EFFECTS OF RESOURCE MANIPULATION ON SELECTED PRIMARY AND SECONDARY CONSUMERS IN TWO DETRITUS-BASED SOUTHERN APPALACHIAN STREAMS

by

BRENT ROBERT JOHNSON

(Under the Direction of J. BRUCE WALLACE)

ABSTRACT

This research was conducted in the context of a larger experiment aimed at assessing the effects of long-term litter exclusion in an Appalachian headwater stream ecosystem. The exclusion experiment provided an opportunity to examine effects of resource reduction on consumers. Mark-recapture and dietary studies were conducted to determine the effects of litter exclusion on the larval salamander *Eurycea wilderae*, a vertebrate stream predator. Litter exclusion resulted in significantly reduced salamander density, individual growth, biomass, and production. Reduced density in the treatment stream likely results from drift of hatchlings, whereas a dietary shift may be responsible for reduced growth. Salamanders in the treatment stream had fewer prey per gut and switched from primarily consuming copepods to relying more on non-tanypodine midges. There was no difference in salamander movement among streams. Results from a downstream recovery reach were often intermediate for measured parameters, indicating residual effects from upstream treatment.

*In-situ* chambers were used to measure effects of litter exclusion on growth of two dominant insect detritivore groups. Larval *Tallaperla* spp. were chosen as representative

shredders and non-Tanypodinae Chironomidae were selected as representative collector-gatherers. Comparison of significant regression lines showed growth of both taxonomic groups were significantly lower in the litter exclusion stream. *Tallaperla* spp. mortality was also higher in the treatment stream. These results show that reduced growth is partially responsible for declines in detritivore production in the treatment stream and that chironomid production is actually lower than previously reported.

Nutrients were added to another headwater stream to measure effects of enrichment in a detritus-based ecosystem. A mark-recapture study was conducted on larval *E. wilderae* to measure effects of enrichment on growth of a representative vertebrate predator. Compared to pre-treatment, growth rates were significantly higher in both reference and treatment streams during the treatment period, indicating interannual growth variation. Growth rates in the nutrient addition stream, however, were significantly higher than the reference stream during treatment. Larvae prey heavily on copepods and chironomids that would respond quickly to enrichment. These preliminary results indicate that enrichment in detrital systems may indirectly affect higher trophic levels in ways similar to living-plant based systems.

INDEX WORDS: Stream, Detritus, Nutrients, Growth, Production, *Eurycea wilderae*, Larvae, Chironomidae, Salamander, Diet, Movement, Macroinvertebrates, Mark-recapture, Enrichment, Limitation, Density

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M.S., Marshall University, 1995

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

2002

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ACKNOWLEDGEMENTS

I am first very grateful to Dr. J. Bruce Wallace for giving me the opportunity to be a part of such wonderful research projects. His knowledge and enthusiasm are truly inspiring.

Thanks to the rest of my committee members for their guidance and reviews of this dissertation: Drs. Judy Meyer, Darold Batzer, Wayne Berisford, and Cathy Pringle. Very special thanks go to John Hutchens and Wyatt Cross for their manuscript reviews, assistance in the field and lab, and for the many helpful discussions. I am also very grateful for the wonderful artwork provided by Erica Chiao.

Many others assisted with various aspects of these projects, including Eric Romanizyn, Sue Eggert, John Benstead, Allison Roy, Danny Fendley, and Alex Huryn. I appreciate the financial support from National Science Foundation and the Departments of Biology and Entomology. Thanks also to everyone and in the Wallace lab and the Entomology Department for making my stay at Georgia so enjoyable. I am especially thankful for the love and support of my wife Andrea and the rest of my family.

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# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

Detritus, consisting of non-living organic matter and its associated microflora, is a pervasive component of aquatic and terrestrial ecosystems. In fact, from 70 to 90% of all global primary production eventually enters detrital pathways (O'Neill and Reichle 1980, Pomeroy 1991, Wetzel and Ward 1992). As a result, detritus often provides the major fuel driving ecosystems because most consumers rely either directly or indirectly on detritus as a food resource (Odum and de la Cruz 1963, Fisher and Likens 1973, Wetzel 1995). Numerous studies have demonstrated tight linkages between detritus and consumers in agricultural systems (Hendrix et al. 1986, 1992), forests (Blair et al. 1994, Arpin et al. 1995, Chen and Wise 1999), intertidal zones (Bustamante et al. 1995, Bustamante and Branch 1996), oceanic islands (Polis and Hurd 1995), and streams (e.g., Wallace et al. 1982, Webster 1983, Cuffney et al. 1984, Cuffney et al. 1990, Richardson 1991, Wallace et al. 1997, 1999).

Despite the prevalence of detritus in ecosystems, the foundations of food web theory are grounded in the traditional grazing food chain (Hairston et al. 1960, Fretwell 1977, Oksanen et al. 1981). Primary production has been implicated as the major factor determining the number of trophic levels and consequently whether plants or consumers have primacy in structuring food webs (Hunter and Price 1992, Power 1992*a*). Many enrichment and limitation experiments have assessed the relative strengths of resources (bottom-up) and consumers (top-down) in structuring grazing food webs. For example, numerous studies have investigated the effects of nutrients in autotrophic streams

(e.g., Peterson et al. 1985, 1993, Hart and Robinson 1990, Rosemond et al. 1993, Harvery et al. 1998, Biggs 2000) and lakes (see Elser et al. 1990 for review). In general, nutrient increases result in increased primary production (e.g., Peterson et al. 1985, Hart and Robinson 1990, Rosemond et al. 1993, Biggs 2000) that can potentially increase production at higher trophic levels (e.g., Johnston et al. 1990, Deegan and Peterson 1992, Peterson et al. 1993, Harvey et al. 1998).

Manipulative experiments in autotrophic systems have also provided examples of strong indirect effects that result in trophic cascades in both aquatic (e.g., Power 1990*a*, 1990*b*, Carpenter and Kitchell 1993, Flecker and Townsend 1994, Kohler and Wiley 1997, Huryn 1998, Nakano et al. 1999) and terrestrial (e.g., Spiller and Schoener 1990, Carter and Rypstra 1995, Hartvigsen et al. 1995, Chase 1996, Floyd 1996, Moran et al. 1996, Letourneau and Dyer 1998) ecosystems, thus lending further support to conventional theory and predictive models developed for grazing food webs. It has been argued, however, that such cascades may be the exception rather than the rule (Strong 1992, Polis 1994, Polis and Strong 1996, Polis et al. 1997), and ecologists now realize that both bottom-up and top-down effects are important in shaping community structure and that these forces can vary in space and time (Hunter and Price 1992, Power 1992*b*, Peterson et al. 1993, Rosemond et al. 1993, Batzer 1998).

Manipulative experiments in detritus-based ecosystems are far fewer in number. Therefore we know less about indirect effects and the relative strength of resources and consumers in shaping community structure. Detritus-based ecosystems are fundamentally different from plant-based systems because they are typically donor-controlled, with the basal resource subsidized from outside the system. Consumers in

detrital food webs therefore have no direct control over the quantity or quality of the food resource entering the system. In addition, omnivory, multiple food web links, and low interaction strengths may be common in detrital food webs and thus prevent the simplified cascading trophic interactions inherent in traditional grazing models (Polis 1994, Polis and Strong 1996).

Increasing evidence suggests that all trophic levels in detritus-based ecosystems can be resource limited (Richardson 1991, Wallace et al. 1997, 1999, Batzer 1998). Short-term nutrient addition experiments have shown increases in microbial production (e.g., Suberkropp and Chauvet 1995, Suberkropp 1998, Tank and Webster 1998), increased organic matter decomposition rates (e.g., Elwood et al. 1981, Meyer and Johnson 1983, Robinson and Gessner 2000), and increases in invertebrate abundance and biomass (Pearson and Connolly 2000, Robinson and Gessner 2000). Manipulations of detritus quantity have also shown to directly affect invertebrate consumer growth, abundance, and biomass (Rasmussen 1985, Richardson 1991, Polis and Hurd 1995, Wallace et al. 1997, 1999, Chen and Wise 1999), thus further supporting the theory of bottom-up limitation. However, the effects of nutrient enrichment on vertebrates in detritus-based systems remain unknown.

Few studies in detritus based systems have focused specifically on bottom-up effects of resource manipulation on higher trophic levels. Densities of predaceous arthropods have been shown to increase following enhancement of detritus in a forest floor community (Chen and Wise 1999), and on oceanic islands (Polis and Hurd 1995). In a long-term ecosystem level experiment, Wallace et al. (1997, 1999) found that reduction of detritus dramatically altered benthic community structure in an Appalachian

headwater stream and resulted in the lowest secondary production reported for streams. However, the mechanisms underlying the observed decrease in production remain uncertain. Wallace et al. (1997, 1999) also showed that predator production closely tracks prey production, indicating that top-down forces also play a key role in structuring the benthic community.

In addition, Wallace et al. (1997, 1999) documented reduced abundance, biomass, and production of larval salamanders, the top predators in high-gradient Appalachian headwaters. However, these studies combined all salamander species rather than considering responses of individual species, and the mechanisms underlying the reductions in larval salamander populations also remain unclear. Reduced abundance may be due to excessive drift from the study reach, reduced adult oviposition, or increased mortality, while lower secondary production can be due to reduced abundance, biomass, or individual growth rate. Furthermore, the results of Wallace et al. (1997, 1999) were based on benthic core samples that may not accurately assess larval salamander populations due to their mobility and difficulty of detection.

The research presented in this dissertation encompasses a range of studies dealing with the effects of resource manipulation on benthic fauna in detritus-based streams. The studies were conducted in three headwater streams (Catchments [C] 53, 54, and 55) at the Coweeta Hydrologic Laboratory in the southern Appalachian Mountains. Much of the research is a continuation of studies by Wallace et al. (1997, 1999) dealing with long-terms effects of detritus limitation in C55, while one project focused on the effects of nutrient enrichment in C54. An attempt to understand specific mechanisms

responsible for changes in the benthic community is the underlying theme of this work and all of the studies therefore include measurements of individual growth rate.

In Chapter 1, I provide the methodology I used to measure population parameters for larval *Eurycea wilderae* Dunn, the Blue Ridge two-lined salamander. *E. wilderae* was chosen as a representative vertebrate stream predator. Mark and recapture studies are the best way of estimating amphibian growth, movement, and density. This method provides the additional benefit of yielding growth estimates for free ranging larvae without possibility of chamber effects. However, when I started this project there was no practical method for long-term marking of *E. wilderae* larvae due to their small size, habitat, ability to regenerate, and the delicate nature of their skin. Subcutaneous injection of acrylic polymers has been used successfully in long-term marking of fish (Kelly 1967, Lotrich and Meredith 1974) and adult amphibians (Ireland 1989, Wooley 1973). This method was tested for *E. wilderae* in the laboratory and then used in subsequent field trials (C53) to assess individual growth rates.

Chapter 2 is a comprehensive study of effects of long-term detritus exclusion on larval *E. wilderae*. A mark-recapture study was used to assess effects of litter exclusion on larval growth, production, density, and individual movement. Gut content analyses were also conducted to analyze effects of detritus limitation on larval diets and the results were used to calculate the trophic basis of production. The trophic basis of production estimates the prey groups that are most important for larval production, thus providing useful information on the stream food web and energy flow pathways. An additional aspect of this study was a comparison of *E. wilderae* population estimates generated

using the mark-recapture technique versus those generated by conventional benthic core sampling.

In Chapter 3, I focus on growth measurements for two numerically dominant insect detritivores, larval *Tallaperla* spp. (Plecoptera: Peltoperlidae) and non-Tanypodinae chironomids (Diptera: Chironomidae), in an attempt to determine the mechanism for reduced production resulting from litter exclusion treatment (Wallace et al. 1997, 1999). Though both groups are detritivores, *Tallaperla* spp. feed on coarse particulate organic matter (CPOM), while chironomids feed on fine particulate organic matter (FPOM). Both CPOM and FPOM were significantly reduced following treatment and in-stream growth chambers were used to assess the effect of food limitation on larval insect growth rates.

Finally, Chapter 4 provides preliminary observations on effects of nutrient enrichment on larval *E. wilderae* growth. Nutrient enrichment studies are lacking for detritus-based ecosystems and the potential indirect effects on higher trophic levels are unclear. Nutrient addition could stimulate biofilms and lead to increased growth and production of invertebrate prey items. Alternatively, enrichment could lead to faster organic matter decomposition that could deplete food resources and ultimately limit production at higher trophic levels. Individual growth rates were measured for *E. wilderae* larvae to assess if it is an indicator of nutrient enrichment effects.

These studies will document how both limitation and enrichment of the detrital resource can affect species occupying higher trophic levels, and will therefore provide much needed information on how consumers respond to resource manipulation in detritus-based ecosystems. Results will also provide a valuable comparison for previous

manipulative studies in living-plant based systems and will thus add to our knowledge of food web theory.

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# CHAPTER 2

# *IN SITU* MEASUREMENT OF LARVAL SALAMANDER GROWTH USING INDIVIDUALS MARKED WITH ACRYLIC POLYMERS[1]

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[1]Johnson, B.R., and J.B. Wallace. 2002. *Herpetological Review*. 33:29-32. Reprinted here with permission of the publisher.

ABSTRACT

Long-term marking of larval salamanders has been problematic due to their small size and ability to regenerate appendages. We marked larvae of the Blue Ridge two-lined salamander, *Eurycea wilderae* Dunn, by subcutaneous injection of acrylic polymers. Individual growth rate was measured as part of a mark-recapture study in a 100-m headwater stream reach at the Coweeta Hydrologic Laboratory, North Carolina, USA. Larvae were collected monthly from November 1997 to April 1999 by sampling the entire wetted area at night using a headlamp and aquarium dip net. On each sample date, captured larvae were anesthetized with 0.1% MS-222, snout-vent length was measured, and each larva was given a unique mark. Mark duration was also tested in a laboratory aquarium. A total of 428 larvae were marked and released during the study period and 98 larvae were recaptured for growth estimates. Growth was nearly constant over the course of the year and the individual growth rate was 0.0024 mg $AFDM^{-d}$. Marking had no apparent effects on growth and survival in either the field or laboratory. Acrylic polymer injection provides a practical way to conduct field studies on amphibian larvae under natural conditions. The method can also be used to estimate population size, individual movements, and secondary production.

Mark-recapture studies are often used to provide valuable life history information for animal populations. However, long-term marking of larval amphibians has been problematic because of their small size, delicate skin, and ability to regenerate tissues (Cecil and Just 1978; Donnelly et al.1994; Seale and Boraas 1974). Procedures that have been used to mark larvae include fin-clipping (Turner 1960), whole-body staining with neutral red (Guttman and Creasey 1973; Herreid and Kinney 1966), injection of mineral oil and petroleum jelly mixtures (Seale and Boraas 1974), application of fluorescent pigments with gas pressure (Ireland 1989; Taylor and Deegan 1982) or heat brands (Ireland 1973), and application of a Congo red and dimethyl sulfoxide paste (Ireland 1989). However, these methods are cumbersome, inhibit growth (Travis 1981), do not produce unique marks, or are only useful for short-term studies. Passive integrated transponder (PIT) tags have been used to mark newts and spadefood toads (Fasola et al. 1993; Jehle and Hodl 1998) as well as metamorphic ambystomid salamanders (Ott and Scott 1999). However, these tags are too large for many larval amphibians. Toe-clipping has been the traditional method for marking amphibians (Ferner 1979), but some larvae may regenerate toes too quickly (<1 mo.) for use in long-term studies. In addition, larvae sometimes suffer toe injuries that make identification difficult (Ott and Scott 1999). As a result of these problems, field experiments that require the individual identification of amphibian larvae have been limited.

Subcutaneous injection of acrylic polymers is a method that has been successful for long-term marking of fish (Kelly 1967; Lotrich and Meredith 1974), adult salamanders (Ireland 1989; Wooley 1973), and tadpoles (Anholt et al. 1998; Cecil and

Just 1978). These polymers are non-toxic and the marks can remain visible for a year or more. Each individual can also be given a unique mark by using several different colors and multiple mark locations. This method is efficient and cost-effective. However, it has yet to be tested on larval salamanders.

Larval salamanders are an important component of first order high-gradient streams because they are often the top predators in these fishless systems. The Blue Ridge two-lined salamander, *Eurycea wilderae* Dunn, is the most abundant salamander larva in the headwaters of the study region (Bruce 1985) and is the focus of our study. In these streams, hatchlings of *E. wilderae* appear in April or May, and the larval stage lasts one or two years (Bruce 1982, 1985, 1988; Voss 1993). The specific objectives of our study were to evaluate the polymer injection method in field trials and to use mark-recapture data to determine individual growth rates of larval *E. wilderae*.

This study was conducted in a first order stream that drains catchment 53 (C53) at the Coweeta Hydrologic Laboratory, Macon County, North Carolina. Vegetation consists of mixed hardwoods with a dense riparian understory of rhododendron (*Rhododendron maximum*, L.). This stream was treated with the insecticide methoxychlor in 1980 to examine the role of invertebrates in processing organic matter (Cuffney et al. 1984; Wallace et al. 1982; Wallace et al. 1986). However, following treatment, the stream quickly recovered to reference conditions ( Lugthart and Wallace 1992; Wallace et al. 1982; Wallace et al. 1997). Detailed descriptions of the Coweeta basin are provided by Swank and Crossley (1988). In addition to *E. wilderae*, four other salamander species are common in the headwaters at Coweeta: *Desmognathus quadramaculatus*, *D. monticola*, *D. ocoee*, and *Gyrinophilus porphyriticus*.

The entire wetted area of a 100-m reach was sampled for *E. wilderae* larvae approximately every month from November 1997 through June 1999. Flagging tape was placed at 5 m intervals to determine capture location. The stream was sampled at night with a head lamp and aquarium dip net (15.5 × 12 cm). Only loose cover objects (e.g. cobble, wood, leaves) were turned over when searching for larvae to minimize disturbance to the stream. Captured larvae were placed in individual 20 mL plastic vials filled with stream water. Vials were labeled with the nearest meter mark and placed on ice in a cooler.

In an on-site laboratory, each larva was anesthetized in Petri dishes containing 0.1% tricaine methylsulfonate (MS-222) (Beachy 1994). Snout-vent length (SVL) was measured to the posterior margin of the vent to the nearest 0.5 mm using a dissecting scope and vernier calipers. The anesthetized larvae were then placed on a damp paper towel under a dissecting scope. A Liquitex ® acrylic polymer emulsion (available at most art supply stores) was injected into the tail using a 3 cc syringe with a 26 gauge 16 mm needle as described by Cecil and Just (1978). Initially, six different colors were used: blue, green, red, white, purple, and yellow. However, yellow was abandoned because it was difficult to see. Marks were inserted just under the skin of the tail immediately behind the hind legs. This insertion point left a mark that was visible if the tail was subsequently lost. Two discrete marks could be placed on either side of the tail, a feature that allowed the same color to be used twice on one side (Fig. 2.1). Marking newly hatched larvae (<14 mm SVL) was more difficult but still possible. After some practice, each larva could be measured and marked within five min. The five colors and four mark locations allowed for ca. 3,000 unique combinations. Following placement of a mark,

larvae were revived in stream water, returned to their plastic vials, refrigerated overnight, and released at the point of capture the following morning or evening.

To determine growth, SVL was first converted to ash-free dry mass (AFDM) using a length-weight regression derived for *E. wilderae* in the study stream:

$$M = 0.0023L^{3.09} \quad (r^2=0.96, p<0.001, n=22)$$

where M is larval mass (mg AFDM) and L is SVL (mm) (Lugthart 1991). Ash-free dry mass is a more accurate measure of growth than SVL or wet weight because it accounts for inorganic matter in guts. Individual daily growth rate (g) was then calculated as:

$$g = \ln(M_2/M_1)/t$$

where $M_1$ = initial larval mass, $M_2$ = final larval mass, and t = time interval in days (Romanovsky and Polishchuk 1982). Exponential growth was assumed. Average growth was also determined by plotting the mean biomass by sample date beginning after the hatchlings appeared in May 1998. In addition, we tested for differences in mean SVL of marked and unmarked larvae for each sample date throughout the study period using t-tests. We used a Mann-Whitney U-test when data failed to meet the assumption of normality.

