MODELING THE CURRENT AND FUTURE ROLES OF STREAM SALAMANDERS IN HEADWATER STREAMS

by

JOSEPH ROSS MILANOVICH

(Under the Direction of John C. Maerz)

ABSTRACT

Communities are shaped by environmental conditions and the interactions within those environments, and changes in community composition or dynamics will in turn affect the environment through alteration of ecosystem processes. With humans rapidly altering ecosystems, predicting how environmental changes may lead to species losses, and how those biotic changes will feedback on ecosystem processes has emerged as a critical challenge. The southern Appalachian Mountains are a global hotspot for stream plethodontids, which are the numerically dominant vertebrate predators of high-elevation first order streams, and as such are hypothesized to be influential in a number of ecosystem processes. The objectives of this dissertation were to determine how projected shifts in salamander-community composition resulting from climate change may affect the retention of nutrients within first-and second-order streams. To meet this objective, elemental mass and excretion rates of salamanders was quantified to estimate the amount of nutrients captured and exported by a stream-salamander community, predictive models of species loss under climate-change scenarios were generated, and experiments were used to determine whether predicted species losses altered nutrient retention or whether compensation by other salamander species may occur. Research was conducted within the Coweeta Hydrological Laboratory basin (NC, USA). Plethodontids showed variation in elemental stoichiometry and relationships between body stoichiometry, body size, and excretion stoichiometry. Larval plethodontids were significant nutrient reservoirs when compared to other stream taxa, which implies that they are important to stream nutrient retention and nutrient cycling within streams. Excretion rates were low, but densities were high, and preliminary evidence indicates that plethodontids play a large role in re-release of nutrients. Models project a decline in suitable habitat associated with climate change scenarios for most currently dominant salamander species within the Coweeta basin, potentially affecting their role in nutrient retention. Furthermore, our results suggest that subordinate plethodontid species are able to compensate for species losses with respect to nutrient retention if their densities increase. Since plethodontids exhibit high diversity in the region and are significant contributors to a number of ecosystem processes, these projections identify a significant potential change to ecosystem function in southern Appalachian headwater streams.

INDEX WORDS: Stream, Headwater, Plethodontid, Ecological Stoichiometry, Excretion, Density, Global Climate Change, Species Distribution Models, Compensation

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BA, Adrian College, 2002

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

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2010

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August 2010

ACKNOWLEDGEMENTS

I deeply thank the large number of people who have helped throughout this dissertation. First and foremost, I would like to thank my major professor, John Maerz, for his support, guidance, and friendship over the past four years. I feel very fortunate to have worked with John, as he not only did his best to help develop this dissertation, but also myself as an ecologist and teacher. I would also like to thank the other members of my committee, Carlos Camp, Nate Nibbelink and Amy Rosemond. Carlos Camp deserves special thanks for always being available for advice and for spending a significant amount of time with me in the field working on unrelated projects. I thank Nate Nibbelink for putting so much time and thought into the study design, analysis and writing of this dissertation, in particular Chapter 3. Amy Rosemond played an integral portion of the development of this study, and I thank her for her time and effort – especially for offering the course *Ecosystem Concepts*. I owe a great debt of gratitude to Jim Peterson, Shannon Albeke, Andy Davis, Bill Peterman and Luke Worsham for statistical and GIS assistance, and Tom Maddox and Lisa Dean for laboratory assistance. My lovely wife, Janice, was the most supportive person anyone could ask for, and I thank her for all her support throughout my graduate career. I look forward to the rest of our “non-graduate school” life together. My family - my mother Marie, brothers John and Jason, and sister-in-law, Amy, also deserve special thanks for their support throughout the years. I thank many people for assistance in the field, including: Kyle Barrett, Kristen Cecala, Brian Crawford, Andy Davis, Jayna DeVore, Matt Erickson, Andrew

Ferreira, Andrew Grosse, Vanessa “Nessa” Kinney, Lincoln Larson, Amanda Perofsky, Bill Peterman, Kate Mowbary, Sean Sterrett, Steve and Sara Samoray, Matt Truslow, and Josh Wisdom. Lastly, I thank the members of the Maerz Lab, Warnell, and Ecology schools for countless antics that often ended up in rough mornings.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

General context

The importance of biota to the functioning of ecosystems has been noted in several recent reviews (Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006, Thompson and Starzomski 2007, Gamfeldt et al. 2008, Duffy 2009, Lecerf and Richardson 2009, Woodward 2009, Vaughn 2010). Although much of the past research has focused on biodiversity of primary producers and microbial communities, the effect of biodiversity loss on consumers is gaining attention (Duffy 2002). Consumers can often have disproportionate influence over ecosystem function and their loss often constitutes greater change to ecosystems than a loss of lower trophic levels (Duffy 2003). However, not all consumers are equal. A loss of vertebrate consumers could have a larger impact than a decline in invertebrate consumers. For example, using a meta-analysis, Shurin et al. (2002) found that small vertebrate predators had significantly stronger effects on plant biomass than invertebrates. Furthermore, a number of fish species have been found to significantly influence rates of recycling and flow of nutrients in freshwater streams (Vanni 2002, Vanni et al. 2002, Taylor et al. 2006, McIntyre et al. 2007, McIntyre et al. 2008). A fundamental challenge for ecologists, therefore, is to determine which species are likely to show population declines or become locally extirpated, and to predict the effects of those species losses to ecosystem function.

Because climate is a major determinant of species distributions, global climate change is projected to have a considerable affect on regional biodiversity including the loss of species that may currently be dominant in particular ecosystems (Kappelle et al. 1999, Chapin et al. 2000, Peterson et al. 2002, Malcolm et al. 2006, Huntley et al. 2008, de Chazal and Rounsevell 2009, Heller and Zavaleta 2009). Ecological responses to recent climate change have already resulted in changes to ecological function in terrestrial systems, such as net primary production (reviewed in Kappelle et al. 1999, McCarty 2001, Walther et al. 2002, Traill et al. 2010). In aquatic ecosystems, climate change is predicted to directly alter mixing regimes in lakes, stream runoff, and nutrient loading (Meyer et al. 1999). Furthermore, changes to the ecosystem processes controlled by biota in both aquatic and terrestrial ecosystems are expected, such as alteration in rates of nutrient recycling and primary production (Traill et al. 2010). Therefore, there is a need to understand potential changes to biodiversity as a consequence to global climate change, and to test how those changes may alter ecosystem processes.

Headwater streams and their importance.

Headwater streams comprise at least 75% of the stream and river channel length in the United States (Meyer and Wallace 2001). Headwater streams in temperate areas are detrital based, have little, if any, primary productivity and depend on allochthonous detritus from terrestrial inputs as the primary source of organic energy (Wallace et al. 1999, Gomi et al. 2002). Study of detritus-based systems is important because they represent the dominant pathway of energy flow in most ecosystems. Greater than 80% of plant biomass ultimately ends up in detritus-based food webs (Wetzel and Ward 1992, Cebrian 1999). In addition, these streams serve important ecological functions within the landscape including, sediment retention, water purification, flood control, cycling of nutrients, and transfer of energy to downstream areas. Downstream movement of material from headwater reaches can be important to support downstream food webs (Meyer and Wallace 2001, Wipfli and Gregovich 2002, Wipfli et al. 2007) and, collectively, these streams create a network structure for the watershed which links headwater and downstream systems (Gomi et al. 2002). Lastly, stream systems have contributed significantly to understanding the relationship between biodiversity and ecosystem function (Lecerf and Richardson 2009). Of particular significance to this study, headwater streams are also home to a wide diversity and large abundance of plethodontid salamanders (Petranka 1998, Lannoo 2005). Terrestrial and semi-aquatic forms of plethodontid salamanders are hypothesized to be important to a number of ecosystem processes, thus given the importance of headwater streams, studies involving stream-dwelling plethodontids are of particular importance.

Amphibian Declines and Plethodontid salamanders

Amphibian populations are undergoing significant declines worldwide (Beebee and Griffiths 2005, Lannoo 2005, Sodhi et al. 2008). There has been no shortage of efforts to determine the causes of amphibian declines (reviewed in Lannoo 2005), but there remains a paucity of studies addressing the consequences of amphibian population declines. Recent studies in the tropics predicted and found that large-scale amphibian declines caused by disease significantly altered algal richness as well as ecosystem-level processes such as changes to algal richness, primary productivity, and macroinvertebrate production (Whiles et al. 2006, Connelly et al. 2008, Colon-Gaud et al. 2009).

Furthermore, with respect to pond-dwelling salamanders in North America, egg deposition by species in the genus *Ambystoma* had a net influx of up to 761 g AFDM/year into ponds in southern Illinois, and these same systems only exported between 21 to 125 g (AFDM/year) of *Ambystoma* via emergence of metamorphic animals (Regester and Whiles 2006). A decline associated with amphibian taxa in these systems could lead to a significant loss of energy input into temporary ponds.

The family Plethodontidae contains 28 genera and about 395 species (AmphibiaWeb 2010, http://amphibiaweb.org). All are lungless, and although most species are terrestrial, semi-aquatic forms are abundant and diverse in the southeastern United States, particularly the Appalachian Highlands (Petranka 1998). Semi-aquatic species (e.g., most species of *Desmognathus* and all of *Eurycea* spp., *Pseudotriton* spp., and *Gyrinophilus* spp.) possess a biphasic life history and have aquatic larval periods that range from a few months to five years (Petranka 1998). Davic and Welsh (2004) in their review of the role of salamanders in ecosystems compiled a number of studies and created strong hypotheses concerning the function of semi-aquatic plethodontids. In particular, they hypothesize plethodontids contribute to ecosystem stability as mid-level predators that connect aquatic and terrestrial habitats, act as a slowly available high-quality nutrient resource for higher consumers, and regulate both decomposition and the composition and numbers of invertebrate taxa. These salamanders are considered the top vertebrate predators in many headwater systems and are hypothesized to affect the release and transfer of nutrients (Davic and Welsh 2004). Plethodontids have low metabolic rates and efficient rates of assimilation (Fitzpatrick 1973a, Fitzpatrick 1973b), thus, plethodontids can convert and store resources very effectively; they are also a high-quality source of energy, as protein makes up more than 50% of their wet weight biomass (Burton and Likens 1975). Furthermore, semi-aquatic species of *Desmognathus* and *Eurycea* occur in high densities (Peterman et al. 2008a, Peterman et al. 2008b, Nowakowski and Maerz 2009), have small home ranges (Peterman et al. 2008a), and are susceptible to changes in microclimatic conditions (Dodd 1990, Welsh and Droege 2001) – all of which are associated with metrics used to examine changes to ecosystems (Welsh and Droege 2001) Climate-change models for southern Appalachia predict increases in temperatures and aridity (Burkett et al. 2001). Because plethodontid ecology is strongly regulated by temperature and moisture (Feder 1983), and much of the diversity of the group is distributed in cool, moist climatic zones, plethodontids are considered particularly vulnerable to a warming, drying climate (Kozak and Wiens 2010).

Dissertation

This dissertation represents the initiation of a long-term project at the Coweeta Hydrologic Laboratory Long-Term Ecological Research Site focused on understanding the ecological role of plethodontid salamanders in the southern Appalachian Highlands and consequences to ecosystem processes of their projected loss due to climate or land use change.

Chapter 2 presents a study quantifying the role of larval stream plethodontids as a standing crop and recycler of nutrients in Appalachian headwater streams. One of the key functions biota may play in flowing, freshwater ecosystems is the uptake and storage of nutrients (Small et al. 2010). Biotic uptake slows the rate at which nutrients move down stream, and vertebrate biota are predicted to lead to long-term storage of nutrients in freshwater streams. I combine ecological stoichiometry with robust estimates of density to describe the stoichiometry of the larval stages of three species of stream-dwelling plethodontids, test what factors, such as body size, body nutrient content, species, and location influenced the whole-carcass and excreta stoichiometry of plethodontids, and estimate the standing crop of limiting nutrients in larval plethodontids in five headwater streams at the Coweeta Hydrological Laboratory. This chapter is a first step toward understanding the importance of both individual species and plethodontids as a whole to headwater nutrient dynamics, and is key to identifying the consequences of a potential decline in plethodontids to ecosystem processes in headwater streams.

Chapter 3 uses species distribution modeling to project a range of future climatic distributions for Appalachian plethodontids. I utilized the program Maxent to model the suitable climatic habitat of 41 plethodontid salamander species that currently inhabit the Appalachian Highland region, and then used an ensemble of global circulation models and CO_2 emissions scenarios to project the future climatic distributions of species for 2020, 2050, and 2080. I also created accumulated richness maps for each of the scenarios to examine how projected changes in individual species “distributions” might affect patterns of diversity within the Appalachian Highlands region over time. It was my hope to identify which species of plethodontids may be locally or regionally at risk of extirpation, and identify which areas are likely to see significant losses of species that currently occupy the region. This chapter will be among the first studies to predict the response of plethodontid salamanders to global climate change in the area of their highest richness.

Predicting how biological communities may change in response to future climates is a challenge. Extrapolating those changes to how they will impact ecosystem processes is equally challenging. In chapter 4, I report on a field experiment to measure the capacity for potential future salamander communities to compensate for the projected loss of blackbelly salamanders (*Desmognathus quadramaculatus*), which currently dominate the larval salamander guild in southern Appalachian headwaters. I used field mesh cages to manipulate larval salamander communities to determine whether the future communities would produce similar biomass and standing crops of limited nutrients compared to the current community. This study is a first step towards merging our current understanding of species' effects on ecosystems, with predictions from species distribution models and field experiments, to project whether future communities might have the capacity to compensate for the loss of currently dominant species. This study draws attention to the importance of intraguild predation, and illustrates that while two species may not be ecologically redundant with regards to their interactions with other species within the community, a currently subordinate species has the capacity to compensate for the loss of the current dominant species with regards to the storage of nutrients.

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CHAPTER 2

ASSESSMENT OF LARVAL SALAMANDERS STOICHIOMETRY, BIOMASS, AND ROLE IN THE STORAGE AND RECYCLING OF NUTRIENTS IN APPALACHIAN HEADWATERS

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[1] Milanovich, J.R., J.C. Maerz, and A.D. Rosemond. To be submitted to *Oikos*

Abstract

Ecological stoichiometry merged with estimates of abundance or biomass shed light on a consumer's influence within an ecosystem. To help understand the role of larval stream salamanders in headwaters, we examined the stoichiometry and measured the standing crop of nutrients of three species of stream-dwelling plethodontid salamanders across five streams within the Coweeta Hydrological Laboratory located within the Appalachian Highlands of the eastern United States. First, we compared carcass and excreta stoichiometry among different species, determined whether size influenced larval stoichiometry, and estimated the degree of stoichiometric homeostasis for larval plethodontids. Next, we estimated larval density and biomass, and compared the standing crop of limiting nutrients in larval plethodontid salamanders among streams. Finally, we estimated the aggregate excretion of our dominant species, *Desmognathus quadramaculatus*. Whole-carcass %C, %N, and %P stoichiometry differed among species. We found negative relationships between body size and whole-carcass %N, %C, C:N, and C:P, and the excretion rate of N:P, and positive relationships between body size and whole-carcass %P, and excretion rate of TDN and TDP. Whole-carcass Ca content was positively correlated with body size and whole-carcass %P, which suggests that increasing %P with increasing larval size was associated with bone formation. Nutrient concentrations, most notably %P, and ratios of excreta were positively correlated with body concentrations and ratios, for example, P excretion rate was positively related to whole-carcass N:P and %P, respectively; while excretion rate of N was negatively related to whole-carcass %N. This suggests that despite low P availability in their prey, larval plethodontids may not be P limited. Mean larval salamander density was 58 larvae · m^{-2}

and mean dry biomass was 2812 mg · m^{-2}. Estimated standing crops of P and N were 0.09 and 0.40 g · m^{-2} respectively, which was 4.0 and 1.2 times greater than amounts reported for invertebrate taxa in similar habitats. Estimated aggregate *D. quadramaculatus* excretion of N and P was 1.48 μg NH$_4$ m^{-2} min^{-1} and 1.14 μg TDP m^{-2} min^{-1}, which is ~7 % of stream N demand, and 19% -33% of stream P demand.. Our results suggest that, compared to reports of freshwater fish, larval salamanders do not supply significant amounts of N and P demand; however, they do represent standing stocks of N and P in headwater streams.

Introduction

Understanding the impact of biota on ecosystem processes, such as the uptake, retention and recycling of nutrients, is gaining increasing attention in light of declines in the abundances of many species (Hooper et al. 2005, Long et al. 2007, Gamfeldt et al. 2008, Vaughn 2010). Consumers can impact ecosystem-level processes through top-down influence (see reviews in Sergio et al. 2008 and Woodward 2009), liberate nutrients via facilitating decomposition and excretion (Parmenter and Lamarra 1991, Regester and Whiles 2006), and stabilize nutrient dynamics by storage of limiting nutrients (e.g., Kraft, 1992, Vanni et al. 2002). Aquatic ecosystems have been a major focus of study with respect to consumer influences on ecosystem processes. In aquatic systems, the mobility and high biomass of invertebrate and fish consumers yield considerable ecosystem-level effects. For example, mobile, long-lived consumers that are also high in biomass can be significant in nutrient fluxes (Schaus and Vanni 2000, Glaholt and Vanni 2005), recycling nutrients via excretion (Kitchell et al. 1979, Elser and Urabe 1999, Vanni et al.

2002, Hall et al. 2003, McIntyre et al. 2008), storing large amounts of nutrients for long periods of time, or slowing down the movement of nutrients through ecosystems (Meyer et al. 1983, Hessen et al. 1992, Hassett et al. 1997, Cross et al. 2005, Small et al. 2009). For example, invertebrates constitute large standing crops of limiting nutrients in temperate and tundra streams (Peterson et al. 1997, Cross et al. 2005), and fish have been shown to be important in supplying N and P demand in tropical and temperate streams (Vanni 2002, McIntyre et al. 2008, Small et al. In press). Variation in fish densities across stream reaches create "hotspots" of fish excretion that have higher levels of N and P recycling (McIntyre et al. 2008).

Consumer-driven effects on stream nutrient dynamics is often a function of the consumer's biomass (Hall et al. 2003, McIntyre et al. 2008), though may also be affected by relationships between the consumer's stoichiometry and other factors such as diet or body size (Sterner and Elser 2002). If a single species dominates other members of a guild in terms of biomass or excretion of limiting nutrients, this species can exert a large influence on consumer-driven nutrient recycling. For example, in low-nutrient neotropical streams, Small et al. (In press) show that because *Astyanax aeneus* excrete P at 10 times the rate of other species, this one species supplies 90% of stream P demand despite representing only 18% of total fish biomass in a 43 species community. There are a number of reasons that consumer's may differ in stoichiometry (Sterner and Elser 2002). For example, Pilati and Vanni (2007) show that whole-carcass stoichiometry and nutrient excretion varied significantly across the life cycle of the fish, *Dorosoma cepedianum*. Specifically, larger larvae excreted lower N:P than adults, thus having a different impact on nutrient recycling. They attributed these differences to changes in both the allocation of P to bone formation, a process that has been found in other studies (Hendrixson et al. 2007), and ontogenetic variation in diet. Understanding what factors influence stoichiometric variables of a consumer and their excreta is essential to estimating a consumers' influence on biogeochemical cycles.

The influence of organisms on nutrient dynamics can be especially strong in low productivity ecosystems such as forested headwater streams. Forested headwater streams are considered low in situ nutrient systems that derive almost all nutrients from nutrient poor allochthonous detritus (Fisher and Likens 1973, Cummins 1974, Webster and Meyer 1997). These streams compose over 75% of stream length of larger basins (Leopold et al. 1964, Wallace 1988, Meyer and Wallace 2001) and are particularly important to cycling of nutrients and transfer of energy downstream due to their spatial area within the landscape and their ratios of benthic surface area to water volume (Meyer and Wallace 2001). Despite their importance to landscape level processes, little is about the influence of most headwater stream biota on nutrient dynamics in temperate detrital streams (see Cross et al. 2005). Evidence suggests invertebrates make up approximately 0.1% of direct contribution to C storage, but contribute 1.3 % and 2.2% to storage of N and P in detrital streams. Greater than 50% of particulate C, N and P export to downstream systems has been attributed to invertebrate consumptive processes (Cross et al. 2005). We are aware of no studies addressing the effects of vertebrates on headwater stream nutrient dynamics. Plethodontid salamanders are the most abundant vertebrates of forested ecosystems in the eastern and northwestern United States (Peterman et al. 2008a,b; see Davic and Welsh, 2004 for review). Southern Appalachian streams are a hotspot of diversity for biphasic (those species with aquatic larval phases) plethodontids where a single stream may include seven or more species and have larval densities averaging 60 per m^2 (Peterman et al. 2008a,b). Despite their dominance in vertebrate biomass of eastern North American forests (Burton and Likens 1975b), very little is known about plethodontid effects on ecosystem processes. Because of their remarkable abundance and metabolic efficiency (Fitzpatrick 1973a, Fitzpatrick 1973b), terrestrial plethodontids are hypothesized to be important nutrient sinks (Davic and Welsh 2004).

The goal of this study was to estimate the role of larval salamanders to the storage and supply of key limiting nutrients in headwater streams. First, we examined factors that influenced the whole-carcass and excreta stoichiometry of three plethodontid species. We predicted that larval stream salamanders are homeostatic and that they would show similar relationships between body size and whole-carcass stoichiometry as have been reported for freshwater fishes. As with fishes (Vanni et al. 2002, Hendrixson et al. 2007, Pilati and Vanni 2007), we believed variation in P content within and among species would be driven by increased use of P in skeletal development of larger individuals. Second, we measured the excretion rate and excreta nutrient stoichiometry of larval Black-bellied Salamanders, *Desmognathus quadramaculatus*. Stoichiometric theory assumes that consumers that exhibit elemental homeostasis will excrete nutrients in an inverse relationship with their demand (Sterner and Elser 2002). Because we predict that larger larval salamanders will use more P for skeletal development, we hypothesized that the %P in excreta would decline with increasing larval size and increasing %P of the larval tissues. Third, we estimated larval abundance and biomass in five headwater streams, and using those abundance estimates, we estimated standing crops of nutrients in larval salamanders and the proportion of N and P demand supplied by salamander excreta. We predicted that larval plethodontids would prove to be large standing crops of N and P; however, because of their high metabolic efficiency and associated low excretion rates, we predicted larval plethodontid excreta would contribute little to stream nutrient demand.

Methods

Study site

The study was conducted in five streams located within the Coweeta Hydrological Laboratory in Macon County, North Carolina. Coweeta is a large (2185 ha) basin located within the Blue Ridge physiological province within the southern Appalachian highlands (Swank and Crossley 1988a). The basin is dominated by mixed hardwoods and a dense understory of *Rhododendron*. The five headwater streams drain catchments 21, 22, 34 and two unnamed catchments west of C22 (Swank and Crossley 1988b). Headwater streams at Coweeta are heterotrophic, detrital fed streams where up to 90% of the energy base for microbial and invertebrate production is derived from detrital sources (Wallace et al. 1997, Hall et al. 2000). Headwater streams at Coweeta have low levels of N and P (29 μg of [NH4 + NO3]-NN/L and 7 μg/L of soluble reactive phosphorus; Cross et al. 2003). These streams are generally fishless, and the dominant predatory vertebrates are the larvae of stream plethodontid salamanders. Streams at Coweeta contain up to seven species of plethodontids across four genera. The five most common species of stream plethodontids found at Coweeta are *Desmognathus quadramaculatus, Desmognathus ocoee, Desmognathus monticola, Eurycea wilderae,* and *Gyrinophilus porphyriticus*.

Numerically, streams are dominated by *E. wilderae* and *D. quadramaculatus*, with *D. quadramaculatus* composing the overwhelming majority of larval salamander biomass.

Salamander stoichiometry and homeostasis

We analyzed the nutrient content of the larvae of three plethodontid species across the five streams that varied in size and length of larval period (Table 2.1). From 1 June 2008 to 30 August 2008, we collected 13-15 *Desmognathus quadramaculatus*, *Desmognathus ocoee*, and *Eurycea wilderae*. During this time period of collection, samples of *D. ocoee* represented larvae that were closer to metamorphosis, samples of *E. wilderae* represented newly hatched individuals, and *D. quadramaculatus* samples represented the full range of larvae from recently hatched to three or four years of age (Table 2.1). We euthanized larvae by immersion in a 0.5% solution of neutral pH-buffered MS-222 (ethyl m-amino-benzoate methanesulfonate). Immediately after euthanasia, animals were thoroughly rinsed with deionized water. We measured each animal's length (snout-vent length [SVL] from the tip of the snout to the posterior portion of the vent to the nearest mm) and wet mass (to the nearest 0.1 mg), and then we dissected the animal to remove the stomachs and intestinal tract, and we froze the remaining carcass. Later, carcasses were oven dried at 60°C to a constant weight, homogenized, weighed into tin capsules and analyzed for whole-carcass %C and %N using Micro-Dumas Combustion using a Carlo Erba 2NA 1500 CHN analyser (Carlo Erba, Milan, Italy). To measure whole-carcass %P and %Ca, samples were weighed into acid-washed and preashed ceramic crucibles, ashed at 500 C, acid digested and analyzed spectrophotometrically (ascorbic acid method). Ground pine needles (US National

Institute of Standards and Technology, 1575a) and poplar leaves (Analytical Chemistry Laboratory, University of Georgia) were used as external standards for P and Ca analyses. For *E. wilderae*, two individuals were ground together to obtain enough tissue for sampling. We did not measure Ca for all specimens. We measured %P for each individual captured, while %Ca was only measured for 15 *E. wilderae*, 15 *D. ocoee*, and 20 *D. quadramaculatus*. Because *D. quadramaculatus* larvae represent animals across a 3-4 year larval lifespan, for %Ca analysis we randomly sampled 6-8 *D. quadramaculatus* from each of three size classes: 18-25 mm SVL, 26-33 mm SVL, and 33-40 mm SVL. All data are presented as either %C, %N, %P or %Ca of dry mass or as molar ratios.

