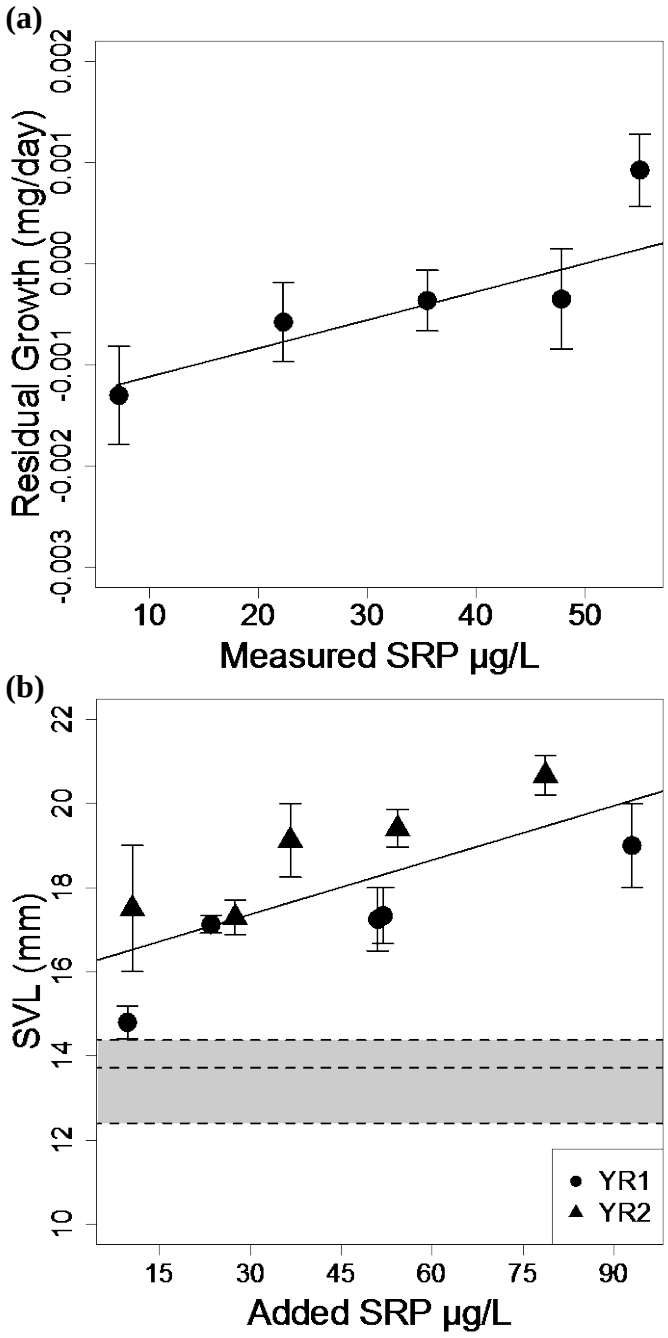

(a)
Residual Growth (mg/day)
0.002
0.001
0.000
-0.001
-0.002
-0.003
0
15
30
45
60
75
90
Added SRP µg/L
Spring 1
Fall
Spring 2
Control
Residual Growth (mg/day)
75
150
225
300
375
450
525
Added DIN µg/L
Residual Growth (mg/day)
3
11
20
39
120
Log (Added N:P)
Spring 1
Fall
Spring 2
Control

Fig 2.2


(a)
Residual Growth (mg/day)
-0.003
-0.002
-0.001
0.000
0.001
0.002
10
20
30
40
50
Measured SRP µg/L
(b)
SVL (mm)
10
12
14
16
18
20
22
15
30
45
60
75
90
Added SRP µg/L
YR1
YR2

Appendix 2.1

Table 1. Dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) concentrations (μg/L) in each of the five treatment streams. Values represent the background concentration plus added nutrients. Target indicates the DIN or SRP concentration that was added to reach the desired N:P ratio. Values for YR1 and YR2 are the estimated concentration being dripped into the stream. This value incorporates known added concentrations of N and P plus a stage-discharge estimation relationship.

		Stream				
		2:1	8:1	16:1	32:1	128:1
DIN	Target	81.3	243.9	365.8	487.7	650.3
	YR1	109.7 ±10.2	232.4 ±18.5	440.3 ±41.2	373.3 ±21.2	427.8 ±30.7
	YR2	82.2 ±5.1	151.2 ±6.7	376.6 ±21.0	380.6 ±10.0	516.9 ±21.7
SRP	Target	90.0	67.5	50.6	33.8	11.3
	YR1	93.0 ±5.2	51.9 ±4.6	55.9 ±6.2	23.5 ±1.8	9.7 ±0.9
	YR2	76.7 ±4.2	36.6 ±1.5	54.3 ±3.0	27.5 ±0.9	10.5 ±0.4
N:P	Target	2.0	8.0	16.0	31.9	127.4
	YR1	2.5 ±0.2	11.3 ±1.1	18.2 ±0.6	38.3 ±2.4	103.5 ±3.7
	YR2	2.6 ±0.2	10.1 ±0.9	15.4 ±0.2	31.4 ±0.8	109.0 ±2.4

Table 2. Time in days that *Dq* larvae were left in the stream, and the number of individuals recovered from each stream during each of the three seasons of enclosure experiments. CMR Recaps is the number of recaptures in each of the 5 treatment streams for which growth rates were calculated over the 2 years of enrichment.

Stream	Days in Enclosure (# of larvae recovered)			
Ratio	Spring 2012	Fall 2012	Spring 2013	CMR Recaps
2:1	98 (10), 103 (4)	101 (3), 116 (8)	80 (13)	19
8:1	93 (7), 92 (4), 104 (3)	114 (5), 101 (1)	80 (11)	21
16:1	100 (14)	114 (6)	80 (13)	26
32:1	103 (13)	116 (6), 101(3)	80 (10)	20
128:1	103 (3), 98 (3)	116 (2), 101 (3), 76 (5)	80 (7)	16
Ref 32:1	105 (14)	114 (7), 98 (1)	80 (8)	NA
Ref 8:1	NA	NA	80 (9)	NA

Table 3. AIC model output for *Dq* enclosure residual growth. Models included in the 95 % CI are included. RG is the residual growth rate, Added is the added P concentration or N:P ratio of enrichments, 3MOAvg and 6MOAvg are the average measured concentration or ratio for the duration of the enclosure study plus the preceding 3 months or 6 months, respectively.

Model	K	AICc	ΔAICc	W_i	Cum W_i	Loglik
RG~ Added P, Season	4	-220.62	0.00	0.37	0.41	115.74
RG~ 3MOAvg N:P, Season	4	-219.15	1.47	0.18	0.55	115.01
RG~ 6MOAvg N:P, Season	4	-218.76	1.87	0.15	0.70	114.81
RG~ Added N:P, Season	4	-218.16	2.47	0.11	0.81	114.51
RG~ 6MOAvg P, Season	4	-217.61	3.01	0.08	0.89	114.23
RG~ 3MOAvg P, Season	4	-216.87	3.75	0.06	0.95	113.87
RG~ Season	3	-215.44	5.18	0.03	0.98	111.52

Table 4. AIC model output for CMR residual growth. RG= Residual Growth rates, Measured is the 2-yr average measured concentration or ratio of dissolved nutrient. Added is the 2-yr averaged added concentration or ratio dosed in the stream.

Model	K	AICc	ΔAICc	W_i	Cum W_i	Loglik
RG~ Measured P	3	-42.01	0.00	0.92	0.92	36.00
RG~ Added Ratio	3	-34.74	7.27	0.02	0.94	32.37
RG~ Added N	3	-34.66	7.35	0.02	0.97	32.33
RG~ Measured Ratio	3	-34.01	7.99	0.02	0.98	32.01
RG~ Added P	3	-32.95	9.06	0.01	0.99	31.48
RG~ Measured N	3	-32.54	9.47	0.01	1.00	31.27

Table 5. Stream-averaged SVL (mm) ± 1 SE for *Ew* in each of the 5 streams. Hatch represents the size of *Ew* soon after larvae appeared in the streams for that year and Metamorph is the average size of the cohort just before metamorphosis and leaving the streams. A given cohort begins with the Hatch date and corresponds to the next calendar year spring (i.e., Sep 2010 and May 2011 are the same cohort). Parentheses represent the number of individuals captured for that date and stream. The grey bars represent pre-treatment measurements.

		Stream (Target Ratio)				
		2:1	8:1	16:1	32:1	128:1
Hatch	Sep 2010	14.5 ± 0.5 (2)	12.7 ± 0.49 (9)	13.4 ± 0.54 (10)	13.1 ± 0.30 (9)	13.2 ± 0.2 (5)
	July 2011	10.3 ± 0.42 (20)	10.7 ± 0.29 (30)	9.8 ± 0.33 (34)	10.2 ± 0.28 (33)	10.5 ± 0.45 (26)
	July 2012	12.4 ± 0.21 (20)	12.0 ± 0.24 (15)	11.4 ± 0.13 (34)	11.4 ± 0.08 (52)	12.1 ± 0.48 (15)
Metamorph	May 2011	12.4 ±1.1 (5)	13.6 ± 0.65 (17)	14.4 ± 1.1 (13)	13.5 ±0.47 (17)	14.9 ± 1.4 (8)
	May 2012	19 (2)	17.3 ±0.67 (9)	17.3 ± 0.75 (8)	17.1 ± 0.21 (40)	14.8 ± 0.39 (10)
	May 2013	20.7 ± 0.46 (9)	19.1 ± 0.88 (4)	19.4 ± 0.45 (10)	17.3 ± 0.40 (14)	17.5 ±1.5 (2)

Figure 1. Photograph showing the nutrient injection system. A) Stream water was collected from upstream and held in tanks where it was mixed with the nutrient solution and injected into an irrigation line. B) Stream 8:1 with two mesh enclosures in which larvae were placed for ~3 months. Enclosures allowed water and prey to move freely. Photo Credit: P.M. Bumpers

CHAPTER 3

LARVAL SALAMANDER DIETS SHIFT IN RESPONSE TO NUTRIENT ENRICHMENT THROUGH UNEXPECTED FOOD WEB PATHWAYS[1]

[1]Bumpers, P.M., A.D. Rosemond, J.C. Maerz, and J.P Benstead. To be submitted to *Oecologia*

Abstract

Nutrient enrichment alters the quantity and quality of basal resources in streams, potentially affecting primary and higher-order consumers. Nutrient effects on top predators are mediated through effects on prey. We tested for changes in the quantity of prey consumed and/or changes in prey identity of two common larval salamanders, *Desmognathus quadramaculatus* and *Eurycea wilderae*, in response to experimental nutrient enrichment. We continuously enriched five streams with different concentrations of dissolved nitrogen (N) and phosphorus (P), creating a gradient of N:P (2:1-128:1), for two years. Nutrient addition resulted in greater quantity of prey consumed, as well as shifts in diet for *D. quadramaculatus* across all streams. Among prey categories, biomass of collector-gatherers and scrapers increased the most under enrichment in *D. quadramaculatus* guts; biomass of both groups were significantly higher in Dq guts in year 2 than during pretreatment (P=0.04). There were no changes in prey composition due to shredders or predators, but biomass of collector-filterers declined in years with nutrient enrichment. The proportion of collector-gatherer and scraper prey consumed was weakly but positively correlated with algal biomass in the study streams. We found no evidence for a change in prey biomass or composition in the guts of *E. wilderae.* These results suggest that nutrient effects on salamander diets may largely occur via algal pathways, despite the dominance of detrital carbon our study streams. Our results further suggest that responses to enrichment may depend on life history characteristic of top predators (i.e. size or habitat).

Introduction

Anthropogenic mobilization of nitrogen (N) and phosphorus (P) to freshwater ecosystems is occurring globally and is a major source of impairment to streams and rivers (USEPA 2013). Nutrient enrichment creates complex responses in stream structure and function that can affect multiple trophic levels. For example, algal biomass is commonly stimulated by increased nutrient availability (Dodds et al. 2002). Moreover, leaf-litter breakdown and fine particulate organic matter export accelerate in response to nutrient enrichment, decreasing the temporal availability of detrital carbon for consumers in headwater streams (Benstead et al. 2009; Suberkropp et al. 2010). In addition to effects on the quantity of basal resources, low levels of enrichment can rapidly change periphyton stoichiometry (i.e., C:N:P) via increased uptake of nutrients (Stelzer and Lamberti 2002; Taylor et al. 2014) and alter detrital resource stoichiometry via microbially mediated increases in nutrient content (Cross et al. 2003; Scott et al. 2013). Such reductions in C:nutrient ratios decrease the resource imbalance that many primary consumers face, potentially increasing growth rates and secondary production (Cross et al. 2006). Community composition and species richness of primary consumers can also in part be influenced by nutrient concentrations (Wang et al. 2007; Evans-White et al. 2009). Additionally, body-size distributions of primary consumers and predatory invertebrates are affected by enrichment (Davis et al. 2010b). Responses of primary consumers to nutrient-driven changes to basal resources may propagate to top predators, or energy may be diverted to a few taxa, creating trophic dead-ends (Davis et al. 2010a). However, research is lacking in understanding the specific mechanisms by which nutrient effects are propagated through food webs to top predators.

In order to improve our predictive ability and management of nutrient effects on aquatic ecosystems, we need to better understand ecosystem-wide responses to nutrient enrichment

(Dodds 2007; Jarvie et al. 2013). Thus, understanding nutrient-driven effects through the entirety of a food web is essential. A few studies in autotrophic streams have documented increased growth rates of fish in response to nutrient enrichment (Slavik et al. 2004 and refrences therein). Additionally, Johnson et al. (2006) reported increased growth rates of a larval salamander in response to enrichment of a detritus-based stream. Generally, such studies do not report explicit mechanisms for increased growth rates, but hypothesize that concomitant increases in potential prey production are likely responsible, (but see Johnson et al. 2006 for hypothesized quality effects as well). Consequently, it is not known by which mechanisms nutrient enrichment propagates through food webs. A way to assess this is to determine food web pathways to predators in response to enrichment. Changes in gut biomass in predators may infer that predators are consuming prey biomass at different rates, altering energy intake. Changes in diet composition may result from changes in community composition or reflect carbon availability to consumers (Johnson and Wallace 2005; Huntsman et al. 2011). Furthermore, shifts in diet composition or size structure of prey (Back and King 2013) may result in changes of the average stoichiometry of diet items (i.e. changes in composition that reduce stoichiometric constraints), altering the elemental imbalance that a consumer may face (Cross et al. 2007). Thus, diet analysis of top predators should illuminate a more mechanistic understanding of the propagation of nutrients through food webs.

The purpose of this study was to quantify prey composition and biomass of prey consumed by two larval salamander species in response to nutrient enrichment of detritus-based headwater streams. Larval salamanders are top predators in many forested headwater streams in the eastern U. S. They primarily consume aquatic macroinvertebrates and play key roles in shaping benthic invertebrate community composition and nutrient storage and cycling (Keitzer

and Goforth 2013a; Keitzer and Goforth 2013b). Two of the most common and dominant salamanders in the southern Appalachians are *Eurycea wilderae* and *Desmognathus quadramaculatus*, respectively. In a previous study, we found significant increases in growth rates of larval *D. quadramaculatus* and increased average cohort size of larval *E. wilderae* in response to nutrient enrichment of forested headwater streams (Bumpers et al. *in prep*). Here, our primary goal was to explore mechanistic pathways by which this response occurred by quantifying total gut biomass and prey composition in response to enrichment. We hypothesized that positive effects of enrichment on salamander growth could be mediated via three main pathways: 1) increased consumption of prey (i.e., increases in gut biomass); 2) changes in prey composition that result in reduced stoichiometric constraints; and 3) individual prey change in nutrient content due to altered detrital stoichiometry. We determined the amount and type of prey consumed by quantifying biomass of gut contents for both salamander species prior to and following two years of experimental enrichments of five streams with N and P.

Methods

Study site

This study was conducted at the Coweeta Hydrologic Laboratory, a Long Term Ecological Research site in Macon County, North Carolina, USA. Coweeta is a heavily forested experimental basin (2185 ha) in the Blue Ridge physiographic province of the southern Appalachian Mountains (Swank and Crossley 1988). Forests surrounding our study streams were dominated by chestnut oak, red maple, and tulip poplar, with a dense understory of *Rhododendron maximum* shading the streams year-round.

We used five streams located in the 559-ha Dryman's Fork watershed of Coweeta. Streams were similar in regards to chemical and physical characteristics pre-enrichment (i.e., pH, gradient, temperature, elevation, discharge) and were geographically close (< 0.5 km). Pre-treatment soluble reactive phosphorus (SRP) concentrations were very low and similar across streams (mean across 5 streams; 2.8 μg/L; range 2.5-3.1 μg/L), while ambient nitrogen concentrations were more variable, but still relatively low (NO_3-N mean, 74 μg/L; range 10.4-179 μg/L; NH_4-N mean, 8 μg/L; range 6.6-8.9).

Focal Species

We studied two larval salamander species common to Coweeta and much of the southern Appalachians. *Eurycea wilderae* has a larval period of approximately 12 months (mo), metamorphosing at 18-24 mm snout-vent length (SVL) (Bruce 1988). Observations made prior to our study indicated that *E. wilderae* were most commonly found in sandy depositional areas of our streams (P.M. Bumpers *personal observation*, Keitzer and Goforth 2013a). In contrast, *D. quadramaculatus* has a larval stage of 36-48 mo and metamorphoses at 40-45 mm SVL (Bruce et al. 2002). Larval *D. quadramaculatus* most commonly inhabit faster flowing riffle areas with cobble substrate, but were regularly found throughout all habitats in our streams. The disparate size between the two species indicates that *E. wilderae* has the higher potential to face gape limitation among its prey. Several studies of *E. wilderae* and their congeners indicate that non-*Tanypodinae* chironomids and copepods are the most abundant prey items, while other aquatic macroinvertebrates generally dominate gut biomass (Johnson and Wallace 2005; Barrett et al. 2012). Studies that exist on *D. quadramaculatus* diets indicate that they are generalist feeders, consuming a myriad of aquatic macroinvertebrates (Davic 1991; Trice 2011).

Experimental enrichment of five headwater streams

We conducted a 2-year continuous experimental nutrient addition (July 2011-July 2013) of five streams in the Dryman's Fork watershed. Beginning in July 2011, dissolved nitrogen (as 21% liquid NH_4NO_3) and phosphorus (as 85% liquid H_3PO_4) were continuously added to 70-m treatment reaches with a solar-powered, flow-proportional pump. Nutrients were added to ambient stream water and dripped approximately every five meters via a gravity-fed irrigation line to ensure a well-mixed treatment. Different concentrations of N (low to high) and P (high to low) were added to each stream to create a gradient of target N:P ratios (Table 3.1). The nature of our design created an inverse relationship with the concentrations of N and P being added. Streams were elevated above background concentrations by 2.5-31× and 3-10 × for SRP and DIN respectively (Bumpers et al. 2014).

Diet sampling

Larvae were collected for diet analysis before and during experimental enrichment. Larval *D. quadramaculatus* were collected pre-treatment in June 2010, April 2011, and July 2011. *Eurycea wilderae* were collected in April 2011 and July 2011 for pre-treatment diets. Pre-treatment diets were grouped across streams to improve replication. During enrichment collection occurred during the spring and summer in each year of enrichment for both species in all five streams (YR1: March 2012, June 2012; YR2: April 2013, June 2013). All larvae were collected at night-when salamanders are most active- using fine-meshed dipnets and turning only loose-cover objects. Larvae were too small to use gastric lavage; therefore, lethal sampling was necessary. Larvae were kept on ice, transported back to the lab and immediately euthanized in neutral-buffered 0.5% Tricaine methanesulfonate (MS-222). Larvae were then rinsed with

deionized water and immediately dissected and their stomachs placed in Kahle's solution or the entire body was preserved in Kahle's solution until later dissection.

Stomach contents were identified using a dissecting microscope to the lowest taxonomic level possible (typically genus; Merritt et al. 2008), except for the family Chironomidae, which were classified as either non-*Tanypodinae* or *Tanypodinae*. Prey items were measured to the nearest 0.5 mm using an ocular micrometer or 1-mm graph paper placed on the dissecting microscope stage, underneath the prey item. Prey biomass (AFDM) was then estimated using established length-mass or head width-mass regressions (J.B Wallace *unpublished data*; Benke et al. 1999)

Statistical Analyses

Linear regression was used to determine if salamanders expressed gape-limitation with respect to prey consumption. Gut biomass (only salamanders that had prey items) was combined across all sampling dates and streams, and regressed against salamander snout-vent length (SVL) for each species of salamander separately. Due to low pre-treatment sample size in the spring, further analyses were conducted with only summer sampling dates. Patterns in prey consumption (biomass, prey number, prey size) were assessed using linear regression. We initially analyzed the data with stream as a random effect but found that it did not improve our models so we removed stream as a random effect. We used treatment year and SVL as our explanatory variables.

Stomach contents were summarized for each salamander species and each stream by calculating percent AFDM and abundance based on estimated biomass from length-mass regressions. To more clearly understand what the average diet of a salamander contained, we

also calculated the estimated contribution to biomass (ECB) per stomach for a given taxa by incorporating average biomass of a prey item, the probability of that item occurring in a stomach, and the average number of that item when it did occur:

$$ECB=P[i]*N*B$$

Where P[i] is the probability of taxa *i* being present in a stomach (composed of the total proportion of stomachs with that taxon multiplied by the total proportion of stomachs containing any prey); N is the average number of individuals in a stomach when present, and B is the average biomass of that taxa consumed (mg AFDM $\cdot$ gut^{-1}). This calculation represents the expected biomass of a given taxon in any randomly sampled stomach. To determine changes of importance to diet biomass for individual prey items we compared changes in ECB to the pre-treatment average ECB for each taxon. We calculated the pre-treatment range of ECB across all streams and dates for each taxon *i* by calculating the pretreatment 95% confidence interval (CI) around each mean ECB for taxa *i.* The mean was then centered on zero and the difference of mean treatment ECB (each stream and date) and pre-treatment ECB was plotted in comparison to the 95 % CI to determine how treatment ECB compared to the natural variation pre-treatment. If ECB fell outside of the CI or all sample points were consistently high or low in the CI that would suggest that enrichment caused a response outside of the pre-treatment variation.