To assess mark duration, 20 larvae were collected from an adjacent headwater stream and returned to the laboratory. These larvae were marked following the procedure described above and maintained in an aerated 37.9 L aquarium in a laboratory cold room at 5ºC. Though this temperature is lower than the average annual temperature in the stream, these temperatures are encountered in winter and the larvae remain active. Moss-covered stones were collected from the stream and added to the aquarium for refuge. Water was changed weekly and larvae were fed freeze-dried *Daphnia* sp. Larvae were

checked regularly for any fading of marks or mortality. After one year, surviving larvae were returned to the stream where captured.

A total of 428 *E. wilderae* larvae were captured, marked, and released during this study. Ninety-eight individuals were recaptured and used for growth determination (~23% of the total marked). Of the 98 recaptured larvae, 32 were recaptured on more than one sample date. Though growth slowed slightly in the winter, growth was nearly linear over the year (Fig. 2.2). The average individual daily growth rate for *E. wilderae* larvae was 0.0024 $d^{-1}$ (± 0.0002 S.E).

Given their delicate appearance, the larvae were surprisingly durable during marking. They typically recovered from the anesthetic within ten minutes of returning to vials of stream water. A poorly placed mark resulted in the death of one larva, but all others were alive at the time of release with no apparent change in behavior from pre-marking. Larvae were recaptured up to eight months after marking (Fig. 2.3). The average biomass and SVL of marked larvae was not significantly different from unmarked larvae on all but one date ($p>0.05$)(Fig. 2.2) when recapture sample size was low (n=1). This indicates that marking had little, if any, effect on growth. In the laboratory aquarium, all 20 marked larvae survived at least six months and 17 survived for a full year. Fading of marks was sometimes evident in both the field and laboratory, but individual identification was always possible.

Amphibian larvae typically show reduced growth during winter (Beachy 1997), but our results show that *E. wilderae* growth was nearly linear throughout the year. Beachy (1997) found a similar linear growth pattern for larval *E. wilderae* in cage experiments. Our daily individual growth rate of 0.0024 $d^{-1}$ agrees closely with a

previous study that determined *E. wilderae* larval growth using chambers in a nearby headwater stream. In that study, the average individual growth rate from three different field trials was 0.003 $d^{-1}$ (Lugthart 1991). However, results from chamber experiments must be interpreted with caution because larval densities and prey availability can influence growth rate (Petranka and Sih 1986; Scott 1990; Smith 1990).

By using acrylic marks, we were able to determine growth rates for free-ranging larvae. Travis (1981) found that whole body staining with neutral red reduced growth of tadpoles and warned that any marking method might adversely affect growth. However, the acrylic polymers are non-toxic and there were no discernable differences in SVL or biomass between marked and unmarked larvae by sample date. There is therefore no evidence to indicate that marks affected growth.

The high survival rates of marked larvae both in the field (up to eight months after marking) and laboratory (up to one year) also indicated the marks had a negligible effect on survivorship. The laboratory survival rate of 85% after 1 yr was much higher than those of investigators who have worked with *Eurycea* spp. in artificial streams over shorter time periods (27% over ~ 4 mos. in Resetarits 1991; 39% over ~2 mos. in Gustafson 1993; and 31% over ~3 mos. in Gustafson 1994). However, our higher survival rate could be partially attributed to reduced metabolic demand associated with lower laboratory temperature. The recapture time interval curve (Fig. 2.3) is indicative of the Type III survivorship curve (Deevey 1947) that has been previously documented for *E. wilderae* (Bruce 1988). The slope of this line may therefore be attributed to natural mortality or emigration rather than mark effects.

This study demonstrates the effectiveness of subcutaneous acrylic polymer injection for long-term monitoring of larval salamander populations. This method provides a practical and cost-efficient way to conduct field studies of the top predators in small, fishless streams. Because unique marks can be generated quickly, the method can easily be used not only to assess larval growth, but also population size and individual movements. Individual growth data can also be used to determine secondary production (Benke 1984; Waters 1977). Mark-recapture studies offer several additional advantages over cage experiments. For example, growth can be determined under natural conditions with realistic prey levels. In addition, natural movements can be measured and thus add to our understanding of species colonization and dispersal.

According to Ferner (1979) an ideal mark or tag should: 1) not affect individual survivorship or behavior, 2) uniquely identify individuals, 3) remain over the individual' s lifetime, 4) be easily discernable, and 5) be easily applied in both field and laboratory studies. In addition, the marking or tagging method should be cost-effective and limit handling time of an individual (Ott and Scott 1999). Acrylic polymer injection meets most of these criteria and is currently the only viable method for *E. wilderae* larvae. A potential problem with this method is that the marks may increase larval visibility and thereby increase predation pressure from larger salamanders such as *D. quadramaculatus, D. monticola,* and *G. porphyriticus*.

Choice of an appropriate marking or tagging method should depend on the individual species, life history stage, and duration of the study period. Any one or a combination of polymer injection, PIT-tagging, and toe-clipping should be suitable for most amphibians. Polymer injection has proven an effective method for long-term

marking of amphibian larvae and small-bodied adults, while PIT-tagging and toe-clipping remain effective in marking larger animals. The latter two methods have the added benefit of near invisible marks. In some cases, it may be desirable to use a combination of marking methods on the same individuals. For example, polymer injection could be used to mark individuals throughout the larval stage and, following metamorphosis, PIT-tags or toe-clips could replace the polymer marks. Such a long-term study design would provide valuable life history information and potentially aid amphibian conservation efforts.

*Acknowledgements* **-** We thank E. Chiao for her artwork and J. Hutchens and W. Cross for their comments on earlier versions of the manuscript. This research was supported by National Science Foundation grant DEB-9629268.

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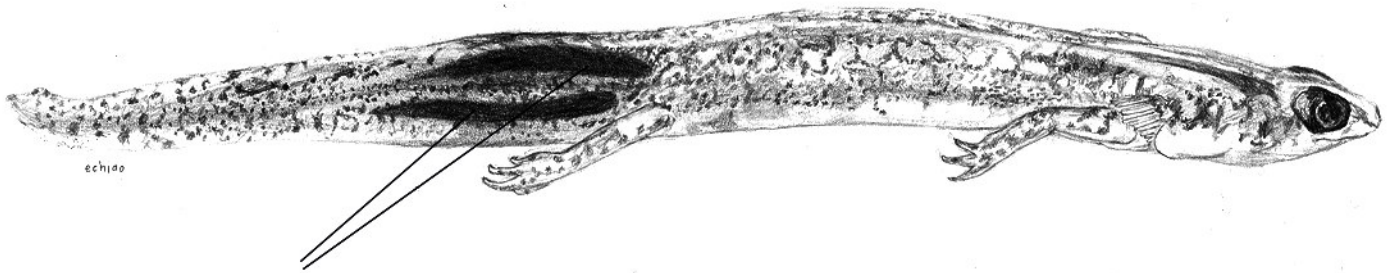
Figure 2.1. A marked *Eurycea wilderae* larva demonstrating the location of color bands.




Color Bands

1 cm

Figure 2.2. Average biomass (mg) for all marked and unmarked (± 95% C.I.) *Eurycea wilderae* larvae by collection date beginning when hatchlings first appeared in spring. Stream temperature is plotted as the average between sample events.

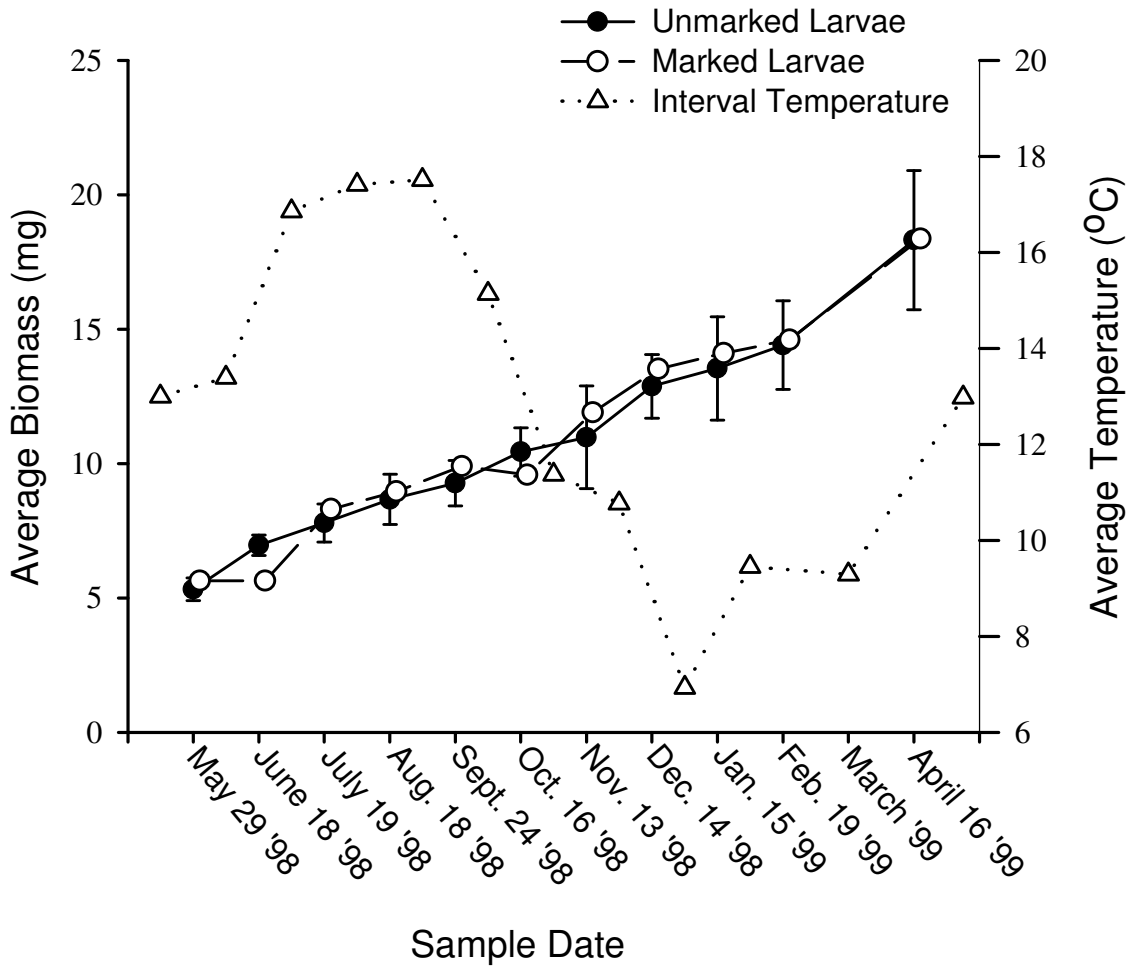
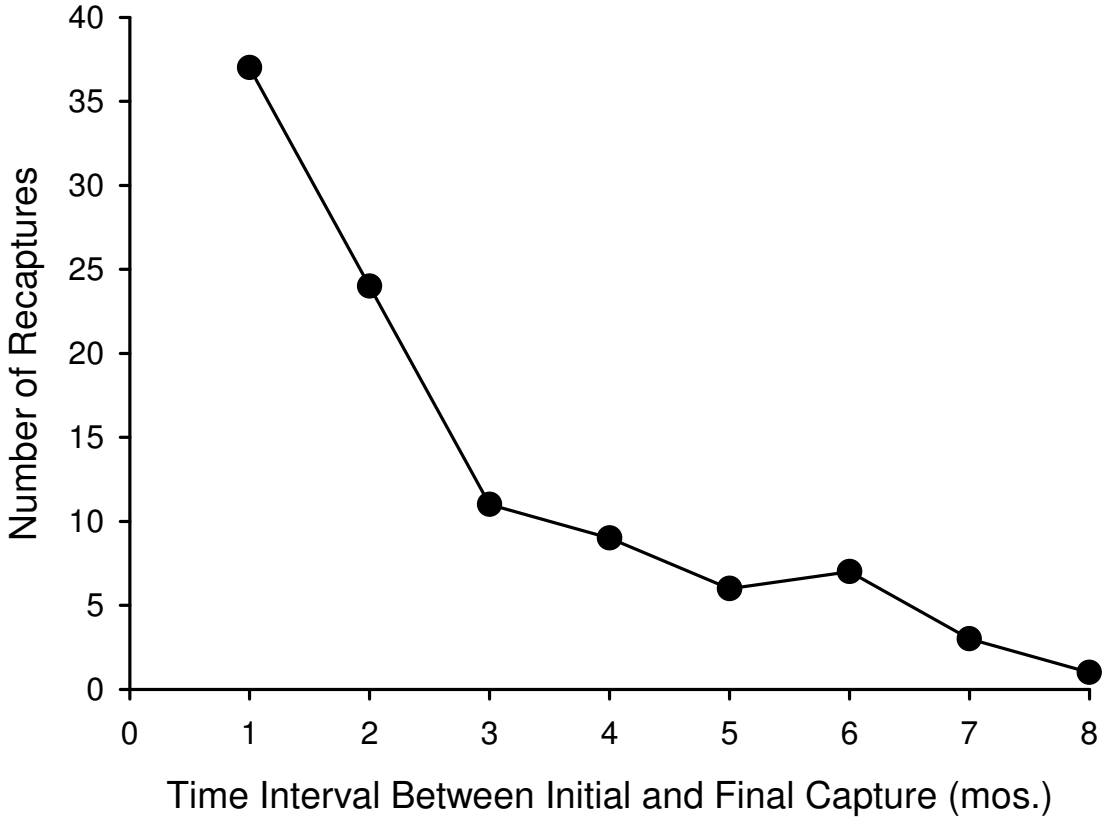

Unmarked Larvae
Marked Larvae
Interval Temperature
Average Biomass (mg)
Average Temperature (°C)
0
5
10
15
20
25
6
8
10
12
14
16
18
20
May 29 '98
June 18 '98
July 19 '98
Aug. 18 '98
Sept. 24 '98
Oct. 16 '98
Nov. 13 '98
Dec. 14 '98
Jan. 15 '99
Feb. 19 '99
March '99
April 16 '99
Sample Date

Figure 2.3. Time interval between initial marking and final recapture for all *Eurycea wilderae* collected from November 1997 to June 1999.


Number of Recaptures
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
Time Interval Between Initial and Final Capture (mos.)

# CHAPTER 3

# LONG-TERM DETRITUS LIMITATION AFFECTS A STREAM PREDATOR[1]

[1]Johnson, B.R., and J.B. Wallace. To be submitted to *Ecology*

ABSTRACT

Allochthonous organic matter was excluded from a southern Appalachian headwater stream beginning in fall 1993 to assess the role of detritus in stream ecosystems. Previous studies have shown that litter exclusion reduces abundance, biomass, and secondary production of benthic macroinvertebrates. We conducted a repeated mark-recapture study that examined growth, population density, production, and movement of a top predator, larval *Eurycea wilderae*, the Blue Ridge two-lined salamander. Salamander larvae were collected monthly from November 1997 to April 1999 from the litter exclusion stream, a nearby reference stream, and a "recovery" reach immediately below the exclusion stream. We collected *E. wilderae* larvae seasonally from each stream for one year for gut content analysis and compared diet and trophic basis of production among streams. In addition, we compared larval sampling methods by comparing mark-recapture estimates for *E. wilderae* abundance, biomass, and density with benthic core sampling estimates over the same period.

Daily growth rates for recaptured larvae were significantly lower in the litter exclusion stream than in the reference stream, and larvae in the litter exclusion reach were an average of 52% smaller than larvae in the reference stream after nearly one year. Even though *E. wilderae* production is low in these streams, production in the reference stream was more than five times higher than in the litter exclusion stream. Larval density and biomass were also significantly lower in the litter exclusion stream. Reduced density in the treatment stream is likely the result of excess drift of hatchlings in the spring due to

reduced prey availability. Marked larvae that were recaptured moved an average of only around 2 m in each stream and there were no movement differences among streams.

There was little similarity in overall diet among streams. Larvae in the litter exclusion stream switched from consuming mostly copepods to relying on more available non-Tanypodinae (Dipetera: Chironomidae) midge larvae. Larvae in the treatment stream also had significantly more nematodes and terrestrial insects in their diets, suggesting larvae had difficulty in finding prey. Midges alone accounted for more than half of *E. wilderae* production in the treatment stream whereas production in the reference and recovery reaches was more evenly distributed among other prey groups. *E. wilderae* larvae consumed very little of total annual production and therefore exert little top-down pressure on the benthic community. Larvae from the litter exclusion stream had significantly fewer prey items per gut than larvae from the reference stream, but there was no difference in prey biomass per gut among streams. Reduced growth of larvae in the treatment stream must therefore result from differences in prey quality or differences in energetic demands.

The downstream recovery reach was intermediate between reference and litter exclusion for many parameters (density, growth, biomass, production, P/B, and prey abundance) and indicates upstream treatment is affecting salamander larvae downstream. Mark-recapture and benthic core sampling produced very similar estimates of *E. wilderae* abundance, biomass, and production. However, the mark-recapture method was more accurate and provided better resolution in following larval population dynamics through time. This is the first comprehensive study of bottom-up limitation of a predator in a

detritus-based ecosystem and it provides further evidence of the tight linkage between detrital inputs and stream ecosystem structure.

# INTRODUCTION

Detritus, consisting of non-living organic matter and its associated microflora, is a pervasive component of aquatic and terrestrial ecosystems. In fact, as much as 70 to 90% of all global primary production eventually enters detrital pathways (O'Neill and Reichle 1980, Pomeroy 1991, Wetzel and Ward 1992). As a result, detritus often provides the major fuel driving ecosystems because most consumers rely either directly or indirectly on detritus as a food resource (Odum and de la Cruz 1963, Fisher and Likens 1973, Wetzel 1995). Numerous studies have demonstrated tight linkages between detritus and consumers in agricultural systems (Hendrix et al. 1986, 1992), forests (Blair et al. 1994, Arpin et al. 1995, Chen and Wise 1999), intertidal zones (Bustamante et al. 1995, Bustamante and Branch 1996), oceanic islands (Polis and Hurd 1995), and streams (e.g., Wallace et al. 1982, Webster 1983, Cuffney et al. 1984, Cuffney et al. 1990, Richardson 1991, Wallace et al. 1997, 1999).

Despite the prevalence of detritus in ecosystems, the foundations of food web theory are grounded in the traditional plant-based food chain (Hairston et al. 1960, Fretwell 1977, Oksanen et al. 1981). Primary production has been implicated as the major factor determining the number of trophic levels and consequently whether plants or consumers have primacy in structuring food webs (Hunter and Price 1992, Power 1992*a*). Many enrichment and limitation experiments have assessed the relative strengths of resources (bottom-up) and consumers (top-down) in structuring grazing food webs. These experiments have provided examples of strong indirect effects that result in trophic cascades in both aquatic (e.g., Power 1990*a*, 1990*b*, Carpenter and Kitchell 1993, Flecker

and Townsend 1994, Kohler and Wiley 1997, Huryn 1998, Nakano et al. 1999) and terrestrial (e.g., Spiller and Schoener 1990, Carter and Rypstra 1995, Hartvigsen et al. 1995, Chase 1996, Floyd 1996, Moran et al. 1996, Letourneau and Dyer 1998) ecosystems, thus lending further support to conventional theory and predictive models developed for grazing food webs. It has been argued, however, that such cascades may be the exception rather than the rule (Strong 1992, Polis 1994, Polis and Strong 1996, Polis et al. 1997), and ecologists now realize that both bottom-up and top-down effects are important in shaping community structure, and that these forces can vary in space and time (Hunter and Price 1992, Power 1992*b*, Peterson et al. 1993, Rosemond et al. 1993, Batzer 1998).

Manipulative experiments in detritus-based ecosystems are fewer in number; therefore we know less about indirect effects and the relative strength of resources and consumers in shaping community structure. Detritus-based ecosystems are fundamentally different from plant-based systems because they are typically donor controlled with the basal resource subsidized from outside the system. Consumers in detrital food webs therefore have no direct control over the quantity or quality of the food resource entering the system. In addition, omnivory, multiple food web links, and low interaction strengths may be common in detrital food webs and thus prevent the simplified cascading trophic interactions inherent in traditional grazing models (Polis 1994, Polis and Strong 1996).