We estimated C:N, C:P, and N:P stoichiometric homeostasis for *D. quadramaculatus*, *D. ocoee* and *E. wilderae* by using the homeostasis coefficient H (eta):

$$\log(y) = \left(\frac{1}{H}\right) \times \log(x) + \log(c)$$

where x is the resource stoichiometry (e.g., C:P of *D. quadramaculatus* prey), y is the consumer stoichiometry in the same unit as x (e.g., C:P of *D. quadramaculatus*) and c is a constant derived from the y-intercept of x and y (Sterner and Elser 2002). This method was first proposed by Sterner and Elser (2002) and later applied in a meta-analysis by Persson et al. (2010) to estimate homeostasis across auto-and heterotropic organisms and by Small and Pringle (2010) to estimate deviation from homeostasis in invertebrates across tropical streams. Values of $H > 1$ are considered homeostatic. Although controlled feeding experiments are more commonly used to precisely calculate H values, H values can be estimated based on field data (G.E. Small. *personal communication*). Each species of salamander was assigned a value for C:P, C:N, and N:P for their prey based on published diet records for those species (Huheey and Brandon 1973, Davic

1991, Johnson and Wallace 2005) and published C:P, C:N, and N:P values for those taxa in their diet (Cross et al. 2003). 1/H is the slope of the regression between log (x) and log (y) and thus takes values between zero and one. Organisms that are strictly homeostatic should have values approaching zero. In their meta-analysis, because organisms that are considered strictly homeostatic would have an H of infinity, Persson et al. (2010) classified all datasets examined in their study with significant regressions. We followed the classification of homeostatic coefficients from Persson et al. (2010) as $0 < 1/H < 0.25$ "homeostatic," as $0.25 < 1/H < 0.50$ "weakly homeostatic," $0.50 < 1/H < 0.75$ "weakly plastic," $1/H > 0.75$ "plastic."

Desmognathus quadramaculatus excretion rate and stoichiometry

Stoichiometric models predict the rates or ratios of excreted nutrients can be influenced by body nutrient content (Sterner 1990, Elser and Urabe 1999). To examine the relationship between whole-carcass and excreta nutrient content we measured excretion rates of larval *D. quadramaculatus* using modified methods from Vanni et al. (2002) and McIntyre et al. (2008). Plethodontids are notably smaller and have lower metabolic rates than other vertebrates (Fitzpatrick 1973a, Feder 1976, Facey and Grossman 1990); therefore, we expected their excretion rates to be slower than those reported for other vertebrates (e.g., fish). To insure we could measure excreta, we incubated animals in smaller volumes of water and for a longer period of time than is typical of protocols for fish. Individual *D. quadramaculatus* (mean SVL = 31.6, n = 18) were collected using dip nets on 27 August 2008 from stream C34 at Coweeta and were immediately placed in plastic bags filled with 0.25 liters of pre-filtered stream water

(Gelman AE 1.0 um filters, Ann Arbor, MI). Bags were secured within the wetted portion of the stream to maintain similar environmental conditions. After 24 hours animals were removed, measured (snout-vent length [SVL] from the tip of the snout to the posterior portion of the vent to the nearest mm), weighed (to the nearest 0.1 mg) and sacrificed. Contents of the bag were filtered within four hours through Gelman AE filters to remove feces and other particles. A 20 ml subsample of each filtered sample was placed in acid washed scintillation vials and immediately frozen. Samples were analyzed by automated colorimetry (Alpkem or Technicon) for total Nitrogen (particulate and dissolved), ammonium-N and total dissolved phosphorus (orthophosphate-P) (Koroleff 1983, Qualls 1989, Association 1998) after persulphate digestion. Analyses were performed at the University of Georgia Analytical Chemistry Laboratory. Excretion rates of N and P were calculated as the change in total N, ammonium-N (NH_4) and total P per unit time (nutrient concentration of control samples minus nutrient concentration of excreted samples). We rejected two measurements that differed by more than two standard deviations from mean excretion rates based on all other conspecifics. We assumed these samples were contaminated or processed incorrectly, and were unreliable.

Estimating salamanders densities

To estimate nutrient storage and excretion by stream plethodontids, we combined estimates of biomass and whole-carcass nutrient composition. We used a combination of leaf litterbags and dip netting to estimate the density of larval stream salamanders (Chalmers and Droege 2002, Peterman et al. 2008b, Nowakowski and Maerz 2009). From 10 May to 28 July 2008 larval and newly metamorphic salamanders were sampled for three consecutive days (secondary sampling occasions) every 14 days, for a total of six primary sampling periods. Within each stream, ten one-meter plots were created every 10 m starting approximately 20 m from the confluence of the stream and road, or stream and weir, and continuing upstream. Within each plot, we placed one leaf litter bag (55 x 25 cm made with 1.3 cm^2 mesh) filled with deciduous litter from the surrounding forest in a wetted portion of the stream channel with a large rock on top to prevent dislodging. To sample litter bags, we removed each trap from the stream and immediately placed it in a plastic bin. We poured stream water over each trap and agitated the trap to dislodge animals. We then poured the contents of the bin through a fine mesh net and searched through the litter and substrate. In addition to checking traps, we thoroughly dip netted the 1 m^2 plot by dislodging substrate parallel to the stream bank across the width of the stream. Animals were identified, weighed, and measured (snout-vent length, SVL, from the tip of the snout to the posterior portion of the vent).

We used zero-inflated abundance models based upon models developed in Wenger and Freeman (2008) to estimate abundance of larval and newly metamorphic plethodontids at each stream across six sampling periods using count data (Appendix A). The Wenger and Freeman (2008) population model combines N-mixure models (Royle 2004, Royle et al. 2005) and zero-inflated binomial occupancy models of MacKenzie et al. (2002), and thus carries the assumptions of both abundance and occupancy models, such as: (1) the sample population is closed to immigration, emigration and state change between samples, and (2) individuals are independent and equally available for capture. If these assumptions are violated, estimates can be inflated due to reduced estimates of detection or increased variation in counts at a particular site. Since the probability of detection, and thus estimates of abundance of plethodontids, may vary seasonally or geographically (by stream), we modeled the abundance and presence as a function of stream, sampling period, and both (Appendix A and B). The best supported model for each species was then selected from multiple competing models using Akaike's Information Criterion adjusted for small sample size (Burnham and Anderson 2002). Abundance estimates provided are for those individuals that were available for capture (at the surface) and the capture probability represents unconditional capture probability. The surface area (1 m^2) of each plot was combined with estimates of abundance to estimate density and biomass per square meter.

Salamander biomass and standing crop.

Standing crop of nutrients for each individual of each species across all streams was determined by multiplying the whole-carcass %C, %N, and %P of each individual by their ash-free dry mass (AFDM [g]). Mean percent C, N, and P was multiplying by salamander biomass (AFDM/m^2) for each species within each stream to estimate standing crop of C, N and P within the larval salamander guild. Numbers were compared to established values of stream litter (fine particulate organic matter [FPOM] and coarse particulate organic matter [CPOM]), and invertebrate standing crop of C, N, and P found in Cross et al. (2005). Ash-free dry mass was estimated by assuming the percent of AFDM in our *Eurycea* and *Desmognathus* species was similar to that found for *Eurycea bislineata* and *Desmognathus fuscus* studied in a New Hampshire stream by Burton and Likens (1975). Burton and Likens (1975) found the percent ash was 13% and 15% of whole-carcass dry mass for *E. bislineata* and *D. fuscus*, respectively, therefore, we took the product of dry mass and 0.85 (for *Desmognathus* spp.) and 0.87 (for *E. wilderae*) to estimate the ash-free dry mass of each individual prior to calculating the standing crop.

Statistical analysis

We used separate general linear models (GLM) for each dependent variable to test for the effects of species (categorical variable) on whole-carcass %C, %N, %P (arcsine square root transformed) and elemental molar C:N, C:P, and N:P (dependent variables). Stream was included in the model as a categorical (blocking) variable to account for the possibility of differences between streams. A two-way analysis of variance (ANOVA) was used to test for the effects of stream and species on the standing crop of C, N and P by using standing crop of C, N, and P (e.g., grams of *E. wilderae* C/m^2) as dependent variables, and stream and species as categorical variables. In both the GLM and two-way ANOVA, Tukey's Honestly Significant Difference ($\alpha < 0.05$) was used as a post hoc test to make pair wise comparisons between streams or species. We note that we chose not to use a multivariate approach to our analysis after finding significant correlations between a number of the dependent variables in both stoichiometric (Table 2.2) and elemental storage analysis. Significant correlations between dependent variables and unevenness within replicates compromises multivariate analysis of variance tests (Scheiner and Gurevitch 2001).

We used linear regression to examine the effect of body size (whole-carcass dry mass) on whole-carcass %C, %N, and %P and elemental ratios of C:N, C:P, and N:P. To remain consistent with other studies, these regressions were not conducted with ash-free dry mass values, rather raw dry mass (mg) of whole-carcasses. We also used linear regression to examine the effect of body size (whole-carcass dry mass [mg]) and whole-carcass nutrient content on excretion rates of N and P, and the ratio of excreted N:P.

We assumed that variation in whole-carcass %Ca would largely reflect differences in bone among individual salamanders; therefore, we used a general linear model to regress whole-carcass %P (arcsine square-root transformed) on whole-carcass %Ca (arcsine square-root transformed) to determine whether variation in %P among individual salamanders was potentially related to bone content (Hendrixson et al. 2007, Pilati and Vanni 2007). We included species as categorical predictor variable to determine whether there were differences in %P among individuals and species and whether those differences were a function of differences in bone content among species. All data were found to be normally distributed.

Results

Salamander stoichiometry and homeostasis

There were significant differences in whole-carcass %P, %C, %N, and elemental C:N, C:P, and N:P among species (Table 2.3; Fig. 2.1; Appendix C). *Eurycea wilderae* had the highest mean values of whole-carcass %N, %C, N:P, and C:P, and the lowest mean whole-carcass %P and C:N; while *D. quadramaculatus* had the highest mean whole-carcass %P and the lowest mean whole-carcass %N, %C, N:P, and C:P. *Desmognathus ocoee*, the species intermediate in size, was also intermediate in all nutrient values and ratios (except for C:N; Fig. 2.1; Appendix C). Whole-carcass %C, %P, C:P, and N:P differed among species, and stream was a significant blocking variable in all pair-wise species comparisons. Species differences in whole-carcass %N, C:N, and

C:P differed among streams (significant interaction between stream and species). Whole-carcass %N was significantly different between *E. wilderae* and the *Desmognathus* species, and whole-carcass C:N was significantly different between *E. wilderae* and *D. quadramaculatus*, but not for *E. wilderae* and *D. ocoee*.

Allometric patterns were observed between whole-carcass nutrient content and body size (Fig. 2.2). We found no relationship between body size and elemental C:N, but found a negative relationship between body size and whole-carcass %C, %N, C:P and N:P content (Fig. 2.2) and a positive relationship with body size and whole-carcass %P ($r^2 = 0.50$, $P < 0.001$; Fig. 2.2). Heavier salamanders were lower in C and N content and higher in P.

The model of species and whole-carcass %Ca explained 53% of variation in whole-carcass %P. Whole-carcass %P (df = 1, MS = 0.002, F = 5.156, $P = 0.028$) was positively correlated with whole-carcass %Ca, and once we accounted for %Ca, we found that %P was not different among species (df = 2, MS = 0.0002, F = 0.375, $P = 0.690$) and the relationship between %Ca and %P was similar among species (species X %Ca interaction: df = 2, MS = 0.001, F = 1.425, $P = 0.251$). Linear regression showed whole-carcass %Ca increased significantly with increasing salamander dry mass and whole-carcass %P (Fig. 2.3A and Fig. 2.3B).

Based upon the classification proposed by Persson et al. (2010) plethodontids appear to be “weakly homeostatic” to “homeostatic” with respect to C:N, C:P, and N:P. Values of 1/H for *D. quadramaculatus* (C:N = 0.50, C:P = 0.46, and N:P = 0.25), *D. ocoee* (C:N = 0.50, C:P = 0.50, and N:P = 0.40), and *E. wilderae* (C:N = 0.33, C:P = 0.50, and N:P = 0.50) were qualitatively similar.

Desmognathus quadramaculatus excretion rate and stoichiometry

Excretion rates of total N, total P excretion, and N:P excretion were significantly correlated with whole-carcass %N, %P content, and body size (Fig. 2.4). Excretion rate of total N was negatively correlated with whole-carcass %N and positively correlated with body size. Excretion rate of total P was positively correlated with whole-carcass %P and body size. Excretion N:P was positively correlated with whole-carcass N:P and negatively correlated with body size (Fig. 2.4). Excretion of N and P by *D. quadramaculatus* larvae had a mean excretion rate of 0.017 µmol NH4 · hr^{-1} (SD = 0.007, range = 0.007 - 0.033;), 0.096 µmol TDN · hr^{-1} (SD = 0.053, range = 0.038 - 0.217;), and 0.008 µmol TDP · hr-^{1} (SD = 0.004, range = 0.0003 - 0.014; value taken from total P). This equals an average of 3.06 µg N · hr^{-1} for NH$_4$, 13.38 µg N · hr^{-1} for total N, and 2.359 µg P · hr^{-1} for total P. Mass specific excretion of N and P (calculated as excretion rate/dry mass, mg) by *D. quadramaculatus* larvae had a mean excretion rate of 0.16 µmol NH4 · g^{-1}dry mass · hr^{-1} (SD = 0.07, range = 0.08 - 0.31;), 0.83 µmol TDN · g^{-1} dry mass · hr^{-1} (SD = 0.43, range = 0.14 – 1.3; value taken from total N), and 0.064 µmol TDP · g^{-1} dry mass · hr-^{1} (SD = 0.065, range = 0.0007 - 0.21; value taken from total P).

Plethodontid biomass and standing crop of nutrients

Estimates of mean *D. quadramaculatus* and *E. wilderae* larval densities were 29 · m^{-2} and 27 · m^{-2} respectively. In contrast, *D. ocoee* mean density was < 2 larvae · m^{-2}. The variation in density estimates across streams was highest for *E. wilderae* and lowest for *D. quadramaculatus* (Appendix D). Estimates of total larval densities varied among streams. Stream 3 had the highest estimated total larval of 85 · m^{-2}, while Stream 1 had the lowest estimated larval density of 37 · m^{-2}.

Standing crop of limiting nutrients for all larvae combined was 0.637 g C · m^{-2}, 0.402 g N · m^{-2}, and 0.089 g P · m^{-2}. Mean standing crops of C, N and P differed among species (Fig. 2.6; Table 2.7). Compared to larval *E. wilderae* and larval *D. ocoee*, larval *D. quadramaculatus* was a 1,457% and 8,357% larger standing crop of C, 225% and 15,200% higher standing crop of N, and 607% and 38,800% higher standing crop of P respectively (Fig. 2.5). Standing crops of C, N, and P in *D. quadramaculatus* also varied across streams as a function of larval density. For example, *D. quadramaculatus* standing crop of C was 62% higher in Stream 5 than Stream 1, standing crop of N was 78% higher in Stream 5 than Streams 1 and 3, and standing crop of P was 82%, 54%, and 117% higher in Stream 5 than Streams 1, 4, and 3, respectively. Standing crops of C, N, or P did not differ for *D. ocoee* or *E. wilderae* across streams. In total, larval salamanders represent an estimated 0.13%, 2.0%, and 12.8% to the total pools of C, N, and P in Coweeta streams (Fig. 2.6).

Discussion

Salamander stoichiometry

Stream salamander stoichiometry varies among species largely as a function of differences in larval size (Fig. 2.1; Table 2.3). Taxonomic differences in stoichiometry have been found in a number of aquatic taxa, and have been attributed to differences in body size, ontogeny, or bone allocation (in vertebrates) among families (e.g., Vanni et al. 2002, Cross et al. 2003, Woods et al. 2004, Evans-White et al. 2005, Moe et al. 2005, Hendrixson et al. 2007, Pilati and Vanni 2007). Patterns of variation in nutrient content of our study species are similar to those described for some freshwater fish and tadpoles. Whole-carcass nutrient content in fish and tadpoles range from 20 – 58 %C, 6 – 15 %N, and 1.8 – 5.5 %P (Sterner and George 2000, Vanni et al. 2002, Dantas and Attayde 2007, Hendrixson et al. 2007, Pilati and Vanni 2007, Torres and Vanni 2007), which is similar to values we report (Appendix C and D). Because we only investigated species within the same family, stoichiometric differences in our study are likely a result of differences in growth, size, or morphology (i.e., skeletal investment) among species. Both larval plethodontids and most freshwater fish show negative relationships between whole-carcass %C, %N, C:P, and N:P versus body size (Davis and Boyd 1978, Sterner and George 2000, Higgins et al. 2006, Dantas and Attayde 2007, Hendrixson et al. 2007, Pilati and Vanni 2007), which suggests body size influences whole-carcass stoichiometry in plethodontids in similar ways to other freshwater vertebrates and is a significant driver of the interspecific variation among our study species.

Interspecific variation in whole-carcass %P within and among plethodontids was strongly related to body size and is likely related to ontogenetic and interspecific differences in larval skeleton development. In plethodontids, whole-carcass %P varied significantly between species i.e., larger species and large individuals within a species had higher whole-carcass %P compared to smaller species and individuals (Figs. 2.1 and 2.2). A similar relationship has been reported for freshwater fishes and is ascribed to the allocation of P to bone (Davis and Boyd 1978, Sterner and George 2000, Higgins et al. 2006, Pilati and Vanni 2007). For example, Hendrixson et al. (2007) utilized whole-carcass %Ca as a proxy for the level of bone found in freshwater fish and estimated as much as 81% of total P is associated with bone (i.e., Ca). Pilati and Vanni (2007) used a similar technique in freshwater lake fish and estimated 71% of total P is associated with bone. We found whole-carcass %Ca explained nearly 50 % of the variation in whole-carcass %P among larval plethodontids, suggesting that increased mineral skeleton among larger larvae drives much of the variation in P content within and among larval plethodontid species. We acknowledge that we are assuming that variation in %Ca is a function of bone, and we lack empirical data on how species vary with regard to the total amount or density of their mineral skeletons. Members of the genus *Desmognathus* are thought to have re-evolved the aquatic larval stage from direct developing ancestors (Chippindale and Wiens 2005, Vieites et al. 2007) and thus have retained synapomorphies adapted to head-first burrowing, including more robust skulls (Schwenk and Wake 1993). *Desmognathus quadramaculatus* larvae, our species with the highest whole-carcass %Ca and P, are considered to have the strongest skulls within the genus (Rubenstein 1971); while members of the genus *Eurycea* (including *E. wilderae*), our species with the lowest values of whole-carcass %Ca and %P, have been characterized to have “light bone density” (Martof and Rose 1962). Though it is likely that variation in P content within and among larval plethodontid species is driven by differences in skeleton formation, we note that other factors could also contribute to ontogenetic variation in P stoichiometry. For example, Deegan (1986) showed an increase in whole-carcass %P after a diet shift in the fish *Brevoorita patronus* and evidence from some invertebrate taxa support dietary influence on whole-carcass %P (e.g., Find and Von Elert 2006, Shimizu and Urabe 2008). However, Pilati and Vanni (2007) failed to show a dietary shift in the fish *D. cepedianum* resulted in changes to whole-carcass stoichiometry. Stream plethodontids are generalist predators and diet studies indicate there is significant overlap in prey resources among size classes and species (Huheey and Brandon 1973, Davic 1991, Johnson and Wallace 2005); therefore, we have no empirical support to suggest a variation in diet is responsible for the P variation.

Consumer-resource homeostasis is a central tenet of ecological stoichiometry (Sterner and Elser 2002). The elemental composition and growth rate of a particular consumer is determined by the elemental composition of its prey and its elemental growth requirements. Stoichiometric theory assumes that individuals maintain relatively constant whole-carcass nutrient content (i.e., maintain fixed elemental composition or strict elemental homeostasis) regardless of variation in diet (Sterner and Elser 2002), thus, an individual species will incorporate nutrients, and excrete excess nutrients, to maintain a constant whole-carcass nutrient content. Therefore, an individual species with a lower requirement for a particular nutrient (i.e., low whole-carcass %P) will excrete that nutrient in excess compared to an individual species with a higher requirement for the same nutrient. How well an animal maintains homeostasis will also influence the rates and ratios of nutrient excretion. Other studies suggest aquatic vertebrates show modest elemental homeostasis (Sterner and George 2000, Frost et al. 2002, Sterner and Elser 2002, Vanni et al. 2002), and our estimates using diet nutrient content values from the literature suggest stream salamanders in detrital streams are no different. Homeostatic coefficients of all nutrient ratios showed plethodontids to be “weakly homeostatic” to “homeostatic” and were similar to those found for other aquatic heterotrophs, including fish (Persson et al. 2010). Larval salamander elemental composition was less variable than values reported for the invertebrates at Coweeta that are common larval salamander prey (Cross et al. 2003; Appendix C). Because our study species differed in their nutrient ratios and appear relatively homeostatic, we would expect these species to vary in their N and P demand and in the rates and ratios at which they recycle nutrients.

Desmognathus quadramaculatus excretion rate and excretion stoichiometry

Theory predicts and many studies confirm that homeostatic consumers excrete nutrients consumed but not used for growth, so a consumer with a low ratio of a nutrient [compared to other nutrients] in its body should be excreting that nutrient at a higher ratio (Glaholt and Vanni 2005, Pilati and Vanni 2007). Our results support this expectation for excretion of N by larval *D. quadramaculatus*, but not excretion of P. The C:N and C:P ratios of *D. quadramaculatus* prey suggest their prey are more limited in P than N (Cross et al. 2003; Appendix A), thus plethodontids should show P limitation. As a result, we would expect *D. quadramaculatus* to excrete a higher N:P compared to their whole-carcass composition and N:P of excreta should increase with size as the demand for P increases with larval size (as a result of skeleton formation). Instead, our data suggests as plethodontids grow they increase the amount and ratio of P in their excreta. This relationship suggests that larval plethodontids are not P limited despite the low content of P in their diet. Consistent with our result, Pilati and Vanni (2007) found that excreta N:P of larval *D. cepedianum* declined with increasing body size, which was attributed to an ontogenetic diet shift between larval and juvenile/adult fish. Larval *D. quadramaculatus* are known to increase consumption of larger, leaf shredding macroinvertebrates as they grow (Davic 1991), but we do not know whether this affects the N:P ratio of their diets or excreta. In the absence of an ontogenetic shift in the stoichiometry of their diet, our results may indicate that larval *D. quadramaculatus* are well-adapted to the limited amounts of P available in their diet. Glaholt and Vanni (2005) found *Lepomis macrochirus* altered their assimilation efficiencies in response to low-rationed food, which in turn altered their whole-carcass and excretion stoichiometry. Plethodontids are noted for extremely high assimilation efficiencies (Fitzpatrick 1973a, Fitzpatrick 1973b), which could lead to mechanisms for more efficient assimilation of nutrients. Frost et al. (2006) proposed organisms with a high threshold elemental ratio for C:P most likely reflects physiological adjustments to process low P food. Although we have no empirical data to test such a hypothesis, plethodontids may simply have different threshold elemental ratios for P limitation, or adjust their elemental metabolism and body elemental composition to compensate for poor quality food (Frost et al. 2005). A better understanding of plethodontid physiology and is needed to fully interpret mechanisms that may drive uncommon patterns in plethodontid stoichiometry.