Prey items were also assigned to functional feeding groups (FFG) (Cross 2004; Merritt et al. 2008) to determine if changes in basal resources affected pathways of energy flow to salamanders. We used ANOVA to assess treatment year (PRE, YR1, YR2) differences for the ECB of each FFG. Linear regression was used to compare changes in FFG with basal resource availability. All statistical analyses were performed in R version 3.0.2 (R Core Team 2013).

Results

General patterns in larval consumption

We collected 409 *D. quadramaculatus* (mean=14, min=9 max=18) and 349 *E. wilderae* (mean=13, min=6, max=17) from all streams and sampling dates. Prey were found in 373 (91%) and 291 (83%) stomachs for *D. quadramaculatus* and *E. wilderae*, respectively. Stomachs contained an average of 4.3 (SE ± 0.14) prey items · gut^{-1} for *D. quadramaculatus* (1.3 ± 0.11 mg AFDM · gut^{-1}) and 6.4 ± 0.33 items · gut^{-1} for *E. wilderae* (0.12 ± 0.01 mg AFDM · gut^{-1}). Average prey size was 3.1 ± 0.11 mm and 1.5 ± 0.06 mm for *D. quadramaculatus* and *E. wilderae*, respectively. Prey biomass in stomachs was positively related to SVL for *D. quadramaculatus* (Fig. 3.1a, R^2= 0.18, P < 0.001) and *E. wilderae* (R^2= 0.22, P =0.002), indicating that both species can be gape limited. Number of prey items in *D. quadramaculatus* stomachs was not correlated with SVL (R^2=0.001, P = 0.4) but average prey size was (R^2=0.13, P < 0.001). Conversely, in *E. wilderae,* the number of prey items was weakly correlated with SVL (R^2=0.07, P < 0.001), while average prey size was not (R^2=0.008, P =0.12).

Collectively, *D. quadramaculatus* consumed 56 unique prey taxa, while *E. wilderae* consumed 42 prey taxa. Across all streams and dates for *D. quadramaculatus, Tallaperla* (Plecoptera: Peltoperlidae) was the most important prey item in terms of biomass, accounting for 19.8 % of ECB and 9.9 % of abundance, but was variable across streams and years (Table 3.2, Appendix 3.1 Table 2). *Stenonema* (Ephemeroptera: Heptageniidae) and *Wormaldia* (Trichoptera: Philopotamidae) were the next most important prey items (9.4% and 6.1% ECB; 2.5% and 4.5% abundance, respectively). Non-Tanypodinae chironomids accounted for 22% of all prey items (< 4% of ECB; Table 3.2) in *D. quadramaculatus* stomachs. The most dominant taxa in stomachs of *E. wilderae* were *Leuctra* (Plecoptera: Leuctridae) (18.3% ECB, 4.5 %

Abundance, Table 3.3) non-Tanypodinae chironomids (16.1% ECB, 24% Abundance) and Paraleptophlebiid mayflies (8.3% ECB, 0.7% Abundance). Copepods accounted for 45% of all prey items in *E. wilderae* stomachs but only 1.2% of ECB (Table 3.3, Appendix 3.1 Table 3)

Larval diet responses to enrichment

Due to low sample size for spring pretreatment, only summer dates were used to examine patterns in prey consumption compared among treatment years. Prey biomass in stomachs of *D. quadramaculatus* increased with enrichment and was significantly higher for a given SVL in YR2. There was a significant interaction with SVL with larger individuals eating larger prey (Fig. 3.1a, Table 3.4). Average prey size consumed increased in both treatment years for a given SVL but the interaction between treatment year and SVL did not change (Fig 3.1b, Table 3.4). Average number of prey items per stomach did not change in YR1 but increased in YR2 for larger individuals, as there was a significant interaction with SVL (Table 3.4, Fig. 3.1c).

Prey biomass in *E. wilderae* stomachs did not change in response to enrichment and there was no significant interaction with SVL (Fig. 3.2a, Table 3.4). Average prey size was significantly larger in YR2 in both years of enrichment (Fig. 3.2b, Table 3.4). Prey size increased from an average of 1.45 (± 0.14 mm) and 1.34 (± 0.06 mm) in PRE and YR1, respectively, to 2.17 (± 0.16 mm) in YR2. Average number of prey items per stomach increased significantly in YR1 but decreased (non-significantly) in YR2 compared to PRE (Fig 3.2c, Table 3.4).

For functional group analysis we grouped taxa into five main FFG. Across all functional groups in *D. quadramaculatus* stomachs, collector-filterers (CF) were the only group that significantly changed, decreasing with enrichment ($F_{2, 12} = 8.22$, $P = 0.006$, Fig. 3.3a). Scrapers (SC) and collector-gatherer (CG) functional groups exhibited increasing trends during both years

of enrichment, however these increases were non-significant ($F_{2, 12}$ = 2.17 P =0.16; $F_{2, 12}$ = 2.83, P = 0.09, respectively, Fig. 3.3a). However, these two FFG were the only groups that had taxa consistently at the high end or above the pre-treatment 95% CI of ECB (Appendix 3.1 Fig 1). All SC and CG taxa that increased in ECB consumed biofilm as part of their diet (Cross 2004, Merritt et al. 2008). Therefore, we analyzed these two groups together as a biofilm-consuming group. In YR2 of enrichment, the combined ECB of CG and SC increased significantly ($F_{2, 12}$ = 4.02, P = 0.04, Fig. 3.3b). Increases in ECB of these two biofilm consuming groups were positively but non-significantly ($F_{1, 13}$ = 3.84, P = 0.07, Adj R^2=0.17, Fig. 3.4) correlated with a significant increase in peak biofilm biomass in response to enrichment ($F_{2,12}$ = 10.06, P = 0.002).

Functional group analysis in *E. wilderae* stomachs revealed that the contribution to ECB of SH and CG generally increased each year of enrichment however; no significant changes in the ECB of any FFG were detected (Fig. 3.5). There was no detectable pattern among any individual taxa according to the 95% CI ECB (Appendix 3.1 Fig 2).

Discussion

Our study revealed that effects of nutrient enrichment on larval salamander prey consumption may differ among species and can occur through unexpected resource pathways. Enrichment of the five study streams was previously shown to result in increased growth rates of *D. quadramaculatus* and *E. wilderae.* We conducted this study to more mechanistically understand how nutrients propagated through a detritus-based food web, and increased growth rates of a top predator. We hypothesized three main mechanisms by which salamanders would increase growth rates in response to nutrient enrichment. In this study, we were not able to

address changes in individual salamander prey stoichiometry, which would reduce elemental imbalances that larval salamanders face. We were able to assess whether changes in prey biomass consumed or prey composition occurred in response to enrichment.

Mechanism 1- Increased biomass of prey in salamander stomachs

Support for this mechanism was found for *D. quadramaculatus* but not *E. wilderae.* Larval *D. quadramaculatus* increased prey consumption in both years of enrichment compared to pre-enrichment, significantly so in YR2. However, the effect size was dependent on size of larvae indicating that smaller larvae may have been gape limited. Average prey size increased for a given SVL in both years of enrichment and average number of prey decreased in YR1 but generally increased in YR2. Thus, increased total biomass consumed likely resulted from a combination of both increased prey size and number of prey items in the stomachs of *D quadramaculatus*.

Changes in prey production would first have to occur for changes in prey consumption by salamanders. A previous enrichment study of a single stream (N:P=16:1) at Coweeta resulted in increased secondary production of primary consumer invertebrates (Cross et al. 2006). Further, fertilization of a tundra stream in Alaska increased the densities of invertebrate prey important for Arctic grayling, a vertebrate top predator (Slavik 2004 and references therein). In our streams, preliminary analysis shows that biomass of important prey resources (e.g. *Tallaperla* and *Leuctra*) for *D. quadramaculatus* increased during the first year of enrichment, as well as *Chironomidae,* an important prey item for both *D. quadramaculatus* and *E. wilderae* (L.M Demi, *unpublished data*). Increased biomass in stomachs of *D. quadramaculatus* occurred despite an ~ 77% (0.23×) decrease in collector-filterer biomass consumed over both years of enrichment

compared to pre-enrichment. Decreased collector-filterer biomass was replaced by a 1.7× and 2.2× increase in collector-gatherer biomass and a 1.1× and 5.0× increase in scraper biomass consumed in years one and two, respectively. Increased prey densities or prey size, could allow larvae to meet their energetic demands more efficiently (Charnov 1976). Larval salamanders are ambush predators that engulf prey through suction feeding (Lauder and Shaffer 1986) therefore, they should encounter prey more frequently and spend less time foraging, investing more energy in growth. Alternatively, increased prey densities could actually lead to more active feeding which as been linked with increased growth rates in larval salamanders (Maurer and Sih 1996; Bernardo and Agosta 2003).

Other studies have also linked changes in basal resources to changes in growth rates of larval salamanders (Johnson and Wallace 2005; Johnson et al. 2006; Huntsman et al. 2011). Hunstman et al. (2011) found that differing in organic matter inputs in two cave systems led to differences in invertebrate production, with higher production corresponding to higher growth rates and population size of the cave salamander *Gyrinophilus palleucus.* The difference in growth was partially attributed to higher consumption by salamanders in the cave receiving higher allocthonous inputs. In our study, increased production of primary consumers that could lead to increased consumption by salamanders would result from increased quality of detrital carbon or increases in autotrophic biomass. Johnson et al. (2006) found increased larval salamander growth rates in response to nutrient enrichment and attributed this to an increase in copepods and chironomids, two of the most common prey items in *E. wilderae* stomachs. In addition, the authors of this study further hypothesized that changes in quality of prey could also contribute to increased growth rates.

Mechanism 2-Changes in prey composition that reduces stoichiometric constraints

Our study indicated that changes in the composition of functional groups in the diets of *D. quadramaculatus* occurred in our study, but we found no support for composition changes in *E. wilderae.* Over both years of enrichment the biomass of scraper and collector-gatherer functional groups increased in the diets of *D. quadramaculatus*, while the biomass of shredders did not change with enrichment. Taxa that increased in these two functional groups all consume at least some biofilm as part of their diet in Coweeta streams (Cross et al. 2005). Increases of these functional groups in the diet of *D. quadramaculatus* is surprising considering the minor role that biofilms have in overall carbon availability in our shaded headwater streams. Biofilm biomass did increase in response to enrichment. However, even with this increase, biofilms still made up a very small fraction of the total carbon available to primary consumers (<1 % of carbon, A.D. Rosemond *unpublished data*). Regardless, the increase in collector-gatherer and scraper biomass was correlated with increases in biofilm availability. This was coupled with concomitant decreases in the availability of detrital carbon (both spatially and temporally; A.D. Rosemond *unpublished data*), likely resulting in a greater need for consumers to utilize increases in biofilm resources. Further, biofilm biomass peaked in the late spring and early summer in our streams coinciding with periods of very low detrital standing stocks.

Changes in the composition of prey in the diets of larval *D. quadramaculatus* may result in reduced stoichiometric changes as well. In reference conditions scrapers and collector-gatherer invertebrate functional groups are generally higher in nutrient content than shredder taxa, though this pattern is highly variable (Cross et al. 2003; Frost et al. 2006). Cross et al. (2003) found the average scraper and collector-gatherer body C:P was 369 and ~ 250 compared to 498 for shredders. Therefore, larvae that increase their consumption of these taxa that are higher in

nutrient content because they become more abundant compared to shredder taxa are likely reducing stoichiometric constraints on growth.

Differences between D. quadramaculatus and E. wilderae

We found markedly different responses in the diets of our two study species. No discernable change occurred in quantity of prey consumed by *E. wilderae* despite increased prey size, yet we saw both prey quantity and composition changes in *D. quadramaculatus.* There are several possible explanations for the apparent lack of response in *E. wilderae.* No apparent change in prey biomass in stomachs of *E. wilderae* could be the result of insufficient sampling of this particular species. We assumed that larvae feed predominately at night and therefore targeted our sampling during that time only. If this assumption is false, prey biomass in stomachs may not change with enrichment despite increased consumption if *E. wilderae* are feeding more frequently throughout the day. Increased frequency of feeding would not likely show up in our sampling methods of only sampling at night (Petranka 1984). Moreover, a lack of biomass response may also indicate that *E. wilderae* were specifically responding to changes in the stoichiometry of their prey and not increased prey availability.

Our findings are similar to that of Johnson and Wallace (2005) who found that growth rates of *E. wilderae* decreased in response to a reduction in detrital resources from litter exclusion, despite no change of quantity of prey consumed. Instead they concluded that changes in prey quality or changes in energetic demands were responsible. Prey compositional changes in *D. quadramaculatus* in this study likely lead to reduced stoichiometric constraints which would likely increase growth rates. We were not able to assess changes in the stoichiometry of prey in

this study, but ongoing studies will indicate if salamander prey shifted their stoichiometry in response to nutrient enrichment.

Differences in microhabitat preference between our two study species likely led to the difference in composition changes. The primary habitat of *E. wilderae* in our study streams and similar ones are sandy depositional areas. Sandy depositional habitat is less likely to contain hard stable substrates that biofilms could attach to. Therefore, *E. wilderae* would not be able to take advantage of an increase in biofilm consuming prey because they would have a much lower probability of encountering those taxa. In comparison, *D. quadramaculatus* were commonly found throughout our study streams and are more likely to encounter invertebrates that feed on biofilm. These results indicate that microhabitat preferences among individual species may partially determine the response to nutrient-driven changes in basal resources and prey.

Our study partially revealed the mechanisms by which salamanders exhibited increased growth rates. We show that multiple mechanisms can work together to propagate nutrient-driven changes to basal resources in detritus-based streams. Moreover, this study reveals that variation among species life history (i.e. gape limitation or microhabitat selection) influences the mechanisms by which they are affected. Our results also indicate that surprising food web pathways can arise as a result of nutrient enrichment and suggests that forested streams that have more open canopies or undergo habitat alteration that results in a more open canopy, may result in even greater invertebrate community changes in response to nutrient enrichment that could affect salamander predators. Finally, this study reveals the need to understand threshold elemental ratios for consumers so that we can better predict how consumers will respond to nutrient enrichment.

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Table 3.1. Target concentrations of dissolved nitrogen and phosphorus added to the treatment reaches to achieve target N:P ratio. Streams are labeled as the target N:P ratio. Values represent the targets injected into the treatment streams via an irrigation line during the 2-year enrichment. DIN an SRP are in μg/L.

		Stream (Target Ratio)				
		2:1	8:1	16:1	32:1	128:1
DIN	Target	81.3	243.9	365.8	487.7	650.3
SRP	Target	90.0	67.5	50.6	33.8	11.3

Table 3.2. Mean expected biomass (mg AFDM gut^{-1}) and abundance (no. Individuals gut^{-1}) of taxa that accounted for at least 1.0% of biomass in *D. quadramaculatus* stomachs for all streams and sampling dates. Capital letters before taxa indicate order. C=Coleoptera, D=Diptera, E=Ephemeroptera, H=Hymenoptera, O=Odonata, P= Plecoptera, T=Trichoptera, VC= Vertebrate Chordata.

Taxa	ECB gut^{-1}	% Total ECB	% Total Abundance	Exp # gut^{-1}
P-Tallaperla	0.237	19.76	9.94	0.371
E-Stenonema	0.113	9.44	2.45	0.089
T-Hydropsychidae	0.103	8.63	2.76	0.113
T-Wormaldia	0.073	6.12	4.23	0.149
E-Paraleptophlebia	0.067	5.57	3.80	0.142
E-Serratella	0.065	5.44	4.54	0.152
E-Epeorus	0.064	5.36	0.12	0.008
P-Leuctra	0.055	4.57	12.52	0.430
VC-Eurycea	0.044	3.67	0.18	0.007
D-Non-Tanypodinae	0.043	3.57	22.70	0.820
P-Amphinemura	0.037	3.13	6.50	0.210
E-Baetis	0.032	2.66	2.09	0.073
O-Cordulegaster	0.019	1.57	0.25	0.009
H-Formicidae	0.018	1.46	0.12	0.004
P-Beloneuria	0.015	1.22	0.25	0.009
Lepidoptera	0.014	1.16	0.06	0.002
T-Lepidostoma	0.012	1.04	1.72	0.085

Table 3.3. Mean biomass (mg AFDM gut^{-1}) and abundance (no. individuals gut^{-1}) of taxa that accounted for at least 1.0% of biomass in *E. wilderae* stomachs for all streams and sampling dates. Capital letters before the taxa name indicate taxa order. C=Coleoptera, D=Diptera, E=Ephemeroptera, P= Plecoptera, T=Trichoptera.

Taxa	ECB gut^{-1}	% Total ECB	% Total Abundance	Exp # gut^{-1}
P-Leuctra	0.0240	18.33	4.58	0.2903
D-Non-tanypodinae	0.0172	16.11	24.01	1.2828
E-Paraleptophlebia	0.0301	8.38	0.72	0.1986
E-Serratella	0.0267	8.36	0.61	0.1057
P-Tallaperla	0.0155	8.09	1.32	0.1276
D-Ceratopognia	0.0125	6.10	1.43	0.1366
D-Hexatoma	0.0299	5.20	0.33	0.1177
E-Stenonema	0.0656	4.56	0.11	0.0788
P-Amphinemura	0.0097	3.71	0.99	0.1447
D-Tanypodinae	0.0037	3.10	6.51	0.3894
D-Rhabdomastix	0.0158	1.65	0.17	0.0851
T-Lype	0.0229	1.59	0.11	0.0857
D-Tipulidae	0.0225	1.57	0.11	0.0690
T-Lepidostoma	0.0071	1.48	0.44	0.1042
T-Psilotreta	0.0207	1.44	0.11	0.0690
T-Fattigia	0.0200	1.39	0.22	0.1250
Copepoda	0.0012	1.19	45.31	2.2070
P-Isoperla	0.0055	1.14	0.44	0.0946

Table 3.4. Parameter estimates (SE) and P values for prey consumption in *D. quadramaculatus* (DQUAD) and *E. wilderae* (EWILD) relating size of larvae and treatment year to total biomass consumed, average prey size consumed, and number of prey items consumed. Significant interactions between treatment year and SVL are indicated, when interactions were not significant, main effects are shown.

Parameter	Estimate (SE)	P	Parameter	Estimate (SE)	P
DQUAD			**EWILD**		
Total Biomass: $F_{5, 215} = 11.25$			**Total Biomass:** $F_{3, 146} = 1.19$		
Intercept	-0.45 (0.78)	0.56	Intercept	-0.025 (0.08)	0.74
Year 1	-1.03 (1.13)	0.36	Year 1	0.02 (0.04)	0.55
Year 2	-3.42 (1.17)	0.004	Year 2	0.04 (0.05)	0.40
SVL	0.06 (0.03)	0.019	SVL	0.01 (0.01)	0.17
Year 1: SVL	0.04(0.04)	0.34			
Year 2: SVL	0.14 (0.04)	0.001			
Prey Size: $F_{3, 217} = 10.27$			**Prey Size:** $F_{3,146} = 4.10$		
Intercept	0.52 (0.51)	0.31	Intercept	1.06 (0.49)	0.03
Year 1	0.53 (0.27)	0.04	Year 1	-0.08 (0.24)	0.74
Year 2	0.56 (0.27)	0.04	Year 2	0.81 (0.30)	0.006
SVL	0.09 (0.02)	<0.001	SVL	0.03 (0.04)	0.49
No. Prey: $F_{5, 215} = 2.68$			**No. Prey:** $F_{3,146} = 6.78$		
Intercept	5.15 (1.03)	< 0.001	Intercept	2.58 (1.39)	0.07
Year 1	0.14 (1.49)	0.93	Year 1	1.89 (0.68)	0.006
Year 2	-4.09 (1.55)	0.008	Year 2	-1.55 (0.85)	0.07
SVL	-0.03 (0.04)	0.33	SVL	0.11 (0.12)	0.35
Year 1: SVL	-0.03 (0.05)	0.55			
Year 2: SVL	0.15 (0.06)	0.008			

Figure Legends

Fig. 3.1 Patterns in prey consumption regressed against size of larvae (SVL) for *D. quadramaculatus* during summer sampling dates for (a) total prey biomass consumed, (b) average prey size consumed, and (c) average number of prey items in stomachs. Points represent the average across all streams in a given year. Dashed lines = pre-treatment, grey lines = year 1 of enrichment, and black lines = year 2 of enrichment.

Fig. 3.2 Patterns in prey consumption regressed against larval size (SVL) for *E. wilderae* during summer sampling dates for (a) total prey biomass consumed. Points represent the average across all streams in a given year. (b) Average prey size consumed in each year and (c) number of prey items in stomachs by year.

Fig. 3.3 (a) Estimated contribution to biomass (ECB) for functional groups in *D. quadramaculatus* stomachs. Letters above bars represent statistical differences within a functional group determined by Tukey's post hoc test. SH = shredder ($F_{2, 12}$= 0.25, P = 0.78), CG = Collector-gatherer ($F_{2, 12}$= 2.83, P = 0.09), CF = Collector-filterer, SC = Scraper ($F_{2, 12}$= 2.17, P = 0.16), and P = Invertebrate Predator ($F_{2, 12}$= 0.82, P = 0.46). (b) Combined estimated contribution to prey biomass of scraper and collector-gatherer taxa. Letters above bars represent statistical differences among years determined by Tukey's post hoc test.