Increasing evidence suggests that all trophic levels in detritus-based ecosystems can be resource limited (Richardson 1991, Wallace et al. 1997, 1999, Batzer 1998), but few studies have focused specifically on bottom-up effects of detritus on higher trophic

levels. Densities of predaceous arthropods have been shown to increase following enhancement of detritus in a forest floor community (Chen and Wise 1999), and on oceanic islands (Polis and Hurd 1995). In a long-term ecosystem level experiment, Wallace et al. (1997, 1999) found that reduction of detritus dramatically altered benthic community structure in a headwater stream and resulted in the lowest secondary production reported for streams. They also found that detritus limitation adversely affected predatory larval salamanders. This finding may be significant because stream predators can be heavily subsidized from outside the system and thus rely less on in-stream resources (Mason and MacDonald 1982, Nakano et al. 1999).

In high-gradient, fishless streams of the southern Appalachians larval salamanders are an important component of the food web because they are the top predators. Wallace et al. (1997, 1999) demonstrated reduced abundance, biomass, and production of larval salamander populations. However, these studies combined all salamander species rather than considering responses of individual populations. Additionally, the mechanisms underlying the reductions in larval salamander populations remain unclear. Reduced abundance may be due to excessive drift from the study reach, reduced adult oviposition, or increased mortality, while lower secondary production can be due to reduced abundance, biomass, or individual growth rate. Furthermore, the results of Wallace et al. (1997, 1999) were based on benthic core samples that may not accurately assess larval salamander populations due to their mobility and secretive nature.

The purpose of this study was to provide a comprehensive examination of the effects of long-term detritus reduction on salamanders using a mark and recapture design. The Blue-Ridge two-lined salamander, *Eurycea wilderae*, is the most common species in

headwater streams of the southern Appalachians (Bruce 1985), and is therefore the focus of this study. Mark-recapture studies can provide more accurate information about populations than benthic sampling because they can focus on individual responses over the duration of the larval period. Our specific objectives were to assess the effects of detritus limitation on *E. wilderae* population density, biomass, individual growth rate, movement, and secondary production. We also assessed *E. wilderae* diet and trophic basis of production to better understand energy flow and the mechanisms underlying any differences in the populations among streams. Finally, we compared the effectiveness of mark-recapture sampling with that of conventional benthic core sampling by comparing our estimates of annual density, biomass and production with benthic estimates covering the same period (J.B. Wallace, unpublished data).

### *Study Sites and Litter Manipulation*

This study was conducted in two perennial first-order streams at the Coweeta Hydrologic Laboratory (U.S. Forest Service) in Macon County, North Carolina. Coweeta is in the Blue Ridge Province of the southern Appalachian Mountains. The streams drain forested catchments dominated by mixed hardwoods including oaks (*Quercus* spp.), hickories (*Carya* spp.), and yellow poplar (*Liriodendron tulipifera*). Dense growths of rhododendron (*Rhododendron maximum*) result in heavy shading of the streams throughout the year. Detailed descriptions of the Coweeta basin are given by Swank and Crossley (1988).

The study streams drain catchments (C) 53 (reference), 55 (litter exclusion), and 56 (recovery). The 100-m reference stream (C53) was treated with the insecticide methoxychlor in 1980 to examine the role of invertebrates in organic matter processing

(Wallace et al. 1982, Cuffney et al. 1984), but following treatment the stream quickly returned to reference condition (Wallace et al. 1982, Lugthart and Wallace 1992, Wallace et al. 1997, 1999). Leaf litter inputs were excluded from the first 170 m of the treatment stream reach (C55) with an overhead net canopy (1.2-cm mesh) beginning in August 1993. Riparian vegetation was left in place during canopy installation to prevent changing the natural light regime. Plastic lateral drift fences (20-cm high with 1-cm mesh) were placed along each side of the treatment reach to prevent lateral inputs of coarse particulate organic matter (CPOM). A large space (>1-m) was left between the drift fence and overhead net canopy to allow aerial colonization by insects. Leaves were removed from the canopy once each week in autumn and when needed in other seasons. Small woody debris (<10-cm dia.) was then removed from the treatment reach in summer 1996 followed by removal of large woody debris (>10-cm dia.) in summer 1999. As a result of these manipulations, organic matter standing crop in the treatment reach has now been reduced by approximately 95% compared to pretreatment and to the reference stream (Wallace et al. 1999). The recovery reach (C56) extends 65-m immediately below the litter exclusion reach so this catchment includes that of the treatment reach (C55). This reach was included to assess how the *E. wilderae* population responds to upstream detritus exclusion and how quickly measured parameters return to reference conditions. The three stream reaches compared in this study have similar physical characteristics, including catchment size, substrate composition, discharge, and thermal regime (Table 3.1).

### *Biology of E. wilderae*

The southern Appalachians are home to a highly diverse salamander assemblage that resulted from an extensive species radiation within the family Plethodontidae (Hairston 1986, Bruce 1996, Tilley and Bernardo 1993). As a result of this radiation as many as seven salamander species can be found in the vicinity of headwater streams in this region. Species commonly encountered include *Desmognathus quadramaculatus*, *D. monticola*, *D. ocoee*, *Gyrinophilus porphyriticus*, and *Eurycea wilderae*. Larvae of these species are restricted to the stream where they feed on a wide variety of aquatic invertebrates (Caldwell and Houtcooper 1973, Burton 1976, Taylor et al. 1988, Lugthart 1991). In these streams *E. wilderae* has a one or two year larval stage with hatching and metamorphosis of older larvae occurring in late spring and early summer (Bruce 1982, 1985, 1988, Lugthart 1991). *E. wilderae* larvae are sometimes preyed upon by larvae of larger salamander species such as *D. quadramaculatus* and *G. porphyriticus* (Bruce 1979, Beachy 1993, 1994).

## METHODS

### *Salamander Sampling and Marking*

The entire wetted areas of the three study reaches (reference, treatment, and recovery) were sampled approximately every month from November 1997 through April 1999. Flagging tape was placed at 5-m intervals throughout each reach to determine capture location. Stream reaches were sampled at night, when salamander larvae are most active, using a small aquarium dip net (1-mm mesh) and headlamp. Due to several ongoing studies in these streams, only loose cover objects (e.g. cobble, wood, leaves) were turned over when searching for larvae to minimize stream disturbance. Captured

larvae were placed in individual 20-ml plastic vials filled with stream water. Vials were labeled with the point of capture to the nearest one meter and placed in a cooler with ice.

In the on-site laboratory, each larva was anesthetized in Petri dishes containing 0.1% tricane methylsulfonate (MS-222) (Beachy 1994). Snout-vent length (SVL) was measured from the tip of the snout to the posterior vent margin to the nearest 0.5-mm using a dissecting microscope (12X magnification) and vernier calipers. Anesthetized larvae were then placed on a damp paper towel under the dissecting scope for marking. Each larva was given a unique mark by injecting acrylic polymers (Liquitex® brand, Binney and Smith Inc., Easton, PA) into the tail using a 3-cc syringe with a 26 gauge 16-mm needle as described by Cecil and Just (1978). Up to two discrete marks could be placed on either side of the tail and five different colors were used: blue, green, red, white, and purple. The 5 color and 4 mark locations allowed for ca. 3,000 unique combinations. Duplicate marking combinations were used in the reference stream because there was no possibility of larval movement between catchments. Marks were inserted under the skin of the tail immediately behind the hind legs. This insertion point left the mark visible if the tail was subsequently lost. This marking procedure has proven to be an effective method for long-term marking of larval *E. wilderae* and has no adverse effects on growth or survival (Johnson and Wallace 2002). After marking, larvae were revived in stream water, returned to their plastic vials, refrigerated overnight, and released at the point of capture the following morning or evening. All larval salamander species were initially captured and marked by this method, but *E. wilderae* proved to be the only species with sufficient recaptures for subsequent population analyses.

*Population Density*

Mark-recapture data were used to generate monthly population size estimates for each stream reach using the Jolly-Seber full model (Jolly 1965, Seber 1965) included in the software package POPAN-5 (Arnason et al. 1998). The Jolly-Seber model is an open population model and therefore offers greater biological realism than closed models because it allows for additions and losses within the population during the study period. Accuracy is often sacrificed for realism in open population models because errors associated with population size estimates are generally larger than in related closed population models that have stricter assumptions (Arnason et al. 1998). However, because open models are more robust, investigators can have greater confidence that the true population size lies within in the error range. Monthly population size estimates were converted to density (individuals/$m^2$) by dividing by mean wetted area of the study reaches over the sampling period.

*Larval Growth*

We measured larval growth by two independent methods. We first compared mean biomass of all larvae collected on each sample date after hatchlings appeared in May 1998. Biomass was calculated by converting SVL to ash-free dry mass (AFDM) using the length-weight regression derived for *E. wilderae* in the study streams:

$$M=0.0023L^{3.09}$$

where M is larval mass (mg AFDM) and L is length (mm) (Lugthart 1991). Ash-free dry mass is necessary for production calculations and is a more accurate measure of growth that SVL or wet mass because it accounts for inorganic matter in guts.

Larval growth rates were also assessed using mark-recapture data for the entire study period. Individual growth rates ($g$) were calculated for all recaptured larvae as follows:

$$g = (\ln(M_f)-\ln(M_i))/t$$

where $M_i$ = initial larval mass (AFDM), $M_f$ = final larval mass (AFDM), and t = time interval in days (Romanovsky and Polischuk 1982). Only the initial and final weights were used for those larvae that were recaptured on multiple sample dates.

*Biomass and Secondary Production*

Secondary production is a valuable measure of energy flow through populations because it incorporates abundance, biomass, growth, reproduction, and survival (Benke 1984). However, studies of salamander production are rare (Spight 1967, Huryn and Wallace 1987, Lugthart and Wallace 1992, Wallace et al. 1997, 1999) due to difficulties with sampling and larval identification. Previous estimates at Coweeta have come from benthic studies (Huryn and Wallace 1987, Lugthart and Wallace 1992, Wallace et al. 1997, 1999). This study provided the unique opportunity to calculate salamander production based on mark-recapture data. *E. wilderae* production was calculated for the 1998-1999 cohort using the instantaneous growth method (Waters 1977, Benke 1984). This actual cohort method may provide more accurate measures of production than the non-cohort methods traditionally used (Benke 1984). Individual growth and density estimates were based on mark-recapture results whereas biomass (mg AFDM $m^{-2}$) was obtained by multiplying the population density estimate for each sample date by the mean weight of all larvae actually captured in the stream on the same date. Mean biomass, mean daily growth rate, and time interval in the sample period were then multiplied to

calculate interval production. Interval production estimates were then summed to get annual production (mg AFDM $m^{-2}$ $yr^{-1}$). Annual production was then habitat-weighted to account for the fact that larvae were not found on bedrock outcrops (Lugthart and Wallace 1992).

*Diet and Tropic Basis of Production*

Larval *E. wilderae* were not collected for diet analysis until the mark-recapture study was completed in spring 1999 to prevent depletion of populations. Approximately five larvae of the same cohort were collected seasonally from each stream reach beginning July 21, 1999 (summer). Other collection dates were October 22, 1999 (autumn), February 12, 2000 (winter), and May 23, 2000 (spring). Samples sizes were kept relatively small due to ongoing population studies in the reference stream. Seasonal samples were subsequently combined in each stream to increase statistical power for comparisons among streams. Larvae were collected at night and immediately placed in vials of Kahle's solution. In the laboratory, guts were removed under a dissecting microscope and their contents teased out and mounted on a slide with CMC-10 (Masters Company, Inc., Bensenville, IL). Insect taxa were identified to genus when possible except for chironomids, which were identified as either non-Tanypodinae or Tanypodinae. Non-insect taxa were identified to order. All prey items were measured to the nearest millimeter using an ocular micrometer. Prey biomass (AFDM) was then estimated using established length-mass or head width-mass regressions (Sample et al. 1993, Benke et al. 1999).

Percent similarities of larval diets were calculated using the method described by Whittaker (1975) because they provide a simple diet comparison among streams.

However, statistical differences among streams were assessed using the multi-response permutational procedure (MRPP). MRPP is a non-parametric multivariate test that has proven useful in comparing species composition data (Biondini et al. 1985, Zimmerman et al. 1985), and is included in the PC-ORD software package (McCune and Mefford 1999).

*E. wilderae* larvae are predators and their diet therefore consists of 100% animal prey. Because we lacked information on specific assimilation and production efficiencies of the different prey taxa, we assigned all prey groups an assimilation efficiency (AE) of 90% and a net production efficiency (NPE) of 75% based on previous studies of salamander energetics (Merchant 1970, Fitzpatrick 1973a, 1973b, Burton and Likens 1975). Although assimilation and production efficiencies can differ among species (Sweeney and Vannote 1981), these values should provide reasonable estimates for *E. wilderae* larvae. Gross production efficiency (GPE) was calculated as:

$$GPE = AE \times NPE$$

Trophic basis of production for *E. wilderae* was then estimated using calculations from Benke and Wallace (1980). Our calculation of trophic basis of production is different from most studies (e.g., Benke and Wallace 1980, 1997, Johnson et al. 2000, Hall et al. 2000) in that we only have animal prey items in the diet and all are considered to be of equal quality. The value of this approach is that it highlights prey groups that contribute most to salamander production and allows for a comparison of energy flows from prey to predator among streams.

### *Larval Movement*

Individual movement data can provide useful insight into larval behavior such as dispersal and foraging effort. The locations of recaptured larvae were compared to their original capture location to measure movement to the nearest meter.

### *Experimental Design and Statistical Analysis*

Ecosystem level experiments have an advantage over studies conducted on smaller scales because they more accurately account for environmental complexity and therefore have a level of biological realism that cannot be obtained from cage or mesocosm experiments (Carpenter et al. 1995, Schindler 1998). This is especially true for studies dealing with predators because enclosures can inhibit movement, provide unnatural prey densities (Cooper et al. 1990), and alter competitive interactions. Unfortunately, reduced statistical power is often the tradeoff for realism in ecosystem studies (Hurlbert 1984, Carpenter et al. 1989, Schindler 1998).

Because we lacked the appropriate replication required for most statistical tests, we compared stream reaches in most cases by using bootstrapped 95% confidence intervals (Johnson 1999). Bootstrapping is a nonparametric resampling method (Manly 1997, Effron and Tibshirani 1993) that is used to estimate the uncertainty of variables with unknown or complex frequency distributions and for situations in which logistical constraints do not allow sufficient replication. Data sets were bootstrapped by random resampling with replacement until 1000 data sets were produced. These recombined data sets were used to produce vectors of 1000 estimates for each parameter. The mean and approximate 95% confidence intervals were then calculated for each vector of estimates. If the estimates followed a normal distribution, normal 95% confidence intervals were

used. However, if the vector of estimates did not follow a normal distribution, we ordered the estimates and used the $25^{th}$ and $975^{th}$ estimates as the 95% interval boundaries (Blank et al. 1999). This often results in confidence intervals that are not symmetrical around the mean, but the intervals accurately represent variation in the data. Errors associated with annual secondary production estimates were calculated by bootstrapping all included parameters for each sampling interval (individual growth rate, biomass, and time) (Morin et al. 1987, Huryn 1996, 1998). Additional information about bootstrapping and its use in production studies are provided by Huryn (1996, 1998). Differences between mean values were considered significant ($p<0.05$) when 95% bootstrapped confidence intervals did not overlap, a conservative test for differences (Zar 1996, Johnson 1999). This method of comparison, however, prevents us from definitively stating that any differences between streams are due to treatment effects alone (Hurlbert 1984).

## RESULTS

### *Field Studies*

A total of 1018 *E. wilderae* larvae were captured in the three streams during the study period. The salamanders remained active in the streams throughout the year and there was no evidence of mark effects on larval behavior (B. Johnson, pers. obsv.). Most larvae were captured in shallow depositional areas; none were found on bedrock outcrops. Larvae were recaptured up to ten months after initial marking, a period covering the majority of the larval stage for most individuals (Bruce 1988, Lugthart 1991). The reference stream had the highest number of both initial captures and subsequent recaptures (Table 3.2). The litter exclusion stream yielded the fewest initial

captures and recaptures, and the same larvae were recaptured more frequently, despite the fact that it has the largest wetted area of the three streams (Table 3.1). The recovery reach was intermediate in capture frequency. Recapture rate was approximately 30% in each stream.

*Population Density*

The litter exclusion stream had the lowest population size and density of the three study streams. Jolly-Seber population size estimates showed that on most sampling dates (9 of 13) population size was greater in the reference stream than in the litter exclusion reach, while the recovery reach was typically intermediate (Fig. 3.1A, recovery reach omitted for clarity). On certain dates recapture frequency was not sufficient to generate standard errors associated with population size estimates. Estimates for these dates were therefore excluded from analyses (n=2 for reference and recovery streams, n=3 for litter exclusion stream). Recaptures frequencies were low in the initial months of the study and in spring after hatchlings entered the stream because only a small portion of the populations had been marked during those times. Mean population size estimates for the study period helped to clarify differences between streams (Fig. 3.1B). The reference stream had a significantly larger population size (153 [98-233, 95% C.I]) than the litter exclusion stream (65 [48-86, 95% C.I.]), while the recovery reach was intermediate (96 [± 32, 95% C.I.]) and not significantly different from either reference or treatment streams. Differences between stream reaches were even more apparent when population size estimates were converted to densities (individuals/$m^2$) to account for differences in stream size. Monthly larval densities in the litter exclusion reach remained well below those of reference and recovery reaches throughout the study period (Fig. 3.2A). Mean

larval densities in both reference (1.16 $m^{-2}$ [0.74-1.75, 95% C.I.]) and recovery reaches (1.08 $m^{-2}$ [± 0.36, 95% C.I.]) were significantly greater than larval density in the litter exclusion reach (0.26 $m^{-2}$ [0.19-0.35, 95% C.I.]) (Fig. 3.2B). Despite a larger wetted area, the litter exclusion stream therefore had a larval density less than 25% of reference and recovery streams.

*Larval Growth*

Newly hatched larvae were collected in early May and metamorphosing larvae were collected in late May and June in all three streams. Plots of mean biomass for the 1998-1999 cohort revealed linear growth patterns in each stream, indicating growth was nearly continuous over the year (Fig. 3.3A). The pattern of biomass accumulation also indicated that most larvae in these streams have an approximate one year larval period. The slopes of the resulting significant growth rate regressions were compared among streams using analysis of covariance (ANCOVA) with time as the covariate. Regression slopes were significantly different ($p<0.001$) among all streams (reference> recovery> litter exclusion). Hatchling biomass in May was very similar between streams, but differences became greater over time. By the following April, larvae from the reference stream were an average of 52% larger than larvae from the litter exclusion stream. Time of year explained approximately 70% of the variation in larval biomass in reference and recovery reaches, but explained only about 50% of the variation in larval biomass in the litter exclusion stream (Fig. 3.3A).

Individual growth rates based on recaptured larvae showed a similar trend as above. Growth rates for the entire study period were compared together because there was little evidence of seasonal influence on growth rate. Mean daily growth rates based

on recaptured salamanders were 0.0024 (± 0.0004, 95% C.I.), 0.0021 (± 0.0005, 95% C.I.), and 0.0014 (± 0.0004, 95% C.I.) $d^{-1}$ for the reference, recovery, and litter exclusion reaches, respectively (Fig. 3.3B). By this method based on recaptured larvae, growth rate differences between the reference and litter exclusion reach was significant while the recovery reach was not significantly different from either.