Salamander biomass and standing crop

One way animals can affect nutrient dynamics is by ‘storing’ limited nutrients in freshwater ecosystems (Vanni et al. 2002, Cross et al. 2005). Using a series of nutrient spiraling simulation models, Small et al. (2009) shows consumers that are high in biomass can have differential effects on the retention and recycling of limiting nutrients in headwater streams. For example, as the standing stock of consumers increases, the chance of a nutrient atom entering the consumer’s biomass increases. Further, there is slower turnover of top consumers, particularly vertebrates, so nutrients that enter top consumers are slower to recycle to the stream in inorganic form. Small et al. (2009)

propose that uptake of nutrients into top consumers significantly delays the velocity at which a nutrient atom travels downstream. Much of the data to support this hypothesis is focused on fish (Kitchell et al. 1979, Andersson et al. 1988, Kraft 1992) and invertebrates (Vaughn and Hakenkamp 2001) in lake ecosystems (but see Cross et al. 2005). We suggest that given their high biomass, limited dispersal (Lowe (2003) (Ashton 1975, Camp and Lee 1996, Peterman et al. 2008a), and low excretion rates, larval plethodontids are important to the retention [slower downstream movement] of N and P in headwater streams. Our results show that the standing crop of N and P in larval salamanders is 1.2 and 4 times more than what is described for macroinvertebrate assemblages found in Coweeta streams (Cross et al. 2005). Because of their muscle and bone, salamander larvae contain higher concentrations of key limiting nutrients (N and P) within their bodies compared to invertebrates. Assimilation efficiencies for larval Northern two-lined salamander (*Eurycea bislineata*) and Allegheny dusky salamander (*D. ochrophaeus*) were > 88% (Fitzpatrick 1973a). In contrast, assimilation efficiencies for detritivorous stream macroinvertebrate range between 5-25% (see reviews in Benke and Wallace (1980) and Berrie (1976)). The metabolic efficiency of plethodontids limits nutrient turnover and recycling from salamander populations compared to macroinvertebrates. The attributes described above set salamanders apart from invertebrates by potentially maintaining a consistent (i.e., non-fluxing), stable, long-term stock of nutrients to headwater streams.

Our results differ from that of Burton and Likens (1975a) who concluded stream salamanders store very little N and P and have little effect on nutrient cycling at the Hubbard Brook Experimental Forest in New Hampshire. Using their estimates of density

$\cdot$ m^{-2}, dry mass, and standing crops of nutrients in stream salamander species (Burton and Likens 1975b, a), we estimate the standing crop of P and N within the stream salamander guild from their study is 0.01 g $P^{-1}m^{-2}$ and 0.005 g $N^{-1}m^{-2}$, respectively. This is equivalent to 9 and 74 times lower than our estimates, respectively. Admittedly, they studied slightly different species at a different location; however, we do not believe this accounts for the differences between studies. The percentages of nutrients per gram of salamander tissue were similar between the two studies; therefore, the differences between our studies likely results from differences in estimated salamander abundance. We believe that their methods used to calculate density grossly underestimated true salamander abundance. Burton and Likens (1975b) used surface counts to estimate population density of plethodontid salamanders, and those methods have been shown to significantly underestimate total population size (Bailey et al. 2004, Peterman et al. 2008b, Nowakowski and Maerz 2009). A re-examination of population estimates from Burton and Likens (1975b) that incorporate new methods of detection and quantification of density would likely result in larger nutrient standing crop values.

Compared to tropical stream fish, the excretion of N and P by larval plethodontids does not appear to contribute high amounts of N and P to the nutrient demand of headwater streams (Vanni et al. 2002, McIntyre et al. 2008, Small et al. In press). For example, to evaluate the importance of *D. quadramaculatus* excretion to supplying N and P for nutrient demand at Coweeta, we calculated per capita N and P excretion by taking the product of the mean excretion rate of N (NH_4-N) and P (TDP) for larval *D. quadramaculatus* and the population density of larval *D. quadramaculatus* at each stream. The mean per capita N and P excretion from all streams was used to estimate the aggregate excretion of N and P by larval *D. quadramaculatus* and was compared with estimates of stream nutrient uptake rates in Webster et al. (2000) (N and P uptake from C53 at Coweeta) and Mulholland et al. (1997) (P uptake rates from Hugh White Creek at Coweeta). We estimate aggregate excretion of N and P by *D. quadramaculatus* equals 1.48 µg N m^{-2} min^{-1} (NH_4) and 1.14 µg P m^{-2} min^{-1} (TDP). Based on these calculations, *D. quadramaculatus* alone provide 7 % of stream N demand, and 19% (using uptake rates from Webster et al. 2000) or 33% (using uptake rates from Mulholland et al. 1997) of stream nutrient demand for P. In contrast, in tropical headwater streams Vanni et al. (2002) estimated the fish assemblage could provide 48 and 126% of algal demand for N and P, respectively, and McIntyre et al. (2008) found the fish assemblage could provide > 75% of stream dissolved inorganic nitrogen demand. Invertebrates in headwater streams and lakes have been documented to provide between 11.5 to 70% of N demand and 4 to 58%P demand (see review in Vanni 2002).

The southern Appalachian Mountains is a global hotspot for plethodontid salamander diversity, and this study is a first step toward understanding the ‘roles’ these organisms play in the headwater ecosystems that characterize much of the region. We have established that larval plethodontids are likely relatively homeostatic, and variation in the composition of N and P within and among species is consistent with an increase in skeletal investment with increasing size. Larval plethodontids do not appear to be P limited despite consuming prey with relatively limited P content in an ecosystem that is highly P limited. We have also established that larval plethodontids are a significant standing stock of N and P compared to published reports for stream macroinvertebrates in the same system. Because they are vertebrates, their bony skeleton makes larval plethodontids a particularly rich stock of P compared to invertebrates; however, larval plethodontids appear to be a small source of stream demand for N and P through their excreta. Until we have measures of subsidies in the form of annual deposits of eggs produced from feeding on terrestrial prey, in-stream production of larval tissues, and ultimately export of materials with metamorphosis, we will not fully understand the effects of plethodontids on headwater nutrient dynamics. However, our results suggest that the role is significant, and that declines or losses of plethodontids from headwater systems would lead to changes in nutrient dynamics within that system.

Acknowledgements

We would like to thank J. Peterson for significant statistical assistance and K. Cecala, J. Denney, J. DeVore, K. Holcolmb, V. Kinney, W.E. Peterman, A. Perofsky, J. Truslow, G. Small, and J. Wisdom for field and/or statistical assistance. Earlier drafts of this manuscript were significantly improved by K. Barrett. This project was partially supported by the Coweeta Hydrologic Laboratory, D.B. Warnell School of Forestry and Natural Resources, the American Museum of Natural History Theodore Roosevelt Memorial Grant, and the North Carolina Herpetological Society.

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Table 2.1. Life history characteristics of *E. wilderae* (n = 75), *D. quadramaculatus* (n = 200), and *D. ocoee* (n = 25) from Coweeta. Mean SVL (SD) and mass (SD) were taken from abundance dataset in this study. Data from previous studies were used for length of larval period (Bruce 1982, 1985, 1988, 1989).

Species	Mean SVL (mm)	Mean mass (g)	Larval period length (months)	Typical hatching date
D. quadramaculatus	30.13 (6.01)	0.89 (0.55)	36 – 48	July - November
D. ocoee	15.35 (3.54)	0.10 (0.04)	9 - 10	August - November
E. wilderae	12.60 (1.32)	0.06 (0.03)	12 - 24	June - August

Table 2.2. Correlation matrix between whole-carcass %C, %N, %P, C:N (molar), C:P (molar) and N:P (molar). Values given are correlation coefficients (*r*). * represents statistical significance (α = 0.05).

Variable	% N	% C	% P	C:N	C:P	N:P
%N	–	0.79*	- 0.25*	- 0.53*	0.33*	0.39*
% C	0.79*	–	- 0.30*	0.10*	0.39*	0.37*
% P	- 0.25*	- 0.30*	–	- 0.00	- 0.79*	- 0.79*
C:N	- 0.53*	0.10*	- 0.00	–	0.00	- 0.11*
C:P	0.33*	0.39*	- 0.79*	0.00	–	0.99*
N:P	0.39*	0.37*	- 0.79*	- 0.11*	0.99*	–

Table 2.3. Results from general linear model's investigating the differences between whole-carcass nutrients among streams and species. Elemental ratios are molar.

Effect	%C				%N			%P		
	df	MS	F	*P*	MS	F	*P*	MS	F	*P*
Stream	4	0.004	7.112	<0.001	<0.001	4.8	<0.001	0.002	3.901	0.004
Species	2	0.019	36.901	<0.001	0.004	34.4	<0.001	0.032	70.011	<0.001
Species X Stream	8	0.001	1.623	0.123	<0.001	2.03	0.050	<0.001	0.973	0.459
Error	207	0.001	–	–	<0.001	–	–	<0.001	–	–
	C:N				C:P			N:P		
Stream	4	0.426	19.341	<0.001	1133.912	3.593	0.007	59.292	3.328	0.011
Species	2	0.278	12.621	<0.001	19594.032	62.094	0.001	1093.313	61.379	<0.001
Species X Stream	8	0.056	2.535	0.012	680.422	2.156	0.032	33.731	1.894	0.063
Error	207	0.022	–	–	315.643	–	–	17.814	–	–

Table 2.4. Mean (SD) of density/m^2, dry mass (AFDM), and biomass/m^2 of the three species of salamanders in this study and invertebrates from the Coweeta LTER site. Mean biomass (mg^{-1} m^{-2}) of invertebrates at the Coweeta basin were derived from Hall et al. (2000).

Taxon or taxa	Mean density/m^2 (SD)	Mean AFDM mg^{-1} (SD)	Mean biomass AFDM mg m^{-2} (SD)
D. quadramaculatus	29.23 (3.14)	92 (63.0)	2689 (1841)
D. ocoee	1.81 (0.20)	7 (3.0)	13 (5.4)
E. wilderae	27.42 (18.43)	4 (1.0)	110 (28.0)
All salamander spp.	58.47	102	2812
Invertebrates	–	–	1334.5 (AFDM)*

Table 2.5. Results from two-way analysis of variance tests investigating the differences in standing crop of nutrients among five Appalachian headwater streams and across three salamander species.

Effect	Carbon				Nitrogen			Phosphorus		
	df	MS	F	*P*	MS	F	*P*	MS	F	*P*
Stream	4	< 0.001	2.226	0.067	< 0.001	2.490	0.044	< 0.001	1.830	0.125
Species	2	0.024	133.207	< 0.001	0.003	138.039	< 0.001	< 0.001	68.405	< 0.001
Stream X Species	8	< 0.001	2.138	0.034	< 0.001	2.350	0.020	< 0.001	1.806	0.078
Error	206	< 0.001	–	–	< 0.001	–	–	< 0.001	–	–

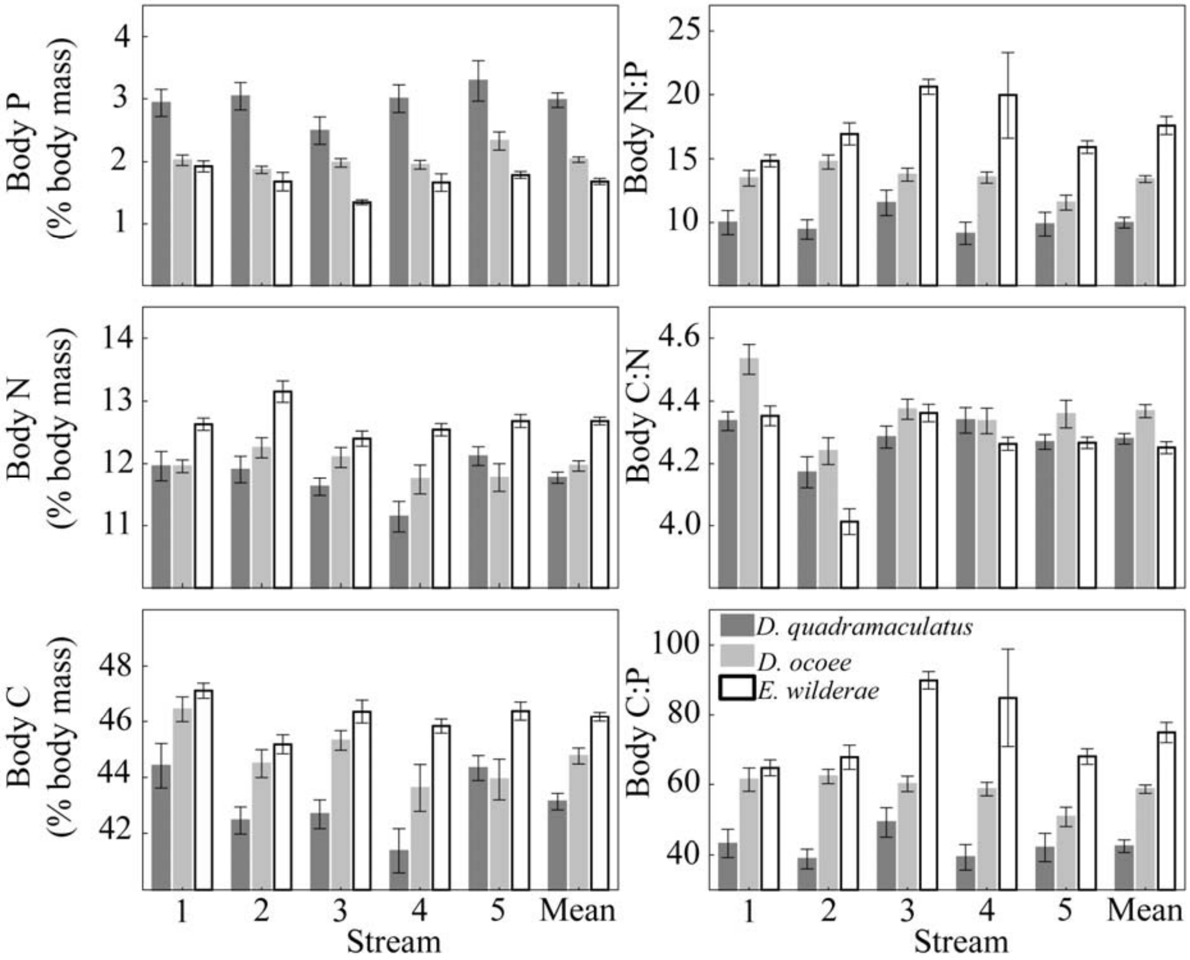

Figure 2.1. Mean (SE) of whole-carcass nutrient content and elemental ratios (molar) of three plethodontid salamanders across five streams from 2008 data. Mean values on horizontal axis represent mean (SE) across all streams. Mean (SE) size data for each species are as follows: *D. quadramaculatus*; mean SVL = 28.97 (0.74) mm, *D. ocoee*, mean SVL = 13.76 (0.35) mm, *E. wilderae*, mean SVL = 12.60 (0.15).

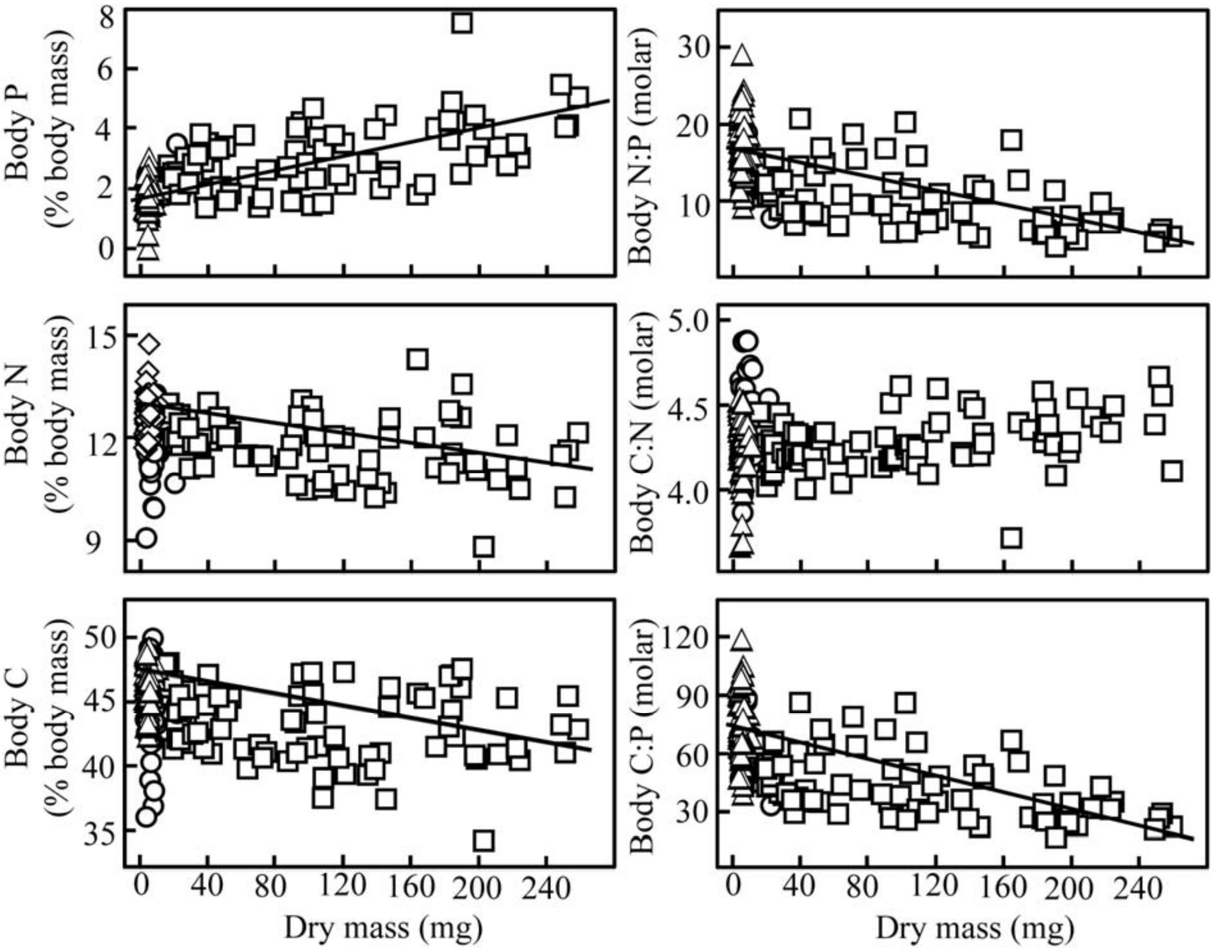

Figure 2.2. %C, %N, %P, and elemental C:N, C:P, and N:P versus body size (dry mass) for all species; triangles represent *E. wilderae*, circles represent *D. ocoee*, and squares represent *D. quadramaculatus*.. Linear equations for the relationships between C, N, P, C:N, C:P, and N:P and body dry mass are as follows: C = $r^2 = 0.159$; $P < 0.0001$; y = 45.345 - 0.017*x; N = $r^2 = 0.134$; $P < 0.0001$; y = 12.321 - 0.005*x; P = $r^2 = 0.497$; $P < 0.0001$; y = 1.824 + 0.010*x; C:N = $r^2 = 0.002$; $P = 0.519$; y = 4.297 + 0.0001*x; C:P = $r^2 = 0.277$; $P < 0.0001$; y = 66.207 - 0.188*x; N:P = $r^2 = 0.369$; $P < 0.0001$; y = 15.121 - 0.042*x.

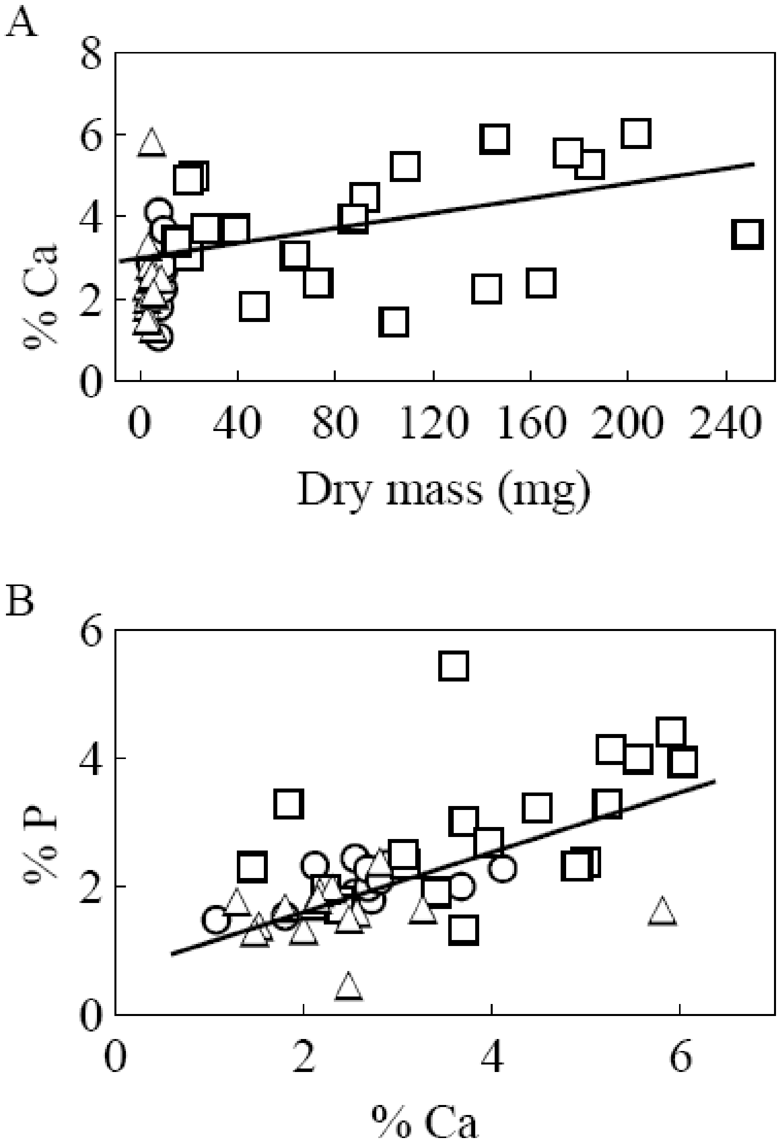

Figure 2.3. (A) Whole-carcass %Ca versus body size (dry mass, mg; $r^2 = 0.25$; *P* < 0.001; y = 2.608 + 0.010*x) and (B) Whole-carcass %Ca versus whole-carcass %P ($r^2 = 0.37$; P < = 0.0001; y = 0.916 + 0.432*x) for *E. wilderae* (triangles), *D. ocoee* (circles), and *D. quadramaculatus* (squares).

7

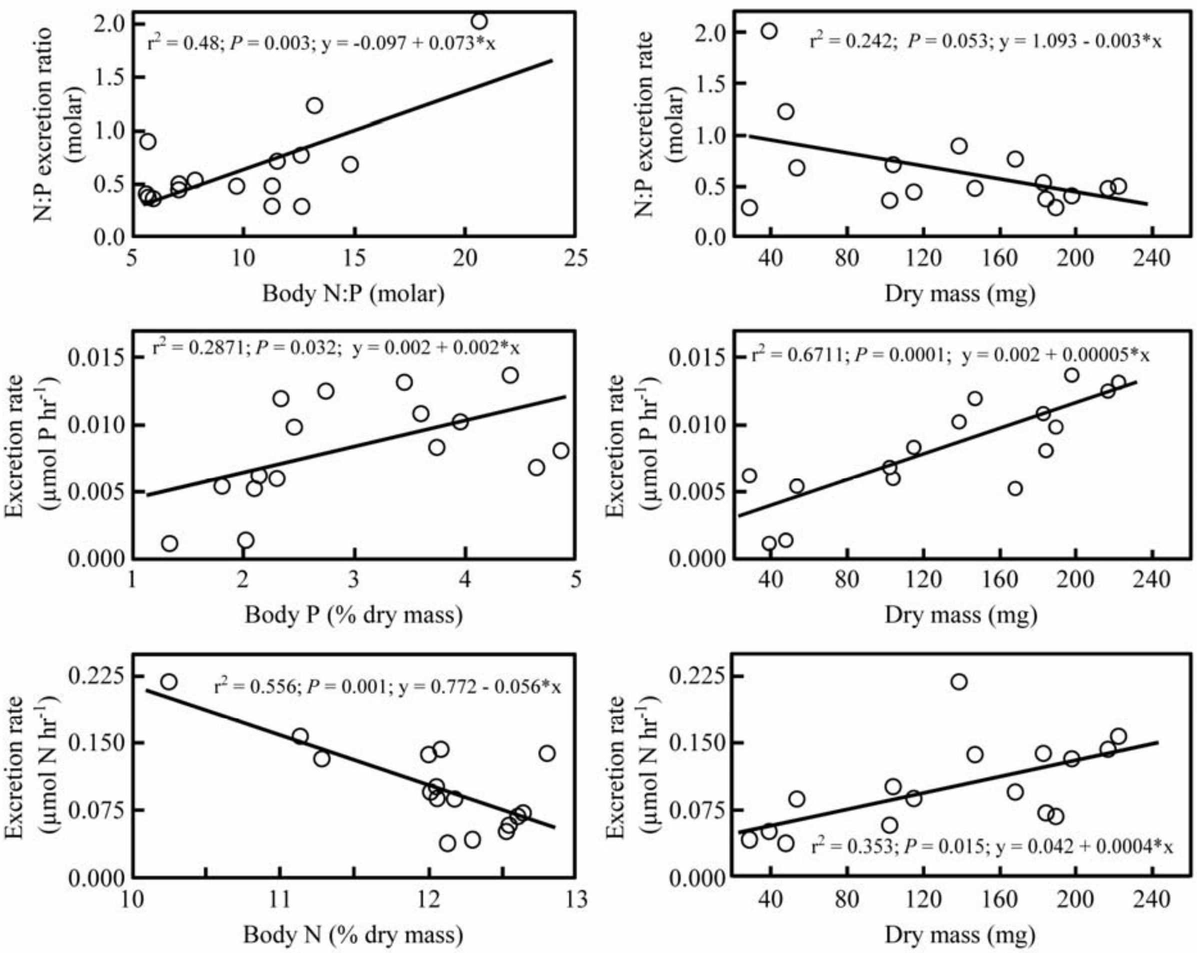

Figure 2.4. Regressions of excretion rates and ratios versus whole-carcass nutrient content and body size (dry mass) for *D. quadramaculatus*. Two animals were excluded from all analysis as the standard deviation of excreted nutrients was greater than two standard deviations from the mean.