Fig. 3.4 The relationship between biofilm biomass and scraper plus collector-gatherer biomass across treatment years. Biofilm biomass is the mean 3-month peak biomass (April-June) for each

treatment stream. Invertebrate biomass is the combined estimated contribution to prey biomass for each stream and year.

Fig. 3.5 Estimated contribution to biomass (ECB) for functional groups in *E. wilderae* stomachs. There were no statistical differences among treatment years for any functional group. SH = shredder ($F_{2, 12}$= 0.11, P = 0.90), CG = Collector-gatherer ($F_{2, 12}$= 0.46, P = 0.64), CF = Collector-filterer ($F_{2, 12}$= 1.08, P = 0.37), SC = Scraper ($F_{2, 12}$= 1.0, P = 0.40), and P = Invertebrate Predator ($F_{2, 12}$= 1.0, P = 0.38).

Fig. 3.1

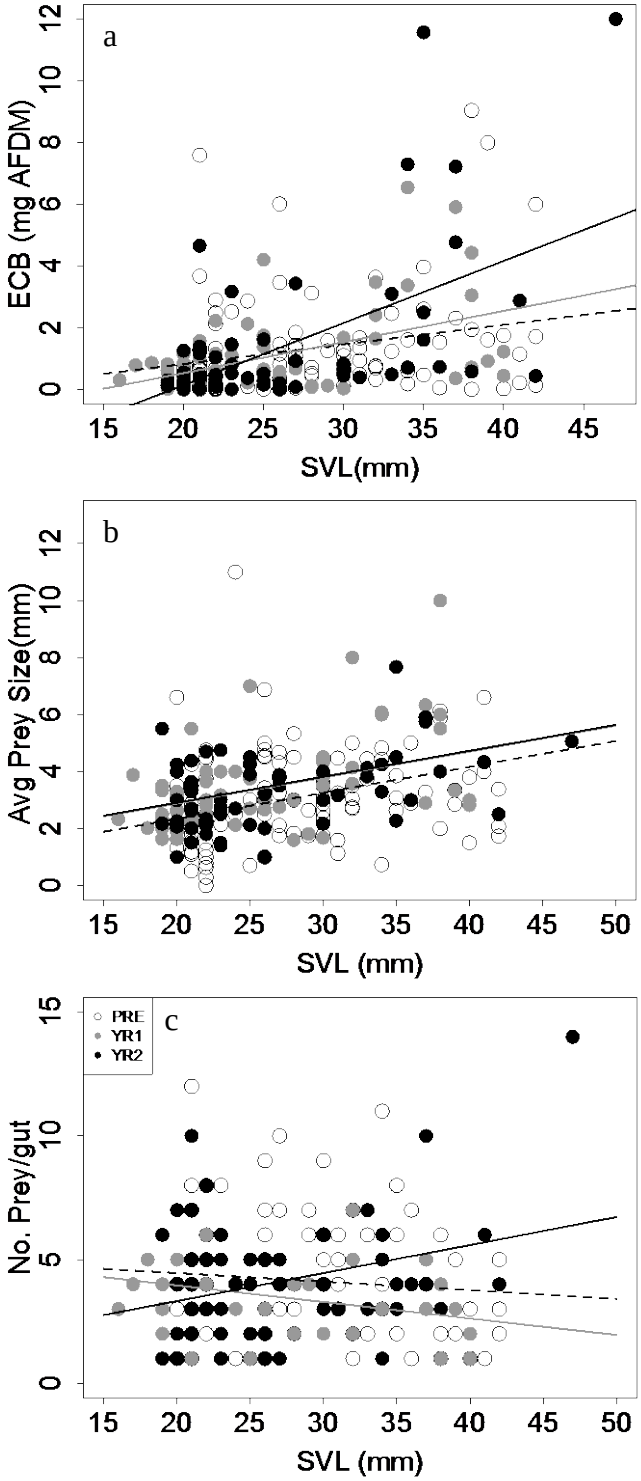

a
ECB (mg AFDM)
SVL(mm)
b
Avg Prey Size(mm)
SVL (mm)
c
PRE
YR1
YR2
No. Prey/gut
SVL (mm)

Fig. 3.2

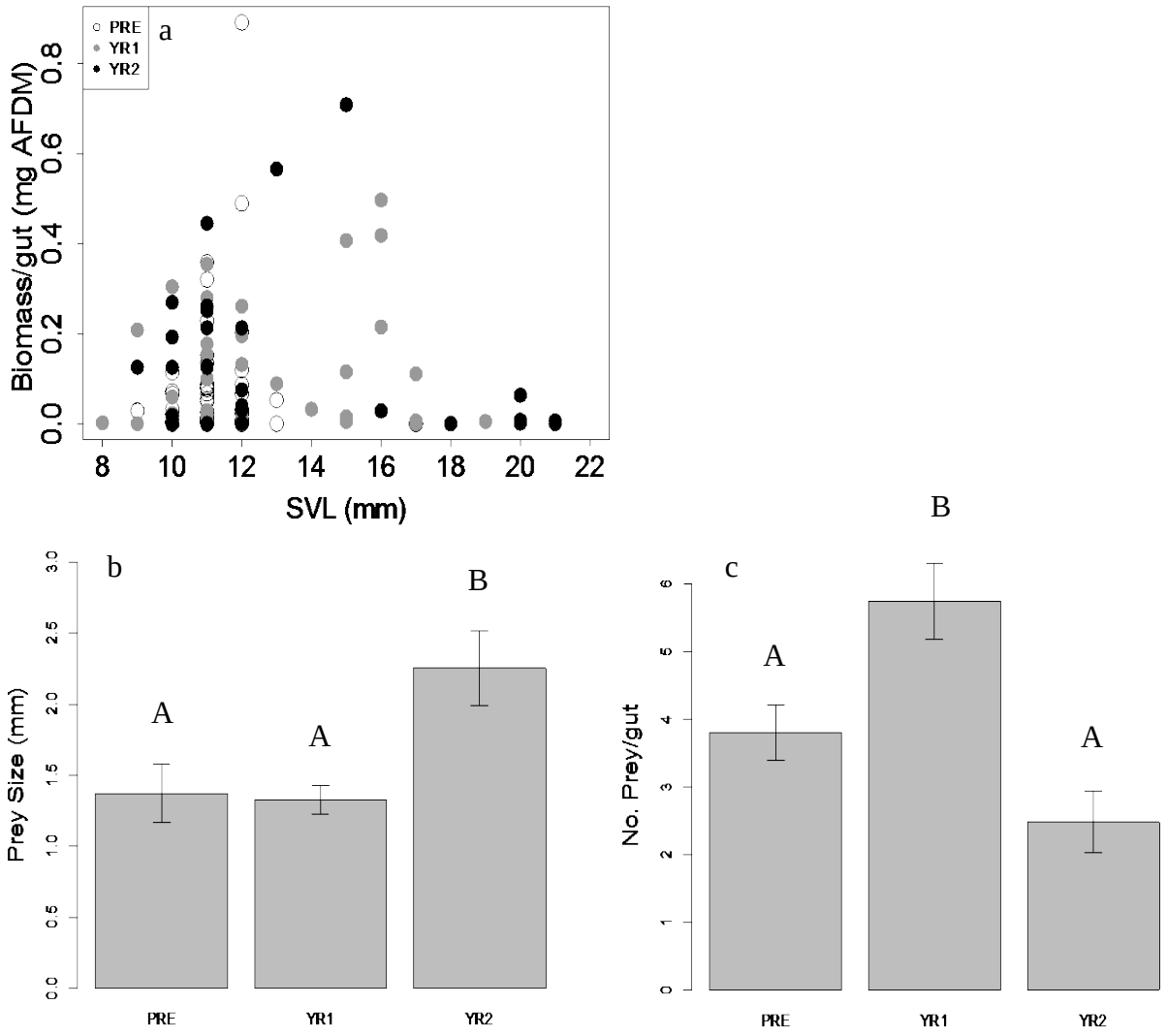
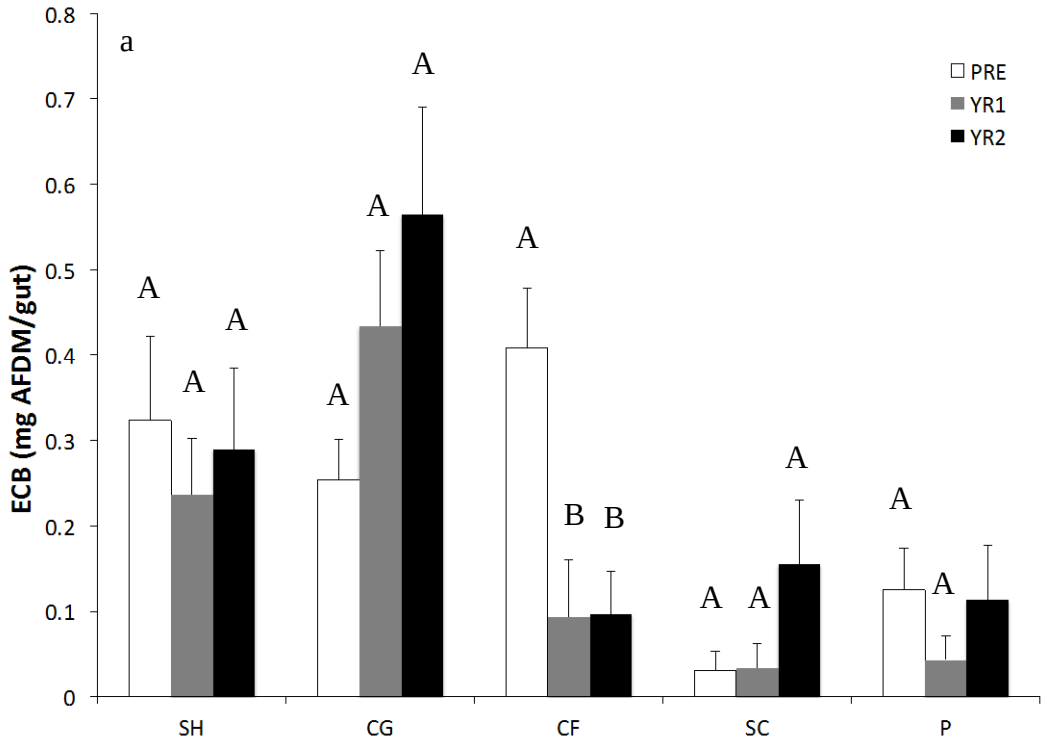

a
PRE
YR1
YR2
Biomass/gut (mg AFDM)
0.0
0.2
0.4
0.6
0.8
8
10
12
14
16
18
20
22
SVL (mm)
b
Prey Size (mm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
A
A
B
PRE
YR1
YR2
c
No. Prey/gut
0
1
2
3
4
5
6
A
B
A
PRE
YR1
YR2

Fig. 3.3


a
ECB (mg AFDM/gut)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
PRE
YR1
YR2
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A
A
A
A
A
SH
CG
CF
SC
P

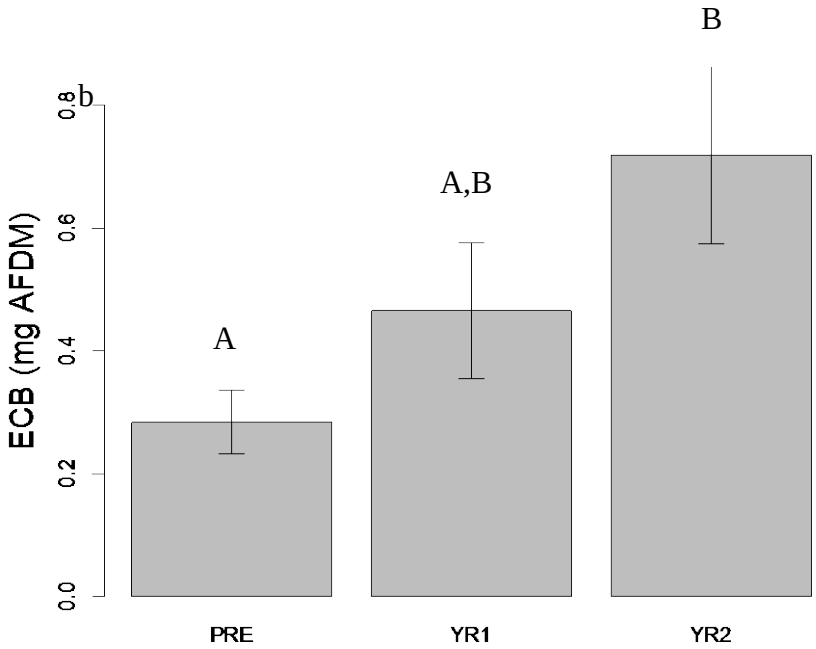

b
ECB (mg AFDM)
0.8
0.6
0.4
0.2
0.0
A
A,B
B
PRE
YR1
YR2

Fig. 3.4

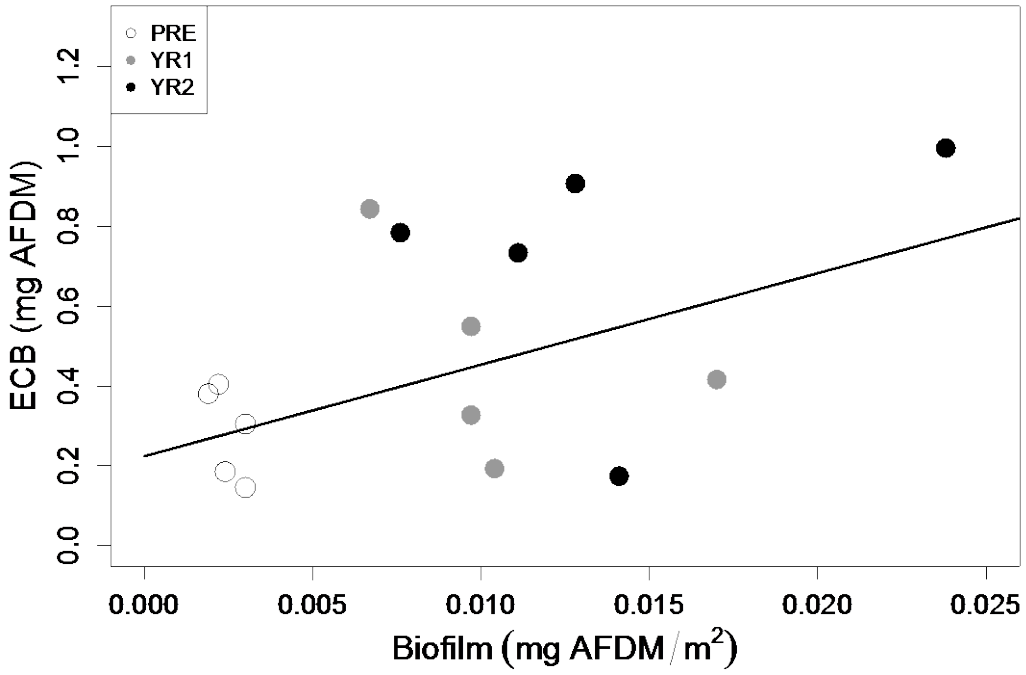
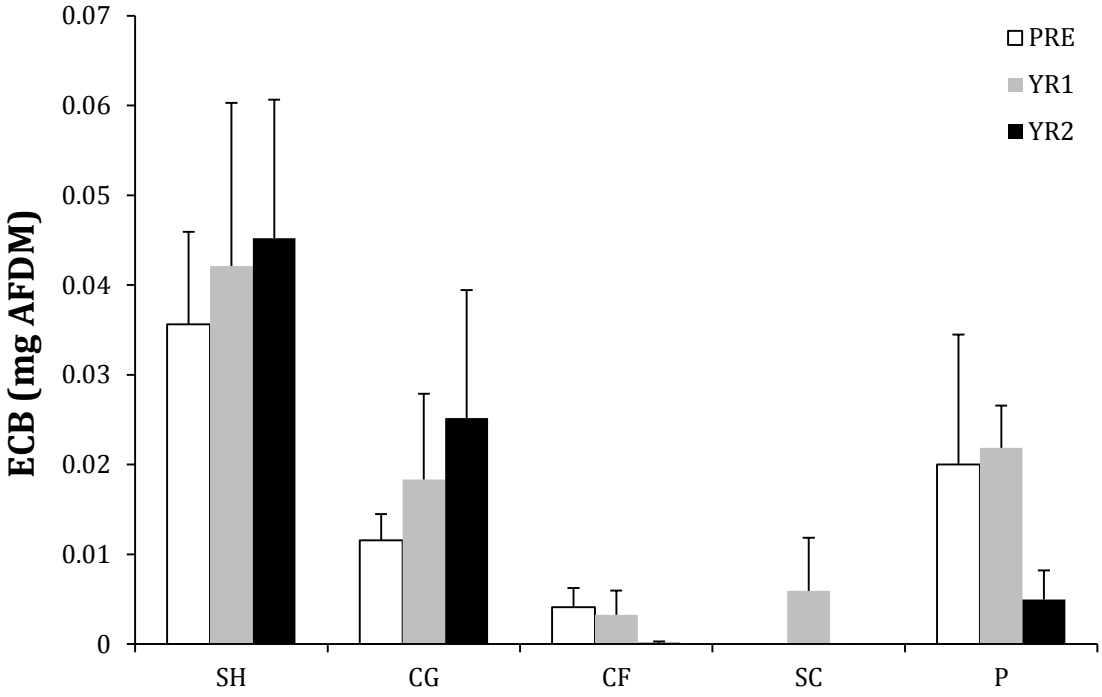

PRE
YR1
YR2
ECB (mg AFDM)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.000
0.005
0.010
0.015
0.020
0.025
Biofilm (mg AFDM / m2)

Fig. 3.5



Appendix 3.1

Table 1. Taxa identified in stomachs of *D. quadramaculatus* combined across all streams and sampling dates (spring and summer; pre-treatment and treatment) that make up < 1% of ECB. Capital letters before taxa indicate order. Taxa are ranked in decreasing order by % ECB. ECB gut^{-1} and Exp # gut^{-1} is the overall expected biomass and number of individuals of that taxa in any randomly sampled stomach.

Taxa	ECB gut^{-1}	% ECB	% Abundance	Exp # gut^{-1}
T-Pycnopsyche	0.012	0.98	0.12	0.004
T-Neophylax	0.012	0.98	2.82	0.091
T-Rhyacophila	0.011	0.92	0.37	0.011
P-Sweltsa	0.010	0.87	0.74	0.031
C-Elmidae (larvae)	0.009	0.74	1.17	0.040
D-Hexatoma	0.009	0.72	0.49	0.016
P-Isoperla	0.008	0.69	0.74	0.026
P-Strophopteryx	0.005	0.44	0.12	0.004
Ostracoda	0.005	0.41	0.43	0.025
T-Fattigia	0.005	0.39	0.98	0.035
T-Polycentropus	0.004	0.36	0.61	0.026
D-Simuliidae	0.004	0.35	0.74	0.033
D-Tanypodinae	0.004	0.35	8.22	0.290
Copepoda	0.004	0.30	0.92	0.040
D-Pericoma	0.004	0.30	0.06	0.007
T-Psilotreta	0.003	0.28	0.25	0.009
D-Ceratopognia	0.003	0.28	0.61	0.025
C-Curculionidae	0.003	0.28	0.12	0.003
T-Lype	0.003	0.25	0.12	0.005
T-Molanna	0.003	0.25	0.06	0.002
C-Elmidae (adult)	0.003	0.23	0.18	0.009
D-Tipulidae	0.002	0.20	0.12	0.004
D-Dixa	0.002	0.15	0.86	0.029
D-Raphium	0.001	0.09	0.31	0.013
D-Rhabdomastix	0.001	0.09	0.12	0.003
D-Pelecorhynchidae	0.001	0.05	0.06	0.002
D-Dicranota	0.000	0.04	0.25	0.011
T-Hydroptila	0.000	0.03	0.06	0.002

Collembola	0.000	0.02	0.37	0.012
C-Ectopria	0.368	0.016	0.805	0.010
D-Cecidomyiidae	0.000	0.01	0.12	0.004
D-Empid	0.000	0.01	0.18	0.006
Hydracarina	0.000	0.00	0.06	0.002
Nematoda	0.000	0.00	0.18	0.007
Ephemeroptera	0.008	0.69	0.25	0.011
Trichoptera	0.029	2.384	0.982	0.046
Plecoptera	0.008	0.71	0.98	0.041
Other	0.000	0.00	0.61	0.024

Table 2. Expected biomass (mg AFDM gut^{-1}) and estimated abundance (# individuals gut^{-1}) found in *D. quadramaculatus* stomachs for each sampling date and stream. Biomass and abundance are calculated separately for each date and stream. Prey items are listed alphabetically by order then family or genus