*Biomass and Secondary Production*

As with growth and density, mean annual biomass was highest in the reference reach, lowest in the litter exclusion reach, and intermediate in the recovery reach (Fig. 3.4A). Mean annual biomass in the reference and recovery reaches were 7.87 (± 0.19, 95% C.I.) and 6.62 (± 0.15, 95% C.I.) mg AFDM/$m^2$, respectively. These estimates were >3x higher than that of the litter exclusion reach (1.96 mg AFDM/$m^2$ [± 0.07, 95% C.I.]). *E. wilderae* production was 8.50 (± 1.25, 95% C.I.), 7.35 (± 1.44, 95% C.I.) , and 1.27 (± 0.33, 95% C.I.) mg AFDM $m^{-2}$ $yr^{-1}$ in the reference, recovery, and litter exclusion reaches, respectively (Fig. 3.4B). Production in the litter exclusion reach however, was one-fifth that of reference and recovery reaches. Annual P/B turnover ratios, which provide another measure of growth, were also significantly higher in the reference (1.1 [± 0.13, 95% C.I.]) and recovery (1.1 [± 0.22, 95% C.I.]) reaches than in the litter exclusion reach (0.65 [± 0.17, 95% C.I.]).

*Diet and Trophic Basis of Production*

Sixty *E. wilderae* larvae were collected for dietary analysis (reference n=18; recovery n=23; litter exclusion n=19), and all larval guts contained at least some prey. A total of 34 prey taxa were identified in the stomachs of *E. wilderae* larvae in the study reaches. This is a conservative estimate of prey taxa richness because, in most cases,

prey were not identified to species. Proportional similarity and multi-response permutational procedures (MRPP) analyses based on abundance of all prey taxa in guts indicated differences in overall dietary composition among reaches. Diets of larvae in the reference reach showed little similarity with larval diets in either recovery (68%) or litter exclusion (53.1%) reaches. MRPP analysis further showed that larval dietary composition in the reference reach was significantly different from both recovery ($p<0.01$) and litter exclusion ($p<0.001$) reaches. Diets in the recovery and litter exclusion reaches showed the highest similarity (78.6%) and were not significantly different ($p=0.135$).

The 34 prey taxa were placed into fourteen major categories for comparison among reaches (Table 3.3). There were differences in the relative importance of prey taxa depending on whether contributions were expressed as mean values or as a percentage of total gut contents. Percentage data revealed more differences between reaches and provided a more accurate assessment of diet because they account for all items in the stomach. The contribution of prey taxa also varied depending on whether the analysis was based on prey abundance or biomass (Table 3.3).

Larvae in the reference reach had significantly more total prey items per gut (22.6 [16.78-29.28, 95% C.I.]) than larvae in the litter exclusion reach (11.95 [9.05-14.58, 95% C.I.]) (Fig. 3.5A). The recovery reach was intermediate in prey abundance (17.53 [14.43-21.17, 95% C.I.]) and was not significantly different from either of the other stream reaches. Prey abundance in each of the reaches was dominated by copepods and non-Tanypodinae chironomids (Table 3.3). These two prey groups together accounted for an average of 77.1% of all prey items in *E. wilderae* diets in the three study reaches. Larvae

in the litter exclusion reach, however, ate fewer copepods and cladocerans and significantly more non-Tanypodinae chironomids, nematodes, and terrestrial insects than larvae in the reference reach (Table 3.3). Larvae in the recovery reach consumed fewer copepods than larvae in the reference reach and fewer nematodes than larvae from the litter exclusion reach (Table 3.3). Differences in total prey abundance between reaches were primarily due to the large number of copepods consumed by larvae in the reference reach.

There was no difference in total prey biomass per gut among reaches (reference 0.28 mg [0.15-0.45, 95% C.I.], recovery 0.55 mg [0.37-0.77, 95% C.I.], litter exclusion 0.28 mg [0.18-0.39, 95% C.I.]) (Fig. 3.5B). Non-tanypod chironomids were by far the dominant prey item by mass. This group alone accounted for nearly a third of total prey biomass in reference and recovery reaches and more than half of prey biomass in the litter exclusion reach (Table 3.3). Not surprisingly, differences in biomass among prey categories were similar to differences in prey abundance. Larvae in the reference reach had greater biomass attributed to copepods and cladocerans than larvae in the litter exclusion reach. However, litter exclusion larvae, as with prey abundance, had significantly higher nematode and terrestrial insect biomass in guts. Recovery reach larvae had lower copepod biomass than larvae from the reference reach, whereas nematode biomass was lower than that from the litter exclusion reach (Table 3.3). Mean prey biomass was slightly higher in the recovery reach due to the presence of a few large stonefly larvae.

Based on prey biomass, non-Tanypodinae chironomids contributed the most to total *E. wilderae* production in each of the study reaches (Table 3.4). In the litter

exclusion reach more than half of *E. wilderae* production was attributed to non-tanypod chironomids alone, while in the other streams chironomids accounted for roughly one third of larval production. Production of larvae from reference and recovery reaches was thus more evenly distributed among other prey categories. Production attributed to copepods in the reference reach was >4x higher than the copepod contribution in recovery and litter exclusion reaches. In the litter exclusion reach larvae had less of their production attributed to common prey taxa such as Plecoptera, Trichoptera, copepods, and other Diptera than in the other streams. Conversely, larvae in the litter exclusion reach had ca. 3x more of their production attributed to nematodes and ca. 4x more production attributed to terrestrial insects. *E. wilderae* larvae consumed very little invertebrate biomass in accounting for annual production (12.5, 10.8, and 1.9 mg AFDM $m^{-2}$ $yr^{-1}$ for the reference, litter exclusion, and recovery reaches, respectively), but larvae in reference and recovery reaches consumed >5x more than larvae in the litter exclusion stream (Table 3.4).

*Movement and Survivorship*

*E. wilderae* larvae moved very little within reaches. Mean larval movements for the entire study period in reference, recovery, and litter exclusion reaches were only 2.38 (1.24-3.75, 95% C.I.), 2.61 (1.41-4.14, 95% C.I.), and 3.37 (0.91-6.45, 95% C.I.) m respectively, and there was no difference between reaches (Fig. 3.6). This comparison is conservative since the litter exclusion reach is the longest of the three reaches and the chance of capturing individuals that moved large distances was therefore greater. Only 35.7% (n=116) of recaptured larvae moved ≥1 m, and of those that moved, the majority (65.5%) were in the downstream direction. No larva moved more than 3 m upstream

(mean = 1.25 m), while the greatest downstream movement was 93 m (mean =11.04 m). In addition, only two recaptured larvae were found to have drifted downstream from the litter exclusion reach into the recovery reach.

We used the time interval between initial marking and final recapture for all recaptured larvae as a surrogate for larval survivorship (Johnson and Wallace 2002) (Fig. 3.7). Larvae in the recovery and litter exclusion reaches were recaptured up to ten months after initial marking whereas reference reach larvae were recaptured up to eight months after marking. In all three reaches recapture probability declined with time. Recapture interval data were transformed (log x+1) and resulting significant regression slopes (reference $p<0.001$, recovery $p<0.01$, litter exclusion $p<0.001$) were compared by ANCOVA with time as the covariate. There were no significant differences in survivorship of marked larvae among reaches (ANCOVA, $p=0.23$).

*Comparison of Larval Sampling Methods*

No larvae were collected in benthic samples from the litter exclusion stream over the study period while the mark-recapture density estimate was 0.26 individuals/$m^2$ (0.19-0.35, 95% C.I.). In the reference stream, the mean mark-recapture density estimate of 1.16 $m^{-2}$ (0.74-1.75, 95% C.I.) was very close to the benthic sampling estimate of 1.03 $m^{-2}$ (0-2.43, 95% C.I.) (Fig. 3.8A). Even though mean annual *E. wilderae* density estimates generated by the two sampling methods were similar, monthly density estimates in the reference stream revealed dramatic differences (Fig. 3.8B). Monthly benthic sampling estimates produced three large peaks in density resulting from the extrapolation of benthic corer area to the entire stream. Each of these peaks was a result of collecting one larva. This variation produced much larger error associated with the

mean density estimate. Mark-recapture estimates, on the other hand, produced a very different pattern and revealed population fluctuations over time. As a result, there was less error associated with the mean mark-recapture density estimate.

Mean biomass and production estimates in the reference stream were also very similar for the two sampling methods (Fig. 3.8A). The mark-recapture estimates for mean biomass (7.87 mg $m^{-2}$ [± 0.19, 95% C.I.]) and production (8.50 mg $m^{-2}$ $yr^{-1}$ [± 1.25, 95% C.I.]) were nearly identical to core sampling estimates (7.69 mg $m^{-2}$ $yr^{-1}$ [0, 20.92, 95% C.I.] and 9.00 mg $m^{-2}$ $yr^{-1}$ for biomass and production, respectively). But again, due to the high variation in monthly benthic samples, the mean biomass estimate based on core sampling had a much larger error than the mark-recapture estimate. Annual production estimates based on benthic core samples were calculated using the size-frequency method and associated errors cannot be generated by that method. However, production confidence intervals (95%) were estimated from mark-recapture data by bootstrapping interval production estimates over the study period.

## DISCUSSION

Reduced larval density in the litter exclusion stream compared to the reference stream can result from greater mortality, emigration from the study reach, or reduced adult oviposition. Of the larvae that were captured and marked, there were no differences in survivorship among streams. Survivorship estimates resulted in typical type III survivorship curves (Deevey 1947) (Fig. 3.7) in all three streams. This survivorship pattern indicates high larval mortality and has been previously documented for *E. wilderae* (Bruce 1988, Beachy 1997). Fewer initial captures in the litter exclusion stream combined with the lack of a difference in survivorship of marked larvae suggests

that lower larval density in the treatment stream is probably due to reduced oviposition or loss of new hatchlings prior to initial marking.

Adult *Eurycea* feed primarily on terrestrial prey rather than stream invertebrates (Burton 1976) and females typically oviposit on the underside of rocks rather than on in-stream woody debris or other organic matter (Dunn 1920, Wood 1949, Baumann and Huels 1982, Bruce 1982). Therefore, treatment effects should have little influence on choice of oviposition sites. Long-term treatment effects (treatment began in 1993) however, may have resulted in fewer adult females returning to the stream. If adults move little and return to the same streams to oviposit, reduced oviposition could contribute to lower densities in the treatment reach. Unfortunately, little is known of adult *E. wilderae* movements and home range size.

Numerous chamber experiments have investigated the effects of predation by larger salamander species on larval *Eurycea* (Resetarits 1991, Beachy 1993, 1994, 1997 Gustafson 1993, 1994, Wiltenmuth 1997). Results from these enclosure studies are equivocal and must be interpreted with caution. While it is possible that salamander predation contributed to greater hatchling mortality in the litter exclusion stream where other prey items are scarce (Wallace et al. 1997, 1999), such strong predatory interactions seem unlikely. Even larger *E. wilderae* larvae (>12 mm) are vulnerable to predation and if these interactions were common they likely would have contributed to reduced survivorship of marked larvae as well.

*E. bislineata* larvae have a strong tendency to drift downstream after hatching as a dispersal mechanism (Johnson and Goldberg 1975, Stoneburner 1978, Bruce 1986). Monthly drift data from the study streams also shows greater spring drift of hatchling *E.*

*wilderae* from the litter exclusion stream during the treatment period (T. Siler and J.B. Wallace, unpublished data). Thus, reduced larval density in the litter exclusion stream likely results from greater drift of hatchlings in spring before initial capture. Hatchlings are restricted to feeding on copepods and other meiofauna (invertebrates <0.5 mm) due to their small gape size (B. Johnson, pers. obs.). Copepods declined dramatically following initiation of litter exclusion treatment (Wallace et al. 1999) and their scarcity could provide the additional drift stimulus for hatchlings.

Even though excess drift response may explain density differences among streams, we found no differences in movement of marked larvae in the streams. Optimal foraging theory (reviewed by Stephens and Krebs 1987) predicts that predators should have higher densities (numerical response) and spend more time (area-restricted search) in prey-rich patches than prey-poor patches. Predators should also forage longer in prey-rich patches before moving to a new patch. This has been referred to as giving up times (McNair 1982). Due to the significant reduction of benthic invertebrate prey in the litter exclusion stream (Wallace et al. 1997, 1999), salamander larvae would likely encounter fewer prey-rich patches, have shorter give up times, and move more in search of prey. However, larvae in all three streams were largely sedentary. Mean larval movement of all recaptures in the study streams was only 2-3 m and nearly 65% of all recaptures moved less than 1-m, often remaining in the same depositional areas for several months. As with larval *E. bislineata* (Bruce 1986), the majority of the movements were in the downstream direction. Upstream movements were short (< 3 m) and took place only in large depositional areas where stream velocity was low. Only two of 104 larvae recaptured in the recovery stream were found to have drifted out of the litter exclusion

stream. This indicates that larvae that did not drift soon after hatching tended to stay in the treatment stream, but at the cost of reduced fitness.

The pattern of larval growth after hatching indicated that growth occurred throughout the year in each of the streams with little influence of temperature (Fig. 3.3A). This linear growth pattern has been previously documented for *E. wilderae* larvae (Lugthart 1991, Beachy 1997, Johnson and Wallace 2002). The majority of larvae in the study streams metamorphose after one year (Lugthart 1991). Our daily growth rate of 0.0024 (± 0.0004, 95% C.I.) for recaptured larvae in the reference stream agrees with a previous study that measured larval *E. wilderae* growth in this stream and in the litter exclusion stream prior to treatment using both in-stream chambers (reference = 0.003 $d^{-1}$ [± 0.001 S.E.; pre-treatment litter exclusion = 0.003 $d^{-1}$ [± 0.001 S.E.]) and biomass regressions from field studies (reference = 0.004 $d^{-1}$; pre-treatment litter exclusion = 0.003 $d^{-1}$) (Lugthart 1991). Even though results were similar, larval growth rates in chambers should be interpreted carefully because amphibian growth rates can be influenced by larval densities and prey availability (Wilbur 1976, Petranka and Sih 1986, Scott 1990, Smith 1990, Van Buskirk and Smith 1991, Walls 1998).

Two independent methods of growth measurement, both free from chamber effects, showed litter exclusion resulted in significantly reduced *E. wilderae* growth. Hatchling biomass in May was very similar among streams, but by completion of the larval stage size, differences were dramatic. Biomass regression slopes indicated growth was significantly different among all three streams. However, the linear relationship between time and larval biomass accumulation was more variable in the litter exclusion reach, indicating differences in biomass were likely the result of treatment rather than

natural variation. Growth estimates based on recaptured larvae also indicated significantly reduced growth as a result of litter exclusion but, unlike biomass regressions, this method indicated larvae from the recovery reach were intermediate in growth and not significantly different from the other streams. Comparison of daily growth rates for recaptured larvae were based on overlap of 95% confidence intervals and is a more conservative comparison than use of regressions (Zar 1996, Johnson 1999). Larval growth differences may have adverse consequences for *E. wilderae* population growth because larval growth can influence timing of and size at metamorphosis and ultimately, adult fecundity (Wilbur and Collins 1973, Bruce 1982, 1988). Mortality also increases with duration of the larval stage (Bruce 1988). There were no obvious differences in timing or size at metamorphosis among streams but sample sizes were too small for thorough analysis because collection of metamorphosing larvae was not an objective for this study.

Gut content analyses were performed after mark-recapture studies to find the mechanism underlying reduced larval growth in the treatment stream. *E. wilderae* are largely specialists, feeding predominantly on copepods and non-tanypodine chironomids. These two prey items accounted for more than 75% of all prey items in the guts in each stream. Similar findings were reported for *E. wilderae* in the study streams (Lugthart 1991) and for larval *E. bislineata* in an Indiana stream (Caldwell and Houtcooper 1973). Burton (1976) found that *E. bislineata* larvae at Hubbard Brook, New Hampshire relied heavily on chironomid larvae but consumed few copepods.

Larval diets in the litter exclusion stream showed little similarity with diets of larvae in the reference stream primarily due to larvae in the treatment stream switching

from copepods to non-Tanypodinae chironomids. Benthic data confirms that copepods have declined by 95% of pretreatment values as a result of litter exclusion, whereas midges remain a relatively available food source (Wallace et al. 1999). Larvae in the litter exclusion stream also consumed significantly more unusual prey items such as nematodes and terrestrial insects. Unlike some studies that have shown stream predators may rely heavily on prey from outside the system (Mason and MacDonald 1982, Nakano et al. 1999), in these streams the terrestrial subsidy of prey was minor and apparently insufficient to support normal larval growth in the litter exclusion stream.

We initially hypothesized that food limitation was responsible for the reduction in *E. wilderae* growth because of the significant reduction in available prey items in the treatment stream (Wallace et al. 1997, 1999). Prey abundance in larval diets supported that hypothesis, but there were no differences in total prey biomass among reaches. The abundance of copepods in larval diets from the reference stream contributed heavily to the overall difference in prey number among streams. The lack of biomass difference resulted from low copepod biomass along with the fact that litter exclusion larvae switched to feeding on larger non-tanypod chironomids. In fact, more than half of *E. wilderae* production was attributed to midges alone, whereas in the other streams production was more evenly distributed among other prey groups (Table 3.5). Given the similarity of prey biomass among streams, the mechanism responsible for reduced growth in the treatment stream must either be a difference in prey quality or a difference in energetic demands.

Copepods may provide a nutritious food source for larvae in the reference stream. Lugthart (1991) found that growth of larval *E. wilderae* actually increased significantly

following insecticide application in catchment 54 at Coweeta. Though not significant, gut content analysis revealed abundance and biomass of copepods were considerably higher in diets of larvae from the insecticide-treated stream compared to the reference stream and pretreatment diets (Lugthart 1991). Lipid reserves are the most efficient energy source for organisms and copepods in pelagic systems can have up to 70% of their dry weight as lipids (Sargent and Falk-Peterson 1988). However, these planktivorous copepods derive their high lipid content from algal food resources (Sargent and Falk-Peterson 1988) which are virtually absent in shaded headwater streams at Coweeta. Further studies are needed to assess lipid content and assimilation efficiencies of stream copepods.

Reduced growth of larvae in the litter exclusion stream could also result from differences in energetic demand. Several studies have demonstrated a positive relationship between activity and larval amphibian growth (Woodward 1983, Morin and Johnson 1988, Lawler 1989, Skelly and Werner 1990, Werner 1991, Maurer and Sih 1996). Few studies, however, have evaluated effects of reduced prey availability on activity levels (Maurer and Sih 1996), and studies linking increased amphibian activity with energetic costs are lacking. The fact that salamanders in the treatment stream ate more nematodes and terrestrial insects strongly suggests they have difficulty finding food. If larvae exert more energy in searching for prey then they may have less energy available for growth. Maurer and Sih (1996) found that larvae of the stream salamander *Ambystoma barbouri* actually reduced activity in response to food deprivation in a laboratory experiment, but the study was conducted for only 9 d and in complete absence of food. Even though we found no movement differences among streams, we measured

movement only to the nearest meter and didn't measure activity rates on a daily basis. If larvae in the treatment stream are expending more energy in finding food by moving more within the same depositional area, then our trophic basis of production estimates would be conservative because we assumed equal energetic efficiencies among streams. *In situ* measurements of assimilation and production efficiencies would be required to verify differences in energetic demand among streams. Unfortunately, such studies are nearly impossible to conduct in the field.

*E. wilderae* production is very low in the headwaters at Coweeta (~ 8 mg AFDM $m^{-2}$ $yr^{-1}$) as a result of relatively low densities and slow growth rates. Yet, biomass and production were still significantly lower in the treatment stream as a result of reduced larval growth and density. The lower P/B turnover ratio in the litter exclusion stream is further evidence of reduced larval growth. This study provides the first reported estimates of larval *Eurycea* production. Wallace et al. (1999) calculated annual production values for all larval salamander species from benthic samples over the period 1992-1997 in the reference and litter exclusion streams. Their reference and pre-treatment salamander production values ranged from 93 to 336 mg AFDM $m^{-2}$ $yr^{-1}$, whereas production during treatment ranged only from 0 to 62 mg AFDM $m^{-2}$ $yr^{-1}$. Based on these estimates, it appears that *E. wilderae* production comprises a small portion of total salamander production and that most salamander production is attributed to *Desmognathus* spp. in Coweeta streams. Huryn and Wallace (1987) estimated larval production of *Desmognathus* spp. was 242 mg AFDM $m^{-2}$ $yr^{-1}$ with P/B values ranging from 1.9-2.7 in catchment 27 at Coweeta. The only production estimate for an individual

species comes from Spight (1967), who found that production of *D. fuscus* was 100-300 mg AFDM $m^{-2}$ $yr^{-1}$ with a P/B ratio of 0.2 in a North Carolina stream.