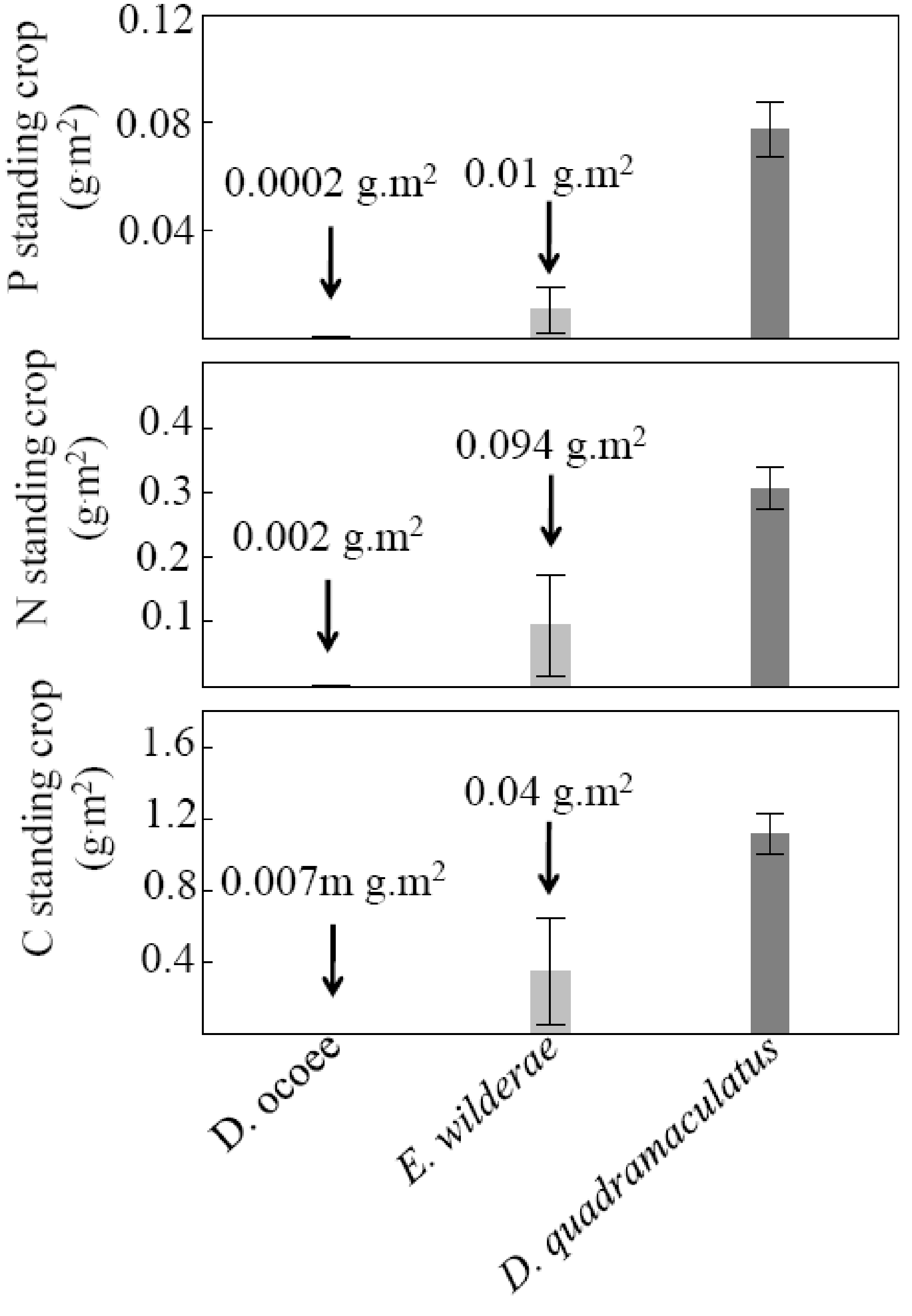

Figure 2.5. Mean (SE) standing crop of carbon, nitrogen, and phosphorus across larval salamander species in five streams at the Coweeta Hydrologic Laboratory, NC.

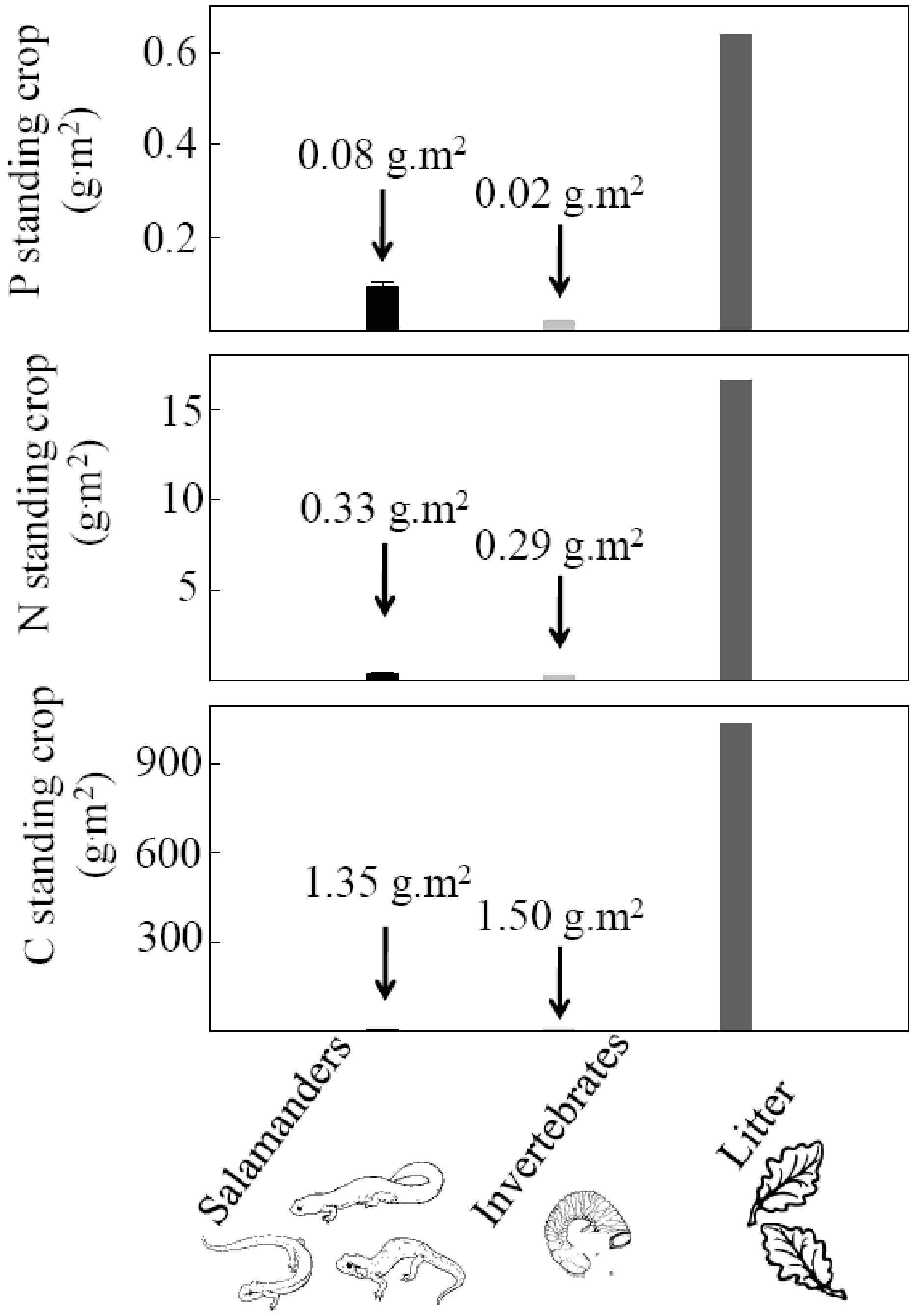

Figure 2.6. Mean standing crop of carbon, nitrogen, and phosphorus of the stream taxa (total salamander guild, invertebrates and leaf litter) at the Coweeta Hydrologic Laboratory, NC. Invertebrate and litter values were estimated from Cross et al. (2005).

Appendix A.

(A)

WinBugs code for modified zero-inflated abundance models which estimate abundance as a function of (A) constant stream and hierarchal sampling period presence and abundance. (B) hierarchal stream and constant sampling period presence and abundance. (C) hierarchal stream and hierarchal sampling period presence and abundance.

```
model{
for (i in 1:samp) {       #specifies # of sample periods, or abundance is modeled as a
function of site (s) and time (t)
                for(h  in 1:site){  #specifies # of sites

                              for (j in 1:occ) {        # k specifies # of samples per period
                                                   data[i,h,j+2] ~ dbin(p[i,z1[i,h]],k[i,h])
                                                          }

                              z1[i,h] <- Pres[i,h]+1
                             k[i,h] <- Pres[i,h]*K[i,h]
                              Pres[i,h] ~ dbern(Psi1[i,h])

# the following line is a workaround to prevent errors
                             Psi1[i,h]  <- min(0.999999,max(.000001,Psi[i,h]))

# conditional abundance drawn from a poisson distribution
                             K[i,h] ~ dpois(lambda[i,h])

# conditional abundance is modeled below, here as a constant among streams but
modeled heir among sampling period
# conditional abundance is the number present, given the species occurs
                            log(lambda[i,h]) <-  a[data[i,h,1]]

 #probability of presence is modeled below, here as a constant among streams but
modeled heir among sampling period
                             logit(Psi[i,h]) <- b[data[i,h,1]]

                            # estimated conditional abundance
                            log(predN[i,h]) <-  a[data[i,h,1]]

                            # estimated probability of presence
                            logit(predPres[i,h]) <- b[data[i,h,1]]

                           # estimated UNCONDITIONAL abundance
                           abun[i,h] <- predN[i,h]*predPres[i,h]
                           }
```

```
            p[i,1] <- 0
```

```
        logit(p[i,2]) <- z[i] # probability of detection as function of constant that varies
among sampling period (i)
            logit(predP[i]) <- z[i] # estimated probability of detection
      }
        log(predNO) <- a0
        logit(predpresO) <- b0
    abunO <-predNO*predpresO

for (x in 1:samp) {
                   a[x] ~ dnorm(a0,a0_tau)
                         b[x] ~ dnorm(b0,b0_tau)
                         z[x] ~ dnorm(0.0,0.001)    # this is the prior for the detection
probability constant
                         }
             a0 ~ dnorm(0.0,0.001)
             b0 ~ dnorm(0.0,0.001)
        b0_tau~ dnorm(0,0.37)I(0,)
        a0_tau~ dnorm(0,0.37)I(0,)

}
```

Appendix A (continued)

(B)

```
model{
for (i in 1:samp) {      #specifies # of sample periods, or abundance is modeled as a
function of site (s) and time (t)
                for(h  in 1:site){  #specifies # of sites

                              for (j in 1:occ) {        # k specifies # of samples per period
                                                   data[i,h,j+2] ~ dbin(p[i,z1[i,h]],k[i,h])
                                                          }

                              z1[i,h] <- Pres[i,h]+1
                              k[i,h] <- Pres[i,h]*K[i,h]
                              Pres[i,h] ~ dbern(Psi1[i,h])  # probability the species occurs
modeled using a bernoulli distribution

# the following line is a workaround to prevent errors
                              Psi1[i,h]  <- min(0.999999,max(.000001,Psi[i,h]))

# conditional abundance drawn from a poisson distribution
                              K[i,h] ~ dpois(lambda[i,h])

# conditional abundance is modeled below, here as a constant among streams but
modeled heir among sampling period
 # conditional abundance is the number present, given the species occurs
                             log(lambda[i,h]) <-  a[data[i,h,2]]

#probability of presence is modeled below, here as a constant among sites but
modeledheir among season
                              logit(Psi[i,h]) <- b[data[i,h,2]]

                             # estimated conditional abundance
                             log(predN[i,h]) <-  a[data[i,h,2]]

                             # estimated probability of presence
                             logit(predPres[i,h]) <- b[data[i,h,2]]

                            # estimated UNCONDITIONAL abundance
                            abun[i,h] <- predN[i,h]*predPres[i,h]
                            }
              p[i,1] <- 0
                    logit(p[i,2]) <- z[i] # probability of detection as function of constant that
varies among sampling period (i)
```

```
        logit(predP[i]) <- z[i] # estimated probability of detection
    }
```

Appendix A (continued)

```
    log(predNO) <- a0
    logit(predpresO) <-b0
    abunO <- predNO*predpresO

# here is where we assign prior distributions
for (x in 1:site) {
            a[x] ~ dnorm(a0,a0_tau)
                b[x] ~ dnorm(b0,b0_tau)
                z[x] ~ dnorm(0.0,0.001)    # this is the prior for the detection
probability constant
                }
        a0 ~ dnorm(0.0,0.001)
        b0 ~ dnorm(0.0,0.001)
    b0_tau~ dnorm(0,0.37)I(0,)
    a0_tau~ dnorm(0,0.37)I(0,)

}
```

Appendix A (continued)

(C)

```
model{
for (i in 1:samp) {       #specifies # of sample periods, or abundance is modeled as a
function of site (s) and time (t)
                for(h  in 1:site){  #specifies # of sites

                              for (j in 1:occ) {        # k specifies # of samples per period
                                                 data[i,h,j+2] ~ dbin(p[i,z1[i,h]],k[i,h])
                                                        }

                              z1[i,h] <- Pres[i,h]+1
                             k[i,h] <- Pres[i,h]*K[i,h]
                              Pres[i,h] ~ dbern(Psi1[i,h]) # probability the species occurs
modeled using a bernoulli distribution

# the following line is a workaround to prevent errors
                             Psi1[i,h]  <- min(0.999999,max(.000001,Psi[i,h]))

# conditional abundance drawn from a poisson distribution
                             K[i,h] ~ dpois(lambda[i,h])

# conditional abundance is modeled below, here as a constant among streams but
modeledheir among sampling period
# conditional abundance is the number present, given the species occurs
                            log(lambda[i,h]) <-  a[data[i,h,2]] + a_s[data[i,h,1]]

#probability of presence is modeled below, here as a constant among streams but
modeledheir among sampling periods
                             logit(Psi[i,h]) <- b[data[i,h,2]] + b_s[data[i,h,1]]

# estimated conditional abundance
                            log(predN[i,h]) <-  a[data[i,h,2]] + a_s[data[i,h,1]]

# estimated probability of presence
                            logit(predPres[i,h]) <- b[data[i,h,2]] + b_s[data[i,h,1]]

# estimated UNCONDITIONAL abundance
                           abun[i,h] <- predN[i,h]*predPres[i,h]
                           }
              p[i,1] <- 0
                    logit(p[i,2]) <- z[i] # probability of detection as function of constant that
varies among seasons (i)
```

```
               logit(predP[i]) <- z[i] # estimated probability of detection
        }
```

Appendix A (continued)

```
          log(predNO) <- a0
          logit(predpresO) <- b0
    abunO <-predNO*predpresO

# here is where we assign prior distributions

for (x in 1:samp) {
                         a_s[x] ~ dnorm(0,as_tau)
                                b_s[x] ~ dnorm(0,bs_tau)
                                }

  rho1 ~ dunif(-1,1)
  as_tau <- a0_tau/(1.-pow(rho1,2))

  rho2 ~ dunif(-1,1)
  bs_tau <- b0_tau/(1.-pow(rho2,2))

for (x in 1:site) {
                         a[x] ~ dnorm(a0,a0_tau)
                                b[x] ~ dnorm(b0,b0_tau)
                                z[x] ~ dnorm(0.0,0.001) # this is the prior for the detection
probability constant
                                }
               a0 ~ dnorm(0.0,0.001)
               b0 ~ dnorm(0.0,0.001)
          b0_tau~ dnorm(0,0.37)I(0,)
          a0_tau~ dnorm(0,0.37)I(0,)

}
```

Appendix B. Composite models depicting the AICc, Δi, wi, and the percent of each model to the model with the highest wi salamander data. Estimates of abundance and presence were modeled as a constant or hierarchal (variable) across streams or sampling periods.

Species	Model	Parameter	Deviance	AICc	Δi	Exp(-0.5) Δ	Wi
D. ocoee	Constant streams, Hierarchal sampling period	10	303	323	0	1	0.931
	Hierarchal streams, Constant sampling period	10	308.3	328.3	5.3	0.071	0.066
	Hierarchal streams, Hierarchal sampling period	14	306.3	334.3	11.3	0.004	0.003
D. quadramaculatus	Hierarchal streams, Constant sampling period	10	443.5	463.5	0	1	0.572
	Constant streams, Hierarchal sampling period	10	444.2	464.2	0.7	0.705	0.403
	Hierarchal streams, Hierarchal sampling period	14	441.8	469.8	6.3	0.043	0.024
E. wilderae	Hierarchal streams, Hierarchal sampling period	14	794	822	0	1	0.690
	Hierarchal streams, Constant sampling period	10	813.6	823.6	1.6	0.449	0.310
	Constant streams, Hierarchal sampling period	10	859	879	57	$4.190E^{-13}$	$2.894E^{-13}$

Appendix C. Mean, standard deviation, and range of values for whole-carcass %C, %N, %P and elemental ratios of C:N, C:P, and N:P for 2008 samples of *D. ocoee* (n = 72), *D. quadramaculatus* (n = 78) and *E. wilderae* (n = 72) and invertebrate prey items (Cross et al. 2003).

Species or prey	C			N			P		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
D. ocoee	44.75	2.65	36.0-49.9	11.95	0.76	9.1-13.3	2.03	0.39	1.4-3.6
D. quadramaculatus	43.18	2.77	34.2-48.0	11.79	0.89	8.8-14.3	2.98	1.09	1.3-7.5
E. wilderae	46.22	1.40	42.4-49.2	12.69	0.55	11.7-14.8	1.70	0.40	0.5-3.6
	C:N			C:P			N:P		
D. ocoee	3.75	0.16	3.3–4.2	22.74	4.12	11.7-34.2	6.06	1.06	3.3-8.5
D. quadramaculatus	3.67	0.14	3.2-4.0	16.46	6.15	6.35-33.3	4.51	1.74	1.81-9.3
E. wilderae	3.65	0.15	3.1-3.9	29.03	10.05	15.2-96.8	7.96	2.77	4.2-26.7
E. wilderae/D. ocoee salamander prey	6.43	0.25	5.2 – 9.0	381.33	111.02	93 - 877	58.33	15.01	14 - 125
D. quadramaculatus salamander prey	6.1	0.70	4.9 – 9.0	341.75	120.35	93 - 877	54.5	14.46	14 - 125

Appendix D. Estimated density (m^2), standard deviation, and 95%Confidence intervals of salamander taxa across five streams at the Coweeta Hydrological Laboratory.

Species	Stream	Density (m^2)	SD	Lower 95%	Upper 95%
D. ocoee	1	2.127	1.152	0.283	4.688
	2	1.823	1.073	0.291	3.961
	3	1.631	0.921	0.253	3.351
	4	1.804	1.149	0.381	3.463
	5	0.910	0.409	0.118	1.415
D. quadramaculatus	1	26.051	22.320	3.981	125.141
	2	31.978	27.983	5.287	148.115
	3	32.231	27.714	4.932	155.720
	4	30.144	26.139	5.517	123.843
	5	25.767	21.639	5.377	87.070
E. wilderae	1	8.984	4.920	0.326	74.248
	2	13.755	7.166	0.418	122.978
	3	52.270	27.113	1.608	457.232
	4	40.748	21.899	1.392	344.424
	5	21.358	11.619	0.732	184.529

CHAPTER 3

PROJECTED LOSS OF A SALAMANDER DIVERSITY HOTSPOT AS A CONSEQUENCE OF PROJECTED GLOBAL CLIMATE CHANGE[1]

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[1] Milanovich, J.R., W.E. Peterman, N. P. Nibbelink, and J.C. Maerz. Provisionally accepted to *Plos One*, 6/1/2010.

Abstract

Significant shifts in climate are considered a threat to plants and animals with significant physiological limitations and limited dispersal abilities. The southern Appalachian Mountains are a global hotspot for plethodontid salamander diversity. Plethodontids are lungless ectotherms, so their ecology is strongly governed by temperature and precipitation. Many plethodontid species in southern Appalachia exist in high elevation habitats that may be at or near their thermal maxima, and may also have limited dispersal abilities across warmer valley bottoms. We used a maximum-entropy approach (program Maxent) to model the suitable climatic habitat of 41 plethodontid salamander species (33 individual species and eight species included within two species complexes) inhabiting the Appalachian Highlands region. We evaluated the relative change in suitable climatic habitat for these species in the Appalachian Highlands from the current climate to the years 2020, 2050, and 2080, using both the HADCM3 and the CGCM3 models, each under low and high CO_2 scenarios, and using two-model thresholds levels (relative suitability thresholds for determining suitable/unsuitable range), for a total of 8 scenarios per species. While models differed slightly, every scenario projected significant declines in suitable habitat within the Appalachian Highlands as early as 2020. Species with more southern ranges and with smaller ranges had larger projected habitat loss. Despite significant differences in projected precipitation changes to the region, projections did not differ significantly between global circulation models. CO_2 emissions scenario and model threshold had small effects on projected habitat loss by 2020, but did not affect longer-term projections. Results of this study indicate that choice of model threshold and CO_2 emissions scenario affect short-

term projected shifts in climatic distributions of species; however, these factors and choice of global circulation model have relatively small affects on what is significant projected loss of habitat for many salamander species that currently occupy the Appalachian Highlands.

Introduction

Understanding how species distributions and patterns of diversity shift with changing climates has been a long-standing theme of ecology that has grown less academic with the specter of rapid climate change. Not surprisingly, there is an increasing effort to project the effects of climate change on species' distributions and regions of high biodiversity (Chapin et al. 2000, Peterson et al. 2002, Malcolm et al. 2006, Huntley et al. 2008, de Chazal and Rounsevell 2009). Knowing whether particular species or hotspots of biodiversity are vulnerable to decline is important to planning management actions and understanding how ecosystem functions may change (Hooper et al. 2005).

Species distribution modeling is one tool for evaluating the potential impact of climate change on the distributions of biota (Elith et al. 2006, Beaumont et al. 2007). Distribution models characterize dimensions, generally mean climatic variables, of the current realized niche of a species based on presence-absence data and then use future climate forecasts to project changes in the distribution of suitable habitat for a species. Climate-driven species distribution models have several limitations including exclusion of other biotic, physiological, and geographic controls on a species' distribution. Additionally, these models cannot mechanistically account for the role of climate in determining species distributions or quantify the limits of species abilities to migrate. Furthermore, this technique ignores the capability of evolutionary change to compensate for species responses to changing climate and they assume reliance upon credible climatic projections by assuming that the “suitable” habitat is saturated and the data input into models is accurate (Pearson and Dawson 2003, Thomas et al. 2004, Guisan and Thuiller 2005, Luoto et al. 2005, Heikkinen et al. 2006, Wiens et al. 2009). Projections from climate distribution modeling are also dependent upon the global circulation model selected, how well that model can be downscaled to predict local climate (Fridley 2009), and assumptions about future atmospheric CO_2 levels. To deal with the potential limitations of model projections, increasingly studies often take an ensemble forecasting approach by modeling a number of future scenarios that bracket ranges of model assumptions or predicted climate change scenarios (Araujo and New 2007). The most common approach is to integrate different global circulation models and CO_2 emissions scenarios and forecast out to multiple future time points.

A potential criticism of forecasts from species distribution modeling is the self-fulfilling nature of the endeavor. Based on relationships between climate variables at sites occupied by a species, climate distribution models such as Maxent (Phillips and Dudik 2008) subsequently provide a continuous probability surface which can be classified (based on a threshold) into suitable or non-suitable climatic space. The user determines the threshold, which is often set to a single value, and then generates a current climate-driven distribution to best fit the known species distribution (Liu et al. 2005). In other words, the user makes the species’ distribution a strict function of the variables that are put into the model (e.g., climate, land cover, soil type). Because the threshold may be a somewhat arbitrary cutoff depicting presence/absence of a species, applying a more liberal threshold in climate distribution models may dampen projected effects of climate change on species' distributions, such as the inability to cross geographic barriers.