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
6/2010										
Cambarus	-	-	-	0.0000	-	-	-	0.1000	-	-
Coleoptera										
Ectopria	-	-	-	-	0.2363	0.3000	-	-	0.0591	0.1000
Elmidae (adult)	-	-	0.0266	0.1000	0.0696	0.2000	-	-	-	-
Elmidae (larvae)	-	-	-	-	0.0106	0.1000	-	-	-	-
Copepoda	-	-	-	-	-	-	-	-	0.0000	0.1000
Diptera										
Ceratopognia	-	-	0.0117	0.1000	0.0011	0.1000	0.0117	0.1000	0.0146	0.3000
Dicranota	-	-	0.0098	0.1000	0.0051	0.1000	0.0020	0.1000	-	-
Dixa	-	-	-	-	-	-	-	-	0.0045	0.1000
Hexatoma	-	-	-	-	-	-	0.0037	0.1000	-	-
Non-Tanypodinae	0.1851	2.3000	0.0746	1.2000	0.0282	0.2000	0.0740	0.6000	0.0123	0.5000
Simuliidae	0.0135	0.1000	0.0499	0.3000	-	-	-	-	0.0337	0.1000
Tanypodinae	0.0009	0.1000	0.0242	0.1000	0.0048	0.2000	-	-	0.0047	0.1000
Ephemeroptera										
Baetis	-	-	0.0039	0.2000	-	-	-	-	-	-
Paraleptophlebia	-	-	-	-	0.1256	0.1000	0.1070	0.4000	0.2227	0.2000
Serratella	-	-	-	-	-	-	0.0908	0.1000		
Stenonema	-	-	0.6008	0.3000	-	-	0.6129	0.3000	0.0627	0.2000
Other	0.0915	0.1	-	-	0.1958	0.2	-	-	0.0023	0.1
Nematomorpha	-	0.4000	-	0.1000	-	-	-	-	-	-

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
Odonata										
Cordulegaster	0.5295	0.1000	-	-	-	-	-	-	-	-
Plecoptera										
Isoperla	0.0154	0.1000	-	-	-	-	-	-	-	-
Leuctra	0.0292	0.2000	0.0117	0.1000	-	-	-	-	0.0290	0.2000
Sweltsa	-	-	-	-	-	-	0.0060	0.1000	-	-
Tallaperla	0.4691	0.4000	0.0489	0.1000	0.0172	0.2000	0.0373	0.2000	0.0811	0.1000
Other	0.0620	0.4	-	-	0.0116	0.2	0.2208	0.2	0.00074	0.1
Trichoptera										
Arctopsyche	0.0777	0.2000	0.2216	0.4000	-	-	0.3389	0.5000	0.0179	0.1000
Diplectrona	0.1149	0.5000	0.0017	0.1000	0.1071	0.1000	0.0010	0.1000	0.0009	0.1000
Fattigia	-	-	-	-	0.1144	0.1000	-	-	-	-
Lepidostoma	-	-	-	-	-	-	-	-	0.0027	0.1000
Neophylax	0.0007	0.1000			-	-	-	-	-	-
Parapsyche	-	-	1.1047	0.5000			0.8723	0.1000	-	-
Polycentropus	0.0291	0.4000	0.0634	0.1000	0.0011	0.1000	0.0483	0.1000	0.0027	0.1000
Psilotreta	0.0278	0.1000	-	-	-	-	-	-	-	-
Wormaldia	0.7316	0.9000	0.0088	0.1000	0.7538	0.1000		-	0.0444	0.1000
Other	0.4024	0.6	0.1071	0.1	0.1550	0.5	0.3176	0.3	0.0168	0.1
Total	2.3782	6.4000	2.2623	3.9000	1.6822	2.3000	2.4267	3.1000	0.5961	2.7000
7/2010										
Coleoptera										
Elmidae (larvae)	0.0065	0.1111	-	-	-	-	-	-	-	-
Diptera										
Ceratopogonia	-	-	-	-	-	-	0.0062	0.1250	-	-
Dixa	-	-	-	-	0.0047	0.1875	0.0057	0.0625	-	-

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
Hexatoma	-	-	-	-	0.0255	0.1250	-	-	-	-
Non-Tanypodinae	0.0066	0.7778	0.0260	1.3333	0.0029	0.3125	0.0120	0.8750	0.0162	1.6000
Raphium	-	-	0.0161	0.1333	-	-	0.0028	0.0625	0.0030	0.0667
Simuliidae	-	-	0.0006	0.0667	-	-			0.0075	0.1333
Tanypodinae	-	-	0.0004	0.4000	-	-	0.0001	0.1875	0.0008	0.2000
Ephemeroptera										
Paraleptophlebia	-	-	-	-	0.0704	0.1250	-	-	-	-
Stenonema	0.0257	0.2222	-	-	0.1018	0.1875	0.0318	0.1250	0.0151	0.0667
Plecoptera										
Amphinemura	-	-	-	-	-	-	0.0330	0.0625	0.0320	0.0667
Isoperla	-	-	0.0124	0.0667	-	-	-	-	-	-
Leuctra	0.2192	0.7778	0.0084	0.0667	0.0330	0.2500	0.0611	0.1875	0.0120	0.1333
Sweltsa	0.0301	0.1111	0.0725	0.1333	-	-	-	-	-	-
Tallaperla	0.5032	0.8889	0.3784	0.4000	0.1951	0.1875	0.1942	0.3750	0.3447	0.7333
Trichoptera										
Diplectrona	-	-	-	-	0.0020	0.0625	-	-	-	-
Fattigia	0.0044	0.2222	-	-	-	-	-	-	-	-
Hydropsichidae	-	-	-	-	-	-	0.0066	0.1875	0.0024	0.0667
Parapscyhe	-	-	0.1360	0.2000	-	-	-	-	-	-
Rhyacophila	-	-	-	-	0.1792	0.0625	-	-	-	-
Wormaldia	-	-	-	-	-	-	0.0001	0.0625	0.2252	0.4000
Total	0.7957	3.1111	0.6508	2.8000	0.6146	1.5000	0.3538	2.3125	0.6590	3.4667

3/2012										
Caudata										
Eurycea	-	-	0.0456	0.0769	-	-	-	-	-	-
Coleoptera										

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
Elmidae (larvae)	0.0016	0.0769	-	-	0.0063	0.0625	0.0009	0.0667	-	-
Collembola	0.0020	0.0769	-	-	-	-	-	-	-	-
Copepoda	0.0003	0.3077	-	-	0.0001	0.1875	0.0001	0.1333	-	-
Diptera										
Ceratopognia	-	-	0.0623	0.0769			-	-	-	-
Dixa	-	-	-	-	0.0057	0.0625	-	-	-	-
Empid	-	-	-	-	-	-	0.0008	0.0667	-	-
Hexatoma	0.1113	0.0769	-	-	-	-	-	-	-	-
Non-Tanypodinae	0.0099	0.6923	0.0023	0.3846	0.0086	0.9375	0.0018	0.8667	0.0037	1.0000
Simuliidae	0.0105	0.0769	-	-	-	-	-	-	0.0002	0.0625
Tanypodinae	-	-	0.0016	0.2308	0.0009	0.1250	0.0006	0.1333	-	-
Ephemeroptera										
Baetis	0.0177	0.1538	0.0104	0.0769	0.0339	0.0625	-	-	0.0754	0.1875
Paraleptophlebia	-	-	0.0546	0.0769	-	-	0.0958	0.3333	0.0344	0.0625
Serratella	0.1149	0.1538	0.0928	0.5385	0.0507	0.0625	0.1633	0.6667	0.0453	0.2500
Stenonema	-	-	-	-	-	-	0.0841	0.1333	0.0298	0.0625
Nematomorpha	0.0000	0.0769	-	-	-	0.0625	-	-	-	-
Nematoda	0.0001	0.0769	-	-	-	-	-	-	-	-
Odonata										
Cordulegaster	0.0325	0.0769	-	-	-	-	0.0282	0.0667	-	-
Ostracoda	0.0000	0.0769	-	-	-	-	0.0467	0.0667	-	-
Plecoptera										
Amphinemura	0.2537	1.1538	0.0156	0.0769	0.0906	0.7500	0.0014	0.0667	0.1379	0.4375
Beloneuria	-	-	0.0992	0.0769	-	-	-	-	-	-
Isoperla	-	-	-	-	0.0071	0.0625	0.0186	0.0667		
leuctra	0.3252	2.2308	0.0024	0.2308	0.1262	1.2500	0.0180	0.6000	0.0470	0.5625
Strophopteryx	-	-	-	-	0.1848	0.1250	-	-	-	-

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
Sweltsa	0.0281	0.3077	0.0993	0.2308	-	-	-	-	-	-
Tallaperla	0.0114	0.2308	0.4263	0.6923	0.7860	0.4375	0.1159	0.4667	0.2013	0.2500
Other	0.0013	0.4615	0	0.0769	-	-	-	-	-	-
Trichoptera										
Fattigia	-	-	0.0137	0.3846	0.0012	0.0625	0.0117	0.1333	0.0095	0.1250
Lepidostoma	0.0051	0.0769	0.0053	0.2308	0.0022	0.0625	0.0006	0.0667	-	-
Neophylax	-	-	0.0041	0.1538	-	-	0.0028	0.0667	-	-
Parapscyhe	0.0041	0.0769	-	-	-	-	-	-	-	-
Psilotreta	0.0047	0.0769	-	-	-	-	-	-	-	-
Pycnopsyche	-	-	0.1034	0.0769	-	-	0.3092	0.0667	-	-
Rhyacophila	-	-	-	-	0.0153	0.0625	-	-	0.0206	0.0625
Wormaldia	0.0862	0.2308	-	-	0.0051	0.0625	-	-	0.0127	0.1250
Total	1.0206	6.7692	1.0387	3.6923	1.3248	4.4375	0.9004	4.0667	0.6177	3.1875

6/2012										
Coleoptera										
Curculionidae	-	-	0.0554	0.0556	-	-	0.0623	0.0625	-	-
Ectopria	-	-	-	-	0.0104	0.0833	-	-	-	-
Elmidae (larvae)	0.0022	0.0714	0.0017	0.0556	0.0025	0.0833	0.0082	0.1250	0.0018	0.0588
Copepoda	-	-	0.0000	0.0556	-	-	-	-	0.0000	0.0588
Diptera										
Ceratopognia	-	-	-	-	-	-	0.0103	0.0625	-	-
Dicranota	0.0006	0.0714	-	-	-	-	-	-	-	-
Hexatoma			0.1053	0.0556	-	-	-	-	-	-
Non-Tanypodinae	0.0045	0.6429	0.0003	0.1667	0.0047	0.4167	0.0024	0.6875	0.0142	0.8824
Pelecorhynchidae	-	-	-	-	-	-	0.0204	0.0625	-	-

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
Rhabdomastix	-	-	0.0284	0.0556	-	-	-	-	0.0076	0.0588
Tanypodinae	0.0001	0.1429	0.0001	0.1667	0.0002	0.1667	0.0000	0.0625	0.0002	0.2353
Tipula	-	-	-	-	-	-	0.0130	0.0625	-	-
Other	-	-	3.333E-05	0.0556	-	-	-	-	-	-
Ephemeroptera										
Baetis	-	-	0.0011	0.0556	0.0384	0.0833	-	-	-	-
Epeorus	-	-	-	-	0.0973	0.0833	-	-	-	-
Paraleptophlebia	0.0219	0.0714	0.1306	0.1111	0.2146	0.3333	0.0260	0.0625	0.0245	0.0588
Serratella	0.1378	0.2143	0.0502	0.1667	0.0897	0.2500	0.1049	0.1250	0.0698	0.2941
Stenonema	0.2405	0.0714	0.0022	0.1111	0.3694	0.0833	-	-	0.3824	0.2353
Hemiptera	-	-	-	0.0556	-	-	-	-	-	-
Hydracarina	0.0012	0.0714	-	-	-	-	-	-	-	-
Plecoptera										
Amphinemura	0.0097	0.2143	0.0753	0.1667	0.0169	0.0833	0.0512	0.1250	0.0570	0.2353
Beloneuria	0.0199	0.0714	-	-	-	-	-	-	-	-
Leuctra	0.0406	0.2857	0.0540	0.4444	0.1128	0.7500	0.0282	0.5000	0.0533	0.5294
Tallaperla	0.3176	0.9286	0.0225	0.1111	0.2546	0.4167	0.1185	0.3333	0.0689	0.2353
Trichoptera										
Diplectrona	0.2122	0.0714	-	-	-	-	-	-	-	-
Fattigia	-	-	0.0009	0.0556	-	-	0.0065	0.1250	-	-
Lepidostoma	0.0003	0.0714	0.0224	0.0556	0.0563	0.0833	0.0059	0.0625	-	-
Parapsyche	0.0018	0.0714	-	-	-	-	-	-	-	-
Polycentropus	-	-	0.0003	0.0556	-	-	0.0052	0.0625	-	-
Psilotreta	-	-	0.0655	0.0556	-	-	-	-	-	-
Rhyacophila	-	-	0.0204	0.0556	-	-	-	-	-	-
Wormaldia	0.1472	0.8571	0.0426	0.0556	0.0142	0.3333	0.0344	0.4375	0.0119	0.1176
Total	1.1580	3.9286	0.6793	2.2222	1.2821	3.2500	0.4973	2.9583	0.6917	3.0000

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
4/2013										
Caudata										
Eurycea	-	-	-	-	-	-	-	-	0.5795	0.0714
Coleoptera										
Elmidae (larvae)	-	-	0.0017	0.0556	-	-	-	-	-	-
Copepoda	-	-	-	-	-	-	0.0000	0.0714	-	-
Diptera										
Dixa	-	-	0.0077	0.1111	-	-	-	-	-	-
Empid	-	-	-	-	-	-	0.0008	0.0714	0.0008	0.0714
Hexatoma	0.0320	0.0625	0.0048	0.0556	-	-	-	-	-	-
Non-Tanypodinae	0.0047	0.5000	0.0015	0.1667	0.2437	2.7692	0.0126	1.3571	0.0260	1.4286
Tanypodinae	0.0001	0.1250	0.0005	0.5000	0.0267	1.9231	0.0030	0.1429	0.0166	1.7857
Ephemeroptera										
Baetis	0.1738	0.2500	0.0039	0.0556	0.3907	0.6154	-	-	0.0644	0.2857
Paraleptophlebia	0.1407	0.2500	0.0597	0.2778	0.0487	0.1538	0.0678	0.6429	0.0507	0.0714
Serratella	0.1242	0.6250	0.2193	0.2778	0.0192	0.0769	0.1795	0.6429	0.0105	0.0714
Stenonema	0.2551	0.3125	0.3561	0.1111	-	-	-	-	-	-
Ostracoda	-	-	-	-	-	-	0.0001	0.2143	-	-
Plecoptera										
Amphinemura	0.0502	0.5625	0.0075	0.0556	0.0557	0.8462	0.0539	0.5000	0.1080	1.1429
Beloneuria	-	-	-	-	-	-	0.0525	0.0714	-	-
Isoperla	-	-	0.0063	0.0556	0.1478	0.2308	0.0515	0.1429	-	-
Leuctra	0.0250	0.5625	0.0490	0.4444	0.0441	0.6154	0.0111	0.1429	0.0217	0.2857
Tallaperla	0.0253	0.1250	0.5732	0.2778	0.0274	0.3846	1.6053	0.5000	0.6444	1.0714
Trichoptera										
Diplectrona	-	-	-	-	-	-	0.0301	0.0714	0.0918	0.0714

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
Lepidostoma	-	-	-	-	-	-	0.0021	0.0714	0.0009	0.0714
Neophylax	0.0585	0.5625	0.0259	0.4444	0.0028	0.0769	0.1268	0.7857	0.0198	0.5000
Parapsyche	-	-	-	-	-	-	0.0944	0.0714	-	-
Rhyacophila	-	-	-	-	-	-	0.0109	0.0714	-	-
Wormaldia	0.0986	0.2500	-	-	-	-	0.0646	0.2143	0.0124	0.0714
Total	0.9882	4.1875	1.3173	2.8889	1.0068	7.6923	2.3670	5.7857	1.6475	7.0000
6/2013										
Caudata										
Eurycea	-	-	-	-	-	-	0.9127	0.0909	-	-
Coleoptera										
Ectopria	-	-	-	-	-	-	-	-	0.0315	0.0769
Elmidae (larvae)	0.0023	0.0769	-	-	0.0039	0.0667	0.0028	0.0909	0.2568	0.3077
Collembola	-	-	-	-	0.0059	0.3333	-	-	-	-
Diptera										
Cecidomyiidae	-	-	0.0033	0.0833	0.0018	0.0667	-	-	-	-
Dixa	-	-	0.0169	0.1667	0.0004	0.0667	0.0083	0.0909	0.0095	0.1538
Hexatoma	0.0188	0.0769	-	-	-	-	-	-	-	-
Molanna	0.1030	0.0769	-	-	-	-	-	-	-	-
Non-Tanypodinae	0.1240	0.9231	0.0071	0.3333	0.0373	1.5333	0.0266	0.7273	0.0120	0.6154
Simuliidae	0.0015	0.0769	-	-	-	-	-	-	-	-
Tanypodinae	0.0417	0.2308	0.0039	0.5833	0.0024	0.9333	0.0076	0.3636	0.0013	0.1538
Tipula	-	-	-	-	-	-	0.0691	0.0909	-	-
Ephemeroptera										
Baetis	0.0409	0.1538	0.2538	0.1667	-	-	0.0016	0.0909	-	-
Paraleptophlebia	0.0546	0.0769	0.2290	0.4167	0.0278	0.0667	0.3439	0.2727	0.0361	0.2308

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
Serratella	0.0444	0.0769	-	-	-	-	0.3022	0.1818	0.3100	0.1538
Stenonema	0.3409	0.0769	0.3037	0.1667	-	-	0.1250	0.0909	-	-
Hymenoptera										
Formicidae	-	-	0.6122	0.0833	0.0006	0.0667	-	-	-	-
Lepidoptera	-	-	-	-	0.4865	0.0667	-	-	-	-
Nematoda	-	-	-	-	-	-	0.0001	0.0909	0.0002	0.0769
Odonata										
Cordulegaster	-	-	0.0667	0.0833	-	-	-	-	-	-
Plecoptera										
Amphinemura	0.0636	0.2308	0.0683	0.1667	0.0988	0.2667	-	-	0.0313	0.1538
Beloneuria	-	-	0.3407	0.0833	-	-	-	-	-	-
Isoperla	0.0049	0.0769	-	-	-	-	-	-	-	-
Leuctra	0.1667	1.0769	0.1601	0.6667	0.0531	0.2000	0.0957	0.8182	0.0705	0.6923
Tallaperla	0.1170	0.4615	0.0756	0.2500	0.0228	0.2000	0.0255	0.1818	0.0216	0.1538
Trichoptera										
Diplectrona	-	-	0.0003	0.0833	0.0062	0.1333	-	-	0.1682	0.0769
Hydroptila	-	-	-	-	-	-	-	-	0.0142	0.0769
Lepidostoma	-	-	-	-	0.0844	0.2000	-	-	-	-
Lype	-	-	0.0563	0.0833			0.0487	0.0909	-	-
Neophylax	0.0108	0.0769	0.0781	0.1667	0.0003	0.0667	0.0361	0.0909	0.0418	0.0769
Psilotreta	-	-	-	-	-	-	0.0205	0.0909	-	-
Rhyacophila	-	-	-	-	-	-	-	-	0.1392	0.0769
Wormaldia	0.0000	0.0769	0.2333	0.5000	0.0354	0.0667	0.0025	0.0909	0.0010	0.0769
Total	1.1350	3.8462	2.5094	4.0833	0.8677	4.3333	2.0288	3.5455	1.1450	3.1538

Table 3. Taxa identified in stomachs of *E. wilderae* combined across all streams and sampling dates (spring and summer; pre-treatment and treatment) that make up < 1% of ECB. Capital letters before taxa indicate order. Taxa are ranked in decreasing order by % ECB. ECB gut^{-1} and Exp # gut^{-1} is the overall expected biomass and number of individuals of that taxa in any randomly sampled stomach.

Taxa	ECB gut^{-1}	% Total ECB	% Total Abundance	Exp # gut^{-1}
T-Wormaldia	0.0072	0.75	0.28	0.0879
T-Neophylax	0.0099	0.69	0.33	0.2167
D-Dixa	0.0022	0.54	0.50	0.0956
Hydracarina	0.0019	0.52	0.66	0.1099
C-Elmidae	0.0073	0.51	0.22	0.2437
Oligochaeta	0.0108	0.38	0.17	0.2000
P-Sweltsa	0.0033	0.34	0.39	0.1833
D-Limonia	0.0092	0.32	0.11	0.1250
Collembola	0.0032	0.32	0.45	0.1973
D-Empid	0.0028	0.29	0.17	0.0614
D-Pilaria	0.0081	0.28	0.06	0.0667
T-Hydropsichidae	0.0074	0.26	0.06	0.0588
Nematoda	0.0004	0.24	1.55	0.1657
D-Raphium	0.0049	0.17	0.06	0.0667
C-Anchytarsus	0.0048	0.17	0.06	0.0588
E-Baetis	0.0047	0.16	0.06	0.0667
Formicidae	0.0039	0.14	0.06	0.0588
T-Polycentropus	0.0007	0.07	0.17	0.0752
T-Rhyacophila	0.0020	0.07	0.06	0.1429
Ostracoda	0.0002	0.07	5.68	0.6463
D-Cecidomyiidae	0.0016	0.06	0.06	0.0588
Plecoptera	0.0003	0.02	0.99	0.5625
T-Diplectrona	0.0002	0.01	0.06	0.0500
Other	0.0030	0.23	0.33	0.2868

Table 4: Expected biomass (mg AFDM gut^{-1}) and estimated abundance (# individuals gut^{-1}) found in *E. wilderae* stomachs for each sampling date and stream. Biomass and abundance are calculated separately for each date and stream. Prey items are listed alphabetically by order then family or genus.