Although predators consume nearly all invertebrate prey in the study streams (Wallace et al. 1999), *E. wilderae* larvae consumed very little in accounting for their annual production. Larvae ate only 10-12 mg AFDM $m^{-2}$ $yr^{-1}$ in reference and recovery streams versus about 2 mg AFDM $m^{-2}$ $yr^{-1}$ in the litter exclusion stream. In comparison, total production of the benthic community in the mixed substrate habitat is approximately 10 g AFDM $m^{-2}$ $yr^{-1}$ in the reference stream and 1 g AFDM $m^{-2}$ $yr^{-1}$ in the treatment stream (Wallace et al. 1999). These data indicate that *E. wilderae* larvae exert little top-down predation pressure on the benthic community. This finding agrees with studies that have shown salamanders play a relatively minor role in these streams in terms of energy flow (Wallace et al. 1997, 1999, Hall et al. 2000) and with others who have shown that vertebrate predators exert less top-down pressure on stream invertebrate communities than invertebrate predators (Allan 1982, Culp 1986, Reice 1991, Wooster 1994). The low rates of total prey consumption by *E. wilderae* may be attributed to their high assimilation efficiencies, low densities, and slow growth rate.

Many of the parameters measured for *E. wilderae* in the recovery reach (population density, growth, biomass, production, P/B, and prey abundance) had values that were intermediate between reference and litter exclusion reaches. These findings indicate larvae in this downstream reach are still affected by the upstream litter exclusion treatment, possibly as a result of reduced prey availability. Benthic samples were not collected in this stream, but Baer et al. (2001) found that invertebrate colonization, secondary production, and FPOM accumulation on artificial substrates were significantly

reduced in the recovery stream after initiation of upstream treatment. Export of fine particulate organic matter (FPOM) from the upstream litter exclusion reach has also declined by more than 81% over the treatment period (Wallace et al. 1999). The fact that *E. wilderae* larvae downstream of treatment had lower density, growth, and production than the reference stream provides additional evidence for the importance of upstream-downstream connections in lotic systems (Vannote et al. 1980, Wallace et al. 1982, Minshall et al. 1983, Cuffney et al. 1990, Baer et al. 2001).

Both mark-recapture and benthic core sampling produced very similar estimates of *E. wilderae* abundance, biomass, and production. The benthic sampling by Wallace et al. (1997, 1999) thus provided reasonable estimates for larval salamander populations in the study streams. However, errors associated with benthic sampling estimates were large due to small monthly sample sizes. The similarity of estimates generated by the two methods may have been serendipitous. Collection of only one more or one less individual in the benthic samples would have produced very different mean values. Mark-recapture is a better method for monitoring *Eurycea* larvae because it provides greater accuracy and allows population dynamics to be closely followed through time. For example, we were able to detect large density peaks after hatching in spring that would have gone unnoticed in benthic data. The accuracy of benthic sampling for *Desmognathus* spp. remains uncertain. *Desmognathus* larvae are better adapted to the stream environment and, unlike *E. wildereae*, often inhabit areas of high stream velocity. *Desmognathus* larvae were often able to evade capture during this study due to their high mobility and recapture rates were insufficient to generate population data for

*Desmognathus* spp. It is reasonable to assume that similar problems occur with benthic sampling given the speed and mobility of these larvae.

### *Conclusions*

Hunter and Price (1992) referred to the effects of resources on higher trophic levels as a "cascade up" and numerous such examples exist for living plant-based systems (e.g. Hart and Robinson 1990, Peterson et al. 1993, Harvey et al. 1998). The inclusion of detritus into the classical food web framework has been largely overlooked (Polis and Strong 1996, Polis et al. 1997) and few experimental studies have demonstrated indirect effects of resources on predators in detritus based ecosystems. In this study, long-term exclusion of the detrital resource produced strong indirect effects that resulted in reduced density, growth, production, and altered diet of larval *E. wilderae*, a vertebrate stream predator. Removal of detritus altered invertebrate community structure such that it was incapable of supporting a typical *E. wilderae* population. These findings agree with Wallace et al. (1997, 1999) who found that removal of detritus resulted in significant reductions in abundance, biomass, and production of the benthic community, including larval salamanders. Our results for larval *E. wilderae*, however, provide the first comprehensive study of bottom-up effects of detritus manipulation on a predatory vertebrate species. Chen and Wise (1999) found that detritus enhancement led to higher densities of predaceous mites in a forest floor community, and Polis and Hurd (1995) found that marine derived detritus facilitated terrestrial spiders on oceanic islands. Further research is clearly needed to gain a better understanding of the strength and prevalence of cascading interactions in detritus based ecosystems.

There are approximately 400 salamander species worldwide and 127 species in the United States (Petranka 1998). As a result of their inconspicuous nature, we know far less about the effects of disturbance on these species compared to more charismatic vertebrate megafauna. Amphibians are generally sensitive to disturbance and recent evidence has suggested global declines in amphibian populations, although most work has focused on frogs and toads (reviewed by Petranka 1998). Whether similar losses are occurring among salamander populations remains largely unknown. Headwater streams are home to many salamander species, including many lungless plethodontids, the largest and most diverse salamander group. Unfortunately, these critical headwater habitats are now being destroyed at alarming rates due to agriculture, mining, urbanization, and forestry practices (Meyer and Wallace 2001). Our findings have important implications for amphibian conservation and recovery efforts because they demonstrate the importance of the detrital resource for salamander populations in these headwater streams. The adverse effects of detritus removal on larval salamanders provide further evidence of the tight linkage between terrestrial organic matter inputs and aquatic ecosystem structure and function.

*Acknowledgements*

We thank John Hutchens, Wyatt Cross, and Darold Batzer, and for providing valuable comments on earlier versions of this manuscript. Litter exclusion aspects of this research were supported by NSF grant #DEB-9629268.

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TABLE 3.1. Physical parameters of streams draining catchments 53 (reference 1), 54 (reference 2), and 55 (litter exclusion treatment) at the Coweeta Hydrologic Laboratory. Elevations were measured at the gauging flumes. Data are from Wallace et al. 1999 and Wallace, unpublished.

| Variable | C53 | C 54 | C 55 |
|---|---|---|---|
| Catchment | | | |
| Area (ha) | 5.2 | 5.5 | 7.5 |
| Elevation (m asl) | 829 | 841 | 810 |
| Channel | | | |
| Length (m) | 145 | 282 | 170 |
| Bankful Area ($m^2$) | 327 | 443 | 373 |
| Discharge (L/s) | | | |
| Average * | 1.19 | 1.45 | 2.39 |
| Maximum * | 47.2 | 35.5 | 40.2 |
| Temperature (°C) | | | |
| Annual Average † | 12.2 | | 12.2 |
| Maximum † | 20.3 | 19.5 | 20.1 |
| Minimum † | 0.7 | 1.1 | 0.7 |
| Annual degree-days † | 4485 | 4440 | 4512 |

* C53 averages from 12 years (1984-1996), C54 averages from 8 years (1985-1992), C55 averages from 5 years (1992-1997)

† C53 and C55 averages from 12 years (1985-1997), C54 averages from 8years (1985-1992)

TABLE 3.2. Larval *Eurycea wilderae* captured from reference (C53), recovery (C56), and treatment (C55) streams at the Coweeta Hydrologic Laboratory from November 1997 to April 1999.

| | Reference (C53) | Recovery (C56) | Litter Exclusion (C55) | Total |
|---|---|---|---|---|
| Total Individuals Captured | 412 | 323 | 283 | 1018 |
| Total No. of Recaptures | 122 | 104 | 99 | 325 |
| No. Larvae Recaptured On Multiple Dates | 99 | 70 | 60 | 229 |
| % Recapture | 29.6 | 32.2 | 35 | 31.9 |

TABLE 3.3. Mean abundance (no./gut), biomass (mg AFDM/gut), and percentage of prey items (number prey / gut and weight of prey gut) in the diets of *Eurycea wilderae* larvae from reference (REF), recovery (REC), and litter exclusion (LE) streams. Significant differences indicated by letters. Values within a prey category that are followed by the same lowercase letter (abundance columns) or capital letter (weight columns) are not significantly different based on overlap of bootstrapped 95% confidence intervals. Absence of letters indicates no significant difference.

| | Abundance | | | Biomass | | | % Abundance | | | % Biomass | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Prey Category** | **REF** | **REC** | **LE** | **REF** | **REC** | **LE** | **REF** | **REC** | **LE** | **REF** | **REC** | **LE** |
| Ephemeroptera | 0 | 0.14 | 0 | 0 | 0.041 | 0 | 0 | 0.84 | 0 | 0 | 5.01 | 0 |
| Plecoptera | 0.34 | 0.56 | 0.31 | 0.042 | 0.146 | 0.019 | 2.75 | 2.85 | 2.10 | 13.03 | 17.76 | 5.75 |
| Trichoptera | 0.45 | 0.53 | 0.32 | 0.049 | 0.056 | 0.038 | 2.33 | 2.85 | 2.67 | 10.38 | 11.65 | 6.88 |
| Chironomidae* | 5.1 | 7.39 | 6.25 | 0.063 | 0.106 | 0.108 | $24.77^{a}$ | $42.07^{a,b}$ | $49.01^{b}$ | 30.80 | 30.26 | 52.86 |
| Tanypodinae | 0.39 | 0.70 | 0.32 | 0.052 | 0.103 | 0.018 | 2.38 | 5.99 | 3.79 | 9.74 | 14.88 | 9.61 |
| Other Diptera | 0.56 | 0.43 | 0.37 | 0.051 | 0.093 | 0.071 | 3.43 | 3.68 | 3.49 | 18.97 | 14.15 | 11.62 |
| Copepoda | $14.37^{a}$ | $6.17^{b}$ | $2.8^{b}$ | $0.015^{A}$ | $0.006^{B}$ | $0.003^{B}$ | $59.49^{a}$ | $34.51^{b}$ | $21.30^{b}$ | $14.06^{A}$ | $3.55^{B}$ | $1.60^{B}$ |
| Coleoptera | 0.11 | 0.05 | 0.05 | 0.002 | 0.001 | 0.0005 | 0.29 | 0.41 | 1.31 | 0.45 | 0.06 | 1.20 |
| Ostracoda | 0.34 | 0.46 | 0.21 | 0.001 | 0.001 | 0.0005 | 1.4 | 1.85 | 1.12 | 0.52 | 0.89 | 0.14 |
| Cladocera | $0.54^{a}$ | $0.04^{a,b}$ | $0^{b}$ | $0.001^{A}$ | $0^{B}$ | $0^{B}$ | $2.52^{a}$ | $0.31^{a,b}$ | $0^{b}$ | $1.45^{A}$ | $0.10^{A,B}$ | $0^{B}$ |
| Acarina | 0.17 | 0.52 | 0.31 | 0.0004 | 0.001 | 0.001 | 0.95 | 3.44 | 4.84 | 0.11 | 0.38 | 1.10 |
| Oligochaeta | 0 | 0 | 0.11 | 0 | 0 | 0.007 | 0 | 0 | 0.27 | 0 | 0 | 2.17 |
| Nematoda | $0^{a}$ | $0.22^{b}$ | $0.42^{b}$ | 0 | 0.001 | 0.009 | $0^{a}$ | $0.47^{a,b}$ | $3.98^{b}$ | $0^{A}$ | $0.06^{A}$ | $2.85^{B}$ |
| Terrestrials | 0.06 | 0.13 | 0.47 | 0.001 | 0.007 | 0.005 | $0.09^{a}$ | $0.88^{a}$ | $5.80^{b}$ | $0.21^{A}$ | $0.87^{A,B}$ | $3.83^{B}$ |

* = non-Tanypodinae Chironomidae

TABLE 3.4. Trophic basis of production and amount of food type consumed for *Eurycea wilderae* in Reference (REF), Recovery (REC), and Litter Exclusion (LE) streams.

| | Biomass (Avg. %) | | | Relative Amount to Production | | | Production Attributed to Food Type (%) | | | Production Atrributed to Food Type (mg $m^{-2}$ $yr^{-1}$) | | | Amount of Food Type Consumed (mg $m^{-2}$ $yr^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Prey Taxa** | **REF** | **REC** | **LE** | **REF** | **REC** | **LE** | **REF** | **REC** | **LE** | **REF** | **REC** | **LE** | **REF** | **REC** | **LE** |
| Ephemeroptera | 0 | 5.01 | 0 | 0 | 3.38 | 0 | 0 | 5.03 | 0 | 0 | 0.37 | 0 | 0 | 0.54 | 0 |
| Plecoptera | 13.03 | 17.76 | 5.75 | 8.8 | 11.99 | 3.88 | 13.07 | 17.83 | 5.77 | 1.11 | 1.31 | 0.07 | 1.63 | 1.93 | 0.11 |
| Trichoptera | 10.38 | 11.65 | 6.88 | 7.01 | 7.86 | 4.64 | 10.41 | 11.7 | 6.91 | 0.88 | 0.86 | 0.09 | 1.3 | 1.26 | 0.13 |
| Chironomidae* | 30.8 | 30.26 | 52.86 | 20.79 | 20.43 | 35.68 | 30.89 | 30.38 | 53.06 | 2.63 | 2.23 | 0.67 | 3.86 | 3.28 | 1 |
| Tanypodinae | 9.74 | 14.88 | 9.61 | 6.57 | 10.04 | 6.49 | 9.77 | 14.94 | 9.65 | 0.83 | 1.1 | 0.12 | 1.22 | 1.61 | 0.18 |
| Other Diptera | 18.97 | 14.15 | 11.62 | 12.8 | 9.55 | 7.84 | 19.02 | 14.2 | 11.66 | 1.62 | 1.04 | 0.15 | 2.38 | 1.54 | 0.22 |
| Copepoda | 14.06 | 3.55 | 1.6 | 9.49 | 2.4 | 1.08 | 14.1 | 3.56 | 1.61 | 1.2 | 0.26 | 0.02 | 1.76 | 0.39 | 0.03 |
| Coleoptera | 0.45 | 0.06 | 1.2 | 0.3 | 0.04 | 0.81 | 0.45 | 0.06 | 1.2 | 0.04 | 0 | 0.02 | 0.06 | 0.01 | 0.02 |
| Ostracoda | 0.52 | 0.89 | 0.14 | 0.35 | 0.6 | 0.09 | 0.52 | 0.89 | 0.14 | 0.04 | 0.07 | 0 | 0.07 | 0.1 | 0 |
| Cladocera | 1.45 | 0.1 | 0 | 0.98 | 0.07 | 0 | 1.45 | 0.1 | 0 | 0.12 | 0.01 | 0 | 0.18 | 0.01 | 0 |
| Acarina | 0.11 | 0.38 | 1.1 | 0.07 | 0.26 | 0.74 | 0.11 | 0.38 | 1.1 | 0.01 | 0.03 | 0.01 | 0.01 | 0.04 | 0.02 |
| Oligochaeta | 0 | 0 | 2.17 | 0 | 0 | 1.46 | 0 | 0 | 2.18 | 0 | 0 | 0.03 | 0 | 0 | 0.04 |
| Nematoda | 0 | 0.06 | 2.85 | 0 | 0.04 | 1.92 | 0 | 0.06 | 2.86 | 0 | 0 | 0.04 | 0 | 0.01 | 0.05 |
| Terrestrials | 0.21 | 0.87 | 3.83 | 0.14 | 0.59 | 2.59 | 0.21 | 0.87 | 3.84 | 0.02 | 0.06 | 0.05 | 0.03 | 0.09 | 0.07 |
| TOTAL | 99.72 | 99.62 | 99.61 | 67.31 | 67.24 | 67.24 | 100 | 100 | 100 | 8.5 | 7.35 | 1.27 | 12.5 | 10.81 | 1.88 |

* = non-Tanypodinae Chironomidae

Figure 3.1. Larval *Eurycea wilderae* population sizes in reference, recovery, and litter exclusion streams from December 1997 to February 1999. (A) Log monthly Jolly-Seber population size estimates (recovery stream omitted for clarity). Error bars are estimate +/- 1 SE. (B) Mean population size estimates per stream. Error bars are mean +/- 95% bootstrapped CI.

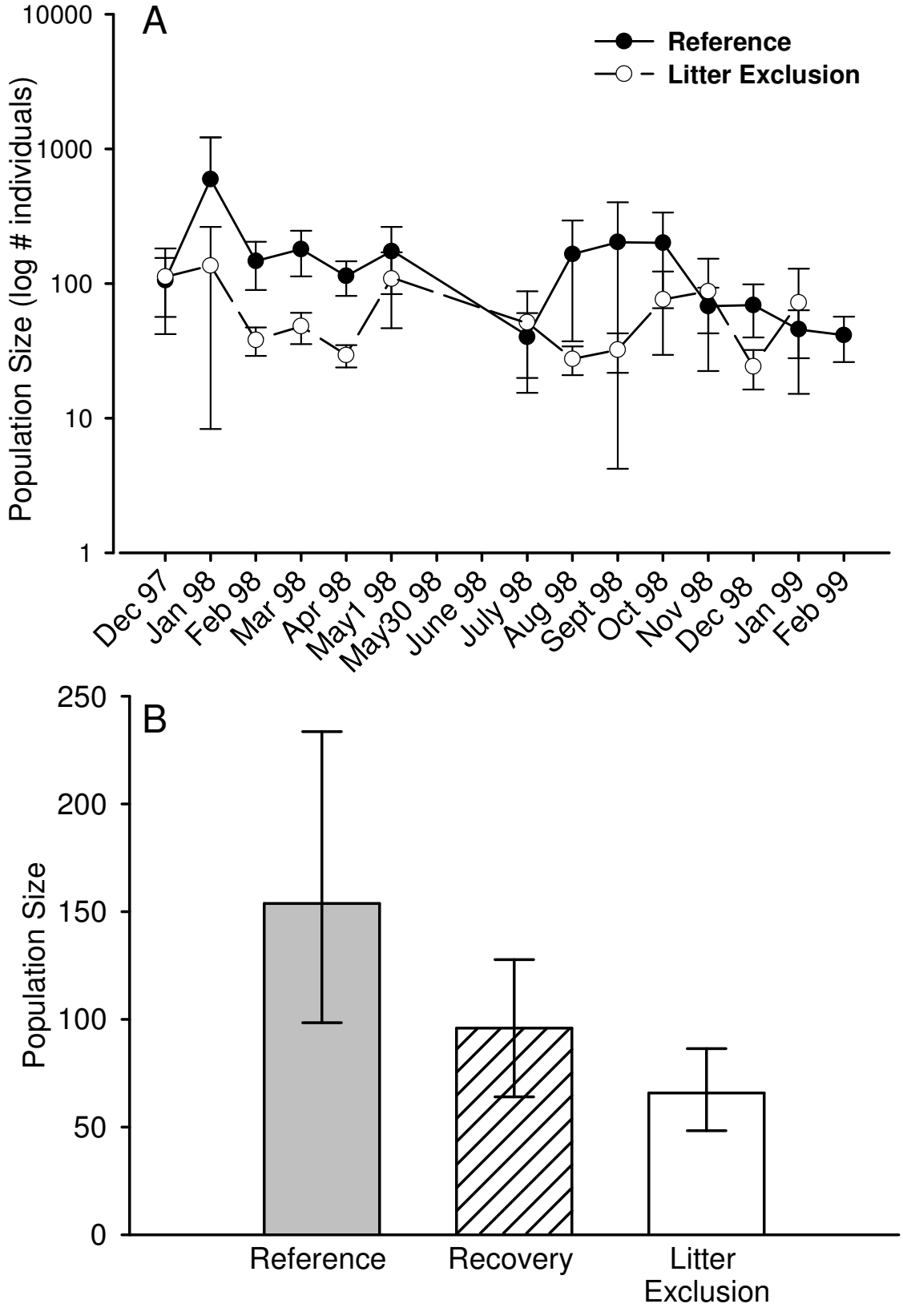
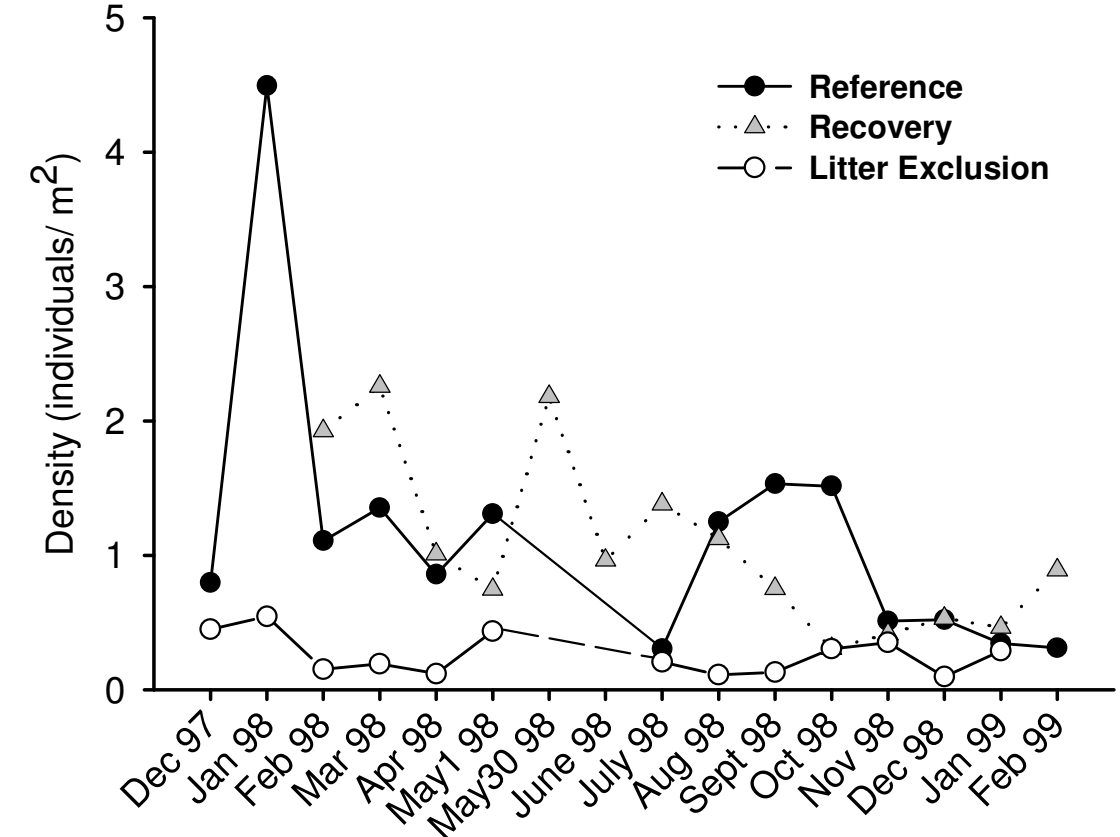