We used a combination of Global Circulation Models (GCM), atmospheric CO_2 scenarios, and both strict and liberal model thresholds to generate a range of projected shifts in potential suitable climatic habitat for plethodontid salamanders in the southern Appalachian region of the eastern United States. Areas with high biodiversity or endemism are of high conservation value, and the Appalachian Highlands are regarded as a biodiversity hotspot with some of the most biologically diverse forests and freshwater systems in the United States (Stein et al. 2000). At broad spatial scales, amphibian diversity is related strongly to the direct and indirect (via net primary production) effects of climate and regional phylogeography (Buckley and Jetz 2007, 2008). The Appalachian Highlands are a global hotspot for salamander diversity, nearly all of which is determined by the family Plethodontidae (Petranka 1998). Plethodontid distributions are determined by a number of factors including land forms (e.g., major river boundaries), history and biotic interactions such as interspecific competition (Highton and Peabody 2000, Rissler et al. 2004); however, because plethodontids are lungless ectotherms, their activity, life-history traits, and consequently geographic distributions and patterns of diversity appear predominantly controlled by climate. (Feder 1983, Bernardo and Spotila 2006, Bernardo et al. 2007) (Marshall and Camp 2006, Kozak and Weins 2010). Consistent with global patterns of amphibian diversity (Buckley and Jetz 2007, 2008), plethodontid species richness throughout the southern Appalachian Highlands is positively linked to the cool, moist montane climate (Marshall and Camp 2006) with most species occupying mid or high elevation climatic zones that were colonized millions of years earlier when those climatic zones occurred in valley bottoms (Marshall and Camp 2006, Kozak and Weins 2010). Recent evidence suggests temperature is a direct limiting factor of dispersal and range size of some species within the family (Bernardo et al. 2007), further supporting the use of climate-based models to examine species distributions within this family. Because plethodontid salamanders are the most abundant vertebrate predators in eastern North American forests and headwater streams and are influential in a number of ecosystem processes (Wyman 1998, Davic and Welsh 2004, Walton and Steckler 2005, Walton et al. 2006), understanding shifts in their distributions or abundance will be important to predicting changes to ecosystem processes.

Methods

Species Distribution Modeling using Maximum Entropy

We developed distribution models using Maxent version 3.30a (Phillips et al. 2006, Phillips and Dudik 2008) for 41 plethodontid species (33 individual species and eight species included within two species complexes) with distributions in the eastern United States that included a portion of the species range within the Appalachian Mountain region (defined by a geographic boundary that includes all ecoregions found within the Appalachian Highland region). The two species complexes were the *Plethodon glutinosus* complex, which was composed of seven species (*P. glutinosus, P. cylindraceus, P. kentucki, P. teyahalee, P. chlorobryonis,* and *P. chattahoochee*) and the *Desmognathus fuscus* complex, composed of two species (*D. fuscus* and *D. conanti*). We treated these groups as complexes because their members were historically identified as one species but were later broken up into parapatric, morphologically cryptic species based on patterns of genetic divergence suggesting that geographic features and isolation promoted speciation (Titus and Larson 1996, Highton and Peabody 2000, Bonett 2002), and they are nearly indistinguishable in hand (although evidence suggest there are differences in body size (Carr 1996)). There are no data indicating that they function differently with regard to ecological factors such as climate. The 35 species (and complexes) represent ~90% of plethodontid species in the southern Appalachian Highlands and ~50% of plethodontid species occurring in the southeastern United States.

Maxent is a machine learning method that utilizes the principle of maximum entropy to model species distributions using presence-only data coupled with environmental data (Phillips et al. 2006). This approach finds a probability distribution of maximum entropy using a set of environmental variables to estimate a species' ecological niche using the defined Maxent probability distribution. For each species or species complex, current species distribution models were created using point data from two natural history databases intersected with georeferenced climatic variables. Salamander presence data were obtained from HerpNET (www.herpnet.org) and Global Biodiversity Information Facility (GBIF; www.gbif.org). To maximize model quality, only species with greater than 30 point locations were used (Wisz et al. 2008). We downloaded 1-km resolution temperature and precipitation bioclimatic layers, which are based on the 30-year period from 1960-1990, from the WorldClim database (Hijmans et al. 2005). We used the 11 bioclimatic layers utilized by Rissler and Apodaca (Rissler and Apodaca 2007) in their bioclimatic distribution modeling of *Aneides flavipunctatus*, a plethodontid species distributed in the western United States. Those 11 bioclimatic layers were winnowed from a larger set of 19 variables using correlations to estimate redundancy between variables and retaining the more biologically meaningful and interpretable variables (e.g., annual mean temperature, mean temperature of the wettest quarter, and precipitation of the wettest quarter). Maxent was run from the command line using the default settings with exception of background points. A total of 4215 target-group background data points representing localities of plethodontid salamanders in the eastern United States were used to develop an initial climatic envelope that represents the range of environmental conditions within the modeled region. In turn, this method is expected to reduce the bias inherent in our sample of museum locality data (Phillips et al. 2009). This approach uses background data (also known as pseudo-absences), chosen with the same bias as the occurrence data used, to develop the models. By using this approach we can produce an unbiased estimate of the geographic distribution of species, since the background data provides an equable sample of the environmental conditions within the region modeled.

We used a threshold approach to designate a location as climatically suitable for a species. When modeling a single species, each location modeled is represented by a probability that the location is climatically suitable for that species; however, it is logistically unfeasible to present each location as a probability of occupancy for every species modeled. Therefore, it was necessary to delineate a threshold at which a location was deemed climatically suitable or un-suitable. As was discussed in the introduction, the use of a single threshold will create a strict relationship between climate and a species' distribution, and thus potentially exaggerating the effect of climate shifts on the species' future distribution. To address this issue, we converted the continuous suitability surface [0–1 from Maxent to presence/absence (1/0)] using two-model output thresholds applied by Maxent; one ‘strict threshold’ that produced a climatic distribution that closely resembled, and at times underrepresented, the species current realized distribution (fixed cumulative value 10) and one ‘liberal threshold’ that predicts a broader climatic distribution than the current realized distribution (minimum training presence). We believe that this two-threshold approach is preferable to using a single threshold because it makes our results comparable to other studies that provide predictions based on strict climatic distributions of species—thresholds that maximize the agreement between observed and predicted distributions (Cramer 2003), and also allows us to present model predictions that relax the assumption of strict climatic control on species’ distributions.

We used null models to test the significance of each species climatic distribution model (Raes and ter Steege 2007). We generated 1000 sets of sample points, which were randomly drawn from the pool 4215 background points without replacement. Since the number of presence localities varied for each species, we generated null data sets with the number of random points per distribution equal to 50, 205, 405, or 695 data points, which represent the range of presence points available to model each species. Maxent was used to calculate the area under the curve (AUC) for the 1000 null data sets to create an AUC frequency distribution. The calculated AUC for each species model was then compared to the 95 percentile AUC value of the null frequency distribution created from the representative number of sample points (50, 205, 405, or 695). A species model performs better than random and is considered significant if the calculated AUC is greater than the corresponding 95 percentile AUC of the null-distribution (Raes and ter Steege 2007).

Projecting Future Species Climate Distributions

Climate projections were downloaded from the WorldClim database (www.worldclim.org). Projections were derived from the IPPC 3rd Assessment (McCarthy et al. 2001) and were calibrated and statistically downscaled using WorldClim Version 1.4 data for current projections. The 11 bioclimatic variables were calculated using the freely available ESRI ArcInfo AML program (available at http://www.worldclim.org/bioclim.htm). We used projections for years 2020, 2050, and 2080 derived from two widely used global circulation models (GCM), the Canadian Centre for Climate Modeling and Analysis Coupled Global Climate Model (CGCM3; (Flato and Boer 2001)) and the Hadley Centre for Climate Prediction and Research (HADCM3; (Johns et al. 2003). For each GCM, we used projections of climate parameters derived from two CO_2 emissions scenarios, A2a (medium to high emissions) and B2a (low to medium emissions) that corresponded to the IPCC Special Report on Emissions Scenarios (Nakićenović and Swart 2000). Therefore, we developed eight spatially explicit climate model scenarios, and used the Maxent climate distribution model developed earlier to project the future climate distribution for each species to 2020, 2050 and 2080.

Quantifying Projected Changes in Species Distributions and Richness

We compared current strict and liberal climate distribution models for each species with known distributions derived from county-level distribution maps to estimate the effect of threshold on over- or under-prediction of current known species distributions (Lannoo 2005). We calculated the percent overlap between modeled and county level distributions using ArcMap version 9.3 (ESRI, Redlands, CA). To measure the change in species distributions under future climate scenarios, we calculated the percentage of predicted area lost between the current and future predicted climate distribution model and compared them using the same Maxent threshold. In order to avoid the common criticism of assuming no potential for dispersal or unlimited dispersal, and to account for disjunct areas of predicted climatic habitat to which species will be unable to disperse to, we clipped all Maxent model predictions for all scenarios by the known county-level distribution buffered by 10 km. The buffer provides opportunities for future expansion by dispersal; however, we note that this is not a mechanistic adjustment and does not account for species-specific dispersal capabilities (Bernardo and Spotila 2006). We know little concerning dispersal capabilities of plethodontid salamanders. Evidence from northern populations of the red-backed salamander (*P. cinereus*) suggests expansion at a rate of only 80 m per year (Cabe et al. 2007), and other recent evidence suggests that some plethodontids may already be dispersal limited by temperature, so it is likely that any warming will further limit dispersal capabilities (Bernardo and Spotila 2006, Bernardo et al. 2007). Our 10 km buffer likely offers a liberal boundary for future migration. Each climatic and species map was projected in the World Geodetic coordinate System of 1984 (WGS84) with a cell size of 0.0083 decimal degrees.

We also examined how well species distribution models predicted known patterns of species richness in the Appalachian Highlands region and whether different climate change model scenarios predict different effects on plethodontid diversity. To estimate patterns of species richness, we made two species-richness maps based on the accumulated modeled distributions of each species or species complex using strict or liberal Maxent thresholds. Next we compared the richness of the two accumulated climate distribution models to known richness from county-based distribution maps by comparing richness values from the different distributions at 250 randomly selected points. We created the same accumulated richness maps for each of the CO_2 X GCM model X threshold scenarios for 2020, 2050 and 2080 to examine how projected changes in individual species distributions might affect patterns of diversity within the Appalachian Highlands region.

To examine the affect of GCM, CO_2 scenario, threshold, current range size, and distribution (latitude) on projected changes in suitable climatic habitat of species', we used a general linear model with percent habitat loss between the current suitable climatic distribution and predicted suitable climatic distributions (square root transformed) as dependent variables and GCM (Hadley or Canadian), CO_2 emissions scenario (low or high), threshold (strict or liberal) as categorical variables, and the size of the current species range and the latitude of the distribution centroid as continuous variables. To reduce over-parameterization of the model and simplify interpretation, we restricted our analysis to main effects and two-way and three-way interaction terms. We conducted a separate analysis for each projected year (2020, 2050 and 2080). We used paired *t*-tests to compare known county-based species richness values and predicted richness values (produced by summing the richness using both the strict and liberal thresholds). Statistical analyses were conducted in STATISTICA 8.0 (Statsoft, Inc., Tulsa, OK).

Results

The mean AUC for plethodontid distribution models based on current climate was 0.911 (range =0.664–0.995; median = 0.940; Table S1), with each species' model AUC being significantly better than random (i.e., model AUC values exceeded the 95 percentile of the null AUC distributions). Model predictions more closely matched current species distributions when the liberal threshold was used (81.04%, sd = 21.25 vs. 62.83% sd = 25.89, for the strict threshold; Table S1).

While projected mean change in salamander suitable climatic habitat size by 2020 varied depending on threshold, assumed CO_2 level, current range size and latitude, even the most ‘optimistic’ model (low threshold, low CO_2, HADCM3) projected at least a 20% reduction in suitable climatic range for more southerly distributed plethodontid species (Fig. 1; Tables S2–S3). There were significant interactions between threshold, assumed CO_2, and current range size and between threshold, assumed CO_2, and centroid latitude (Table 1). Percent of suitable climatic habitat loss was highest for species with small, southerly geographic ranges under models assuming high CO_2, and strict Maxent threshold (Fig. 1; Tables S1–S3). The effects of assumed CO_2 and threshold were small relative to the effects of range size and latitude (Fig. 1). For later projections (2050 and 2080), only threshold and latitude significantly affected mean percent climatic habitat loss (Table 1; Fig. 2). For all time points, the percent climatic habitat loss was greatest among more southerly distributed species (range centroid 32–34˚ north latitude), and slightly greater for models that assume a strict Maxent threshold. The projected percent climatic habitat loss among the most southerly-distributed (range centroid 32–34˚ north latitude) species increased from 50–100% by 2020 to 80–100% by 2050 and 85–100% by

2080 (Fig. 2; Tables S1–S3). For mid-latitude species (range centroid 36–38° north latitude) projected percent climatic habitat loss was 40–70% by 2020 and 70–85% by 2080, and for more northerly distributed species (range centroid 42–44° north latitude), projected percent climatic habitat loss was 0–70% (mean 20–38%) by 2020 and 0–70% (mean 30–40%) by 2080 (Fig. 2; Tables S1–S3).

Richness estimates based on accumulated climate distribution models produced with strict and liberal thresholds differed from each other ($t = 20.458$, $P < 0.001$; Fig. 3) and from current known richness values based on county level distribution records. The liberal threshold over-predicted known richness for the study region ($t = -10.106$, $P < 0.001$, mean county-level = 10.54, mean liberal threshold = 12.47), while the strict distribution models significantly under-predicted richness ($t = 10.968$, $P < 0.001$, mean county-level = 10.54, mean strict threshold = 8.68).

Reflecting the results for species-specific projected climatic habitat losses, even the most ‘optimistic’ projections predict declines in plethodontid richness within the southern portion of Appalachian Highlands as early as 2020 (Fig. 3). 2020 richness projections for the low threshold, low CO_2, HADCM3 model are relatively similar to current richness patterns in the region, with losses predicted only on the south-eastern fringe of the Appalachian Highlands region (Fig. 3); however, all other scenarios predict a significant loss of species in the southern highlands including the loss of all current species in the region by 2020. Over time, all model scenarios predict significant declines in species richness across the southern portion of the Appalachian Highlands with the loss of all current species from some areas under all model scenarios. Models using the more liberal Maxent threshold project the retention of high salamander richness in the central and northern portions of the Appalachian Highlands through 2080 regardless of CO_2 level or GCM. Only the most ‘pessimistic’ models (Canadian GCM3, high CO_2) predicting the greatest amount of warming and reduced precipitation, project a near complete loss of current species from the entire Appalachian Highland region by 2080 (Fig. 3).

Discussion

Our modeling approach shows that depending on model assumptions every plethodontid salamander species currently found within the Appalachian Highlands could experience restricted climatic habitat with climate change. We note that our models do not predict the extinction of the majority of species. Rather, those species with small, southerly ranges are predicted to experience the largest declines in range size including possible extinction. Sixty percent of the species (or complexes) we modeled, which in total comprised approximately 85-90% of plethodontid species richness found within the Appalachian Highlands, have a current range smaller than 115,500 km^2, and our models project the largest declines among those species with small ranges in the southern portion of the region. This scenario likely applies to the handful of range-limited endemics we could not analyze because of insufficient data on distributions. Projected climatic habitat declines are much smaller for species in the central and northern Appalachian Highlands region, and projected species richness remains high in the central and northern regions under a range of model scenarios. These predictions are consistent with a number of studies predicting more significant range contractions or northward shifts as a consequence to global climate change (McCarty 2001).

The robustness of our model predictions depends in part on the on the relative importance of climate versus geographic and biotic limitations on species distributions. Geographic and biotic limits on species distributions may conceal broader climatic tolerances than are reflected by a species' current distribution. Further, if biotic interactions are important in determining species distributions, and those interactions are altered by climate (e.g.), then it may be difficult to predict how a species distribution may respond to climate change, The boundaries of some plethodontid species do clearly coincide with major land formations, such as rivers, or the occurrence of interspecific competitors (Highton and Peabody 2000, Rissler et al. 2004). We are not aware of any data to indicate whether those species can occupy climates not represented by their current distribution. There is also evidence that interspecific competition shapes salamander phenotypes (morphology and behavior), but there is limited evidence that competition is a significant determinant of species distributions (reviewed by Kozak and Wiens 2010). Evidence suggests that some high elevation species, which are strongly climatically restricted, may limit the upslope distribution of lower elevation species, but there is no evidence of the reverse. This would imply that competitive effects on species distributions are biased toward underestimating the cold tolerance of low elevation species, but not the warm tolerance of high elevation species. Therefore, interspecific competition would not confound the use of climatic models to predict range loss from climate warming.

The most compelling evidence is that, with the potential exception of some low elevation species discussed previously, most plethodontids are restricted to their current realized climatic zones. It is true that the species we modeled have persisted through several historic periods of warming, and that historic warming events were associated with periods of plethodontid diversification (Vieites et al. 2007); however, this should not be confused as evidence that historic warming events were not associated with species range contractions and extinctions. Kozak and Wiens (2010) provide phylogeographic evidence that many extant plethodontid species that currently exist at mid and high elevation climatic zones are descended from species that colonized those cool climates when they occurred at lower elevations. They suggest that species have been "strictly confined" to specific climatic zones for millions of years, and have migrated with shifting climatic zones during historic changes in climate (Kozak and Weins 2010). These results are significant for several reasons. First, they suggest that it is unlikely that many plethodontid species have persisted in the same geographic location while that location has undergone significant climatic change. Rather, species migrate with their associated climatic zone during periods of climate change. Second, species currently distributed at mid and high elevations are most vulnerable to climate warming if their current climatic zone is lost because those species have limited ability to disperse through warmer valley bottoms. For example, show that range size and genetic differentiation of southern Appalachian *Desmognathus* species is related to temperature-dependent resting metabolic rate, with many high elevation populations existing near their thermal maxima and significantly limited in their ability to disperse through warmer, low-elevation environments. Collectively, these studies suggest that mid and high-elevation species are generally limited to upslope migration under a warming climate, which will lead to reductions in the area occupied by those species and the extinctions of some species with small, southerly, high-elevation distributions. This is consistent with our model predictions of northward range contractions and some extinctions of southern species with small, high-elevation distributions.

We would note that even though evidence suggests that most plethodontids will be limited in their ability to disperse northward under a warming climate, our study allowed for an optimistic level of dispersal that was still not sufficient to prevent significant declines in most species. Further, we did not account for land cover and other natural or anthropogenic geographic barriers that would limit species migrations in a contemporary landscape. The southeastern United States, including the Appalachian Highlands, are predicted to have one of the largest increases in urban and exurban development in the United States with a projected population increase to more than 360 million by 2030 (White et al. 2009). Large-scale reductions in climatic habitat availability combined with finer scale losses and fragmentation of remaining suitable habitats would reduce the likelihood that species could migrate with climate, increasing the probability of regional extirpations and extinction (Lawton and May 1995, Pimm and Raven 2000).

The inability to account for potential evolutionary change or plasticity within the models is another potential limitation to consider. Although correlative models include variation in traits as a consequence of using occurrence data across a geographical region to model distributions, mechanistic models can be parameterized based on a representative sample of species to include variation. Identifying which traits to incorporate, data sources for model parameterization, and determining the extent of a species adaptability remains challenging (Dormann 2007, Kearney and Porter 2009). Although examples of species adapting to environmental change, particularly global climate change, are increasing (Reznick and Ghalambor 2001, Parmesan 2006), little is known concerning the ability of plethodontids to adapt to changing climate conditions. Our understanding of the evolutionary adaptations or phenotypic plasticity exhibited by plethodontids to new environmental conditions is very limited. Recent studies have found correlations between genetic diversity, species diversity, and environmental variables in *Desmognathus* spp. (Marshall and Camp 2006), a measurable influence of moisture on adaptive phenotypes of *Desmognathus ocoee* (Camp et al. 2007), and morphological changes in *Plethodon cinereus* (Gibbs and Karraker 2006). These studies suggest members of this family are capable of adaptation as a consequence of recent environmental change, but more conclusive evidence is needed to examine their ability to persist through adaptation.

Because of the potential pitfalls associated with species distribution modeling and forecasting, a number of studies have stressed the need to use ensemble modeling in forecasting efforts. A true ensemble approach would consider a range of algorithms to relate species distributions to climate (Araujo and New 2007), and many authors have cautioned that assumptions in species distribution models, such as the use of threshold or selection of GCM, need to be explored in forecasting efforts. Although we did not explore multiple algorithms, a strength of our study was our use of a collection of GCM, CO_2, and threshold scenarios. While we did find that projected CO_2 level and Maxent threshold did affect the magnitude of projected climatic habitat loss in the near term (2020), these effects were relatively small compared to the effects of current range size and latitude. One concern that has been raised regarding the use of thresholds that over-predict the current range of an organism is that the projected loss of suitable climatic habitat may be underestimated; however, our findings did not support that concern. Further, we argue that by utilizing a threshold that slightly over-predicts the current suitable climatic habitat, we allowed the climatic range of each species to be larger than realized ranges. Projected climatic habitat losses were greater for scenarios that assume high CO_2 levels, and we note that the A2a CO_2 emissions scenario from the IPPC 3rd Assessment, which was considered a high-emissions scenario in our modeling effort, is now considered a conservative estimate of emissions (Sokolov et al. 2009). In other words, our 'high CO_2' scenarios using A2a emissions may be the more likely scenario for future forecasts. Remarkably, despite relatively large differences in projected temperature and precipitation changes between the Canadian and Hadley models, the choice of GCM had no measurable effect on our projected climatic habitat loss.

When using species distribution models, there are a number of additional limitations and assumptions that should be addressed (for a review see Guisan and Thuiller 2005, Araujo and Guisan 2006, Heikkinen et al. 2006, Wiens et al. 2009). Biases in the availability of species distribution data, such as points concentrated within national parks or areas likely to be foci for the collection of ecological data, can bias species distribution models (Phillips et al. 2009). Our use of target-group background points has been shown to reduce sample-selection bias (Phillips et al. 2009), and Maxent is considered to be a good conciliation to full ensemble forecasting (Araujo and New 2007). Additionally, correlative, niche-based models that predict distributions solely on the association between climatic variables and species range are not explicitly mechanistic, and as discussed earlier, those models fail to account for the influence of phylogeographic or biotic processes. Mechanistic models incorporate variables of physiological requirements and limitations, and interactions of an organism's functional traits and its habitat (Robertson et al. 2003, Kearney and Porter 2009, Morin and Thuiller 2009); however, unlike correlative models, mechanistic models require a great deal more data. For a number of taxa, data are simply not available to develop mechanistic models. Species distribution models are also affected greatly by the quality of taxonomic resolution and proper identification of species. For example, the family Plethodontidae is currently undergoing significant taxonomic revision, as detailed by the number of studies examining Plethodontidae phylogeny (Chippindale et al. 2004, Mueller et al. 2004, Macey 2005, Frost et al. 2006, Vieites et al. 2007). Revisions are particularly abundant within the genus *Desmognathus* (Titus and Larson 1996, Mead et al. 2001, Crespi et al. 2003, Rissler and Taylor 2003, Kozak et al. 2005, Jones et al. 2006, Beamer and Lamb 2008). Phylogenetic changes to this family in eastern North America have large implications to our study, as species currently analyzed as one single species could soon be broken into two or more species. In turn, this would separate a larger, single-species climatic niche into smaller, multiple-species niches. Based on our current models, which predicted larger percent climatic habitat loss among species with smaller geographic ranges, breaking species with larger distributions into multiple species with smaller geographic ranges and narrower climatic distributions would increase the proportion of species vulnerable to extinction and the estimated richness loss within the Appalachian Highlands.

Finally, we believe that a novel strength of our study is that our models predict measurable declines in species climatic habitat and richness as early as 2020. It is a reasonable criticism of other modeling efforts that they focus on longer-term projections

(2050–2080). While longer-term projections are important for management (Kettleborough et al. 2007), longer-range forecasts are less robust. In addition, formulating testable predictions is fundamental to science and the value of models. A number of studies have demonstrated the value of species distribution models to predicting current species distributions and patterns of richness, and then validated those models through sampling (e.g., (Raxworthy et al. 2007, Williams et al. 2009, Trotta-Moreu and Lobo 2010). To apply the same principle to species distribution model forecasting, we need shorter-term predictions of change to test. Our various 2020 model predictions can serve as testable alternative hypotheses concerning changes in species distribution and richness that will play out in the next 10 years. They also provide the opportunity to determine how other factors such as land cover change, biotic interactions, and other processes affect model projections.