	2		8		16		32		128	
Date/Taxon	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance
7/2011										
Coleoptera										
Anchytarsus	-	-	-	-	-	-	-	-	0.005	0.059
Collembola	-	-	-	-	-	-	0.001	0.067	0.000	0.059
Copepoda	0.001	1.714	0.001	1.333	0.000	0.588	0.000	0.533	0.001	1.529
Diptera										
Ceratopogonia	0.029	0.286	0.001	0.133	0.004	0.059	0.001	0.067	0.000	0.059
Dixa	0.003	0.143	-	-	-	-	0.001	0.133	0.004	0.059
Hexatoma	0.046	0.143	-	-	-	-	-	-	-	-
Non-Tanypodinae	0.022	0.714	0.007	0.867	0.011	0.941	0.007	0.867	0.007	0.824
Raphium	-	-	-	-	-	-	0.005	0.067	-	-
Tanypodinae	0.000	0.571	0.000	0.667	0.001	0.118	0.000	0.067	0.005	0.235
Hydracarina	0.003	0.143	-	-	0.001	0.118	-	-	-	-
Hymenoptera	-	-	-	-	0.004	0.059	-	-	-	-
Nematomorpha	-	-	-	-	-	-	-	-	-	-
Nematoda	-	-	-	-	0.000	0.059	-	-	0.000	0.059
Ostracoda	-	-	-	-	0.000	0.118	0.000	0.867	-	-
Plecoptera										
Leuctra	0.035	0.286	0.024	0.133	0.005	0.059	0.007	0.133	0.047	0.353
Tallaperla	-	-	0.001	0.067	-	-	0.059	0.133	-	-
Trichoptera										
Hydropsichidae	-	-	-	-	0.007	0.059	-	-	-	-
Wormaldia	-	-	-	-	0.005	0.059	-	-	-	-

Total	0.054	1.714	0.034	3.200	0.039	2.235	0.082	2.933	0.068	3.235
3/2012										
Collembola	-	-	-	-	-	-			0.003	0.188
Copepoda	0.004	6.333	0.001	2.700	0.003	5.375	0.002	3.000	0.003	5.438
Diptera										
Ceratopogonia	-	-	-	-	0.011	0.063	0.027	0.200	0.008	0.250
Hexatoma	-	-	-	-	0.020	0.063	0.071	0.067	-	-
Limonia	-	-	-	-	-	-	-	-	0.009	0.125
Non-Tanypodinae	0.009	3.500	0.017	1.500	0.020	2.438	0.011	1.400	0.017	4.500
Tanypodinae	-	-	-	-	0.001	0.125	0.001	0.067	0.001	0.313
Ephemeroptera										
Paraleptophlebia	-	-	-	-	-	-	0.010	0.067	-	-
Serratella	-	-	0.074	0.300	-	-	0.026	0.067	-	-
Hydracarina	-	-	-	-	0.001	0.063	-	-	0.001	0.063
Nematoda	-	-	-	-	0.000	0.125	-	-	0.000	0.125
Ostracoda	-	-	0.000	1.100	0.000	0.375	0.000	1.467	-	-
Plecoptera										
Amphinemura	0.025	0.500	-	-	-	-	0.001	0.067	0.013	0.188
Isoperla	-	-	0.003	0.100	-	-	0.004	0.067	-	-
Leuctra	0.038	0.167	0.002	0.300	0.030	0.313	-	-	0.014	0.063
Plecoptera	-	-	-	-	0.001	0.313	-	-	0.000	0.813
Sweltsa	0.007	0.167	-	-	0.001	0.250	0.002	0.133	-	-
Tallaperla	-	-	-	-	-	-	0.002	0.067	0.002	0.125
Trichoptera										
Fattigia	-	-	-	-	0.039	0.188	-	-	0.001	0.063
Lepidostoma	-	-	0.002	0.200	-	-	0.001	0.067	-	-
Psilotreta	-	-	-	-	-	-	0.001	0.067	-	-
Wormaldia	-	-	-	-	-	-	0.003	0.133	-	-

Total	0.082	10.667	0.100	6.200	0.125	9.688	0.163	6.933	0.073	12.250
6/2012										
Coleoptera										
Elmidae	-	-	-	-	-	-	0.002	0.059	-	-
Copepoda	0.001	2.824	0.001	2.941	0.001	2.125	0.001	2.824	0.001	2.571
Diptera										
Cecidomyiidae	-	-	0.002	0.059	-	-	-	-	-	-
Ceratopognia	0.011	0.059	0.028	0.294	-	-	-	-	0.004	0.143
Dixa	0.001	0.059	-	-	-	-	0.001	0.118	-	-
Empid	-	-	0.002	0.059	-	-	0.001	0.059	-	-
Hexatoma	-	-	-	-	0.004	0.250	-	-	-	-
Non-Tanypodinae	0.002	0.706	0.009	1.471	0.001	1.125	0.001	0.941	0.006	1.000
Rhabdomastix	-	-	-	-	0.031	0.125	0.011	0.059	0.006	0.071
Tanypodinae	0.000	0.176	0.000	0.059	0.000	0.250	0.000	0.176	0.001	0.214
Tipulidae	-	-	-	-	-	-	-	-	0.023	0.071
Ephemeroptera										
Paraleptophlebia	-	-	-	-	-	-	-	-	0.001	0.071
Serratella	0.015	0.059	-	-	-	-	0.001	0.059	-	-
Hydracarina	-	-	-	-	-	-	0.002	0.118	0.002	0.143
Nematoda	-	-	0.000	0.059	0.000	0.125	0.000	0.059	0.001	0.357
Ostracoda	0.000	0.412	-	-	-	-	0.000	0.059	-	-
Plecoptera										
Amphinemura	-	-	0.001	0.059	-	-	0.001	0.059	0.038	0.071
Isoperla	-	-	-	-	-	-	0.004	0.176	-	-
Leuctra	0.028	0.176	0.021	0.176	0.013	0.375	0.009	0.235	0.039	0.357
Tallaperla	-	-	-	-	-	-	0.012	0.118	0.010	0.071
Trichoptera										
Lepidostoma	-	-	-	-	-	-	0.014	0.059	-	-
Lype	-	-	-	-	-	-	-	-	0.008	0.071

Polycentropus	0.000	0.059	-	-	-	-	-	-	-	-
Psilotreta	-	-	-	-	-	-	-	-	0.041	0.071
Wormaldia	-	-	-	-	-	-	-	-	0.014	0.071
Total	0.060	4.529	0.063	5.176	0.050	4.375	0.060	5.176	0.196	5.357
4/2013										
Collembola	0.003	0.133	-	-	-	-	-	-	-	-
Copepoda	0.004	5.267	0.001	2.000	0.001	1.400	0.004	5.400	0.001	2.182
Diptera										
Ceratopogonia	-	-	-	-	-	-	0.008	0.067	0.022	0.091
Dixa	0.004	0.067	-	-	-	-	-	-	-	-
Empid	-	-	-	-	0.005	0.067	-	-	-	-
Hexatoma	-	-	-	-	0.009	0.067	-	-	-	-
Non-Tanypodinae	0.009	1.667	0.009	1.900	0.158	1.800	0.012	1.133	0.070	1.455
Pilaria	-	-	-	-	-	-	0.008	0.067	-	-
Tanypodinae	0.000	0.267	0.004	1.300	0.016	1.267	0.002	0.733	0.032	1.909
Tipulidae	-	-	-	-	0.022	0.067	-	-	-	-
Ephemeroptera										
Baetis	0.005	0.067	-	-	-	-	-	-	-	-
Paraleptophlebia	-	-	0.012	0.200	-	-	0.016	0.200	0.009	0.091
Serratella	0.005	0.067	0.015	0.100	0.017	0.067	-	-	-	-
Stenonema	-	-	-	-	0.000	0.067	-	-	-	-
Hydracarina	-	-	0.000	0.100	-	-	0.004	0.133	-	-
Nematoda	0.001	0.200	-	-	0.000	0.067	0.000	0.133	0.001	0.182
Oligochaeta	0.011	0.200	-	-	-	-	-	-	-	-
Ostracoda	0.000	0.533	0.000	0.200	-	-	0.001	1.333	-	-
Plecoptera										
Amphinemura	0.004	0.200	0.013	0.200	0.006	0.067	-	-	0.004	0.091
Isoperla	0.004	0.067	-	-	-	-	0.001	0.067	-	-
Leuctra	0.012	0.333	0.005	0.300	0.047	0.800	0.012	0.467	0.034	0.636

Tallaperla	-	-	0.002	0.100	0.005	0.267	0.001	0.067	0.006	0.091
Trichoptera										
Lepidostoma	-	-	-	-	0.003	0.133	0.001	0.067	-	-
Lype	-	-	0.037	0.100	-	-	-	-	-	-
Neophylax	0.016	0.333	0.004	0.100	-	-	-	-	-	-
Polycentropus	-	-	0.000	0.100	0.002	0.067	-	-	-	-
Total	0.078	9.400	0.102	6.700	0.290	6.200	0.071	9.867	0.179	6.727
6/2013										
Copepoda	0.000	0.167	0.000	0.300	0.000	0.600	0.000	0.455	0.000	0.889
Diptera										
Dixa	-	-	-	-	-	-	0.001	0.091	-	-
Non-Tanypodinae	0.021	0.333	0.002	0.150	0.000	0.100	0.023	0.182	-	-
Tanypodinae	0.016	0.167	0.000	0.050	0.000	0.200	0.009	0.182	-	-
Ephemeroptera										
Paraleptophlebia	-	-	-	-	-	-	-	-	0.079	0.111
Nematoda	-	-	0.000	0.050	0.000	0.100	-	-	-	-
Ostracoda	-	-	-	-	-	-	0.000	0.182	0.000	1.111
Plecoptera										
Leuctra	0.066	0.333	-	-	0.038	0.300	-	-	-	-
Tallaperla	-	-	0.022	0.050	0.028	0.200	0.051	0.091	-	-
Trichoptera										
Diplectrona	-	-	0.000	0.050	-	-	-	-	-	-
Lepidostoma	-	-	-	-	0.021	0.100	-	-	-	-
Total	0.103	1.000	0.024	0.650	0.088	1.600	0.083	1.182	0.079	2.111

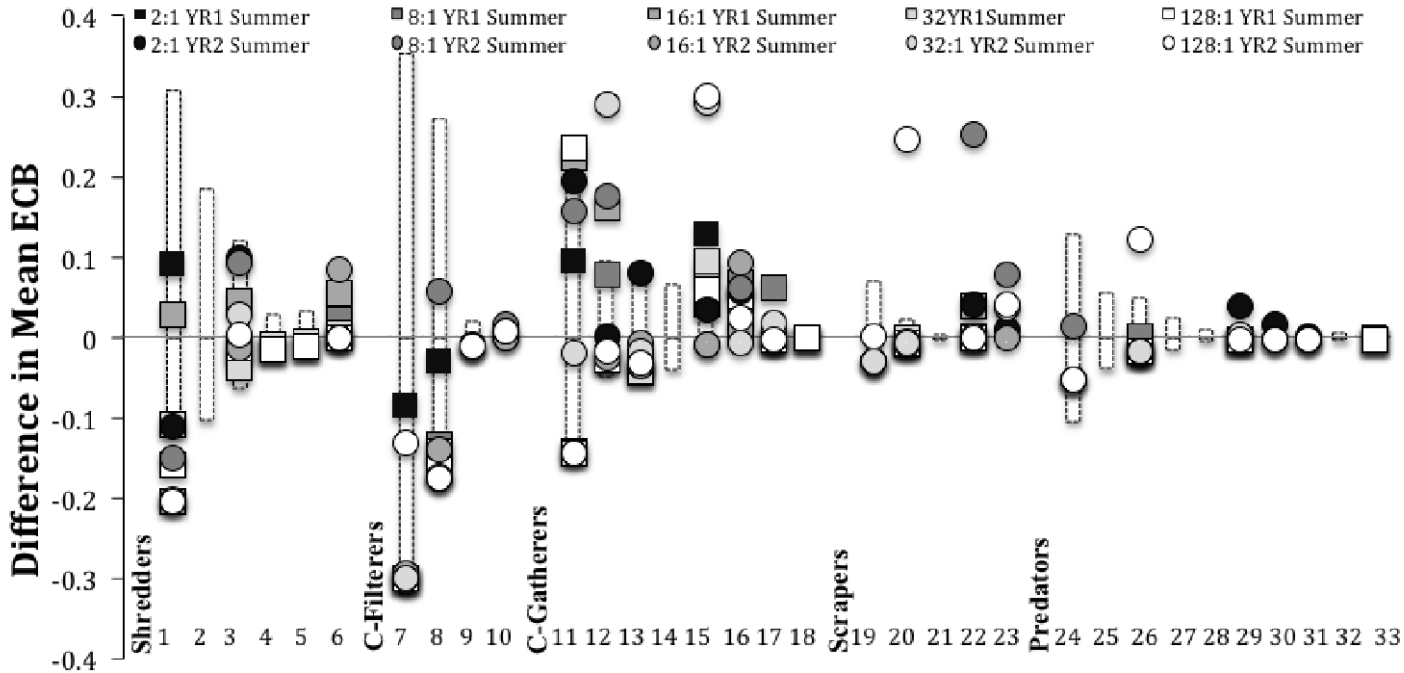


Fig. 1. The difference in mean estimated contribution to biomass (ECB) in *D. quadramaculatus* stomachs for each stream and treatment year compared to pretreatment means. Individual points represent the difference between the average ECB pretreatment. Dotted bars represent the 95% confidence interval fro the pretreatment mean. Taxa are listed from most important FFG to least important by pretreatment levels. Taxa key for numbers on the axis are: 1, *Tallaperla;* 2, other shredders; 3, *Leuctra;* 4, *Polycentropus;* 5, *Fattigia;* 6, Lepidostoma; 7, Hydropscychidae; 8, *Wormaldia;* 9, Simuliidae; 10, Dixa; 11, *Stenonema*; 12, *Paraleptophlebia;* 13, non-Tanypodinae; 14, other scrapers; 15, *Serratella;* 16, *Amphinemura;* 17, *Psilotreta;* 18, Copepoda; 19,

Ectopria; 20, Elmidae (adult); 21, Elmidae (larvae); 22, *Baetis;* 23, *Neophylax;* 24, *Cordullagaster;* 25, Other predators; 26, *Rhyacophila;* 27, *Sweltsa;* 28, Ceratapogonidae; 29, *Tanypodinae;* 30, *Hexatoma;* 31, *Isoperla;* 32, *Raphium;* 33, *Dicranota.*

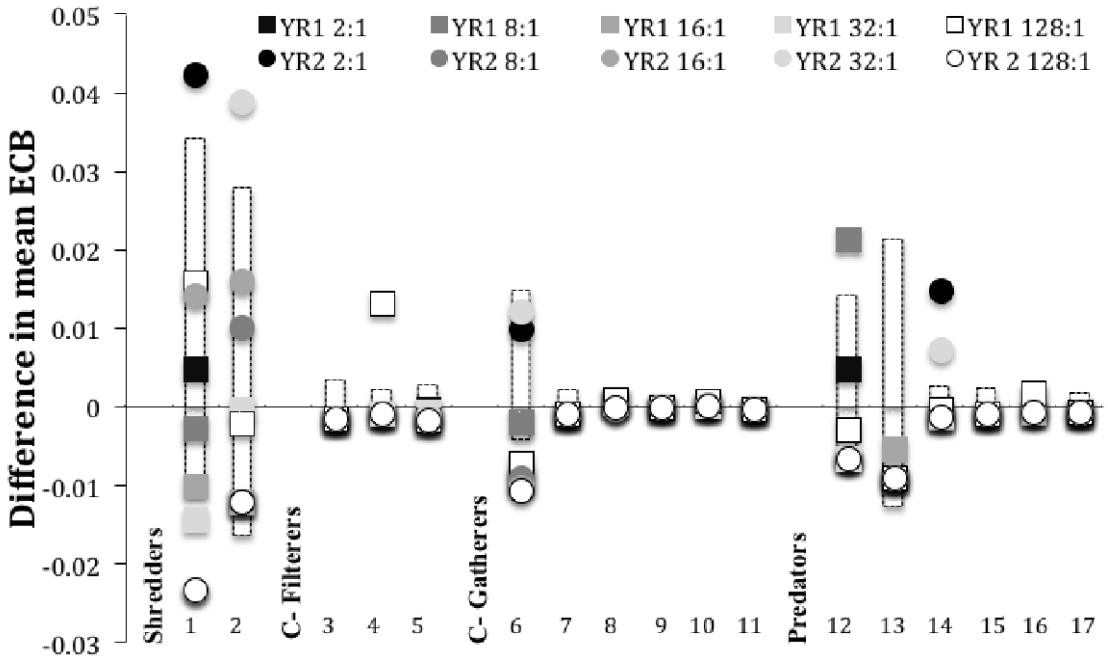


Fig 2. The difference in mean estimated contribution to biomass (ECB) in *E. wilderae* stomachs for each stream and treatment year compared to pretreatment means. Individual points represent the difference between the average ECB pretreatment. Dotted bars represent the 95% confidence interval fro the pretreatment mean. Taxa are listed from most important FFG to least important by pretreatment levels. Taxa key for numbers on the axis are: 1, *Leuctra;* 2, *Tallaperla*; 3, Hydropsychidae*;* 4, *Wormaldia;* 5, Dixidae*;* 6, non-*Tanypodinae*; 7, *Anchytarsus;* 8, Copepoda*;* 9, Ostracoda*;* 10, Nematoda*;* 11, Collembola; 12, Ceratopogonidae*;* 13, *Hexatoma*; 14, *Tanypodinae*; 15, *Raphium;* 16, Hydracarina*;* 17, Formicidae.

CHAPTER 4

POTENTIAL CONSEQUENCES OF STREAM ECOSYSTEM DISTURBANCES ON ELEMENTAL LIMITATION OF LARVAL SALAMANDERS[1]

[1]Bumpers, P.M., J.C. Maerz, A.D. Rosemond, and J.P Benstead. To be submitted to *Functional Ecology*

Introduction

Aquatic ecosystems are undergoing a myriad of complex changes as a result of anthropogenic activities (MEA 2005). Phenomena such as habitat alteration, climate change, and cultural eutrophication can create long-term and complex perturbations to stream ecosystems (Allan 2004; Palmer *et al.* 2009; Smith & Schindler 2009). In addition to the better-known effects that these disturbances create, such as sedimentation, altered flow regime, and algal blooms, global changes can also have more nuanced impacts. For instance global changes could impact organismal growth by altering energetic demands or by altering elemental and/or energetic constraints of consumer maintenance, growth and reproduction. Such changes to nutritional needs can arise from direct changes in the metabolism of an animal (e.g. increased respiration rates caused by increased temperatures) or indirectly through changes in the quantity or quality of its food resources (Cross *et al.* 2003; Bernardo & Spotila 2006; Suberkropp *et al.* 2010). Primary consumers (e.g. shredders, grazers) can be directly affected by disturbances that alter the quantity or quality of basal resources in streams (Cross, Wallace & Rosemond 2007; Stoler & Relyea 2013; Lauridsen *et al.* 2014). In contrast, predators should only be impacted by such changes on basal resources indirectly via effects on their prey (i.e., primary consumers), although energetic demands could still be directly influenced via respiration.

Changes in basal resources could propagate to predators through effects on production of prey resources and/or the quality of prey resources. For example, decreased basal resources may reduce primary consumer production, and therefore result in reduced prey availability (Wallace *et al.* 1997; Johnson & Wallace 2005). In addition, changes in basal resource quality that results from nutrient enrichment (Scott *et al.* 2013) can increase secondary production of primary consumers and potentially alter consumer body stoichiometry (Cross *et al.* 2003; Cross *et al.*

2006). Increased production has obvious potential mechanisms for predator growth; however, changes in prey quality may or may not result in changes of predator growth rates (Malzahn *et al.* 2007; Dickman *et al.* 2008; Malzahn *et al.* 2010). To understand the impacts that quantity and quality of prey may have on consumers, ecologists often consider the elemental imbalances (C:N, C:P) that consumers may face (Sterner 1997; Sterner & Elser 2002 ; Frost *et al.* 2006; El-Sabaawi *et al.* 2012). A more realistic way to understand these relationships is to use threshold elemental ratios (TERs), which are the theoretical point at which elemental limitation switches between one element and another (Frost *et al.* 2006).

Modeling the interactions of food quality in a TER framework can allow us to make predictions or better understand the responses of consumers to ecosystem disturbances (e.g. nutrient enrichment). Such models generally assume that the parameters in the model are fixed (e.g., fixed assimilation of phosphorus, A_p) and may result in altered or unrealistic interpretations of a consumer's limitation. For example, animal respiration is quite variable and changes with temperature. Moreover, changes in food quality could alter assimilation efficiencies of particular nutrients (N or P); however, such parameters are rarely explicitly measured. In vertebrates, a phosphorus assimilation efficiency of 0.8 is often used in the development of TERs. Further, ecological stoichiometry theory predicts that higher order consumers are homeostatic in regards to their body elemental composition (Sterner & Elser 2002). While this is largely true, wide variation in body stoichiometry can be found, and primary consumers have been shown to deviate from strict homeostasis (Cross *et al.* 2003; Kendrick & Benstead 2013). Threshold elemental ratio models work under the assumption of strict homeostasis (Frost *et al.* 2006). Consequently, if a consumer displays some deviation from this assumption, predicting the response of a consumer to changes in nutrient availability becomes more complicated. If this

parameter changes due to changes in prey quality, limitations may be under- or over-estimated due to a flexible parameter.

Here, our goals were three-fold. First, we wanted to characterize larval salamander body stoichiometry and determine if it changed as a result of long-term (2 years) experimental nutrient enrichment. Secondly, we developed TERs for two species of larval salamanders to try and further understand previous research in which we found increased growth rates in response to nutrient enrichment (Bumpers *unpublished*). Lastly, we aimed to better understand the consequences of variation in TER parameters on larval salamanders by exploring the sensitivity of TER models to ranges in parameters. We used known variation in parameters to predict how larval salamanders may respond to nutrient enrichment.