A
Reference
Litter Exclusion
Population Size (log # individuals)
10000
1000
100
10
1
Dec 97
Jan 98
Feb 98
Mar 98
Apr 98
May1 98
May30 98
June 98
July 98
Aug 98
Sept 98
Oct 98
Nov 98
Dec 98
Jan 99
Feb 99
B
Population Size
250
200
150
100
50
0
Reference
Recovery
Litter Exclusion

Figure 3.2. (A) Monthly and (B) mean larval *Eurycea wilderae* density estimates in reference, recovery, and litter exclusion streams from December 1997 to February 1999. Error bars are mean +/- 95% bootstrapped CI.


Density (individuals/ m2)
5
4
3
2
1
0
Reference
Recovery
Litter Exclusion
Dec 97
Jan 98
Feb 98
Mar 98
Apr 98
May1 98
May30 98
June 98
July 98
Aug 98
Sept 98
Oct 98
Nov 98
Dec 98
Jan 99
Feb 99

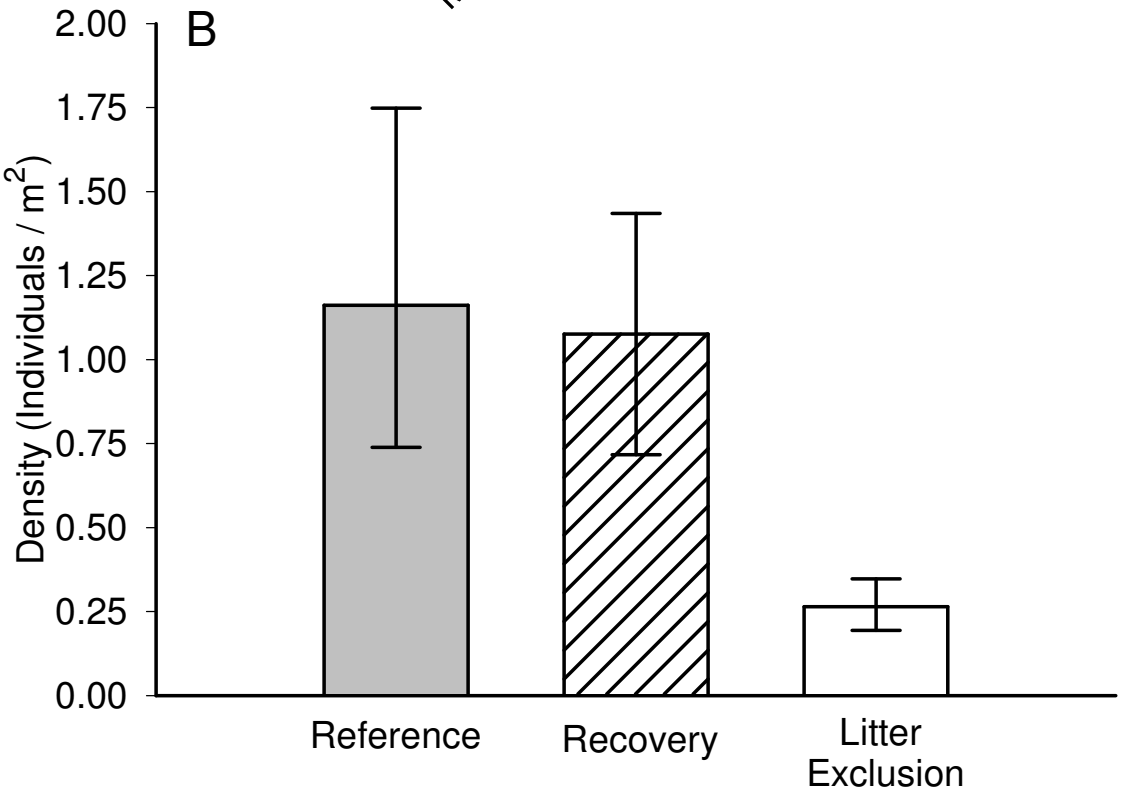

B
Density (Individuals / m2)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Reference
Recovery
Litter Exclusion

Figure 3.3. (A) Relationship between time and *Eurycea wilderae* growth after hatching for the 1998-1999 cohort in reference (y=0.034x + 4.991), recovery (y=0.027x + 5.026), and litter exclusion (y=0.017x + 5.288) streams. Regressions based on biomass of all larvae collected on each sample date (day 0 = May 1, 1998 to day 351 = February 19, 1999). Error bars are +/- 1 SE. (B) Mean individual daily growth rates of all recaptured larvae in reference (n=122), recovery (n=104), and litter exclusion (n=99) streams. Error bars are mean +/- 95% bootstrapped CI.

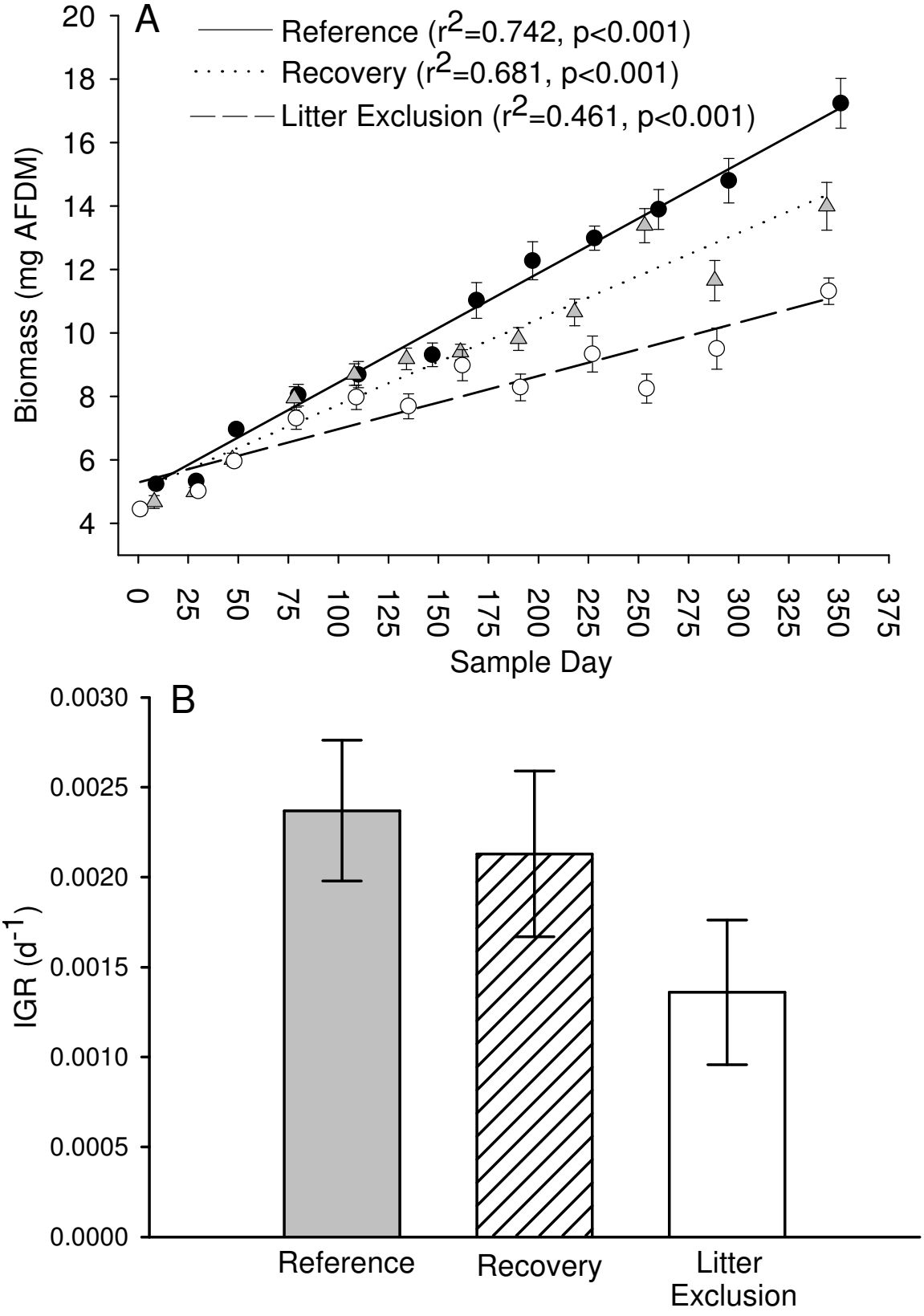
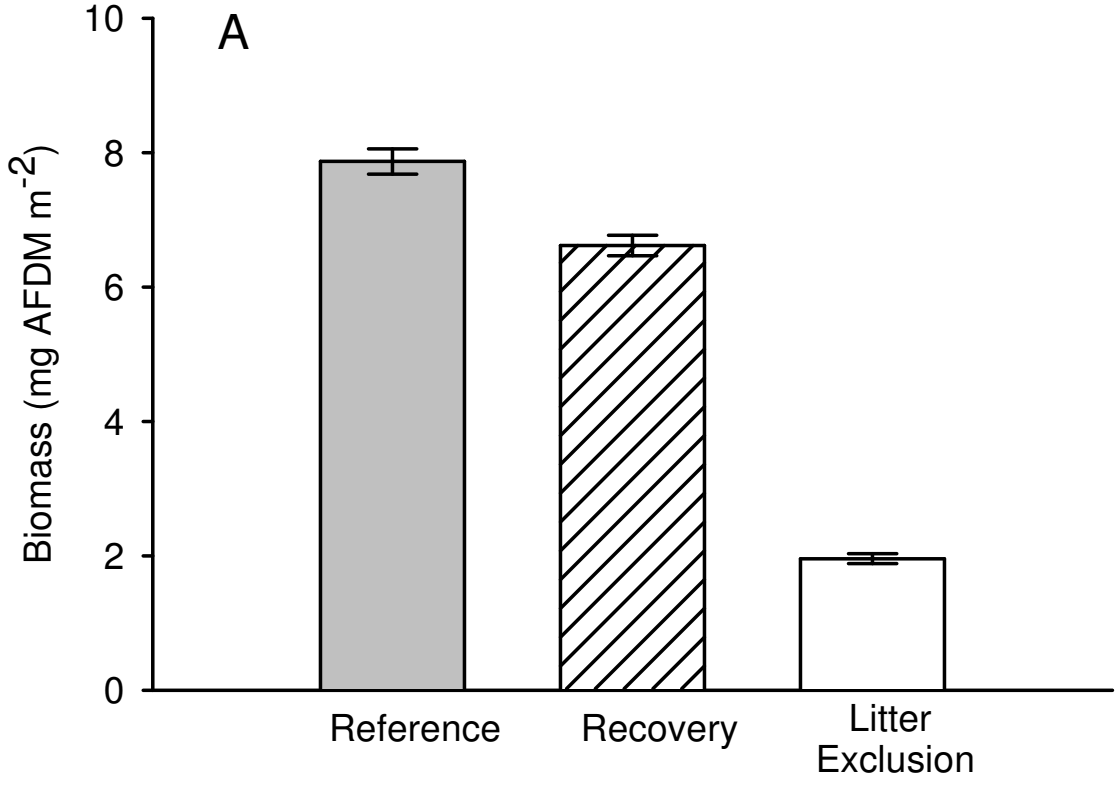

A
Reference ($r^2$=0.742, p<0.001)
Recovery ($r^2$=0.681, p<0.001)
Litter Exclusion ($r^2$=0.461, p<0.001)
Biomass (mg AFDM)
Sample Day
B
IGR ($d^{-1}$)
Reference
Recovery
Litter Exclusion

Figure 3.4. (A) Mean annual biomass and (B) habitat-weighted annual production for the 1998-1999 larval *Eurycea wilderae* cohort in reference, recovery, and litter exclusion streams. Error bars are mean +/- 95% bootstrapped CI.


A
Biomass (mg AFDM m-2)
10
8
6
4
2
0
Reference
Recovery
Litter Exclusion

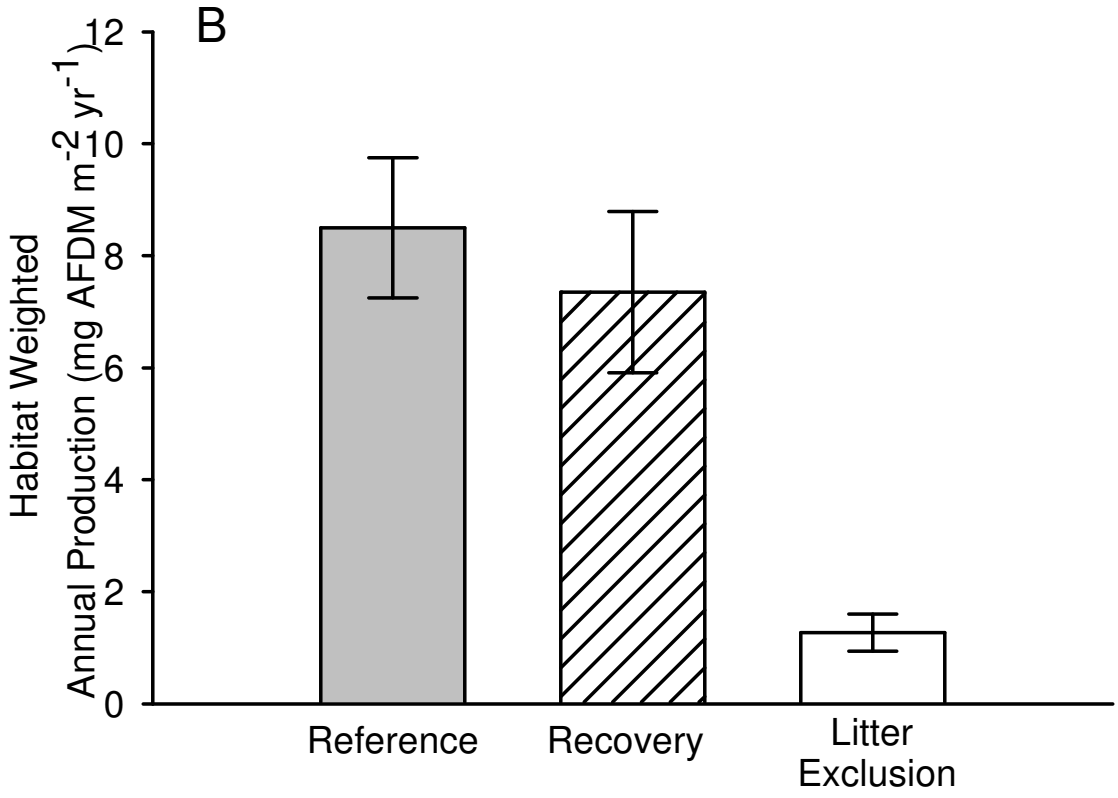
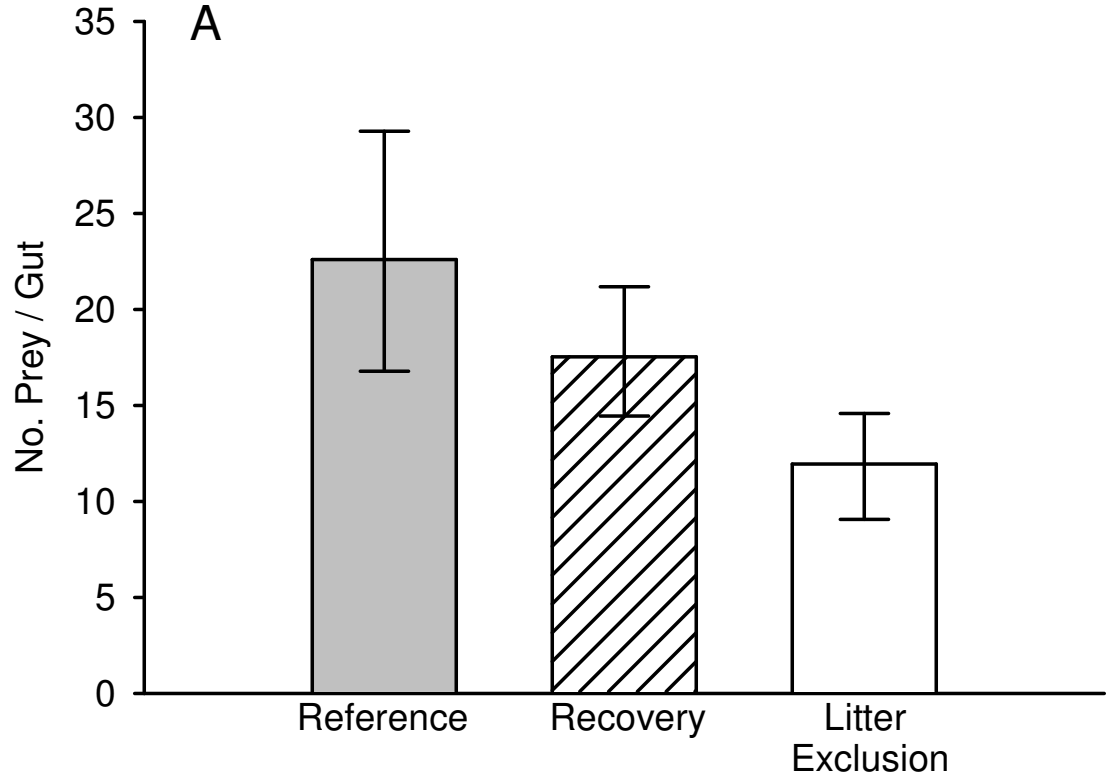

B
Habitat Weighted Annual Production (mg AFDM m-2 yr-1)
12
10
8
6
4
2
0
Reference
Recovery
Litter Exclusion

Figure 3.5. (A) Total number of prey items and (B) Total prey biomass per gut for larval *Eurycea wilderae* in reference (n=18), recovery (n=23), and litter exclusion (n=19) streams. Error bars are mean +/- 95% bootstrapped CI.