The use of species distribution modeling to forecast the effects of climate change has been characterized in some ways as a necessary evil. Despite the potential pitfalls of species distribution modeling, there is a very real practical need to project how climate change may affect the distributions of species and potential losses of diversity in focal regions. We conservatively project the loss of a large proportion of plethodontid species from the southern portion of the Appalachian Highlands, a region that is currently a global biodiversity hotspot of salamander diversity. As salamanders are important in terrestrial and stream ecosystem processes (Wyman 1998, Davic and Welsh 2004, Walton and Steckler 2005, Walton et al. 2006), the decline of species could significantly alter the function of ecosystems in that region.

Acknowledgments

We are grateful for logistical help from S. Albeke and L. Worsham, and R. K. Barrett for constructive comments. The contributions of J. C. Maerz and J. R. Milanovich were partially supported by the Coweeta LTER (http://coweeta.uga.edu/), which is funded by the National Science Foundation and has among its current objectives to understand the role of climate change in patterns of species losses and gains in southern Appalachia. This study was also partially funded by the American Museum of Natural History (http://www.amnh.org/) and the North Carolina Herpetological Society (http://www.ncherps.org/).

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Table 3.1. Results from a general linear model investigating the factors that influenced the percent of suitable climatic habitat lost in 2020, 2050, and 2080. Data were square root transformed.

		2020			2050			2080		
Source	df	MS	F	*P*	MS	F	*P*	MS	F	*P*
Model	1	1.037	0.213	0.645	0.025	0.012	0.914	1.835	1.009	0.316
CO_2	1	80.185	16.467	<0.001	1.505	0.703	0.402	1.119	0.616	0.433
Threshold	1	6.227	1.278	0.259	5.001	2.341	0.127	5.699	3.136	0.078
Latitude	1	0.132	0.0271	0.869	26.626	12.443	<0.001	14.220	7.825	<0.01
Range size	1	19.884	4.085	0.044	2.580	1.206	0.273	5.454	3.001	0.084
Model*CO_2	1	11.932	2.450	0.119	1.125	0.526	0.469	0.081	0.045	0.833
Model*Threshold	1	0.753	0.155	0.694	0.682	0.319	0.573	0.001	0.001	0.982
CO_2*Threshold	1	0.260	0.053	0.817	1.390	0.650	0.421	0.367	0.202	0.653
Model*Latitude	1	1.194	0.245	0.621	0.013	0.006	0.937	1.934	1.064	0.303
CO_2*Latitude	1	74.663	15.333	< 0.001	1.449	0.677	0.411	1.141	0.628	0.429
Threshold*Latitude	1	7.815	1.605	0.206	5.555	2.596	0.108	6.043	3.326	0.069
Model*Range size	1	1.622	0.333	0.564	0.002	0.001	0.974	1.975	1.087	0.298
CO_2* Range size	1	77.874	15.992	< 0.0001	1.466	0.685	0.409	0.006	0.003	0.954
Threshold*Range size	1	4.389	0.901	0.343	1.681	0.785	0.376	2.835	1.560	0.212
Latitude*Range size	1	34.248	7.033	0.009	8.596	4.017	0.046	12.354	6.800	0.010
Model*CO_2*Threshold	1	1.285	0.264	0.608	0.257	0.120	0.729	0.015	0.008	0.927
Model*CO_2*Latitude	1	9.366	1.923	0.167	1.015	0.474	0.491	0.138	0.076	0.783
Model*Threshold*Latitude	1	0.636	0.131	0.718	0.645	0.301	0.584	< 0.001	< 0.001	0.987
CO_2*Threshold*Latitude	1	0.122	0.025	0.874	1.372	0.641	0.424	< 0.001	0.246	0.621
Model*CO_2*Range size	1	9.888	2.031	0.155	0.020	0.009	0.923	0.605	0.333	0.564
Model*Threshold*Range size	1	0.044	0.009	0.925	1.085	0.507	0.477	<0.001	< 0.001	1.000
CO_2*Threshold*Range size	1	0.117	0.024	0.877	0.297	0.139	0.709	0.0315	0.017	0.895

Table. 3.1 (con’t)

Model*Latitude*Range size	1	3.100	0.636	0.426	0.022	0.010	0.920	1.858	1.022	0.313
CO_2*Latitude*Range size	1	76.248	15.658	< 0.0001	1.165	0.544	0.461	0.186	0.103	0.749
Threshold*Latitude*Range size	1	5.447	1.119	0.291	1.856	0.867	0.353	2.989	1.645	0.201
Error	254	4.869	–	–	2.140	–	–	1.817	–	–

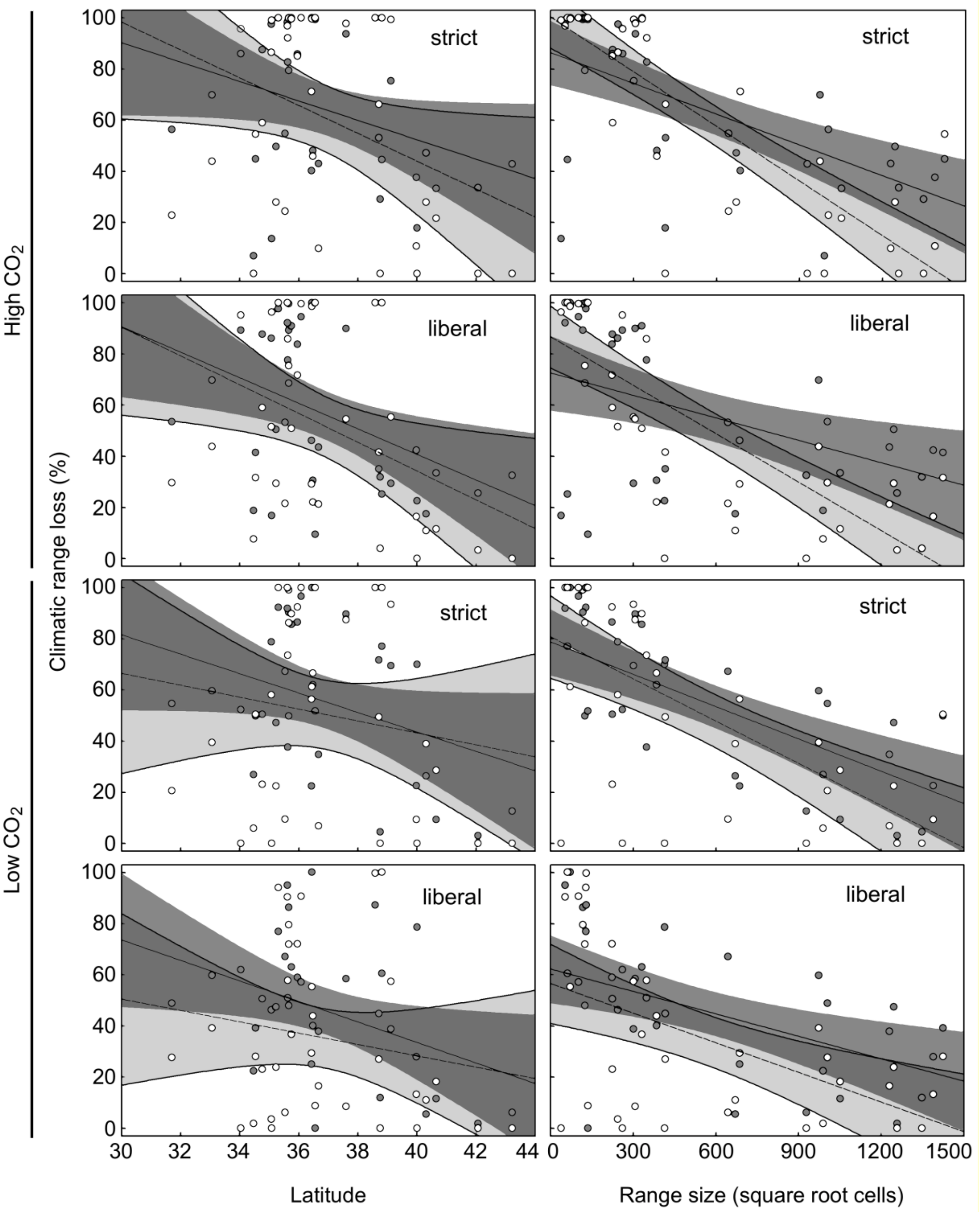

Figure 3.1. Effects of latitude and current range size on projected percent climatic habitat loss by 2020 of 35 plethodontid species/species complexes currently found within the Appalachian Highlands. Presented are percent of suitable climatic habitat losses relative to current climate distribution model for two Maxent thresholds ('strict' vs. 'liberal'), two projected CO_2 levels ('high' vs. 'low'), and two global circulation models (Canadian, CGCM3 = solid regression line with dark grey 95% confidence bands; Hadley, HADCM3 = dashed regression line with light grey 95% confidence bands). Darkest shading indicates regions of overlap between CGCM3 and HADCM3 confidence bands.

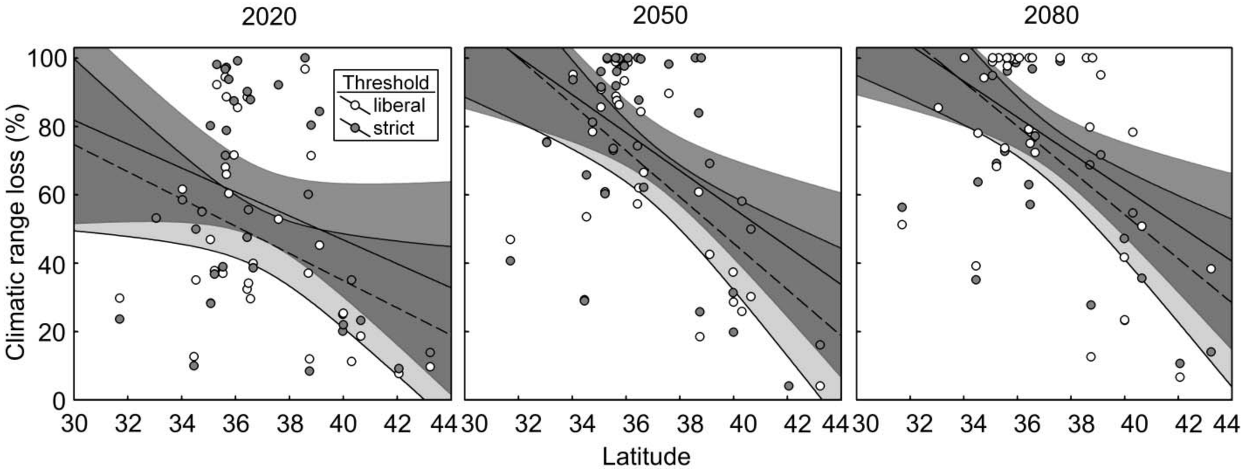

Figure 3.2. Effect of latitude and Maxent threshold on projected percent climatic habitat loss by 2020, 2050 and 2080 for 35 plethodontid species/species complexes currently found within the Appalachian Highlands. Presented are percent suitable climatic habitat losses relative to current climate distribution model for two Maxent thresholds ('strict' = solid points with a solid regression line and dark grey 95% confidence bands; 'liberal' = hollow points with a dashed regression line and light grey 95% confidence bands). Darkest shading indicates regions of overlap between 'strict' and 'liberal' confidence bands.

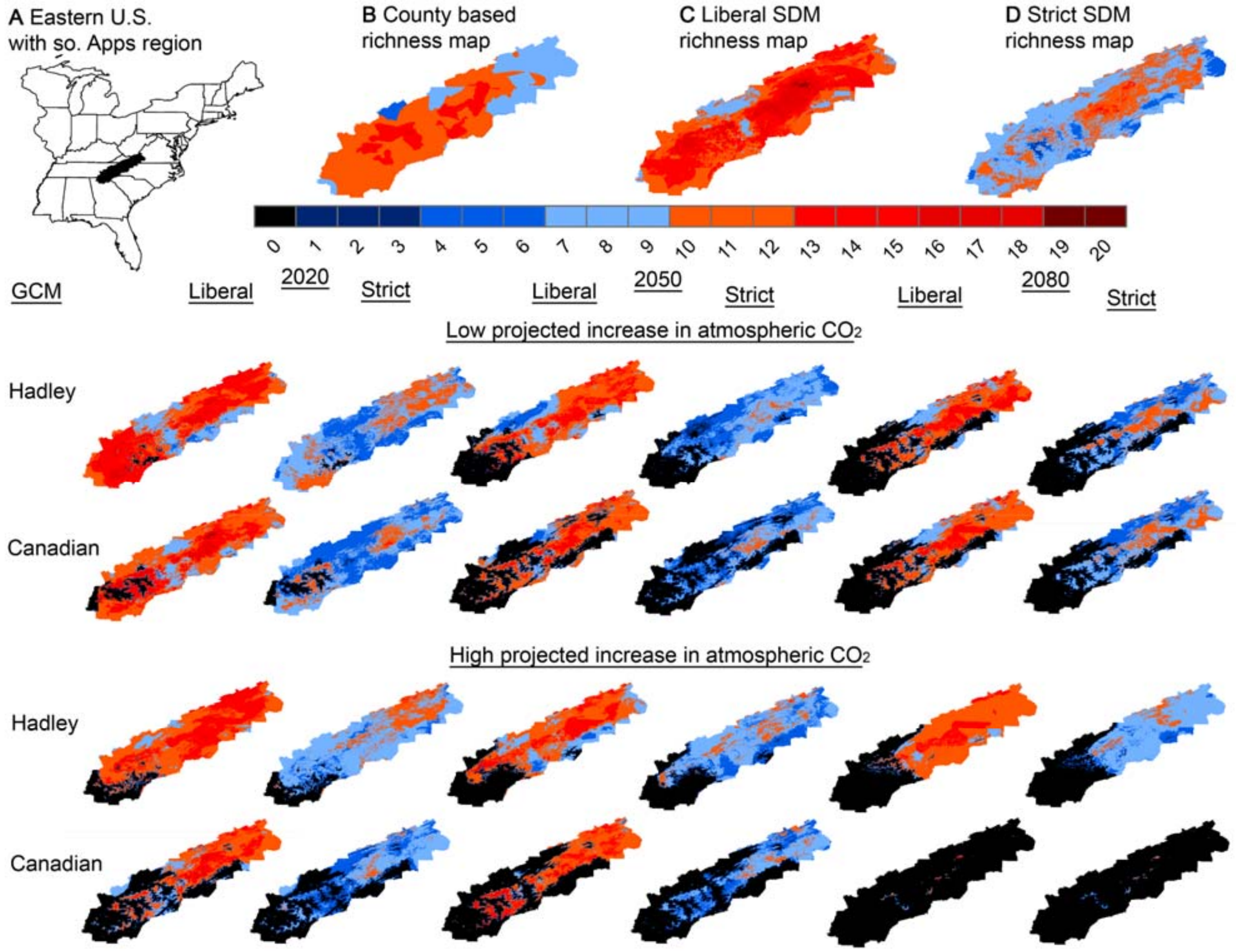

Figure 3.3. Predicted species richness of southern Appalachian plethodontids under 24 scenarios by year, global circulation model, CO_2 emissions scenario and model threshold. A) Shapefile used to create the species richness maps for the southern Appalachians. B) Species richness from county-level shapefiles. C) Current predicted suitable climatic habitat for the liberal model threshold. D) Current predicted suitable climatic habitat for the strict model threshold.

Appendix A. Total distribution size and percent of distribution overlap of current distributions for each species with AUC values for each species to show model fit and life history traits and number of points used to model each species. Mean AUC for all species was 0.911.

Species	Life history	Size of distribution (km^2)	Predicted current distribution		AUC	No. points used to model	Centroid of species range (latitude)
			Model threshold				
			Strict	Liberal			
Desmognathus aeneus	Semi-aquatic	48219	28.32	40.40	0.967	60	34.03
Desmognathus carolinensis	Semi-aquatic	11431	36.19	56.59	0.982	57	35.31
Desmognathus fuscus complex	Semi-aquatic	1524847	87.00	95.45	0.764	923	39.98
Desmognathus imitator	Semi-aquatic	9604	13.29	44.07	0.960	283	35.66
Desmognathus marmoratus	Semi-aquatic	35121	58.07	57.62	0.940	83	34.76
Desmognathus monticola	Semi-aquatic	303109	61.00	93.33	0.797	695	36.43
Desmognathus ochrophaeus	Semi-aquatic	292260	82.26	98.95	0.899	96	40.31
Desmognathus ocoee	Semi-aquatic	42013	37.91	97.44	0.906	378	35.07
Desmognathus quadramaculatus	Semi-aquatic	84413	56.15	83.55	0.880	264	35.63
Desmognathus santeetlah	Semi-aquatic	10752	19.81	47.59	0.957	85	35.66
Desmognathus wrighti	Semi-aquatic	34505	7.54	30.75	0.947	209	35.95
Eurycea bislineata	Semi-aquatic	1198566	83.74	93.44	0.911	403	43.22
Eurycea cirrigera	Semi-aquatic	1153855	94.29	98.86	0.820	258	35.22
Eurycea guttolineata	Semi-aquatic	738309	94.55	98.14	.927	57	33.05
Eurycea longicauda	Semi-aquatic	848917	80.08	99.21	0.892	126	34.46
Eurycea wilderae	Semi-aquatic	76312	32.21	73.33	0.842	668	35.75
Gyrinophilus porphyriticus	Semi-aquatic	764568	66.91	93.14	0.750	432	40.65
Hemidactylium scutatum	Terrestrial	1454021	62.17	87.24	0.914	283	38.75

Appendix A (con’t)

Plethodon cinereus	Terrestrial	1812454	85.94	94.92	0.887	395	42.06
Plethodon dorsalis	Terrestrial	278593	97.70	93.65	0.994	45	35.53
Plethodon electromorphus	Terrestrial	113396	88.89	96.55	0.989	52	40.00
Plethodon glutinosus complex	Terrestrial	1553973	78.08	96.24	0.664	2211	34.53
Plethodon hoffmani	Terrestrial	59988	86.61	96.48	0.953	489	39.11
Plethodon jordani	Terrestrial	1943	49.73	67.47	0.965	319	35.61
Plethodon montanus	Terrestrial	7080	49.17	76.43	0.973	137	36.07
Plethodon punctatus	Terrestrial	11143	16.78	32.77	0.991	35	38.58
Plethodon richmondi	Terrestrial	100793	86.14	95.63	0.955	159	36.48
Plethodon serratus	Terrestrial	137032	47.06	93.38	0.907	291	37.60
Plethodon shermani	Terrestrial	999	60.04	61.30	0.983	81	35.08
Plethodon virginia	Terrestrial	2472	86.49	92.53	0.995	93	38.80
Plethodon wehrlei	Terrestrial	114482	82.02	92.56	0.953	189	38.71
Plethodon welleri	Terrestrial	3540	52.84	73.76	0.990	59	36.44
Plethodon yonhalossee	Terrestrial	12313	69.40	90.24	0.975	173	36.56
Pseudotriton montanus	Semi-aquatic	758984	81.00	96.66	0.911	42	31.70
Pseudotriton ruber	Semi-aquatic	1065948	79.57	96.83	0.747	570	36.66

Appendix B. Percent loss or gain of suitable climatic habitat for each species using the Canadian Centre for Climate Modeling and Analysis Coupled Global Climate Model, two Maxent thresholds (strict and liberal), and two CO_2 emissions scenarios (low-medium and medium-high).

	CGCM2											
	Strict threshold						Liberal threshold					
	B2A			A2A			B2A			A2A		
Species	2020	2050	2080	2020	2050	2080	2020	2050	2080	2020	2050	2080
Desmognathus aeneus	52.31	100	100	85.94	99.96	100	61.92	100	100	89.19	99.95	100
Desmognathus carolinensis	92.18	100	100	100	100	100	76.90	99.40	100	97.64	99.47	100
Desmognathus fuscus complex	22.58	26.87	26.83	37.53	37.77	61.12	27.90	32.93	33.56	42.32	43.11	64.83
Desmognathus imitator	90.23	100	100	99.36	100	100	86.28	99.79	100	89.21	95.27	100
Desmognathus marmoratus	50.47	74.52	92.77	87.53	94.93	99.99	50.57	74.58	92.76	87.64	83.43	99.99
Desmognathus monticola	22.37	62.36	58.31	40.18	50.76	99.94	24.93	50.60	51.88	46.13	56.27	91.18
Desmognathus ochrophaeus	26.30	44.06	60.15	47.12	42.27	94.80	5.45	16.84	30.92	17.43	17.56	65.09
Desmognathus ocoee	78.66	96.44	98.92	97.45	96.70	100	46.20	89.41	93.28	86.12	91.17	99.55
Desmognathus quadramaculatus	37.55	93.80	95.33	82.57	80.26	100	50.89	89.15	92.18	77.58	79.30	100
Desmognathus santeetlah	49.81	97.51	100	79.37	86.36	100	47.90	89.37	98.38	68.46	74.11	99.97
Desmognathus wrighti	86.49	95.97	99.15	85.79	97.29	100	58.92	93.62	97.08	83.73	85.47	100
Eurycea bislineata	12.51	21.51	27.22	42.78	25.90	47.80	6.05	5.10	3.60	32.49	8.09	23.63
Eurycea cirrigera	47.14	54.44	55.82	49.66	70.04	87.32	47.38	54.09	57.07	50.43	70.53	87.68
Eurycea guttolineata	59.58	61.74	78.52	69.86	84.62	96.27	59.76	62.48	78.41	69.68	84.61	96.37
Eurycea longicauda	26.80	25.56	30.87	6.91	49.50	70.38	22.43	30.80	21.49	18.71	37.46	61.86
Eurycea wilderae	85.48	99.19	100	100	100	100	62.93	83.33	98.47	90.92	92.95	100
Gyrinophilus porphyriticus	9.28	39.09	23.39	33.27	34.13	75.65	11.45	22.71	26.17	33.34	33.11	52.15
Hemidactylium scutatum	4.45	91.01	6.81	29.04	11.79	21.24	11.92	14.30	16.04	31.78	23.89	42.90
Plethodon cinereus	3.02	3.32	0	33.52	4.19	16.41	1.77	0	0	25.48	3.61	24.00

Appendix B (con't)

Plethodon dorsalis	67.23	74.88	56.62	54.72	87.14	93.17	67.04	74.17	55.01	53.22	87.03	92.86
Plethodon electromorphus	70.00	32.92	14.40	17.73	25.94	64.55	78.59	35.20	12.76	22.49	34.61	67.34
Plethodon glutinosus complex	49.78	48.41	66.08	44.76	70.43	83.95	39.08	45.40	49.64	41.43	56.49	79.99
Plethodon hoffmani	69.43	37.21	95.44	75.35	88.28	89.29	38.78	10.60	55.32	29.36	49.24	77.01
Plethodon jordani	91.80	100	100	98.30	100	100	94.99	95.79	100	92.10	99.56	100
Plethodon montanus	96.55	100	100	100	100	100	57.15	96.17	99.98	94.47	99.15	100
Plethodon punctatus	100	100	100	100	100	100	87.26	100	100	100	100	100
Plethodon richmondi	61.95	87.38	64.81	48.00	86.04	99.68	40.06	62.72	43.97	30.56	77.33	99.69
Plethodon serratus	89.63	97.73	100	93.62	96.37	100	58.46	90.60	98.09	89.90	92.00	100
Plethodon shermani	0	100	100	13.65	65.90	100	0	100	100	16.77	63.03	100
Plethodon virginia	76.95	100	100	44.46	100	100	60.54	100	100	25.08	100	100
Plethodon wehrlei	71.69	71.95	59.49	52.99	100	100	44.75	47.81	38.65	34.97	64.08	98.30
Plethodon welleri	100	100	100	100	100	100	100	100	99.96	100	100	100
Plethodon yonhalossee	59.19	98.90	100	99.46	100	100	0	68.91	93.54	9.46	71.79	100
Pseudotriton montanus	54.61	50.22	57.55	56.33	75.83	97.11	48.82	56.62	65.03	53.39	78.62	97.53
Pseudotriton ruber	34.67	38.72	41.88	42.96	52.67	73.45	37.79	42.58	45.57	43.51	55.32	76.64

Appendix C. Percent loss or gain of suitable climatic habitat for each species using the Hadley Centre Coupled Model (version 3), two Maxent thresholds (strict and liberal), and two CO_2 emissions scenarios (low-medium and medium-high).