Methods

Site description and salamander stoichiometry collection

We collected larval salamanders, *Desmognathus quadramaculatus* (DQUAD) and *Eurycea wilderae* (EWILD), from five streams at the Coweeta Hydrologic laboratory, in Macon County, NC, USA. Both species are commonly found in southern Appalachian headwater streams and generally comprise the most biomass and abundance of salamander assemblages, respectively (Peterman & Truslow 2008; Keitzer & Goforth 2013). *Eurycea wilderae* has a larval period of approximately 12 months and metamorphoses at 18-24 mm snout-vent length (SVL) (Bruce 1988). In contrast, *D. quadramaculatus* has a larval stage of 36-48 months and metamorphoses at approximately 40-45 mm SVL (Bruce, Castanet & Francillon-Vieillot 2002). Both salamanders are secondary to tertiary consumers, primarily feeding on aquatic macroinvertebrates (Davic 1991; Johnson & Wallace 2005) with DQUAD having a greater

ability to consume larger predatory macroinvertebrates and other larval salamanders. Coweeta is located in the Blue Ridge physiographic province of the southern Appalachians and is characterized by hardwood forests dominated by chestnut oak, maple, and tulip poplar. Streams at Coweeta have naturally low nutrient concentrations (Swank & DA Crossley 1988). Pre-treatment, soluble reactive phosphorus (SRP) was very low in our streams (2.8 μg/L; range 2.5-3.1 μg/L) and dissolved inorganic nitrogen (DIN) was also relatively low, but more variable (NO_3-N mean, 74 μg/L; range 10.4-179 μg/L; NH_4-N mean, 8 μg/L; range 6.6-8.9).

The five streams in this study were experimentally enriched for two years (July 2011-July 2013). Each stream received different concentrations of N and P such that a gradient of N:P ratios was created (N:P range=2:1-128:1). See Chapter 2 for a detailed description of the enrichment experiment. Nutrient concentrations in the streams were elevated 2.5-31× and 3-10× above background for SRP and DIN, respectively. We collected samples to examine variation in salamander stoichiometry and to determine if nutrient enrichment had any effect on the body stoichiometry of our two study species. Samples were collected pre-enrichment (PRE) and twice (Spring, Summer) during each year of enrichment (YR1, YR2).

For each species ~ 5 individuals were collected from each stream and date for C, N, and P composition. Collections occurred in June and August 2010 (PRE), March and June 2012 (YR1), and April and June 2013 (YR2), however, only nine EWILD were collected in the PRE samples from all streams. Larvae were collected by hand, placed on ice and transported back to the lab where they were immediately euthanized in 0.5% neutral-buffered MS-222. Larvae were rinsed with D.I. water, their stomachs removed and then frozen until later analysis. All samples were then dried to a constant weight and homogenized. Samples for C and N analysis were analyzed on a Carlo Erba (Milan, Italy) NA 1500 CHN analyzer at the Odum School of Ecology

Analytical Chemistry Lab, University of Georgia. P content of salamanders was determined using the plant dry ash/acid extraction method followed by spectrophotometric analysis using the ascorbic acid method (APHA 1998). All elemental compositions were determined as % of body dry mass (DM) and all ratios are molar ratios.

TER calculation and sensitivity of parameters

To examine elemental limitations in larval salamanders we estimated TERs for C:P ($TER_{C:P}$; Note, TER and $TER_{C:P}$ are used interchangeably throughout this paper, always referring to $TER_{C:P}$) for both species following the model formulated in Frost *et al.* (2006):

$$TER_{C:P} = A_P / (I_C A_C - R_C / I_C) * Larval_{C:P}$$

Where A_P and A_C are the assimilation efficiencies of P and C, respectively, I_C is the mass-specific max ingestion rate (mg C mg C^{-1} day^{-1}) and R_C is the mass-specific respiration rate (mg C mg C^{-1} day^{-1}). To parameterize the model, we assumed A_P to be 0.8 (Frost et al. 2006). We used mean values of 0.55 and 0.64 for A_C measured for *Gyrinophilus palleucus* and *Ambystoma opacum* (Regester, Whiles & Lips 2008; Huntsman *et al.* 2011). Respiration rates were determined using published mass-respiration rate equations at 5 °C, 15 °C, and 20 °C (Gatten, Miller & Full 1992; Wells 2007). These relationships were generalized to all Urodeles (salamanders). Therefore, we plotted the relationship of measured mass against measured respiration rate for adult salamanders and larval salamanders to determine if they differed (Appendix 4.1). Ingestion rate was determined from gut biomass from larvae collected in a separate study during the same time frame as this one (Chapter 3). We regressed the relationship between maximum gut biomass and snout vent length (SVL) for each species. We assumed this value to be the maximum daily ingestion rate under the assumption that larval salamanders feed

primarily at night and take 24 hours to digest prey. Stomach biomass (mg DM) was converted to mg C assuming a conversion factor of 0.48 and then divided by the salamander C content (mg C).

We explored the sensitivity and potential consequences to the TER model to variation in a range of parameters. Specifically, we varied each parameter between the mean, minimum, and maximum of values we measured or modeled for body stoichiometry (measured), ingestion rate (measured and modeled), and respiration rate (modeled). We also used extreme minimum and maximum values for each parameter to assess changes outside measured natural variation. Parameters we did not directly measure or model (A_P, A_C) were parameterized with known published values (values previously stated). We used the mean of the published values and the minimum and maximum published values for our analysis. These parameters were then varied for extreme minimum and maximums (see Table 4.1 for parameter ranges). To determine the effect of each parameter, we varied one parameter at a time and held all other parameters constant at the mean value. For simplicity of interpretation, we only varied one parameter at a time. We did this for the minimum, mean, and maximum dry mass of salamanders we sampled (DQUAD= 14, 89, 395 mg DM; EWILD= 3.6, 16, 44 mg DM). Similarly to Frost *et al*. (2006) we limited this analysis to C and P only. This was primarily due to the importance of P in animal growth, especially considering the importance of P in bone development in vertebrates (Sterner & Elser 2002).

The elemental imbalance between salamanders and their diets was determined as the arithmetic difference between $TER_{C:P}$ and diet C:P (sensu El-Sabaawi *et al*. 2011). We used the average TER for each size class of individual determined from the parameters that were within the measured variation (i.e., the minimum, mean, and maximum). Biomass-weighted diet

stoichiometry was determined by multiplying the proportion of gut biomass (measured explicitly) of individual taxa by its C:P and then summing all weighted C:P values for each taxon and averaging across streams in each year (PRE, YR1, YR2). Stoichiometry values were determined from Cross *et al.* (2003). When an individual did not have a specific value reported we assigned it the average value reported in Cross *et al*. (2003) for the taxon-specific functional feeding group or order (i.e., when extent of digestion precluded identification beyond order). We used the values reported for un-enriched conditions but also made comparisons using stoichiometric values from enriched conditions in Cross *et al.* (2003) to determine the potential consequence of changes in individual prey stoichiometry, under the assumption that all streams in our study respond similarly to that of Cross *et al.* (2003). Gut biomass and composition was determined in a separate study and detailed methods are outlined in Chapter 3. Negative imbalance values indicate increased potential for P limitation while a positive value would indicate a greater potential for C limitation.

Since salamander stoichiometry and elemental limitations are far less studied than other aquatic organisms we wanted to compare our TER values to similar functional groups that are well studied (i.e. fish). Therefore, we compared the relationship between body C:P and TER for salamanders in this study and fish used in Frost *et al.* (2006).

Statistical analyses

Variability in salamander stoichiometry was assessed using generalized linear models (GLM). Specifically, we used size of larvae (as SVL), treatment year (PRE, YR1, YR2), and species in our models. Treatment year was used to assess if enrichment had any effect on body elemental composition or stoichiometry. Stream was originally included as a factor but did not

contribute significantly to the models so we excluded it from further analysis. We used linear regression to compare the fish data set to our salamanders. We assessed the relationship between body C:P and $TER_{C:P}$ to determine if there was a difference in the slope or intercept between the data sets. All analyses were performed in R version 3.0.3 (R Core Team 2014).

Results

Salamander stoichiometry

We captured 151 and 119 individuals of *D. quadramaculatus* and *E. wilderae*, respectively, for elemental composition across all streams and dates. Salamanders exhibited a wide range of stoichiometric variability (Fig. 4.1, Appendix 4.2 Table 1, Supporting information). % P ranged from ~ 1.8-3.8 % (Fig. 4.1A, Appendix 4.2 Table 1). Snout-vent length was significantly and positively related to % P and significantly but negatively related with % N, C:P, and N:P (Table 4.2, Figs 4.1a,b,d,f). Models indicated that the two species differed in their relationships with SVL and elemental composition for % P, % N, and %C, C:P, and N:P (Table 4.2). The GLM analysis also indicated that there were significant differences in elemental composition between treatment years (Table 4.2). The distribution of sizes of larvae captured across treatment years was variable and biased to smaller individuals particularly in YR1 for DQUAD larvae (Appendix 4.2 Fig. 1)

TER model results to variation in parameters

The ranges in TERC:P were fairly similar between DQUAD and EWILD with variation largely a function of differences in dry mass (Table 4.3). Generally, DQUAD exhibited a lower TER primarily due to its larger body size compared to EWILD. The TER in both species

declined with increasing DM, with the largest DQUAD exhibiting a comparatively lower TER (59) than all other size classes in DQUAD and EWILD (79-132, Table 4.3). The range in sizes for both species we sampled and modeled here represents close to the full range of expected sizes for the larval periods of these salamanders.

Mass-specific ingestion rate (I_C) exhibited a threshold relationship with TER, increasing sharply at very low I_C (Fig. 4.2a). Respiration rate (R_C) was exponentially related to TER for a given body size with the range of values we used (Fig. 4.2b). Varying R_C within a natural range of temperatures (5-20°C) varied the TER by up to ± 15-20 %. (mean ± 12%, Fig. 4.2c). Assimilation of C (A_C) was related to TER negatively and non-linearly (Fig. 3a). The normal range of A_C values (0.55-0.69) came from the literature and showed that even this variation created up to a ± 15% change in the TER, having the greatest effects on smaller larvae (Fig. 4.3a). When considering values beyond the range of published values, the TER increased by up to 52% when A_C was set to 0.45 across both species and all sizes, which is a 0.1 reduction from the lowest published value we used (Huntsman *et al.* 2011). Assimilation of P (A_P) was positively and linearly associated with TER. When A_P was varied by ± 0.1 (0.9 and 0.7 A_P) TER changed by ± 12.5 %. When A_P was decreased to 0.5, TER decreased by 37.5% in both species, creating strong P limitation. Body C (Q_C) was positively and generally linearly related to TERs. The average value for % C was ~ 0.45 mg C mg DM^{-1} with a measured range of 0.42 – 0.51 mg C mg DM^{-1}, creating a change in TER by up to ± 10% from the mean value for a given body size (data not shown). However, varying the observed min and max values by just 0.05-0.08 (0.35-0.55 mg C mg DM^{-1}, Table 4.1) created a change in TER of up ± 25 %. Body P (Q_P) was asymptotically related to TER and indicated that the range of our measured elemental composition (Fig. 4.3b) could create a large range in TERs of 33-168 and 43-194 for DQUAD

and EWILD, respectively, when all other parameters were equal (Fig. 4.3b). This equates to changes in TERs of up to an ~75% increase and an ~40% decrease compared to mean measured values. When parameterized with values outside to the range in our measured individuals the effects were even greater (Fig. 4.3b).

Average diet stoichiometry for DQUAD and EWILD varied with treatment year and generally decreased for DQUAD (Fig 4.4). EWILD diet C:P was usually lower than that of DQUAD but showed less response to enrichment. Compositional changes alone in response to enrichment apparently decreased diet C:P by ~ 120 and 150, for YR1 and YR2, respectively in DQUAD (Fig 4.4a). Diet C:P for EWILD decreased by only ~ 25 in YR1 but increased slightly above PRE in YR2 (Fig 4.4b). Calculating diet stoichiometry using values from Cross *et al.* (2003) that changed due to enrichment decreased diet C:P considerably in both species, though it was more pronounced in DQUAD (Fig 4.4). Elemental imbalances between $TER_{C:P}$ and $Diet_{C:P}$ indicated that both salamanders are likely to face P limitation when food is abundant, exhibiting negative imbalances (Fig 4.5). EWILD exhibited lower imbalances than DQUAD, which was largely driven by the lower average diet C:P of EWILD. However, EWILD imbalances were less sensitive to nutrient enrichment as DQUAD showed markedly larger decreases in the C:P imbalance (Fig 4.5a,b)

The comparison between fish and salamander body C:P and $TER_{C:P}$ revealed that generally for a given C:P larval salamanders have lower TERs (Fig 4.6). Linear regression indicated that the intercepts between the two data sets were significantly different ($F_{2, 12} = 9.56$, $P = 0.007$). The differences in TER were largely driven by lower R_C in larval salamanders compared to fish, however, there was some overlap between juvenile fish and larval salamanders (Fig. 4.6).

Discussion

Our exploration of salamander elemental composition and stoichiometry, and $TER_{C:P}$ revealed that larval salamanders are likely largely limited by P content of their food (under the assumption of saturating food levels), but showed considerable variation in the TER to a moderate range in parameter variation. Our analysis of salamander stoichiometry indicated that there were significant differences between treatment years. Stoichiometric theory and numerous studies suggest that vertebrates are largely homeostatic in regards to their body stoichiometry (Sterner & Elser 2002 ; Vrede *et al.* 2011). Therefore, we would not expect salamander stoichiometry to change in response to nutrient enrichment. All measures of salamander stoichiometry except % C and C:N were significantly correlated with SVL, therefore differences in size distributions of salamanders between treatment years could create differences in elemental composition. In DQUAD, we sampled a greater proportion of smaller individuals in YR1 compared to PRE and YR2 (Appendix 4.2 Fig. 1) which likely biased the outcome of statistical test. We also had uneven samples of larval sizes in EWILD and very low PRE sample numbers. Therefore, it is likely that the reason for statistical differences is due to biased sampling and not a function of our nutrient treatments. Nonetheless, the variability in stoichiometry that we saw appeared to have moderate to large potential effects on salamander $TER_{C:P.}$

Salamander % P exhibited the most variability compared to other elements in our study. This trend is consistent with that of other studies that found the largest variability in % P in fish (Pilati & Vanni 2007; McIntyre & Flecker 2010; El-Sabaawi *et al.* 2012) and also salamanders (Milanovich *et al*. in Milanovich 2010). We also found consistent trends with stoichiometry related to body size that are reported in these same studies. This variability in % P and C:P created relatively large changes in the TER of an individual (Fig 4.3b), indicating that %P alone

can create significant intraspecific and community-level variation in the severity to which salamanders are imbalanced with that of their prey resources. In addition, the patterns with % P and C:P indicate that that P limitation may become more severe in larger larvae. However, as larvae grow they also increase their ability to prey upon larger predatory macroinvertebrates and other larval salamanders, which would reduce their elemental imbalance.

We modeled the maximum ingestion rate using relationships found between size of larvae and the maximum biomass found in salamander guts. Variation of this parameter indicated a threshold relationship with TER, suggesting that natural variation in this parameter or inaccuracies in estimating it could have large effects. The mean prey biomass that was previously found in salamander stomachs was considerably lower than the maximum biomass for a given size. For example in DQUAD, the mean gut biomass for the 89 mg size (mean size sampled) would equate to an ingestion rate of 0.02 (mg C mg C^{-1} day^{-1}), which was the value for the extreme minimum in our parameter ranges. A maximum ingestion rate this low indicated that a DQUAD larvae of that size would have a $TER_{C:P}$ closer to 300, compared to 76 using the maximum biomass which equated to an ingestion rate of 0.08. We believe this highlights the critical importance of accurately measuring ingestion rates explicitly in the field. Further, the model used in this paper operates under the assumption that food is not at limiting quantities. The fact that the mean biomass found in guts of salamanders is up to a third of the maximum biomass they could consume indicates that at least at times, larval salamanders are likely food-limited. However, variation in the parameter exhibited dampened effects on larger individuals (Fig. 4.2a). The dampened effect of lower ingestion rates is due to a combination of both lower mass-specific respiration rates and higher % P content in larger individuals.

We used three respiration-temperature relationships to model the effect of natural variation in temperature on the TER. In our system 5 °C would be near to temperatures found in the late fall and into early spring, while 15 °C represents close to the summer average water temperature and 20 °C would represent a high stream water summer temperature. As expected, increases in respiration temperature increased the likelihood of C limitation (as increased TER) and this effect was largest in smaller individuals. This relationship indicates that the effects of nutrient enrichment on salamander prey will likely vary due to an interaction with climate change. If stream temperatures warm as expected in response to climate change, then salamander carbon demand could increase, lowering the severity of P limitation. This could dampen stoichiometric effects of enrichment (i.e., changes in salamander prey nutrient content), and increase the importance of nutrient enrichment on prey availability. Moreover, salamanders exhibit metabolic depression at high temperatures (Bernardo & Spotilla 2006) indicating that the interaction of warming temperatures and nutrient enrichment could create a non-linear response in the severity of elemental limitations in larval salamanders. Bernardo and Spotilla (2006) showed that adult salamanders exhibit metabolic depression at temperatures as low as 20 °C which was the maximum temperature used in our natural variation scenarios. This indicates that altitudinal and latitudinal variation in populations of salamanders will be important in determining how and to what extent larval salamanders may be affected by nutrient enrichment. We believe this highlights the importance of studying the interactions of global changes on ecosystem structure and function, so we can better predict how organisms will respond to such changes.

We found relatively large differences in the apparent elemental imbalances between the two species in our study. EWILD, which had only slightly higher TERs exhibited markedly

lower elemental imbalances due to their diet being a lower C:P (Figs 4.4, 4.5). Moreover, diet stoichiometry appeared to change more for DQUAD in response to enrichment using values that reflect both no change in prey stoichiometry and changes in prey stoichiometry in response to enrichment. Previously, we found that DQUAD exhibited compositional changes in diet in response to enrichment while EWILD did not (Chapter 3). This apparently is reflected in the diet stoichiometry. We note that we did not measure individual prey stoichiometry in this study, but it is likely that it would be similar to that of Cross *et al.* (2003) given that that study was conducted in a nearby watershed (Shope Fork, Otto, NC). The fact that these two species have fairly different elemental imbalances indicates that they could exhibit varying responses (or at least different mechanisms) to stream perturbations like nutrient enrichment or climate change. We feel this also highlights the importance of measuring the variation in diet contents between similar species in order to accurately assess elemental imbalances as has recently been highlighted by Lauridsen *et al.* (2014), who found considerably different elemental imbalances when using realized diet stoichiometry (based on measured gut content composition) and perceived diet stoichiometry (based on assumed gut content composition).

One of the objectives of this study was to help explain previously documented growth responses of DQUAD and EWILD to experimental nutrient enrichment. We previously found that growth rates and average size of DQUAD and EWILD, respectively, increased and was significantly related to stream water P concentration (Chapter 2). Larval salamanders could increase growth rates in response to enrichment through either increased prey consumption (due to increases in prey availability) or increased prey nutrient content (as either direct changes in individual stoichiometry or compositional changes of taxa dominance). Analyses of TERs for EWILD and DQUAD indicate that it is likely that larvae increased growth rates due to increased

consumption due to the threshold relationship with ingestion rate and the relative accuracy of our estimate of I_C but also that salamanders that aren't food limited should respond to changes in stoichiometry of prey or prey composition due to the low calculated TERs.

Finally, we found that larval salamanders have comparatively lower $TER_{C:P}$ than what has been reported for fish, which represent similar functional roles in streams. This research highlights the importance of increasing empirical measurements of parameters used in the calculation of TERs but also that individuals within a population likely vary in the severity of their elemental imbalance and therefore will create large variation in the responses to stream disturbances that affect quality and quantity of prey. Moreover, this study indicates that growth restrictions in larval salamanders will be complexly (and likely non-linearly) related to interacting global changes like nutrient enrichment and climate change.

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Table 4.1. Mean parameter values (range) used to test the sensitivity of the threshold elemental ratio ($TER_{C:P}$) in 3 sizes of *D. quadramaculatus* (DQUAD) and *E. wilderae* (EWILD). Numbers under the species abbreviations are the dry mass (mg DM) associated with a range of parameters. I_C is the max ingestion rate, R_C is the mass-specific respiration rate, A_C and A_P are assimilation efficiencies of carbon and phosphorus, respectively, and Q_C and Q_P is the mass of carbon and phosphorus per unit of DM of a salamander.

Larval Size (mg DM)	I_C (mg C/mg C/ day)	R_C (mg C/mg C/ day)	A_C	A_P	Q_C (mg C/ mg DM)	Q_P (mg P/ mg DM)
DQUAD						
14	0.3 (0.075-1.8)	0.0202 (0.0001-0.17)	0.62 (0.45-.8)	0.8 (0.5-1)	0.45 (0.35-0.55)	0.021 (0.01-0.05)
89	0.08 (0.02-0.48)	0.0099 (0.00005-0.045)	0.62 (0.45-.8)	0.8 (0.5-1)	0.44 (0.35-0.55)	0.024 (0.011-0.05)
395	0.06 (0.016-0.3)	0.007 (0.00001-0.035)	0.62 (0.45-.8)	0.8 (0.5-1)	0.45 (0.35-0.55)	0.032 (0.015-0.05)
EWILD						
3.6	0.14 (0.035-0.84)	0.0169 (0.001-0.08)	0.62 (0.45-.8)	0.8 (0.5-1)	0.47 (0.35-0.6)	0.015 (0.01-0.03)
16	0.12 (0.031-0.72)	0.0124 (0.001-0.07)	0.62 (0.45-.8)	0.8 (0.5-1)	0.45 (0.35-0.55)	0.021 (0.01-0.035)
44	0.12 (0.03-0.72)	0.0097 (0.001-0.06)	0.62 (0.45-.8)	0.8 (0.5-1)	0.45 (0.35-0.55)	0.022 (0.012-0.04)

Table 4.2. Parameter estimates from GLMs relating variation in elemental composition and stoichiometry to size of larvae (SVL), species, and treatment year (Year 1, Year 2 compared to PRE).