A
No. Prey / Gut
35
30
25
20
15
10
5
0
Reference
Recovery
Litter
Exclusion

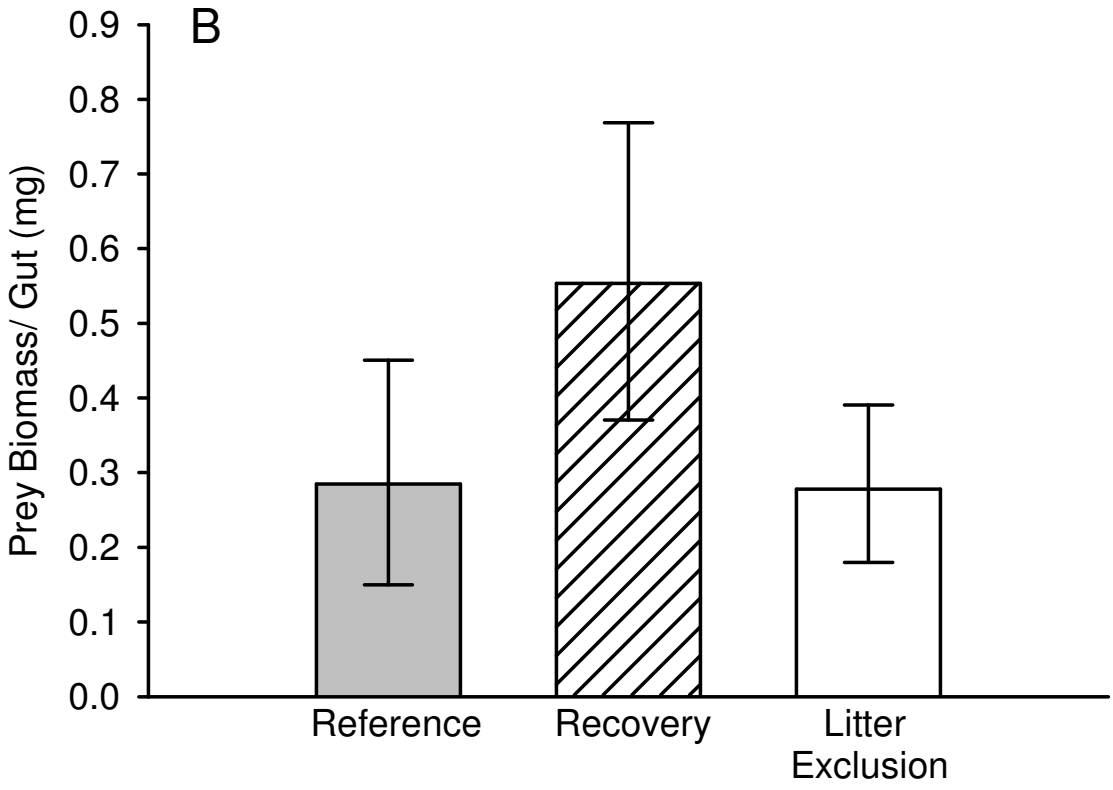

B
Prey Biomass/ Gut (mg)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Reference
Recovery
Litter
Exclusion

Figure 3.6. (A) Distance moved by *Eurycea wilderae* larvae recaptured in reference (n=122), recovery (n=104), and litter exclusion streams (n=99). Error bars are mean +/- 95% bootstrapped CI.

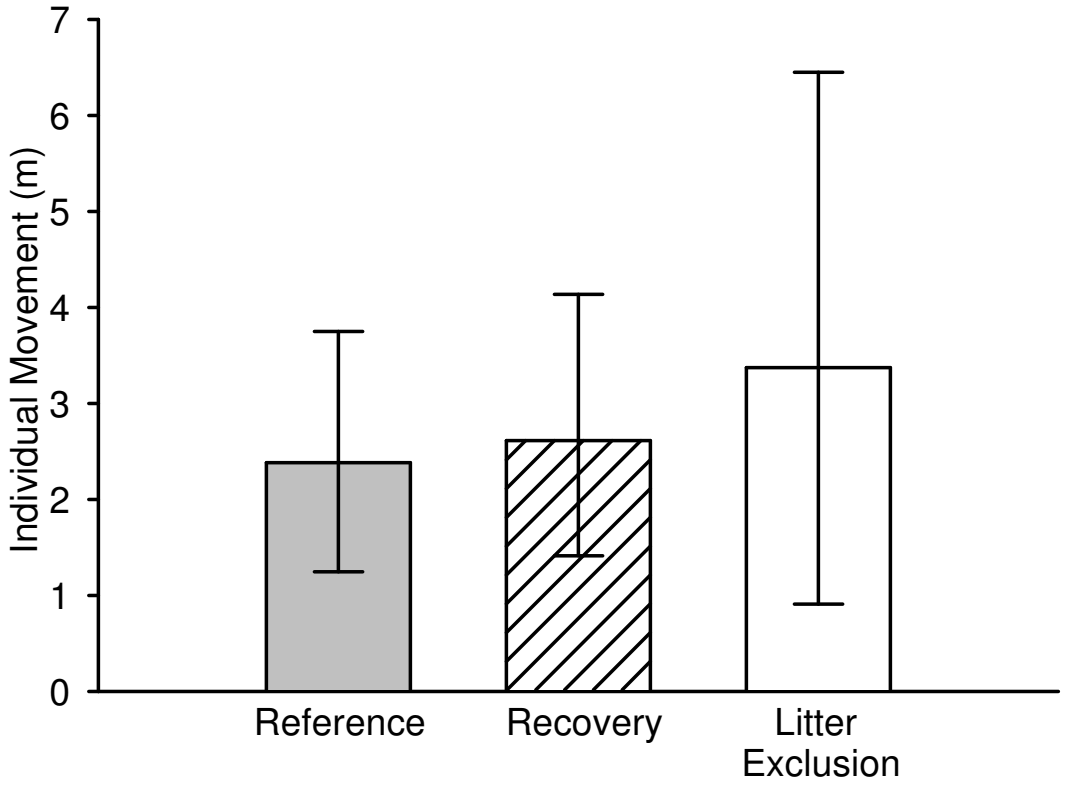

Individual Movement (m)
0
1
2
3
4
5
6
7
Reference
Recovery
Litter
Exclusion

Figure 3.7. Time interval between initial marking and final recapture for all *Eurycea wilderae* larvae recaptured in reference (n=122), recovery (n=104), and litter exclusion streams (n=99).

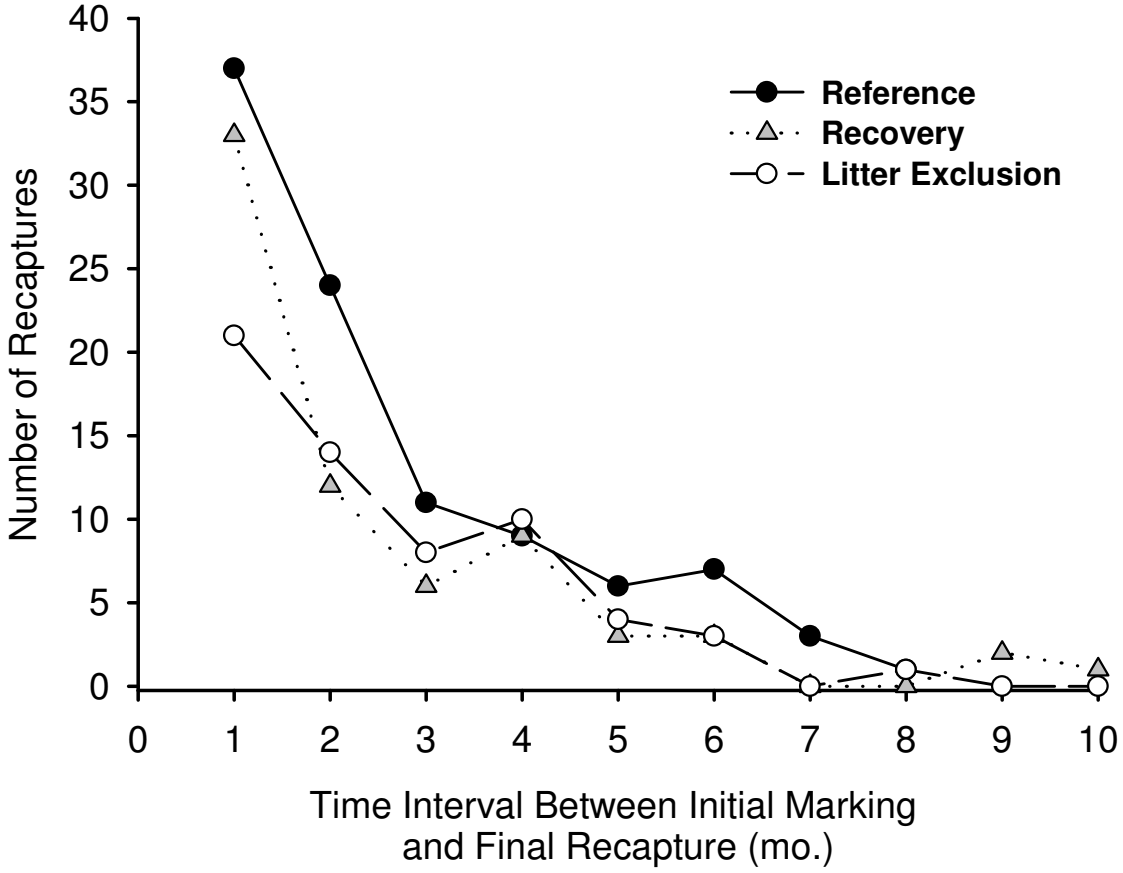
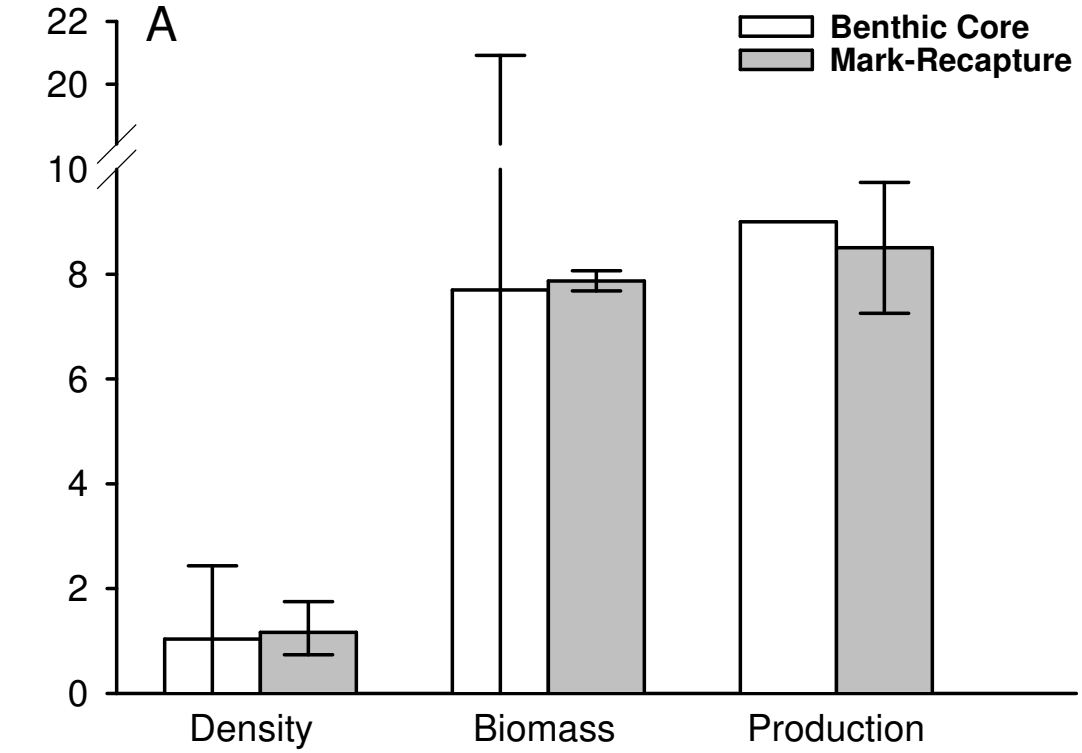
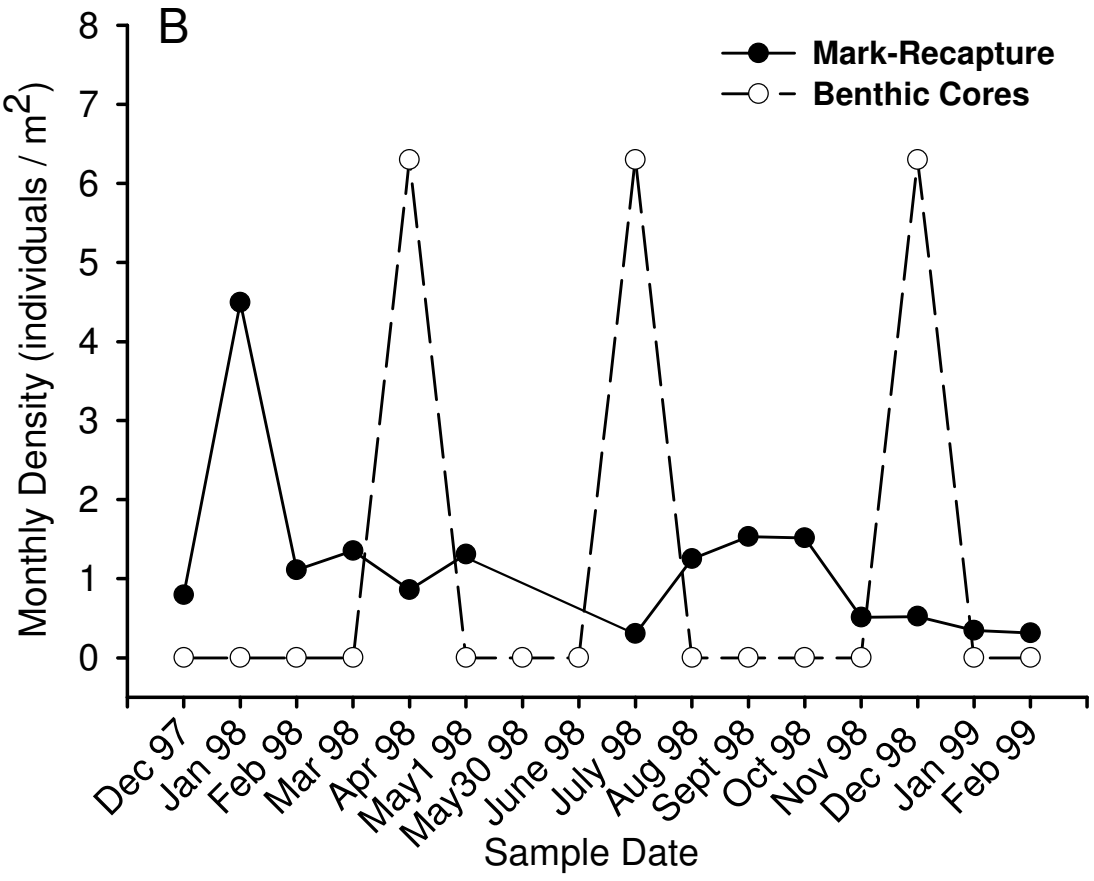

Number of Recaptures
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
Time Interval Between Initial Marking
and Final Recapture (mo.)
Reference
Recovery
Litter Exclusion

Figure 3.8. Comparison of larval *Eurycea wilderae* sampling methods from December 1997 to February 1999. Comparisons are for mean (A) Density (individuals $m^{-2}$), biomass (mg AFDM $m^{-2}$), and habitat weighted annual production (mg AFDM $m^{-2}$ $yr^{-1}$). Error bars are mean +/- 95% bootstrapped CI. (Error could not be calculated for estimates based on size frequency production for benthic core samples). (B) Monthly larval density estimates in reference stream for mark-recapture and benthic core samples.


A
Benthic Core
Mark-Recapture
22
20
10
8
6
4
2
0
Density
Biomass
Production


B
Mark-Recapture
Benthic Cores
Monthly Density (individuals / m2)
8
7
6
5
4
3
2
1
0
Dec 97
Jan 98
Feb 98
Mar 98
Apr 98
May1 98
May30 98
June 98
July 98
Aug 98
Sept 98
Oct 98
Nov 98
Dec 98
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Feb 99
Sample Date

# CHAPTER 4

# LONG-TERM RESOURCE LIMITATION REDUCES INSECT DETRITIVORE GROWTH IN A HEADWATER STREAM[1]

[1]Johnson, B.R., W.F. Cross, and J.B. Wallace. To be submitted to *Journal of the North American Benthological Society*.

ABSTRACT

Terrestrial litter inputs were excluded from a southern Appalachian headwater stream beginning in autumn 1993 to assess the role of detritus in stream ecosystems. Previous studies have shown that litter exclusion reduces abundance, biomass, and secondary production of benthic macroinvertebrates. We measured *in situ* larval growth rates of two dominant stream insect detritivore groups to help determine the mechanism underlying declines in invertebrate production in the treatment stream. Larval *Tallaperla* spp. (Plecoptera) were chosen as representative shredders and non-Tanypodinae Chironomidae (Diptera) were selected as representative collector-gatherers. Instantaneous growth rates (IGRs) were measured in the treatment stream and in two undisturbed reference streams using *in situ* growth chambers. Estimates of daily growth rates were derived from change in mean length of larvae over incubation periods. Initial larval length was a significant predictor of growth in each stream for both taxonomic groups ($r^2$ >0.43-0.72, $p<0.05$). Comparison of significant regression lines showed that growth of both *Tallaperla* spp. and chironomids were significantly reduced in the litter exclusion stream (ANCOVA, $p<0.05$). Lower chironomid growth rates in the treatment stream indicates that production estimates based on the instantaneous growth method are actually lower than previously reported for the site. Mortality of *Tallaperla* spp. was also significantly lower in the treatment stream (ANOVA, $p<0.05$). Reduced growth of these representative taxa apparently results from reduced quantity of organic matter food resources. These results show that reduced growth is partially responsible for observed declines in detritivore production in the litter exclusion stream.

# INTRODUCTION

Terrestrial detritus is the primary energy source in shaded headwater streams of the southern Appalachians (Webster et al. 1983, Webster et al. 1995, Webster and Meyer 1997). Many stream invertebrates feed directly on this detritus and play an important role in organic matter processing (e.g. Cummins et al. 1973, Cummins 1973, 1974, Hynes 1975, Vannote et al. 1980, Wallace et al. 1982, Cushing et al. 1995, Wallace and Webster 1996). In addition, insect detritivores may have life histories that are keyed to autumn leaf fall (Sweeney 1984, Huryn and Wallace 2000). Thus, tight linkages exist between terrestrial detritus inputs, stream detritivores, and ecosystem structure and function (e.g. Fisher and Likens 1973, Hynes 1975, Vannote et al. 1980, Cushing et al. 1995, Wallace et al. 1997, 1999). Disturbances (e.g., logging, mining, urbanization, agriculture) that change the quantity or quality of organic matter entering streams can disrupt aquatic-terrestrial linkages and alter ecosystem dynamics (Webster et al. 1983).

In a long-term ecosystem-level manipulation, Wallace et al. (1997, 1999) excluded terrestrial detritus inputs to a headwater stream at the Coweeta Hydrologic Laboratory in western North Carolina. After 4 y of treatment, organic matter standing crop had declined significantly and invertebrate production in mixed substrate habitats was among the lowest reported for streams (Wallace et al. 1999). More specifically, production of shredder and collector-gatherer functional feeding groups (FFG) declined to 13 and 24% of pretreatment values, respectively. The mechanism responsible for reduced invertebrate production, however, remains uncertain. Reduced larval production can result from reduced abundance, biomass, or individual growth rate (Benke 1984).