							HADCM3					
Species			Strict threshold						Liberal threshold			
		B2A			A2A			B2A			A2A	
	2020	2050	2080	2020	2050	2080	2020	2050	2080	2020	2050	2080
Desmognathus aeneus	0	100	100	95.58	74.36	100	0	100	99.99	95.17	80.47	100
Desmognathus carolinensis	100	100	100	100	100	100	93.99	100	100	100	100	100
Desmognathus fuscus complex	9.31	27.40	26.90	10.67	33.27	51.89	13.15	33.93	33.60	16.40	39.20	56.49
Desmognathus imitator	99.10	100	100	100	100	100	79.46	99.95	100	99.57	100	100
Desmognathus marmoratus	22.99	78.32	92.77	58.91	76.98	91.02	23.05	78.40	92.76	59.01	77.05	91.07
Desmognathus monticola	56.27	90.19	58.31	71.15	93.79	99.86	29.26	51.49	51.88	29.13	70.83	56.76
Desmognathus ochrophaeus	38.86	67.95	60.18	27.92	78.00	97.81	10.94	27.42	30.95	10.90	41.46	91.45
Desmognathus ocoee	58.01	95.75	98.92	86.40	95.04	97.52	3.48	89.89	93.28	51.53	71.88	93.29
Desmognathus quadramaculatus	73.50	95.47	95.33	92.12	97.75	100	57.79	92.16	92.18	85.85	94.48	99.98
Desmognathus santeetlah	86.14	99.94	100	99.86	100	100	71.85	92.03	98.38	75.36	94.45	100
Desmognathus wrighti	92.35	97.39	99.15	85.02	99.72	100	72.03	95.37	97.08	71.66	98.74	100
Eurycea bislineata	0	5.14	27.25	0	11.58	50.89	0	0	3.66	0	2.98	25.01
Eurycea cirrigera	22.34	56.19	55.82	27.86	60.44	73.87	23.84	57.30	57.08	29.41	61.28	74.61
Eurycea guttolineata	39.35	70.15	78.53	43.86	84.40	88.36	39.14	70.26	78.42	43.73	84.15	88.20
Eurycea longicauda	5.90	17.16	30.75	0	23.52	24.49	1.76	27.73	21.49	7.57	21.12	35.62
Eurycea wilderae	89.70	100	100	99.54	100	100	36.64	85.47	98.47	50.87	83.39	100
Gyrinophilus porphyriticus	28.57	63.81	23.42	21.53	62.61	80.60	18.13	20.21	26.19	11.57	44.75	37.59
Hemidactylium scutatum	0	0	6.88	0	0	15.37	0	15.14	16.09	3.93	20.49	35.76
Plethodon cinereus	0	0	0.00	0	8.57	10.06	0	2.16	0	3.25	10.38	18.46
Plethodon dorsalis	9.37	70.02	56.62	24.36	61.91	88.15	6.15	70.28	55.24	21.50	60.51	87.61

Appendix C (con't)

Plethodon electromorphus	0.00	20.19	14.40	0	0	0	0	44.45	12.76	0	0	0
Plethodon glutinosus complex	50.49	60.93	66.08	54.50	83.26	95.73	27.98	50.54	49.66	31.58	61.58	75.33
Plethodon hoffmani	93.34	50.94	95.44	99.24	100	100	57.37	13.78	55.32	55.32	96.34	98.78
Plethodon jordani	100	100	100	96.78	100	100	90.34	100	100	100	100	100
Plethodon montanus	100	100	100	100	100	100	90.70	99.25	99.98	99.59	100	100
Plethodon punctatus	100	100	100	100	100	100	99.70	100	100	100	100	100
Plethodon richmondi	66.49	88.30	64.81	45.88	88.79	70.64	43.85	61.31	43.97	21.96	46.50	40.73
Plethodon serratus	87.36	98.80	100	97.65	99.73	100	8.47	87.64	98.09	54.44	88.14	99.74
Plethodon shermani	0	100	100	99.05	100	100	0	100	100	96.35	100	100
Plethodon virginia	100	100	100	100	100	100	100	100	100	100	100	100
Plethodon wehrlei	49.38	80.29	59.49	66.10	83.23	99.95	26.98	60.48	38.65	41.51	70.66	99.28
Plethodon welleri	61.11	100	100	99.60	100	100	55.30	100.00	99.96	98.55	100.00	100
Plethodon yonhalossee	99.96	100	100	100	100	100	6.00	96.21	93.34	100	99.94	100
Pseudotriton montanus	20.49	59.16	57.57	22.82	63.42	77.10	27.68	63.69	65.05	29.57	67.03	80.93
Pseudotriton ruber	6.85	34.07	41.90	9.87	37.21	47.66	16.46	42.84	45.59	21.20	46.73	57.36

CHAPTER 4

WILL FUTURE SALAMANDER COMMUNITIES PROVIDE SIMILAR NUTRIENT STORAGE FUNCTION FOR APPALACHIAN HEADWATER STREAMS: CONSIDERING THE POTENTIAL FOR COMPENSATORY RELEASE BY SUBORDINATE SPECIES[1]

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[1] Milanovich, J.R. and J.C. Maerz. To be submitted to *Global Change Biology*

Abstract

Climate change is predicted to alter biotic communities and, as a result, cause changes in ecosystem processes. Such predictions assume that future communities will lack species capable of compensating for the loss of other species. In southern Appalachian headwater streams, abundant larval Black-bellied Salamanders (*Desmognathus quadramaculatus*) represent a significant standing crop of nitrogen (N) and phosphorus (P). *Desmognathus quadramaculatus* are projected to be extirpated from the southern Appalachian highlands under most climate change scenarios, which would result in the loss of most salamander standing crop of limiting nutrients unless other species compensate for the loss of *D. quadramaculatus* biomass. *Eurycea cirrigera*, which has an abundant congener *E. wilderae* already in the headwaters, and *Gyrinophilus porphyriticus*, which currently occurs in low densities in the headwaters, are projected to remain within southern Appalachian highlands. We used field cages to measure (1) the amount of compensatory survival and growth *Eurycea* would show in the absence of the larger, predatory *D. quadramaculatus*, and (2) whether replacement of *D. quadramaculatus* by *G. porphyriticus*, which is known to be a more efficient predator, would reduce *Eurycea* and total salamander biomass. In the absence of a larger, predatory species, we did see compensatory survival by *E. wilderae*. In the presence of *G. porphyriticus*, *Eurycea* survival was lower than in the presence of *D. quadramaculatus*, and *G. porphyriticus* survival was lower than *D. quadramaculatus* survival; however, despite the reduced survival, the larger size of larval *G. porphyriticus* resulted in greater biomass than communities dominated by *D. quadramaculatus*. Our results show that the potential for compensation for the loss of *D. quadramaculatus* will

depend on whether larval *G. porphyriticus* abundance increases and the degree to which intraguild predation also increases.

Introduction

As species' ranges and local abundances shift in response to climate change, there may be significant changes in ecosystem processes regulated by biota (Chapin 1997, McCarty 2001, Naeem 2002, Hooper et al. 2005, Kannon and James 2009). However, if current species assemblages are sufficiently diverse such that they include functionally redundant, subordinate species, then shifts in ecosystem function may not accompany shifts in community composition (Walker 1992; Naeem 1998, but see Loreau 2004 and Gamfeldt et al., 2008). Density compensation in response to environmental change was first conceptualized from studies of island ecosystems (MacArthur et al. 1972) and has since been shown in a number studies (reviewed by Gonzales and Loreau, 2009).

Density compensation occurs when environmental change causes a shift in community composition, and the level of aggregate biomass is partially or wholly maintained by subsequent increases in population densities or colonization by other taxa (MacArthur et al. 1972, Cody 1975, Ernest and Brown 2001). On an ecosystem level, whether or not density compensation occurs could have major consequences for aggregate ecosystem variables. Ecosystem homeostasis reflects the ability of an ecosystem to maintain certain properties such as biomass, productivity, and energy or nutrient flux despite environmental change and alterations to community structure (Odum 1969). When investigating the effect of loss of biodiversity on ecosystem function, density compensation could be the significant factor determining whether or not the ecosystem maintains homeostasis. Although density compensation generally makes predictions regarding community or population dynamics, the theory also predicts that species loss will have little effect on ecosystem function due to the expected increases in population abundance by members of the community (Gonzalez and Loreau 2009). Diverse communities can accumulate more biomass by utilizing resources more efficiently (Tilman et al. 1997a) and also contain more species to compete for dominance (Loreau 2000), which can also increase biomass (Tilman et al. 1997b). However, the effects of groups of species may not be predictable or similar in how they impact ecosystems. For example, previous studies have argued that vertebrate species feeding on similar prey do not necessarily have similar effects on lower trophic levels (i.e., they are not functionally equivalent, Chalfcraft and Resetarits 2003b, Resetarits and Chalcraft 2007), and in some systems, a single species may have a disproportionate effect on ecosystem processes (Paine 2002, Bellwood et al. 2003, Solan et al. 2004, Hoey and Bellwood 2009).

Following a decline in a single species, their ecosystem-level role can be compensated by the increased densities of other similar species. This has been termed "functional compensation" by Lawton and Brown (1994) and has been empirically shown in a number of systems (see review in Gonzalez and Loreau 2009). Most notably, for example, in response to acidification in lake ecosystems, Frost et al. (1995) found that communities of lake zooplankton demonstrated functional compensation by maintaining total biomass (the ecosystem function) following the decline of a number of species. Furthermore, using a long-term data set, Ernest and Brown (2001) found that communities of small mammals experienced significant fluctuation in their community composition over a 22 yr period, with a number of species maintaining, losing, and gaining dominance throughout. The aggregate ecosystem variables, however, provided by small mammals (e.g., biomass and energy use) remained relatively homeostatic. In both of these cases, homeostasis was due to the replacement of dominant species within the same community following assemblage shifts as a result of environmental change.

Stream salamanders of the family Plethodontidae represent a group of species that possess certain attributes that predictably make them more susceptible to environmental change (Welsh and Droege 2001). Stream plethodontids are lungless, poikilothermic vertebrates that are adapted to cool, moist, montane habitats (Petranka 1998). Streams in the southern Appalachians are inhabited by up to eight species of semi-aquatic plethodontids; however, typically these streams are dominated by a few taxa (e.g., *Desmognathus quadramaculatus*, *Desmognathus monticola*, *Desmognathus ocoee*, *Gyrinophilus porphyriticus*, and *Eurycea wilderae* or *Eurycea cirrigera*. In small headwater streams in the southern Appalachians, although *G. porphyriticus* is the largest of the semi-aquatic plethodontid species, *D. quadramaculatus* has been found to dominate the biomass of the salamander community; while *E. wilderae* can often dominate density (e.g., Milanovich Chapter 2, Peterman et al. 2008). Because climatic variables have a strong influence on plethodontid life and natural history, the composition of plethodontid communities across the Appalachians is predicted to significantly decline with predicted global climate change (Milanovich Chapter 3). A change in plethodontid stream salamanders, that does not include compensation for lost taxa, could result in changes to headwater stream ecosystem processes. Larval stream plethodontids have been shown to influence richness of lower trophic levels and are hypothesized to be important to a number of ecosystem processes (Davic 1983, Davic and Welsh 2004). They also have comparable roles to invertebrates and fish as reservoirs for limiting nutrients in headwater streams, for example, larval plethodontids store 4 and 1.5 times more P and N than invertebrates in headwater stream (Milanovich Chapter 2).

Forecasts predict the loss of some but not all salamander species that occupy the headwaters of the southern Appalachian highlands. Most models predict the loss of climatically suitable habitat for *D. quadramaculatus* and *E. wilderae* (Milanovich, Chapter 3); however, there is a relatively higher probability that climatically suitable habitat for *Gyrinophilus porphyriticus* and *E. cirrigera*, a sister species to *E. wilderae* that also occurs in the region, will occur in the highlands into 2050 (Fig. 4.1; Milanovich Chapter 3). *Eurycea cirrigera* is the sister taxon to *E. wilderae*, has a significantly larger distribution across the eastern U.S., typically inhabits more lowland, warmer areas, and are often as numerically dominant as *E. wilderae* in regions where the two species are allopatric (Camp et al. 2000, Pauley and Watson 2005). We predicted that *E. cirrigera* could replace *E. wilderae* as the climate in high elevation sites becomes more favorable.

The objectives of this study were to determine whether replacing the current dominant species with a confamilial species with a similar larval life history would have similar effects on other salamander species, total salamander biomass and associated standing crop of limiting nutrients. We hypothesize that a loss of currently dominant *D. quadramaculatus*, concurrent with an expansion of suitable climatic habitat (i.e., warmer temperatures) across the southern Appalachians, will lead to densities of *E. cirrigera* equivalent to current *E. wilderae* densities. Although we do not predict an increase in densities of *G. porphyriticus* as a consequence of warmer temperatures, in this experiment we elevated their densities solely based upon the assumption that *G. porphyriticus* will be the only remaining species able to compensate for *D. quadramaculatus*. An increase in densities and expansion of distributions of these two species could compensate for the loss of the two currently dominant species with respect to biomass and standing crop of limiting nutrients. We tested the hypothesis that the survival of *E. wilderae* will be higher in absence of either *D. quadramaculatus* or *G. porphyriticus*, and lower in the presence of *G. porphyriticus* compared to *D. quadramaculatus*. Furthermore, we hypothesize the total salamander biomass will be higher in communities where *D. quadramaculatus* is present and *G. porphyriticus* is absent, and lowest in communities where both large larval predators are absent. Considering the standing crop of C, N, and P is a function of biomass, we hypothesize the standing crop of limiting nutrients will also be higher in communities where *D. quadramaculatus* is present and *G. porphyriticus* is absent, and where both large larval predators are absent. If the total biomass or standing crop of limiting nutrients in the larval stream-salamander community cannot be compensated (i.e., maintaining equal or greater biomass of standing crop of nutrients across communities), Appalachian headwater streams could face significant future losses in ecosystem function. In the context of climatic extremes, Jentsch et al. (2007) highlighted the importance of experiments testing the effects of climate-change based upon predictive models, thus, we feel our study is an important step to help understand the consequences of global climate change.

Methods

To test our hypotheses, we used 15, 0.5 m^2 Reptarium® cages (1.0 mm mesh size) in a single unnamed stream west of catchment 22 (35.02624 N, 83.26948 W) at the Coweeta Hydrological Laboratory. Coweeta is a 2185 ha experimental forest located in southwestern North Carolina. Our particular stream had similar characteristics to other headwater streams located at Coweeta, as it was surrounded by dense deciduous forest and has a thick understory of *Rhododendron* adjacent to the stream. Cages consisted of a PVC pipe frame covered with nylon mesh and were anchored into the stream. To replicate stream conditions, cages were stocked with enough fine silt and substrate (gravel, rocks and sandy substrate > 2 cm) to cover a mean of two centimeters across the bottom of each cage, one liter of small rocks (> 10 x 10 cm), and five rocks measuring between 15 x 15 to 30 x 30 cm. To provide initial prey, approximately one liter of leaf litter (with invertebrates) from the surrounding stream was added to each cage, with an additional one liter of leaf litter added 30 days into each experiment. Litter and substrate were searched to insure no salamanders were present prior to being placed within the cage. Substrate, rocks, and litter were taken directly from the streambed and margin. The mesh cages allowed for air and water flow while still confining salamanders to each cage.

Estimates of density from previous studies (Peterman et al. 2008a, Peterman et al. 2008b, Milanovich Chapter 2) indicate that the larval salamander community in the southern Appalachian Highlands is typically numerically dominated by two taxa; *Eurycea wilderae* and *D. quadramaculatus*. Larval *Eurycea wilderae* occurs in densities between 27 – 137$\cdot m^{-2}$, but composes only ~4% of total salamander biomass (Milanovich

Chapter 2). *Eurycea wilderae* has a larval period of approximately 12 – 14 months (typically with an snout-vent length [SVL] of approximately 20 mm), at which point transformed larvae leave the stream bed to live the remainder of their lives in riparian or terrestrial habitat only to return to breed. In contrast, larval *Desmognathus quadramaculatus* occur at mean densities of $29 \cdot \mathrm{m}^{-2}$ and makes up ~96% of larval salamander biomass (Milanovich Chapter 2). Larval *D. quadramaculatus* transform from the larval stage between 3-4 years (SVL of approximately 40 mm), and remain in riparian areas following metamorphosis. *Gyrinophilus porphyriticus* occurs at very low densities ($< 1/\mathrm{m}^2$; J.R. Milanovich and J.C. Maerz, unpublished data). *Gyrinophilus porphyriticus* is similar to *D. quadramaculatus* in that it has a multi-year larval period (between 4-5 years); however, *G. porphyriticus* has the has the largest larvae of any species of Appalachian plethodontid, and is considered the species most likely to prey upon other salamander species (Formanowicz and Brodie 1993). Therefore, it is possible that at a similar density, larval *G. porphyriticus* could provide similar function in headwater streams; however, if *G. porphyriticus* preys on other guild members at a higher rate, as suggested by work from Beachy (1994), then its effects on salamander biomass and nutrient dynamics may differ from the effects of *D. quadramaculatus*.

We developed three treatments to represent the current (*E. wilderae* and *D. quadramaculatus*) and two potential future larval salamander communities (*E. wilderae* alone or *E. wilderae* and *G. porphyriticus* at *D. quadramaculatus* density). Even though projections are for *E. cirrigera* to replace *E. wilderae*, because *E. cirrigera* did not currently occur in our study stream, we considered *E. wilderae* as a suitable proxy for *E. cirrigera* (Camp et al. 2000). In each cage we stocked 30 larval *E. wilderae*. In cages with *D. quadramaculatus* or *G. porphyriticus*, we stocked six larvae with two conspecifics of each of three size classes (*D. quadramaculatus*, small = 10-23 mm, medium = 24-30 mm, large = 31-40 mm), *G. porphyriticus*, small = 18-36 mm, medium = 31-44 mm, large = 45-70 mm; Table 4.1). All larval salamanders were collected from streams located within the Coweeta Hydrological Laboratory. Prior to stocking, we measured (snout-vent length, SVL, anterior tip of the snout to the posterior portion of the vent, mm) and weighed (mg) each salamander. We repeated the experiment during two "seasons": 03 May to 03 July 2009 (Experiment 1) and 06 July to 06 September 2009 (Experiment 2). *Eurycea wilderae* hatch in July and metamorphose the following June; therefore, the *E. wilderae* in Experiment 1 were larger and near metamorphosis, while newly hatched *E. wilderae* were used in Experiment 2.

On the 60th day of each experiment, we recovered all the animals remaining within the cages by searching through substrate and litter. We euthanized larvae in a 0.5% solution of tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate (two g per g of MS-222). Salamanders were counted, measured (SVL), and weighed (mg) to obtain estimates of survival and mean biomass for each cage. Because our animals were not individually marked, we could not estimate individual growth. Standing crop of C, N and P for *E. wilderae*, *D. quadramaculatus* and *G. porphyriticus* was determined by calculating the product of salamander biomass of each species/cage and mean whole-body % C, % N, and % P for each species. Mean whole-body %C, %N, and %P of *E. wilderae* and *D. quadramaculatus* values were taken from Milanovich Chapter 2; while mean whole-body %C, %N and %P for *G. porphyriticus* was determined from five animals collected across the Coweeta Hydrological Laboratory site from 1 July 2007 to 1 August 2008 and were processed following previous methods (Milanovich Chapter 2).

Statistical analyses

We used a two-way analysis of variance (ANOVA) to test the hypothesis that *E. wilderae* percent survival (arcsine transformed) would increase with the loss of *D. quadramaculatus*, but would decline with the replacement of *D. quadramaculatus* by *G. porphyriticus*. We included treatment and season as factors in our analysis, and we used planned contrasts to conduct pair-wise comparisons of treatments ($\alpha = 0.05$). Because *E. wilderae* is significantly smaller than the other salamander species, the mean final total salamander biomass in treatments lacking the larger species would inevitably be lower than treatments containing the larger species. Therefore, we restricted our analysis of the larger species treatments to pair-wise comparisons. We used two-way ANOVA to test the hypothesis the percent survival (arcsine transformed) of the larger species and mean final total salamander biomass would be greater in treatments with *D. quadramaculatus* compared to treatments with *G. porphyriticus*. Again we included treatment and season as factors in our analysis. Because differences in nutrient standing crops are primarily a function of total larval biomass and larval size (Milanovich Chapter 2), we did not conduct separate statistical analyses on nutrient standing crops. To do so would be essentially redundant with the analysis of biomass. However, we do present results for nutrient standing crops. All analyses were performed using Statistica version 6.0 (Tulsa, OK).

Results

There was a significant interaction between experiment and treatment on the percent survival of *E. wilderae* (Table 2.2). The presence of *D. quadramaculatus* or *G. porphyriticus* reduced the percent survival of *E. wilderae* during both experiments. In Experiment 1, the percent survival of *E. wilderae* in the treatment containing no predators was 71%, and was 486% higher in the treatment that included *D. quadramaculatus* compared to the treatment that included *G. porphyriticus* (Table 2.3; planned comparisons). In Experiment 2, the percent survival of *E. wilderae* in the treatment containing no predators was 57%, and was 13% higher in treatments that included *D. quadramaculatus* compared to the treatment that included *G. porphyriticus* (Table 2.3; planned comparisons). Survival of predators significantly differed between treatments and experiments, and there was a significant interaction between experiment and treatment on the percent survival of the predators (Table 2.2). During Experiments 1 and 2, the percent survival of *D. quadramaculatus* was 75% and 71% higher than *G. Porphyriticus* respectively (Table 2.3; planned comparisons, $P < 0.0001$).

Total salamander biomass significantly differed between treatments (Table 2.2). In Experiment 1, the total salamander biomass was 2.8 and 4.1 times greater in the *D. quadramaculatus* or *G. porphyriticus* treatments when compared to the *E. wilderae* only treatment respectively, and was 1.5 times greater in the *G. porphyriticus* treatment compared to the *D. quadramaculatus* treatment. In Experiment 2, the total salamander biomass was 3.4 and 5.1 times greater in the *D. quadramaculatus* or *G. porphyriticus* treatments when compared to the *E. wilderae* only treatment respectively, and again was 1.5 times greater in the *G. porphyriticus* treatment compared to the *D. quadramaculatus* treatment. In Experiment 1, when compared to the E. wilderae only treatment, the standing crop of C was 2.6 and 3.8 times greater in the *D. quadramaculatus* or *G. porphyriticus* treatments respectively, and was 1.5 times greater in the *G. porphyriticus* treatment compared to the *D. quadramaculatus* treatment, the standing crop of N was 2.6 and 3.9 times greater in *D. quadramaculatus* or *G. porphyriticus* treatments, respectively, and was 1.5 times greater in the *G. porphyriticus* treatment compared to the *D. quadramaculatus* treatment, and the standing crop of P was 4 and 6.3 times greater in *D. quadramaculatus* or *G. porphyriticus* treatments, respectively, and was 1.6 times greater in the *G. porphyriticus* treatment compared to the *D. quadramaculatus* treatment. In Experiment 2, the standing crop of C was 3.2 and 4.7 times greater in *D. quadramaculatus* or *G. porphyriticus* treatments, respectively, and was 1.5 times greater in the *G. porphyriticus* treatment compared to the *D. quadramaculatus* treatment, the standing crop of N was 3.1 and 4.9 times greater in *D. quadramaculatus* or *G. porphyriticus* treatments, respectively, and was 1.6 times greater in the *G. porphyriticus* treatment compared to the *D. quadramaculatus* treatment, and the standing crop of P was 6 and 9.5 times greater in *D. quadramaculatus* or *G. porphyriticus* treatments, respectively, and was 1.6 times greater in the *G. porphyriticus* treatment compared to the *D. quadramaculatus* treatment (Table 2.3).

Discussion

Our study shows that, with the projected loss of *D. quadramaculatus*, the amount of compensatory survival and standing crop of larval salamanders will depend on whether the density of larval *G. porphyriticus* increases. As expected, we found that in the absence of larval *D. quadramaculatus*, larval *E. wilderae* survival increased significantly; however, this compensatory survival is not sufficient to produce a compensatory amount of larval biomass and associated standing crop of nutrients. This is consistent with other studies (Beachy 1994, 1997). We note that we stocked *E. wilderae* at ~ twice the natural densities typical of our study streams, which provided for nearly a doubling of larval biomass. Further, other studies show limited and inconsistent changes in growth by larval *E. wilderae* in the presence of larval *D. quadramaculatus* (Beachy 1997); therefore, it is unlikely that, with more time for growth or higher larval abundance, that *E. wilderae* would ever compensate for the loss of *D. quadramaculatus* biomass. We also found that shifting from larval *D. quadramaculatus* to larval *G. porphyriticus* significantly reduced *E. wilderae* survival. In fact, in all but a few cases, the presence of *G. porphyriticus* eliminated all *E. wilderae* in both experiments in only 60 days; while the presence of *D. quadramaculatus* had a more measurable influence on the survival of small (e.g., newly hatched) *E. wilderae* (i.e., Experiment 2) compared to larger *E. wilderae* (i.e., Experiment 1). This result is also consistent with previous research (Resetarits 1991, Gustafson 1993, Beachy 1994, Gustafson 1994). Further, larval *G. porphyriticus* showed lower survival than larval *D. quadramaculatus*, and it was only the largest *G. porphyriticus* that generally recovered from cages. These results undoubtedly reflect more intense intraguild predation including cannibalism in the presence of *G. porphyriticus*, and demonstrate that from the perspective of intraguild dynamics, larval *G. porphyriticus* and *D. quadramaculatus* are not ecologically redundant. Diet records confirm that larval *D. quadramaculatus* predation on other salamanders is relatively rare (Camp 1997), while other larval salamanders may account for as much as 16% of larval

G. pophyriticus diets (Bruce 1979). Though *D. quadramaculatus* and *G. porphyriticus* may have different effects on community dynamics and larval abundances, *G. porphyriticus* larvae are significantly larger and therefore, can produce a compensatory amount of larval salamander biomass at a lower larval density.