Model	Estimate (SE)	P	Parameter	Estimate (SE)	P
% P: $F_{4, 276} = 16.85$			**C:N :** $F_{4, 260} = 5.48$		
Intercept	1.53 (0.13)	< 0.001	Intercept	4.09 (0.11)	< 0.001
SVL	0.02 (0.004)	< 0.001	SVL	0.005 (0.003)	0.09
Ewild	0.18 (0.67)	0.008	Ewild	0.057 (0.05)	0.26
Year 1	-0.001 (0.07)	0.9	Year 1	0.16 (0.06)	0.01
Year 2	0.27 (0.06)	< 0.001	Year 2	0.24 (0.06)	< 0.001
% N: $F_{4, 260} = 28.01$			**C:P :** $F_{4, 256} = 28.28$		
Intercept	12.70 (0.31)	< 0.001	Intercept	67.95 (4.11)	< 0.001
SVL	-0.02 (0.009)	0.02	SVL	-0.54 (0.12)	< 0.001
Ewild	-0.33 (0.144)	0.02	Ewild	-6.89 (1.91)	< 0.001
Year 1	0.33 (0.17)	0.06	Year 1	9.49 (2.35)	< 0.001
Year 2	-0.55 (0.17)	0.001	Year 2	-1.71 (2.21)	0.44
% C: $F_{4, 260} = 24.37$			**N:P :** $F_{4, 256} = 36.27$		
Intercept	44.68 (0.79)	< 0.001	Intercept	16.43 (1.04)	< 0.001
SVL	-0.03 (0.02)	0.26	SVL	-0.14 (0.03)	< 0.001
Ewild	-0.89 (0.37)	0.017	Ewild	-1.19 (0.48)	0.01
Year 1	2.76 (0.46)	< 0.001	Year1	2.04 (0.59)	< 0.001
Year 2	0.43 (0.43)	0.33	Year 2	-1.32 (0.56)	0.019

Table 4.3. Average threshold elemental ratios ($TER_{C:P}$) (Range) for three size classes (min, mean, max mg DM) *D. quadramaculatus* (DQUAD) and *E. wilderae* (EWILD). Normal represents the average $TER_{C:P}$ from the minimum, mean, and maximum of measured values used in each parameter. Extreme is the average $TER_{C:P}$ that includes the natural range (normal) of parameters and the extreme minimum and maximum (outside measured variation) for each parameter.

Treat YR	Normal	Extreme
DQUAD		
14 mg	83 (67-140)	109 (33-830)
89 mg	79 (59-130)	106 (36-658)
395 mg	59 (46-85)	88 (36-792)
EWILD		
3.6 mg	132 (81-195)	181 (64-1333)
16 mg	87 (66-120)	129 (64-1333)
44 mg	80 (61-115)	91 (43-352)

Figure Legends

Fig. 4.1. The relationship of body size (SVL mm) and %P (a), % N (b), % C (c), N:P (d), C:N (e), and C:P (f) for *D. quadramaculatus* (DQUAD; circles) and *E. wilderae* (EWILD; triangles). Lines indicate significant relationships.

Fig. 4.2. Model outputs for threshold elemental ratios ($TER_{C:P}$) for variation in parameter values for mass-specific maximum ingestion rate, I_C (a), mass-specific respiration rate, $R_{C,}$ (b), and mass-specific respiration rate at three temperatures that salamanders plausibly encounter throughout a year, for *D. quadramaculatus* (DQ) and *E. wilderae* (EW) larvae at three size classes (minimum, mean, maximum measured DM (mg)).

Fig. 4.3. The relationship of variation in assimilation efficiency of carbon, A_C (a), and body phosphorus content, Q_P, (b), and the threshold elemental ratio ($TER_{C:P}$) for the minimum, mean, and maximum dry mass (mg DM) of *D. quadramaculatus* (DQ) and *E. wilderae* (EW) larvae. Numbers to the left of species abbreviations are the mg DM of larvae.

Fig. 4.4. Average diet stoichiometry (C:P) for (a) *D. quadramaculatus* and (b) *E. wilderae* during PRE, YR1, and YR2. Stoichiometry for No Change is based on reference stoichiometry values from Cross et al. (2003). Diet stoichiometry for "Change" is based on enrichment values from Cross et al. (2003) and represents the potential consequence of changes in individual prey stoichiometry in response to enrichment.

Fig. 4.5. Elemental imbalances between salamander threshold elemental ratios ($TER_{C:P}$) and diet stoichiometry ($Diet_{C:P}$) for (a) *D. quadramaculatus* and (b) *E. wilderae* for the minimum, mean, and maximum of dry mass (mg DM) sampled in our study. Numbers on the top of the x-axis are the DM of the salamander. Imbalances were calculated as the arithmetic difference between the $TER_{C:P}$ and $Diet_{C:P}$. separated by treatment years (PRE, YR1, YR2). YR2 Change represents the imbalance if salamander prey exhibit altered stoichiometry in response to enrichment (values taken from Cross et al. (2003).

Fig. 4.6. Comparison of the relationship of body C:P and threshold elemental ratios ($TER_{C:P}$) between adult and juvenile fish (from Frost *et al.* 2006) and larval salamanders from this study. For a given body C:P, larval salamanders generally have lower $TER_{C:P}$.

Figure 4.1.

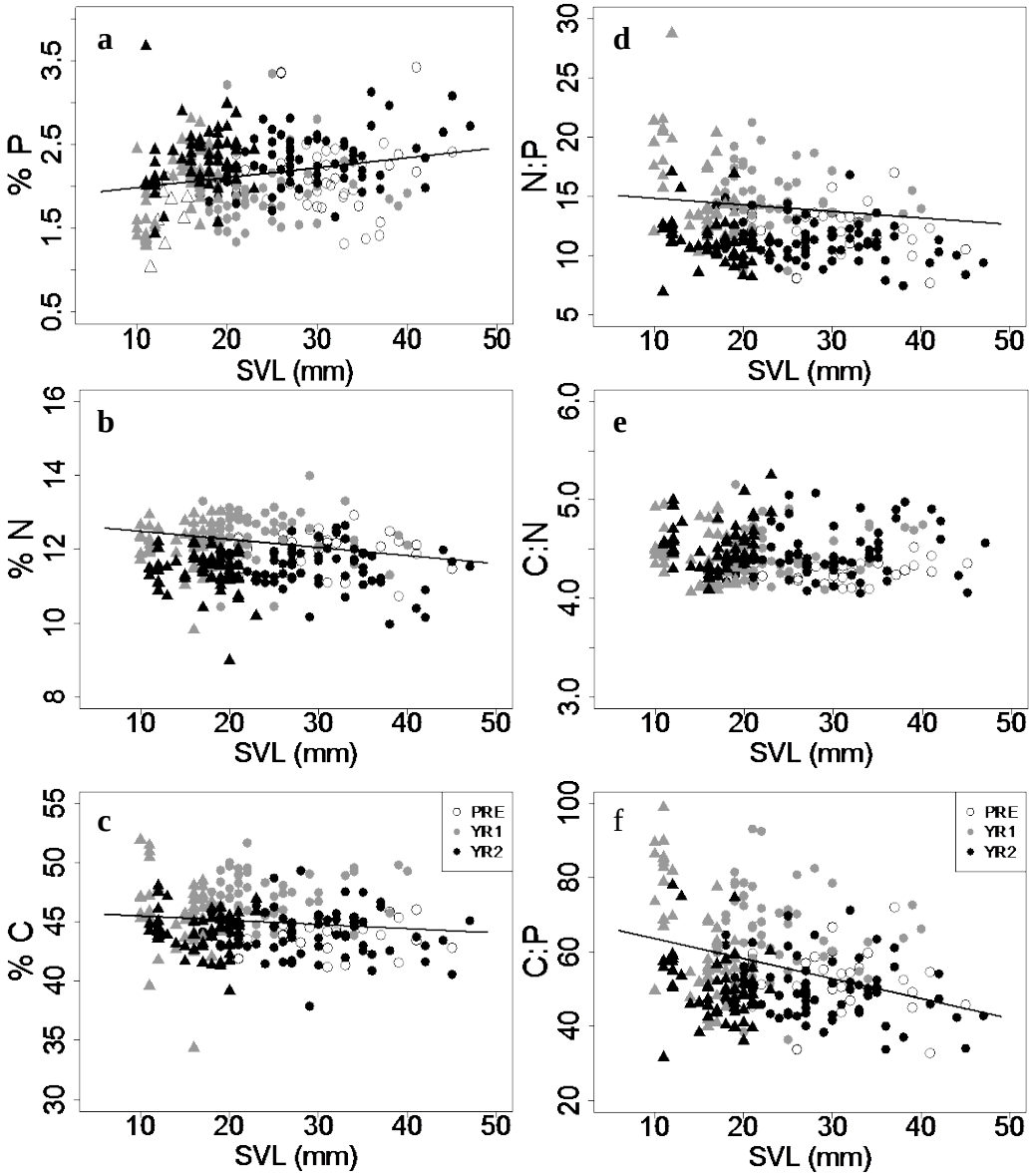

a
% P
SVL (mm)
d
N:P
SVL (mm)
b
% N
SVL (mm)
e
C:N
SVL (mm)
c
% C
SVL (mm)
PRE
YR1
YR2
f
C:P
SVL (mm)
PRE
YR1
YR2

Figure 4.2

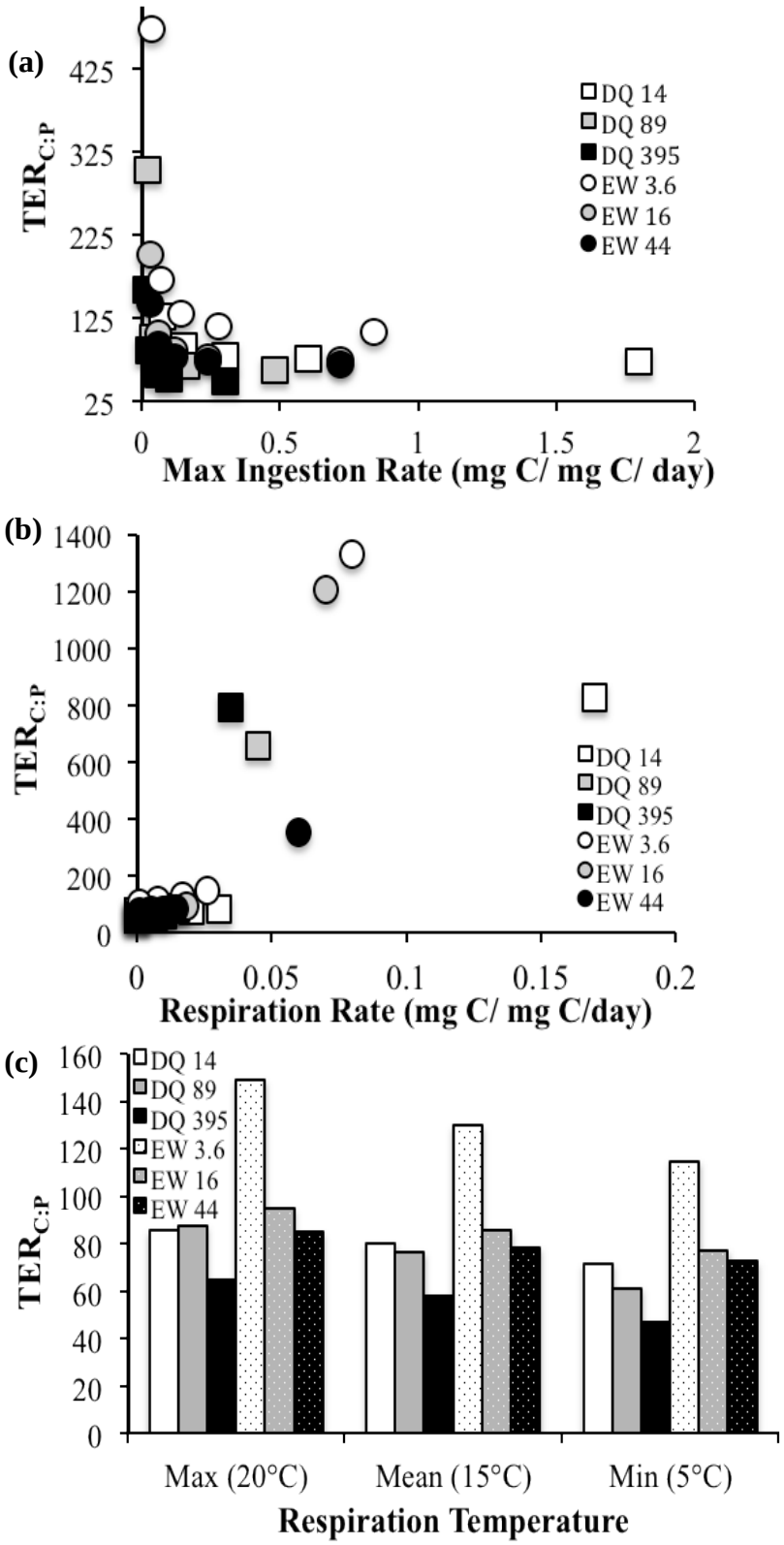

(a)
425
325
225
125
25
TER C:P
0
0.5
1
1.5
2
Max Ingestion Rate (mg C/ mg C/ day)
DQ 14
DQ 89
DQ 395
EW 3.6
EW 16
EW 44
(b)
1400
1200
1000
800
600
400
200
0
TER C:P
0
0.05
0.1
0.15
0.2
Respiration Rate (mg C/ mg C/day)
DQ 14
DQ 89
DQ 395
EW 3.6
EW 16
EW 44
(c)
160
140
120
100
80
60
40
20
0
TER C:P
DQ 14
DQ 89
DQ 395
EW 3.6
EW 16
EW 44
Max (20°C)
Mean (15°C)
Min (5°C)
Respiration Temperature

Figure 4.3

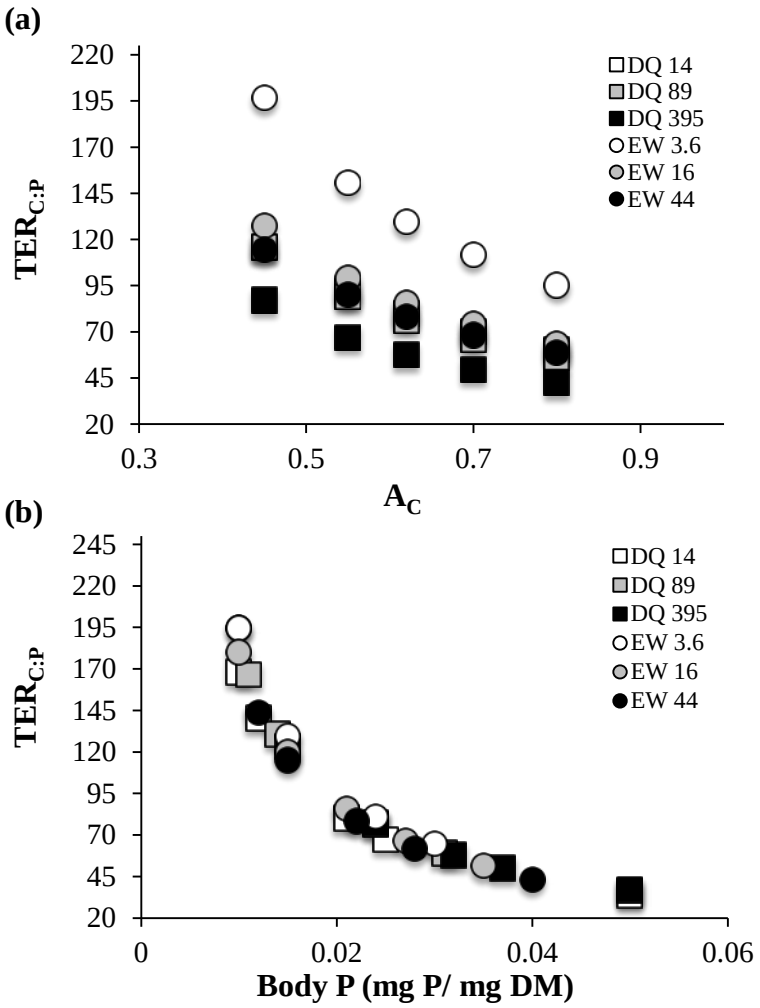

(a)
220
195
170
145
120
95
70
45
20
TER C:P
0.3
0.5
0.7
0.9
A C
DQ 14
DQ 89
DQ 395
EW 3.6
EW 16
EW 44
(b)
245
220
195
170
145
120
95
70
45
20
TER C:P
0
0.02
0.04
0.06
Body P (mg P/ mg DM)
DQ 14
DQ 89
DQ 395
EW 3.6
EW 16
EW 44

Figure 4.4

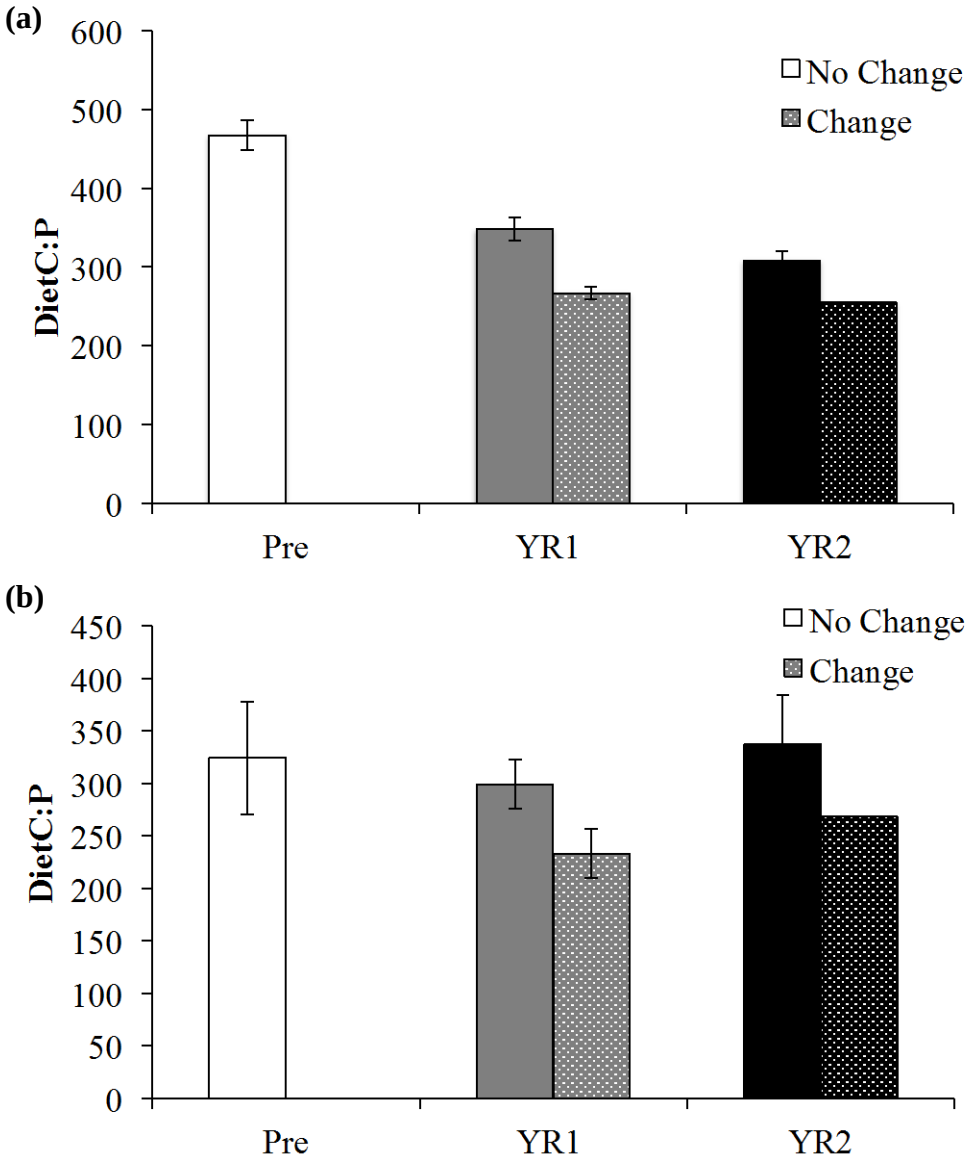

(a)
600
500
400
300
200
100
0
DietC:P
No Change
Change
Pre
YR1
YR2
(b)
450
400
350
300
250
200
150
100
50
0
DietC:P
No Change
Change
Pre
YR1
YR2

Figure 4.5

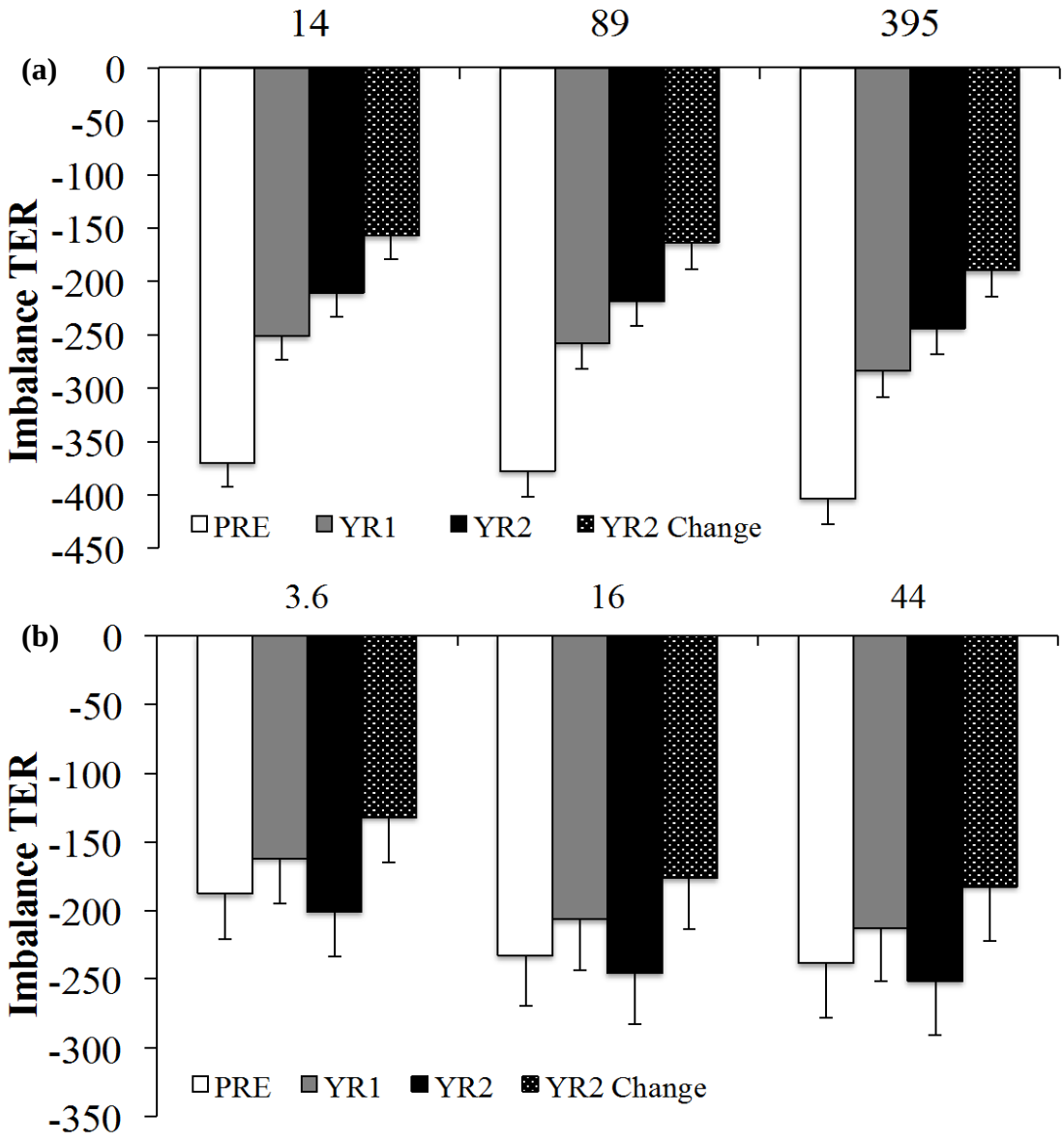
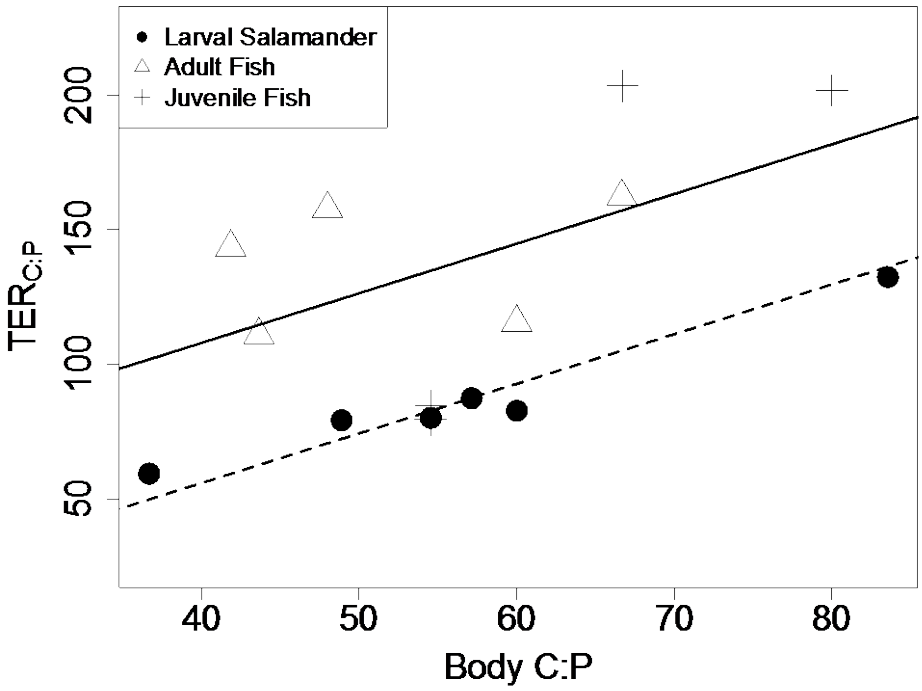

14
89
395
(a)
0
-50
-100
-150
-200
-250
-300
-350
-400
-450
Imbalance TER
PRE
YR1
YR2
YR2 Change
3.6
16
44
(b)
0
-50
-100
-150
-200
-250
-300
-350
Imbalance TER
PRE
YR1
YR2
YR2 Change

Figure 4.6


Larval Salamander
Adult Fish
Juvenile Fish
TER C:P
50
100
150
200
40
50
60
70
80
Body C:P

Appendix 4.1
Relationship between respiration and body mass for adult and larval salamanders

Methods:

Respiration data for larval salamanders are rare, particularly in regards to larvae in the family Plethodontidae, which includes our study organisms. Therefore we used published wet mass to oxygen consumption relationships generalized for all salamanders (Gatten, Miller & Full 1992; Wells 2007):

$$5\ °C: \log Y = 0.81 \log X - 1.69$$

$$15\ °C: \log Y = 0.81 \log X - 1.34$$

$$20\ °C: \log Y = 0.80 \log X - 1.17$$

Where log Y is the respiration rate (mL O_2 hr^{-1}), and X is wet mass (g). These equations are largely created from adult salamander data. Therefore, we compared known wet mass-respiration relationships between adult and larval salamanders to determine if they differed and to ensure that the equations we used would be practical for estimating larval respiration. Linear regression was used to compare adult and larval wet mass to O_2 consumption at 20 °C (Fig. 1a) and 15 °C (Fig. 1b). Model results indicated that the relationships were statistically equivocal for both temperature values among larvae and adults (15: $F_{2,17} = 8.33$, $P = 0.29$; 20: $F_{3,16} = 6.32$, $P = 0.38$, Fig. 1a,b).

The equations we used to estimate larval respiration were based on wet mass and were reported as mL O_2 consumed per hour (mL hr^{-1}). Therefore, to use these values in our TER models, we converted wet mass to dry mass in order to get respiration in terms of mg C of a salamander. We did this using known wet mass to dry mass relationships for *D. quadramaculatus* (Milanovich and Maerz, *unpublished data*). Reliable estimates for wet mass to dry mass were not available for *E. wilderae,* therefore we used known relationships for *E.*

cirrigera, a closely related congener of *E. wilderae* (Trice, Rosemond, and Maerz, *unpublished data*). To ensure the practicality of this we compared the relationships between SVL and wet mass for *E. wilderae* and *E. cirrigera* and found they were very similar. Therefore, we are confident that using *E. cirrigera* relationships for converting wet mass to dry mass is reliable for estimating mass-specific respiration rates. After converting to dry mass we then converted mass in terms of mg C by multiplying by a constant of 0.46, which was the average % C among salamanders. Further, we converted oxygen consumption to carbon respired using a respiratory quotient of 1 (Frost *et al.* 2006).

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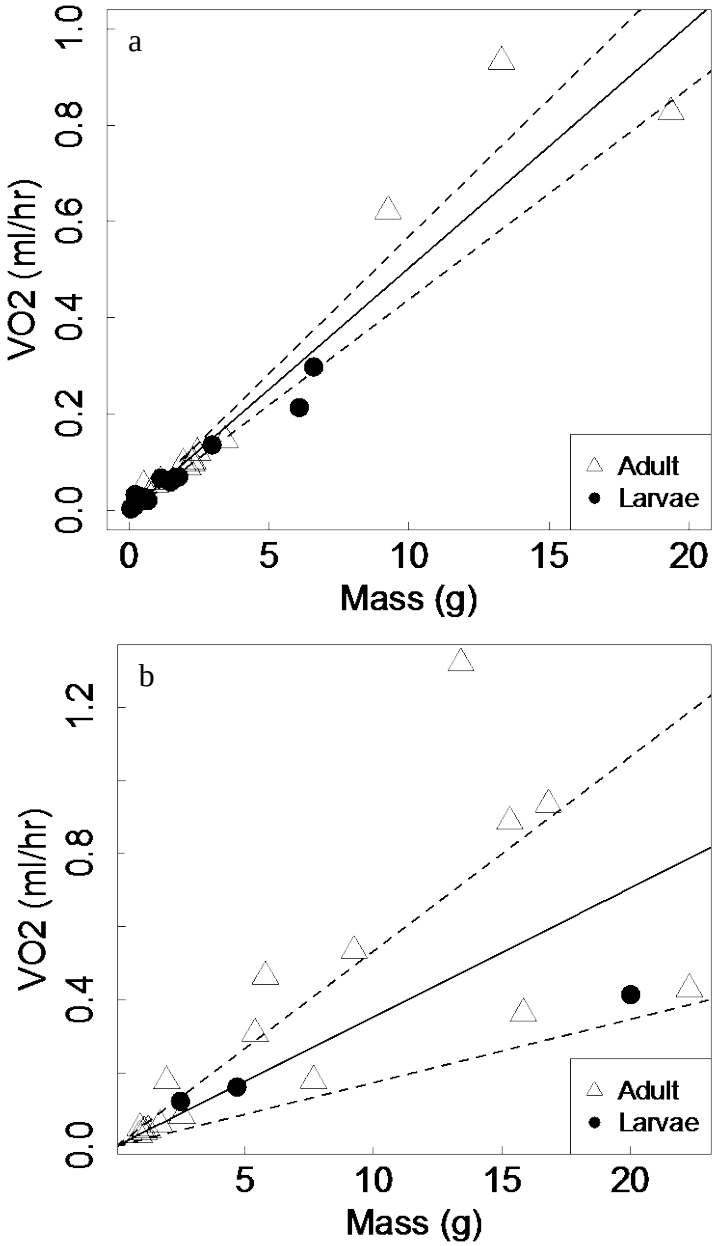

Figure 1. Mass-specific oxygen consumption for larval and adult salamanders at a) 20 ° C and b) 15 ° C. Dashed lines represent the 95 % confidence intervals .

Appendix 4.2
Appendix S2 Supporting Information

Table S1. Elemental and stoichiometric composition of *D. quadramaculatus* (DQUAD) and *E. wilderae* (EWILD) for each treatment year. SVL is snout-vent length (mm). Numbers in parentheses represent ± SE.

	% P	% N	% C	N:P	C:N	C:P	SVL
DQUAD							
PRE	2.15 (0.08)	12.04 (0.09)	43.91 (0.24)	12.16 (0.37)	4.26 (0.02)	51.74 (1.55)	30.45 (1.23)
YR1	1.96 (0.06)	12.43 (0.08)	47.27 (0.25)	14.61 (0.38)	4.43 (0.03)	64.77 (1.72)	24.45 (0.79)
YR2	2.31 (0.04)	11.57 (0.07)	43.99 (0.23)	11.33 (0.22)	4.45 (0.03)	50.34 (0.99)	29.16 (0.86)
EWILD							
PRE	1.69 (0.11)	-	-	-	-	-	13.3 (0.46)
YR1	1.99 (0.05)	12.45 (0.20)	45.70 (0.39)	15.59 (0.63)	4.32 (0.06)	62.18 (2.09)	14.92 (0.41)
YR2	2.31 (0.05)	11.37 (0.07)	44.20 (0.22))	11.12 (0.23)	4.54 (0.03)	50.57 (1.13)	17.05 (0.45)

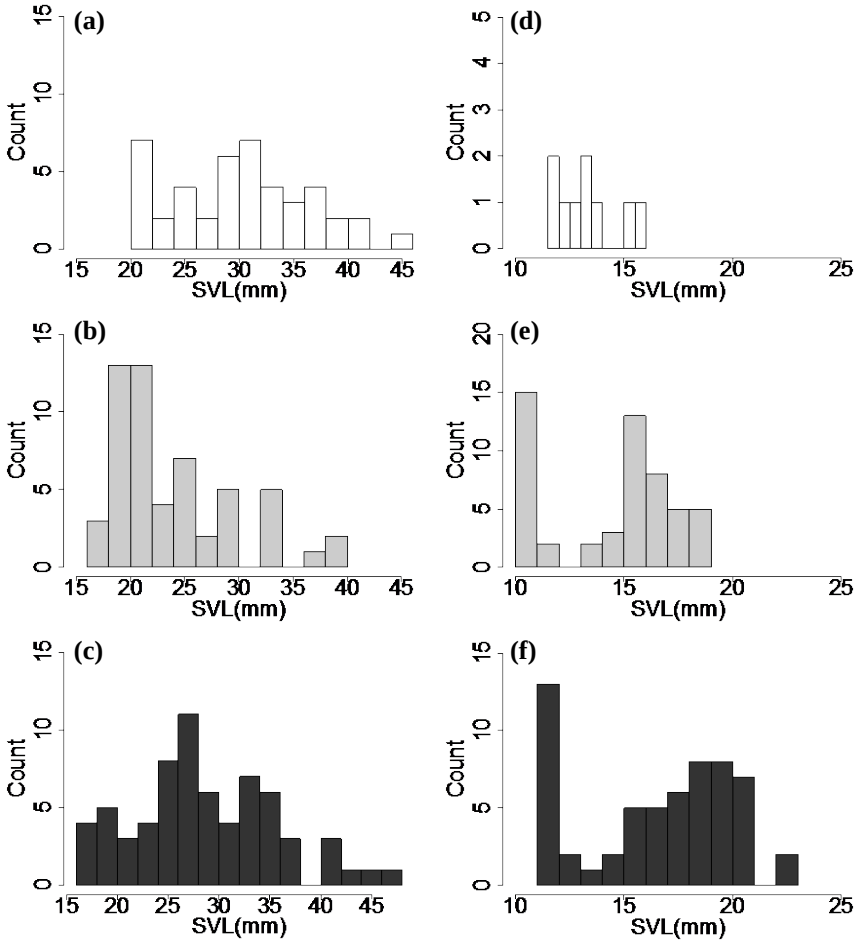

Fig. 1. Size (SVL) distributions for *D. quadramaculatus* (a,b,c) and *E. wilderae* (d,e,f) during each sampling year, PRE (a,d), YR1, (b,e), and YR2, (c,f).

CHAPTER 5

SUMMARY AND CONCLUSIONS

Aquatic ecologists have been charged with the goal of more mechanistically understanding the effects of nitrogen (N) and phosphorus (P) enrichment on aquatic ecosystems to help create effective nutrient criteria in order to maintain stream health and mitigate negative consequences already occurring (US EPA 1998; Evans-White et al. 2013). Despite this charge over a decade ago, the major source of impairment to streams and rivers is enrichment of N and P (US EPA 2013). The goal of this thesis was to determine the effects and relative importance of N and P concentration on larval salamander ecology in detritus-based streams. Further, I wanted to more mechanistically understand these responses by examining diet contents and modeling elemental constraints. Larval salamanders are top predators in fishless headwaters and therefore responses of salamanders to bottom-up perturbations like nutrient enrichment integrate responses of all trophic levels (e.g., fungi/detritus to macroinvertebrates to salamanders). My hope is that understanding how these predators respond to enrichment will inform the development of nutrient policy and aid in a more holistic understanding of stream ecosystem responses to enrichment.

Summary of Chapters

In Chapter 2 I assessed the effects of N and P enrichment on the growth rates of larval salamanders. I did this using *in situ* mesocosms (in stream cages) experiments and capture-mark-

recapture (CMR) of free-roaming individuals. I found that growth rates of caged larvae were stimulated by nutrient enrichment (up to 40% increase) and positively related to phosphorus concentrations. Growth rates of *D. quadramaculatus* increased in all streams compared to reference cage animals. This response was not related to N concentration of our treatments, suggesting that P was most important in driving increased growth rates. Further, free-roaming larvae, which integrated spatially and temporally the response to experimental enrichment showed positive correlation to phosphorus concentration. Additionally, average size of *E. wilderae* increased in all streams in response to enrichment (up to 60% increase) and was positively related to our gradient of P concentration. This response exhibited the potential for moderate additions of nutrients to create relatively large responses at the top of food webs.

After documenting increased growth rates in larval salamanders, I hypothesized that increased growth rates could occur via three main food web pathways: 1) Larval salamanders increase consumption of prey, 2) prey composition of salamanders changes such that the stoichiometric imbalance between predator and prey is reduced, and 3) individual prey exhibit changes in stoichiometry in response to enrichment. I was not able to assess mechanism 3, however, I did find evidence partially supporting *both* mechanisms 1 and 2. Biomass of prey consumed in *D. quadramaculatus* stomachs increased with enrichment and was driven by larvae greater than ~25 mm. Prey size consumed also increased in response to enrichment. The response of *E. wilderae* diets was less clear, though I did find evidence for increased prey size in YR2 of enrichment and increased number of prey consumed in YR1. Additionally, I found compositional changes among functional feeding groups in *D. quadramaculatus* diets. This was driven by a large decrease in collector-filterers and increases in both collector-gatherers and scraper functional groups. There was no change in the proportion of shredder taxa. Surprisingly,

I found that the increase in collector-gatherer and scraper taxa was related to increased peak biofilm biomass. This is surprising given the low availability of biofilms in our streams because of light limitation. Further, I found no evidence of compositional shifts in *E. wilderae*, which may reflect differences in microhabitat preferences between the two species.

Chapter 4 investigated the potential consequences of variation of parameters to the threshold elemental relationships (TER) in larval salamanders. TERs represent the theoretical point at which limitation of growth switches from one element to another (in my case C:P). I found that both species of larval salamanders exhibit remarkably low $TER_{C:P}$ (under the assumption of saturating food levels) and potentially large consequences of the apparent limitation of salamanders to variation in model parameters. This exercise highlighted the importance to accurately measure ingestion rates of salamanders as this parameter exhibited a threshold relationship with the TER output. Further, I found that natural variation in body stoichiometry of both species can create a relatively large range in the $TER_{C:P}$. I also assessed the imbalance of salamanders between their $TER_{C:P}$ and the C:P of their diets. I found that *E. wilderae* has slightly lower imbalances due to lower diet C:P and that compositional changes of *D. quadramaculatus* diets found in Chapter 3 likely lead to reduced elemental imbalances by reducing diet C:P. Lastly, I compared the TER between salamanders in my study and fish and found that generally for a given body C:P larval salamanders have much lower $TER_{C:P}$ suggesting they may be more phosphorus limited when food is saturating than we would predict based on what is known about fish.

Final Conclusions

The findings in this body of work highlight the importance of phosphorus (and N to some extent) in the ecology of larval salamanders in detritus-based systems. The strong relationship I found between growth rates of salamanders and phosphorus concentration suggests that variation in the delivery of nutrients to aquatic systems matters. When N is added to streams without P, enrichment may not propagate up trophic levels to affect larval salamanders (or other top predators limited by P). For instance, the addition of both P and N, which is more likely at low elevations (e.g., from sewage effluent, agriculture), may stimulate food web responses more so than atmospheric deposition of N (without P) at high elevations. Additionally, this thesis highlights that unexpected energetic pathways may emerge as a result of enrichment (Chapter 3), and life-history characteristics of organisms will play an important role in mediating the pathway and magnitude of response to nutrient enrichment.

Other studies have found effects of nutrient enrichment on top predators. A few studies in algal-based streams have shown that enrichment can stimulate fish growth and a single previous study document increased growth rates of *E. wilderae* in response to enrichment (Peterson et al. 1993; Slavik et al. 2004; Johnson et al. 2006). My thesis adds to this body of literature that supports propagation of enrichment to top predators, but goes beyond in highlighting the importance of P concentration and partially determining mechanisms responsible for observed responses.

Larval salamanders are important components of headwater ecosystems (Davic and Welsh 2004). Relatively little is known about the ecological role of salamanders or their response to stream perturbations. However, ecologists are beginning to better understand larval salamander roles in headwaters and the consequences of their loss. Recent studies have shown

that larval salamanders can shape macroinvertebrate communities and strongly control their populations (Keitzer and Goforth 2013a). Moreover, salamanders are likely important in nutrient cycling and storage in headwater streams (Milanovich et al. in Milanovich 2010; Keitzer and Goforth 2013b). The bottom-up effects of nutrient enrichment may alter the ecological function of larval salamanders. Increased growth rates may stimulate the top-down control of macroinvertebrate communities thereby altering energy flow and nutrient dynamics of headwater streams. Additionally, this study demonstrates the potential consequences of global changes to the consumer resource base (Kominoski and Rosemond 2012) on top predators. Overall, this study provides some of the first evidence that P enrichment can create important consequences in stream food webs, propagating to top predators.

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