Though we estimate that communities with larval *G. porphyriticus* could produce similar larval biomass and standing stocks of nutrients, other factors may cause *G. porphyriticus* to have different effects on stream nutrient dynamics compared to *D. quadramaculatus*. *Desmognathus quadramaculatus* prey almost exclusively on invertebrates that are lower in their %N and %P. Because *G. porphyriticus* commonly prey upon other larval plethodontids, larval *G. porphyriticus* stoichiometry should be more similar to their prey. The degree to which a consumer's stoichiometry matches its diet affects the amounts and ratios of the nutrients excreted by the consumer (Sterner and Elser 2002). Consumer excretion can be an important component of ecosystem nutrient demand, and shifts in the stoichiometry of consumer communities are known to alter ecosystem nutrient dynamics (see review in Elser and Urabe 1999).

Whether future salamander communities will compensate for the loss of *D. quadramaculatus* is contingent largely upon the compensatory increase in the abundance of *G. porphyriticus*. Despite a large number of studies exploring community interactions for stream plethodontids (see review in Bruce 2007), there has been relatively little attention given to the processes that regulate the distribution and abundance of *G. porphyriticus*. In particular, it is not clear why, despite its apparent superiority as a larval predator, *G. porphyriticus* occurs at low abundance at high elevations in the southern Appalachian Mountains. Our study shows that larval *G. porphyriticus*, in the absence of

D. quadramaculatus, survive well in streams where they naturally occur at low densities. *Gyrinophilus porphyriticus* occur as far north as Maine, so we presume both adults and larvae are tolerant of the cooler climates at high elevation. We hypothesize that it is competition from *D. quadramaculatus* that may currently limit the abundance of *D. quadramaculatus* in high elevation streams. Competition is frequently invoked as an important factor structuring plethodontid communities, and there is evidence that interspecific competition can affect the performance, phenotypes, and abundance of sympatric species (see review in Bruce 2007). Within the genus *Plethodon*, there is evidence that species are climatically restricted to high elevations can competitively suppress species with broader geographic distributions (Kozak and Wiens 2010). One hypothesis is that climatically restricted species are under stronger selective pressure to be competitively superior. Without the ability to migrate upslope, high-elevation species must resist the upslope expansion of lower elevation species that might displace them. If the abundance of *G. porphyriticus* is currently limited by *D. quadramaculatus*, then it is reasonable to assume that *G. pophyriticus* would respond positively to the loss of *D. quadramaculatus* predicted with a warmer of the southern Appalachian region. We predict that such an increase would likely compensate for the loss of *D. quadramaculatus* with regard to standing crop of limiting nutrients; however, the change is likely to result in altered dynamics within larval salamander communities including the reduced abundance of species vulnerable to predation by *G. porphyriticus*.

Predicting how biological communities may change in response to future climates is a challenge. Extrapolating those changes to how they will impact ecosystem processes is equally challenging. Such predictions are fraught with logistical challenges and dubious assumptions, yet such exercises are necessary. This study is a first step towards merging our current understanding of species' effects on an ecosystem, with predictions from species distribution models and field experiments, to project whether future communities might have the capacity to compensate for the loss of currently dominant species. This study draws attention to the importance of intraguild predation, and illustrates that while two species may not be ecologically redundant with regards to their interactions with other species within the community, a currently subordinate species has the capacity to compensate for the loss of the current dominant species with regards to the standing crop of nutrients. We stress that future studies should explore how community interactions limit the current abundance of subordinate species, identify those species that are likely to respond positively to the loss of a dominant species and are likely to persist in that environment under changing conditions, and then determine whether those subordinate species will have similar or different effects on key ecosystem processes.

Acknowledgements

We thank C.C. Camp, R.K. Barrett, A. Rosemond, and N. Nibbelink for providing critical reviews to earlier drafts of this manuscript. We also thank R.K. Barrett, K. Cecala, B. Crawford, J. Denney, M. Erickson, A. Ferreira, L. Larson, K. Mowbary, and Steve and Sara Samoray for field assistance. This project was partially supported by the Coweeta Hydrologic Laboratory, American Museum of Natural History Theodore Roosevelt Memorial Grant, and the North Carolina Herpetological Society.

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Table 4.1. Mean (SE) of SVL (mm) and wet mass (mg) of animals stocked in cages. Size classes of predators are as follows: *D. quadramaculatus*, small = 10-23 mm, medium = 24-30 mm, large = 31-40 mm), *G. porphyriticus*, small = 18-36 mm, medium = 31-44 mm, large = 45-70 mm. Experiment 1 had six *E. wilderae*, six *D. quadramaculatus* and *E. wilderae*, and three *G. porphyriticus* and *E. wilderae* cages. Experiment 2 had six *E. wilderae*, five *D. quadramaculatus* and *E. wilderae*, and four *G. porphyriticus* and *E. wilderae* cages.

Experiment	Species					
	E. wilderae		*D. quadramaculatus*		*G. porphyriticus*	
	SVL	Mass	SVL	Mass	SVL	Mass
Experiment 1	17 (0.19)	0.07 (0.002)	27 (1.2)	0.65 (0.11)	36 (3.9)	1.28 (0.36)
Experiment 2	12 (0.27)	0.05 (0.002)	29 (1.1)	0.64 (0.09)	40 (3.8)	1.99 (0.37)

Table 4.2. Results from two-way ANOVAs testing for the effect of treatment and experiment (1 or 2) on *E. wilderae* survival (arcsine transformed) and biomass, survival of predators (*D. quadramaculatus* and *G. porphyriticus*), and total salamander biomass in *D. quadramaculatus* and *G. porphyriticus* treatments (per cage).

Dependent variable	Effect	df	MS	F	*P*
E. wilderae biomass	Season	1	0.071	1.486	0.235
	Treatment	2	3.622	75.816	< 0.0001
	Experiment X Treatment	2	0.344	7.208	0.004
	Error	24	0.048	–	–
E. wilderae survival	Season	1	0.001	0.034	0.855
	Treatment	2	0.797	35.566	< 0.0001
	Experiment X Treatment	2	0.175	7.816	0.002
	Error	24	0.022	–	–
Predator survival	Season	1	1.600	49.851	< 0.0001
	Treatment	1	0.673	20.977	< 0.0001
	Experiment X Treatment	1	0.332	10.355	0.006
	Error	14	0.032	–	–
Total salamander biomass	Season	1	21.970	5.125	0.040
	Treatment	1	0.204	0.048	0.830
	Experiment X Treatment	1	0.148	0.035	0.855
	Error	14	4.287	–	–

Table 4.3. Mean survival (SE) in percentage, mean final total biomass (mg per cage), mean estimated standing crop of C, N, and P (mg per cage), and sample size (N) of number of cages for each treatment within each experiment (1 and 2). Treatment labels are as follows: EW = *E. wilderae* only, EW + DQ = *E. wilderae* and *D. quadramaculatus*, and EW + GP = *E. wilderae* and *G. porphyriticus*.

Treatment	N	Community parameters			Ecosystem parameters			
		Survival (SE) in %			Final biomass (mg)	Estimated standing crop (mg)		
		EW	DQ	GP		C	N	P
Experiment 1								
EW	6	71.11 (3.30)	–	–	1.56 (0.13)	0.72 (0.03)	0.20 (0.01)	0.03 (0.001)
EW + DQ	6	45.56 (6.59)	66.67(4.30)	–	4.29 (0.30)	1.86 (0.13)	0.51 (0.04)	0.12 (0.019)
EW + GP	3	7.78 (7.80)	–	38.89 (5.56)	6.39 (2.13)	2.74 (0.18)	0.78 (0.05)	0.19 (0.015)
Experiment 2								
EW	6	56.67 (5.84)	–	–	1.24 (0.07)	0.58 (0.06)	0.16 (0.02)	0.02 (0.002)
EW + DQ	5	26.00 (4.52)	80.00 (8.16)	–	4.22 (0.30)	1.84 (0.13)	0.50 (0.04)	0.12 (0.009)
EW + GP	4	23.33 (4.51)	–	45.83 (7.98)	6.35 (0.43)	2.73 (0.91)	0.78 (0.26)	0.19 (0.064)

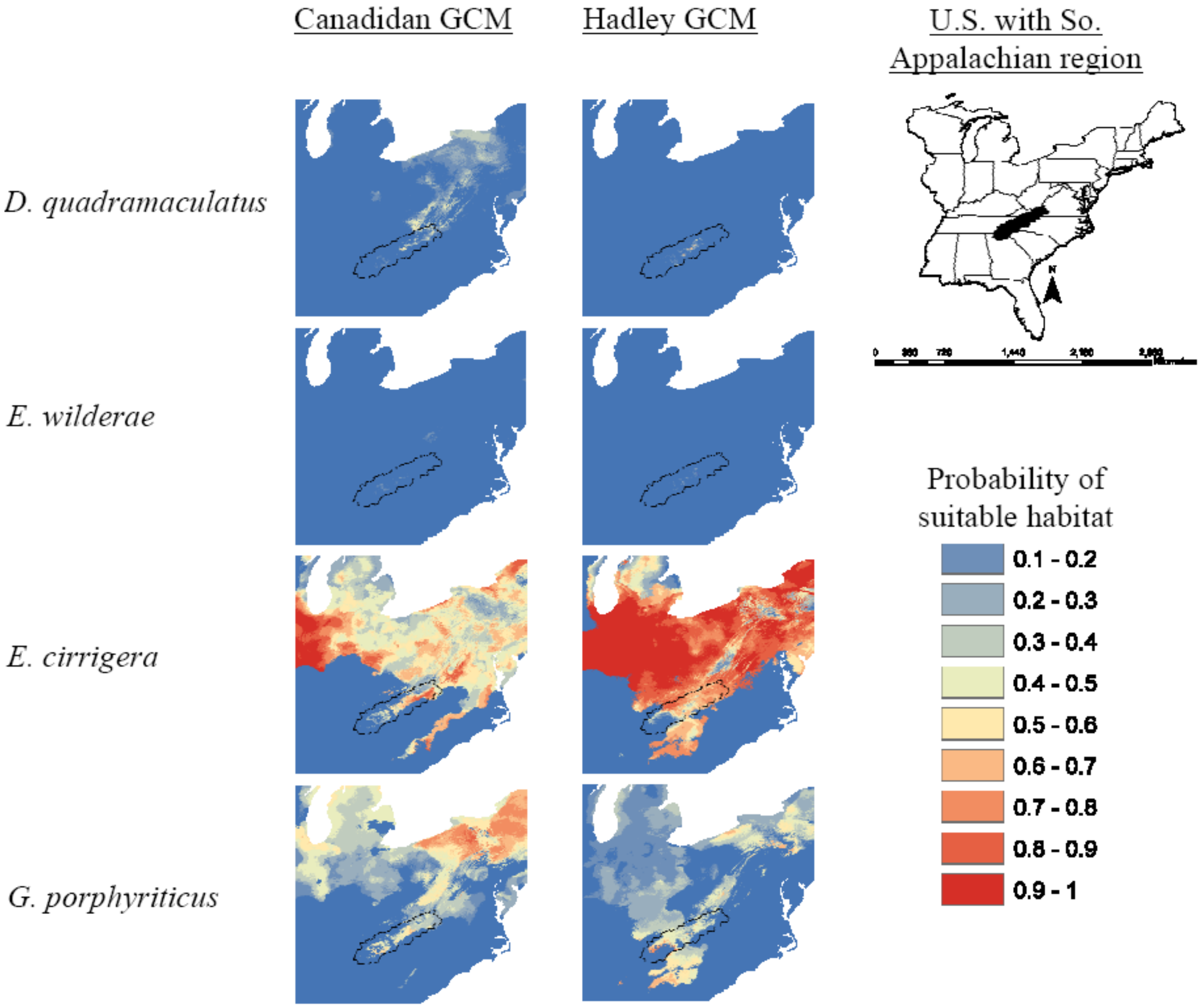

Figure 4.1. A series of maps depicting the probability of suitable climatic habitat of *D. quadramaculatus*, *E. wilderae*, *D. ocoee*, and *G. porphyriticus* for the year 2050. Probability of suitable climatic habitat in 2050 (i.e., 2050 distributions) were taken from Milanovich (Chapter 3) and were modeled using a climatic scenario which utilized both the Canadian Centre for Climate Modeling and Analysis Coupled Global Climate Model (CGCM3; (Flato and Boer 2001) and Hadley Centre for Climate Prediction and Research (HADCM3) assuming a high CO_2 emissions scenario. See Milanovich Chapter 3 for detailed model descriptions.

CHAPTER 5

GENERAL CONCLUSIONS

Despite the prediction that biota in freshwater systems will decline far faster than biota in terrestrial systems (Dudgeon et al. 2006) few attempts have been made to understand how the loss of consumers will affect ecosystem function in headwater streams. Evidence from the tropics suggests that a loss of dominant consumers could significantly alter stream function (Whiles et al. 2006, Connelly et al. 2008, Colon-Gaud et al. 2009), and considerable breakthroughs have been made to help understand the role of consumers and consumer richness in temperate headwater streams (reviewed in Covich et al. 2004, Kominoski et al. 2009, Lecerf and Richardson 2009). However, studies addressing this issue in temperate streams have focused primarily on invertebrates (e.g., Dangles and Malmqvist 2004, Boyero 2007). This dissertation was an attempt to examine the ecological role of a dominant vertebrate consumer group that inhabits headwater streams in Appalachia, and to determine how projected shifts in salamander communities as a consequence to changing climate, may influence their ecological role. My hope was that by conducting studies examining plethodontids in the context of ecosystem function, this dissertation could help clarify the potential consequences of a vertebrate-consumer loss and create a framework for future studies to examine similar issues within the Appalachian Highlands. My objectives were to 1) describe the elemental stoichiometry and estimate the contribution of larval plethodontids to ecosystem processes of headwater streams (Chapter 2), 2) predict the influence of global climate change on plethodontid salamanders (Chapter 3), and 3) to experimentally test the consequence of a change in plethodontid salamander ensembles (primarily the loss of a dominant species) to ecosystem processes (e.g., biomass and standing crop of nutrients) in Appalachian headwater streams (Chapter 4).

In the first study chapter (2), I described the elemental stoichiometry of plethodontid salamanders, estimated the standing crop of nutrients of larval plethodontids in headwater streams, and provided a framework for future studies to estimate the contribution of plethodontids to nutrient recycling in headwater streams. No other studies to date have examined the elemental stoichiometry and ecological role of vertebrate consumers in temperate headwater streams of eastern North America. This research was also the first attempt to fully detail and compare the stoichiometry of plethodontids. Furthermore, this study was the first attempt since Burton and Likens (1975) to estimate the ecological role of plethodontids and is the first attempt to do so in the Appalachian Highlands, the region of their highest diversity. This study also estimated the density of larval plethodontids, which surprisingly has only been done in a select number of Appalachian streams (Peterman et al. 2008a, Peterman et al. 2008b). This research led to a number of significant findings that offer a foundation for future hypothesis testing. First, the stoichiometry of larval stream plethodontids, while varying among species, is similar to fish in that it seems to be driven by allocation of phosphorus for formation of bone. Secondly, the stoichiometry of excretory output for at least one species of stream plethodontid differs from that of many fish species. Larval *Desmognathus quadramaculatus* excrete similar ratio’s of N:P relative to their body N:P, but appear to contribute little to stream demand for N or P. Lastly, by combining estimates of density and ecological stoichiometry, this study provided evidence that vertebrate consumers store a significant level of limiting nutrients. Until we have measures of subsidies in the form of annual deposits of eggs produced from feeding on terrestrial prey, in-stream production of larval tissues, and ultimately export of materials with metamorphosis, we will not fully understand the effects of plethodontids on headwater nutrient dynamics.

Although Chapter 2 significantly adds to our understanding of plethodontids roles in headwater streams, this study is limited in scope by only examining the larval stage three species of stream-dwelling plethodontids located across 5 streams of southern Appalachia. Information regarding more species and life stages of plethodontids, and replication across a more heterogeneous area will undoubtably lead to a better understanding of plethodontids roles across different regions. Furthermore, a more detailed look at other aspects of the ecosystem (e.g., invertebrates, basal resources) coinciding with studies involving the stoichiometry of plethodontids will lead to a more complete understanding of how plethodontids influence ecosystem processes.

The second study chapter utilized species distribution models to predict the response of plethodontid salamanders in eastern North America to global climate change. I examined how various climatic scenarios (e.g., variation in global-circulation model, model threshold, time period, and CO_2 emissions level) might influence the suitable climatic habitat of 41 species of terrestrial and stream plethodontids as well as overall species richness in the Appalachian Highlands. By producing eight projected scenarios for every species and the overall richness, I created a broad spectrum of potential responses of each species to global climate change. Results of this study indicated that choice of model threshold and CO_2 emissions scenario affect short-term projected shifts in climatic distributions of species; however, these factors and the choice of global circulation model have relatively small affects on what is a significantly projected loss of habitat for many current Appalachian species. Furthermore, predictions based upon species richness maps under each projected climate scenario project declines in plethodontid richness in the southern Appalachian Highlands as early as 2020 – with predicted declines increasing over time. However, more optimistic scenarios that include a more liberal Maxent threshold or, for example, climatic scenarios that assume low increase in atmospheric CO_2, predict portions of the central and northern Appalachians to retain richness levels similar to current patterns.

As is often the case, our modeling efforts were confounded by a limitation of data. For example, a number of species could not be modeled accurately simply due to an insufficient number of species locality points. Furthermore, lack of access to “new and improved” global circulation models confounded the results of this study. Although we utilized the best available models, our understanding of the patterns of global climate change is growing rapidly, and as a consequence our predictions would most certainly be improved with “new and improved” models. Lastly, one limitation of this chapter was the lack of data to build mechanistic species distribution models, which incorporate physiologically limiting mechanisms of species to environmental change, and are thus considered a more precise prediction of species responses to climate change (Robertson et al. 2003, Kearny and Porter 2009, Moran and Thuiller 2009).

Chapter 4 describes the use of field cages that were built into a stream to test whether *Eurycea wilderae* would show compensatory survival or growth in the absence of *D. quadramaculatus* and whether a replacement of *D. quadramaculatus* by *G. porphyriticus* would compensate for loss or if it would reduce *Eurycea* or total salamander biomass. In this study following two 60-day experiments, I incorporated the earlier results of Chapter 3 by designing the experiment around predictions of the response of plethodontids at the Coweeta LTER site to global climate change, while utilizing previously obtained nutrient data (Chapter 2) to help quantify the change in the standing crop of nutrients. I hypothesized that the losses of *D. quadramaculatus* and *E. wilderae*, the two currently dominant taxa at Coweeta, could be compensated for by an increase in *G. porphyriticus* and *E. cirrigera*, respectively - two species that were predicted to have a high probability of suitable climatic habitat in the Appalachian Highlands. An increase in *G. porphyriticus* was able to compensate, i.e., maintain equal or greater biomass and standing crop of nutrients, for the loss of biomass and standing crop of nutrients by *D. quadramaculatus*. However, this was entirely dependent upon the predicted increase of *G. porphyriticus* density to equal that of current *D. quadramaculatus* density. Any future scenario with a loss of *D. quadramaculatus* and *E. wilderae* that did not include a significant increase in *G. porphyriticus* density could result in a net loss of salamander biomass and standing crop of limiting nutrients.

Limitations of this chapter stem from a lack of understanding concerning intraguild competition of larval plethodontid salamanders. For example, the design of this study was based upon the prediction that *G. porphyriticus* will increase in density in future decades. However, we have no evidence to support such an increase will occur.

In fact, we currently can only speculate on why *G. porphyriticus* is not as abundant as *D. quadramaculatus*, and have no empirical evidence describing what intraspecific interactions may arise with an increase in *G.* porphyriticus – both of which limit the inferences that can be drawn from this study.

The chapters of this dissertation suggest that vertebrate consumers are significant contributors to the ecological function of Appalachian headwater streams and that the loss of currently dominant species could lead to a significant reduction in certain ecosystem processes. This dissertation should also provide a basic framework for more detailed studies examining the ecological role of plethodontids and the response of plethodontid physiology and stoichiometry to global climate change. It is my hope that this dissertation could be used to inspire conservation measures for headwater streams by accounting for the influence of biota to stream processes and understanding the consequences of a loss to species richness.

Future research potential

This dissertation represents one of a limited number of attempts to improve our understanding of the ecological role of plethodontid salamanders and amphibians more generally. This dissertation has made the first steps towards understanding larval plethodontids role in headwater streams; however, this work only represents a snap-shot of data pertaining to the ecological role of plethodontids in one region across a short time period. Furthermore, although our modeling efforts were conducted with the best available models given our limitation of data, predictions of plethodontid distributions resulting from global climate change scenarios can undoubtedly be improved. I would like to propose a series of “next steps” that could help improve our understanding of plethodontids role in ecosystem function, their predicted response to global climate change, and the influence of global climate change on plethodontid salamander ecosystem function.

First, one set of metrics I believe will greatly enhance our understanding of plethodontid roles in ecosystems, and the larger framework of ecosystem function in headwater streams, would be the addition of ecological stoichiometry data from more biota, across more locations, and various time scales. For example, conducting replicated studies examining the ecological stoichiometry of the same or similar species, their prey, and basal resources across seasons and regions such as the southern and northern Appalachians, northern mixed-hardwood forest, and southern pine forest would improve our understanding of plethodontids roles in ecosystems. For example, with respect to plethodontid salamanders, questions such as: 1) how does diet influence whole-carcass and excreta stoichiometry and does the affect of diet change with season, location, or ontogeny?; 2) do plethodontid species differ in the rates or ratios at which they recycle nutrients and do these rates or ratios change across regions, or seasons?; and 3) are plethodontids important to the subsidies, or transfer of limiting nutrients in headwater streams? These questions should be a priority for ecosystem ecologist. Plethodontids in the eastern United States are extremely abundant and diverse, and are known and predicted to respond to anthropogenic and natural environmental change. By knowing what factors influence plethodontids ecological role (e.g., their rates and ratios of nutrient recycling) and by knowing the overall importance of plethodontids to the transfer and retainment of nutrients in ecosystems can be used to predict the consequence of plethodontid loss.

Another key question is how plethodontids affect nutrient fluxes and whether they are net terrestrial or aquatic subsidies. Researchers have estimated that southern Appalachian plethodontids could consume more kcal/m^2 than soil invertebrates and that by regulating richness and abundance of invertebrates that consume leaf litter salamanders could be important in the retention of nutrients over time (see review in Davic and Welsh 2004). Plethodontids may also serve as a net influx of nutrients into systems as well. For example, Regester and Whiles (2006) found Ambystomatid salamanders imported a significant amount of nutrients compared to what they exported in temporary pond systems. This dissertation shows larvae of stream-dwelling plethodontids occur at high densities in southern Appalachian headwater streams; however, once transformed, many of these larvae leave the stream entirely and remain in terrestrial habitats for much of their lives – only returning to headwater streams to breed. Questions of importance include: 1) do plethodontids input more nutrients into headwater streams via egg deposition compared to what leaves the streams via metamorphisis?; or 2) does the life or natural history of particular species dictate whether or not they are significant terrestrial or aquatic subsidies? Questions of this nature are important to address given the predicted decline in suitable climatic habitat for many Appalachian plethodontids.

Third, much work is still needed regarding the metabolic physiology and physiological response of plethodontid salamanders to temperature. For example, for most species the metabolic response to temperature and that limits affects a species' metabolism or limits its distribution are still unknown (but see Bernardo and Spotilia 2006 and Bernardo et al. 2007). Work of this nature can be utilized in a number of ways to support our understanding of the ecological role of plethodontids and how they may change across climatic gradients or in response to climate change. For example, combining ecological stoichiometry and bioenergetic data can inform our understanding of the threshold elemental ratios of plethodontid salamanders, or the dietary mixture where growth limitation switches from one nutrient to another (Sterner 1997). By calculating TER's of plethodontids for limiting nutrients we can provide data on the imbalances between plethodontids and their prey, which can be used to help predict and quantify plethodontids rates and ratios of nutrient recycling. Our existing, and hopefully growing, stoichiometric dataset on plethodontids can be combined with experimental data showing how temperature influences ingestion, egestion, accumulation, excretion, and physiology (e.g., O_2 consumption and meatabolic rate) of plethodontids to quantify the accumulation and excretion of limiting nutrients by larval stream salamanders as a function of temperature. With this knowledge of how the physiological and population dynamics of larval plethodontids are influenced by temperature, we can remodel the biomass and productivity of larval salamanders as a consequence of temperature. Ecological stoichiometry, metabolic physiology, and mechanistic species distribution models can be combined to create a spatially explicit characterization of nutrient dynamics within the larval plethodontid ensembles under a number of predicted climate scenarios across a number of future decades. This could be used to help predict the gain or loss of plethodontid ecosystem function in response to global climate change.

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