The objective of this study was to gain a better understanding of the mechanism underlying reduced secondary production in the litter exclusion stream by measuring growth rates of two dominant stream detritivore groups. Larval *Tallaperla* spp. (Plecoptera) were chosen as representative shredders. Up to four species of *Tallaperla* potentially co-occur in Coweeta headwaters: *T. maria*, *T. anna*, *T. cornelia*, and *T. elisa* (Stewart and Stark 1993). All these species feed on leaf detritus (Merritt and Cummins 1996) and have similar semivoltine life cycles in these streams (O'Hop et al. 1984 [as *Peltoperla maria*], Huryn and Wallace 1987). Non-Tanypodinae chironomid larvae were chosen as representative collector-gatherers. These midges feed on fine benthic organic matter (FBOM) and undergo rapid growth that results in several generations per year in Coweeta streams (Huryn 1986, 1990). We predicted that litter exclusion would reduce growth rates for these representatives of the shredder and gatherer FFGs.

*Study Sites*

This study was conducted in three first order streams draining catchments (C) 53 (reference 1), 54 (reference 2), and 55 (litter exclusion) at the Coweeta Hydrologic Laboratory in Macon County, North Carolina. These three streams have similar physical characteristics including catchment size, discharge, and thermal regime (Table 4.1). Vegetation consists of mixed hardwoods with a dense understory of rhododendron (*Rhododendron maximum*) that shades the streams throughout the year. The heavy shading results in extremely low in-stream primary production. Additional details of the Coweeta basin are provided in Swank and Crossley (1988). Litter exclusion treatment in C 55 began in August 1993 when an overhead net canopy (2.5-cm mesh) was installed over the uppermost 170-m of the stream. Plastic drift fences (20-cm high with 1-cm

mesh) were installed along each side of the treatment stream to prevent lateral inputs. A large (> 1 m) space between the fence and canopy allowed aerial recolonization by aerial insects. Riparian vegetation was left in place during canopy installation to prevent changing the natural light regime. Leaves were removed from the canopy once a week in autumn and when needed in other seasons. As a result of these manipulations, organic matter standing crop in the treatment stream has been reduced by approximately 95% compared to pretreatment and reference stream values (Wallace et al. 1999).

## METHODS

Growth rates for *Tallaperla* and non-Tanypodinae chironomids were measured seasonally in each stream beginning in November 1999. *Tallaperla* growth was measured in C 55 (litter exclusion) and C 53 (reference 1) through April 2001 (n=6 seasons) and in C 54 (reference 2) through June 2000 (n=3 seasons). Chironomid growth was measured in C 55 and C 53 through September 2000 (n=4 seasons) and in C 54 through June 2000 (n=3 seasons). Fewer growth estimates were made in C 54 because this study only includes pretreatment data for an ongoing experiment in that catchment. Temperature data were collected continuously in each stream using digital recorders. Mean daily temperatures over each incubation period were averaged to obtain a mean temperature during growth incubations.

Litter for reference stream chambers was rinsed through a series of nested sieves using stream water and the sieve contents placed under a dissecting microscope for collection of larvae. Length of each larva was then measured to the nearest 0.01 mm with an ocular micrometer and grouped into 1-mm length classes. Size classes were chosen based on the general size distribution in the stream at the time of collection. Three

chironomid size classes and from two to four *Tallaperla* size classes were used in each stream each season.

Larvae of the same size class were incubated separately in the three streams using the triangular growth chambers described by Huryn and Wallace (1986). Side panels of the growth chambers consisted of 63-μm Nitex mesh. Chambers were placed in the stream with their top extending above the water surface and with the triangular wedge oriented directly into the current to reduce drag and prevent mesh clogging. Chambers were anchored to the stream bottom by placing rocks along external flanges of the chambers. From 15 to 51 *Tallaperla* and from 19-94 chironomids were initially introduced to each chamber. In some seasons two widely disparate *Tallaperla* size classes were placed in the same chamber to increase the number of growth estimates. Chamber densities were well within the range of natural densities found in undisturbed Coweeta headwaters (Wallace et al. 1999).

Each season 8-10 conditioned leaves were collected from the reference steam, rinsed, and placed in each of the reference stream chambers for food and substrate. Leaf species depended on time of year and availability, but consisted primarily of rhododendron (*Rhododendrom maximum* L.), maple (*Acer* spp.), beech (*Fagus gradifolia* Ehrh.), yellow popular (*Liriodendron tulipifera* L.), oak (*Quercus* spp.) and dogwood (*Cornus florida* L.). In the litter exclusion stream where leaves are absent, stream substrate consisting of sand, gravel and FBOM was placed in each chamber to accurately reflect stream conditions. *Tallaperla* were incubated for approximately two months, and leaves in reference stream chambers were replaced midway through incubation. There was no replacement of FBOM in litter exclusion stream chambers because they naturally

accumulated FBOM during incubation periods. Midges were incubated for 7-14 days depending on stream temperature. Chironomids >3-mm were not used in the chambers to prevent emergence during incubation.

At the end of incubation periods, chamber contents were preserved in plastic bags containing Khale's solution. All recovered larvae were then measured again using the method described above. Initial and final length measurements were converted to ash-free dry mass (AFDM) using known length-weight regressions:

*Tallaperla* $W=0.0194\ L^{2.853}$ (Benke et al. 1999)

Midges $W= 0.452\ L^{3.099}$ (Huryn and Wallace 1986)

where W is AFDM in µg (midges) or mg (*Tallaperla*) and L is length in mm. Mean larval growth rate ($day^{-1}$) for each size class was then calculated for both taxa as:

$$g = (\ln(M_f)-\ln(M_i))/t$$

where $M_f$ is final AFDM, $M_i$ is initial AFDM, and t is the number of days of incubation. This method produces community-level growth estimates for *Tallaperla* spp. and non-Tanypodine chironomids. The underlying assumption is that changes in mean weight of similar sized larvae of different taxonomic composition can be used to estimate mean growth rates that are representative of taxa in the streams (Huryn and Wallace 1986, Huryn 1990).

Multiple regression models were initially used to measure the influences of initial length and temperature on larval growth rates. Differences in growth rates among streams were then assessed by comparing slopes of significant regression lines by analysis of covariance (ANCOVA) with initial length as the covariate. Initial length was used as the covariate because small larvae generally grow faster than larger larvae (e.g.,

Huryn and Wallace 1986, Perry et al. 1987, Huryn 1990). Incubation temperatures and larval mortality (% mortality $d^{-1}$) were compared among streams by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Regression models were also used to assess the influence of temperature and initial length on larval survivorship.

## RESULTS

### *Tallaperla* spp.

A total of 33 *Tallaperla* growth measurements were taken in the three streams (C53 n=15; C54 n=6; C55 n=12). Mean incubation temperatures ranged from 5 to 16.8 °C over the six incubation periods, and there were no temperature differences among streams (ANOVA, $p>0.05$). Temperature had little influence on growth in the study streams ($r^2<0.15$, $p>0.05$) and was therefore dropped from the growth model. Initial length, however, was a strong predictor of larval growth rate and resulted in significant regression equations for each stream ($r^2$: 0.50-0.70, $p<0.05$). Growth was negatively correlated with initial length indicating that smaller size classes exhibited higher growth rates during incubations.

Comparison of growth equations showed no significant differences between the two reference streams (ANCOVA, $p<0,05$) so these data were combined to yield a single reference growth equation. The relationship between growth and initial length in the reference streams was best described by the linear equation: $y = -0.001x + 0.0128$ ($r^2=0.596$, $p<0.001$) (Fig. 4.1). The corresponding growth regression equation derived for the litter exclusion stream was: $y= -0.001x + 0.009$ ($r^2=0.502$, $p<0.05$). Reference and litter exclusion regression equations were significantly different (ANCOVA, $p<0.01$),

showing reduced *Tallaperla* growth in the treatment stream (Fig. 4.1). Half the growth measurements in the litter exclusion stream resulted in negative growth rates whereas all 21 measurements showed positive growth in the reference streams.

Larval mortality differed significantly among streams (ANOVA, $p$=0.005) with mortality in the litter exclusion stream significantly lower than both reference streams (Tukey test, $p$<0.05) (Fig. 4.2). Mortality was expressed as % dieing per day to account for differences in time of incubation over the year. Mean daily mortality in the reference streams chambers were both around 0.5% $d^{-1}$ whereas mortality in the litter exclusion stream was nearly 1% $d^{-1}$ (Fig. 4.2). Larval mortality was negatively correlated with mean incubation temperature in the litter exclusion stream ($r^2$=0.417, p<0.05). No larvae were recovered from either chamber incubated in the litter exclusion stream in spring 2000 (April 23, 2000 to June 20, 2000) when the mean stream temperature was 13.6 °C, and only 17% of the individuals survived for the one chamber recovered in summer 2000 (7/26/00 to 9/29/00) when the mean temperature was 16.6 °C (the second growth chamber was destroyed by an animal during the summer 2000 incubation period). There was no relationship between temperature and mortality in the reference streams (combined data: $r^2$=0.003, p>0.05). Initial length was not related to larval mortality in any stream ($r^2$<0.070, p>0.05).

*Non-Tanypodinae Chironomids*

Twenty-nine chironomid growth measurements were taken over the study period (C53=10; C54=8; C55=11). Mean incubation temperatures ranged from 8.5 to 16.3 °C and there were no temperature differences among streams (ANOVA, p>0.05). As with *Tallaperla*, the relationship between chironomid growth and temperature was not

significant in the study streams ($r^2$<0.33, $p$>0.05) so temperature was dropped from growth regression equations. Chironomid growth rates were also negatively correlated with initial length in each stream ($r^2$: 0.43-0.72, $p$<0.05).

Growth rates did not differ between reference streams (ANCOVA, $p$>0.05) so these data were combined to produce the linear equation: y = -0.035x + 0.132 ($r^2$=0.642, $p$<0.001) (Fig. 4.3). In the litter exclusion stream, the corresponding growth equation was: y = -0.023x + 0.073 ($r^2$=0.430, $p$<0.05). Growth rates for all larval size classes were generally lower in the litter exclusion stream than in either reference stream. Comparison of reference and litter exclusion growth equations showed midge growth was significantly reduced in the treatment stream (ANCOVA, $p$<0.001) (Fig. 4.3).

There were no differences in midge mortality among streams (ANOVA, $p$>0.05). Mortality in each of the streams was around 0.5% $d^{-1}$ and mortality was not influenced by either temperature ($r^2$<0.132, $p$>0.05) or initial length ($r^2$<0.068, p>0.05) in any of the study streams.

## DISCUSSION

After four years of litter exclusion, peltoperlid production had declined by approximately 77% compared to a reference stream (C53) and pre-treatment values (Wallace et al. 1999). Coarse benthic organic matter (CBOM) had declined significantly due to treatment, and leaf detritus had been nearly eliminated from the stream (Wallace et al. 1999). The lack of leaf detritus likely led to reduced growth and increased mortality of *Tallaperla* larvae in the litter exclusion stream. *Tallaperla* growth rates were characteristically low in all three streams, as would be expected for long-lived semivoltine taxa (Sweeney 1984). Yet, in the reference stream, growth rates were more

than three times higher. Furthermore, larvae lost mass during incubation in half of the growth chambers in the treatment stream. Interestingly, growth of the smallest size class measured during the study period (winter 2001) was actually greater in the litter exclusion stream than in the reference stream (Fig. 4.1). This indicates that early instar peltoperlids may be able to rely on FPOM as a food resource rather than shredding leaf detritus. *Tallaperla* larvae are adapted to cold streams and are capable of growth through winter months (O'Hop et al. 1984, Huryn and Wallace 1987). The fact that we found no relationship between larval growth and incubation temperature may result from the relatively stable thermal regime in these groundwater-dominated streams. The lack of temperature effect may also indicate that growth in these headwater streams may be influenced more by timing and availability of leaf litter inputs than by temperature alone.

*Tallaperla* mortality was also significantly reduced due to treatment (Fig. 4.2). Mortality declined with increasing stream temperature only in the litter exclusion stream. Greater mortality in the summer likely resulted from lack of available food and increased metabolic demands associated with increased stream temperatures.

Chironomid growth rates in the reference streams were characteristically high and indicative of fast turnover rates. Chironomid growth rates in reference streams were within the range of midge growth estimates previously reported at Coweeta (Huryn and Wallace 1986, Huryn 1991) and in other temperate streams (Berg and Hellenthal 1992). As with *Tallaperla*, there was no relationship between growth and temperature in any of the study streams. This again is not surprising given that midges in the chambers were representative of the entire midge community and thus contained many taxa that may

have different thermal optima (Rempel and Harrison 1987, Berg and Hellenthal 1992), but see Huryn (1990).

In mixed substrate habitats of the litter exclusion stream, non-tanypod chironomid production had declined by approximately 82% compared to reference and pre-treatment values (Wallace et al. 1999). Growth rates in the litter exclusion stream averaged less than half of those in reference streams. FBOM, like CBOM, has declined significantly as a result of treatment (Wallace et al. 1999). FBOM in the litter excluded stream was only 32% of that in the reference stream following three years of treatment (Meyer et al. 1998). With a reduction in the FBOM food resource, midges would likely collect and ingest greater quantities of inorganic sediments. This lower quality diet may explain the reduced individual growth rates. There were no differences in midge survival among streams, suggesting that larvae may survive equally well on a lower quality diet, but at the expense of reduced growth.

Litter exclusion has resulted in some of the lowest secondary production values reported for stream ecosystems. By the fourth year of treatment, Wallace et al. (1999) estimated that total production of the benthic community in mixed-substrate habitats had declined to 1.1 g AFDM $m^{-2}$ $yr^{-1}$ (a decline of 78% from pretreatment values). Chironomids alone accounted for over 16% of that production. In that study chironomid production was calculated by the instantaneous growth method (Benke 1984) using growth rates established for undisturbed Coweeta streams by Huryn and Wallace (1986) and as modified by Huryn (1990). The fact that our results show chironomid growth rates are reduced in the treatment stream means that Wallace et al. (1999) actually overestimated chironomid production and that total benthic production in the treatment

stream is even lower than previously reported. Application of our newly derived chironomid growth rates for C55 to 1998-1999 benthic data lowered chironomid production estimates nearly three fold in mixed substrate habitats, from 194 to 68 mg AFDM $m^{-2}$ $yr^{1}$.

Detritus limitation resulted in significantly lower growth rates for representatives of both shredder and collector-gatherer FFGs. Reduced individual growth is therefore one mechanism, along with lower biomass and density, responsible for the exceptionally low detritivore production in mixed-substrate habitats of a stream with reduced detrital resources.

As result of poor management practices, riparian vegetation is often removed for development, logging, and agricultural activities. Failure to maintain an appropriate riparian buffer zone results in streams that are effectively disconnected from the surrounding landscape. Several studies have now demonstrated the importance of the detrital resource for stream ecosystem productivity (Bilby et al. 1996, Wallace et al. 1997, 1999, Wipfli et al. 1998, 1999). Our results support those findings and further show that reduced growth rate of insect detritivores is yet another consequence of severing the aquatic-terrestrial linkage.

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Table 4.1. Physical parameters of streams draining catchments 53 (reference 1), 54 (reference 2), and 55 (litter exclusion treatment) at the Coweeta Hydrologic Laboratory. Elevations were measured at the gauging flumes. Data are from Wallace et al. 1999 and W. Cross, unpublished.

| Variable | C53 | C 54 | C 55 |
|---|---|---|---|
| Catchment | | | |
| Area (ha) | 5.2 | 5.5 | 7.5 |
| Elevation (m asl) | 829 | 841 | 810 |
| Channel | | | |
| Length (m) | 145 | 282 | 170 |
| Bankful Area ($m^2$) | 327 | 443 | 373 |
| Discharge (L/s) | | | |
| Average * | 1.19 | 1.45 | 2.39 |
| Maximum * | 47.2 | 35.5 | 40.2 |
| Temperature (°C) | | | |
| Annual Average † | 12.2 | | 12.2 |
| Maximum † | 20.3 | 19.5 | 20.1 |
| Minimum † | 0.7 | 1.1 | 0.7 |
| Annual degree-days † | 4485 | 4440 | 4512 |

* C53 averages from 12 years (1984-1996), C54 averages from 8 years (1985-1992), C55 averages from 5 years (1992-1997)

†C53 and C55 averages from 12 years (1985 -1997), C54 averages from 8years (1985-1992)

Figure 4.1. Relationships between instantaneous growth rate ($day^{-1}$) and initial length (mm) for *Tallaperla* spp. in two reference streams (C53 and C54) and the litter exclusion stream (C55). Growth measurements were made seasonally in each stream beginning November 1999 (C53 and C55 through April 2001, n=6 seasons; C54 through June 2000, n=3 seasons).

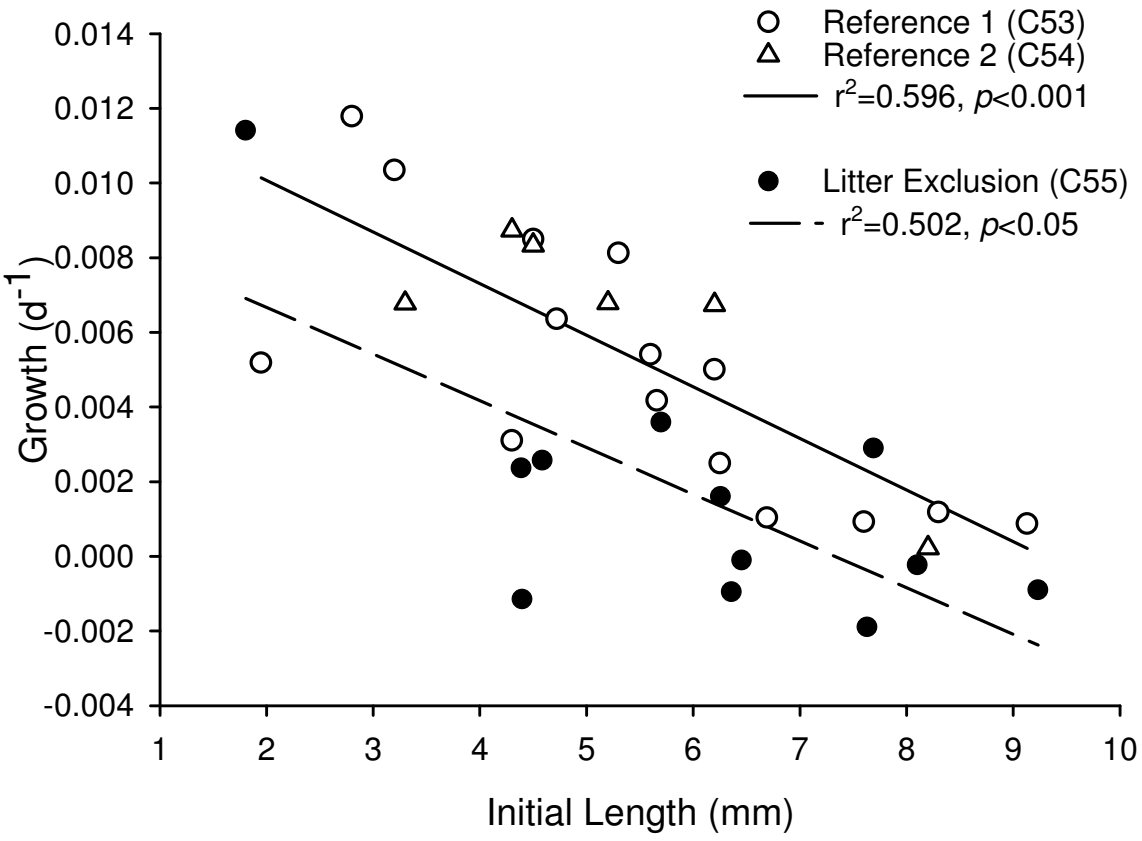

Reference 1 (C53)
Reference 2 (C54)
r²=0.596, p<0.001
Litter Exclusion (C55)
r²=0.502, p<0.05
Growth (d⁻¹)
Initial Length (mm)
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
-0.002
-0.004
1
2
3
4
5
6
7
8
9
10

Figure 4.2. Average daily mortality (expressed as % $d^{-1}$ +/- 1 S.E.) of larval *Tallaperla* spp. from chambers incubated in two reference streams (C53 [n=15] and C54 [n=6]) and the litter exclusion stream (C55 [n=12]).

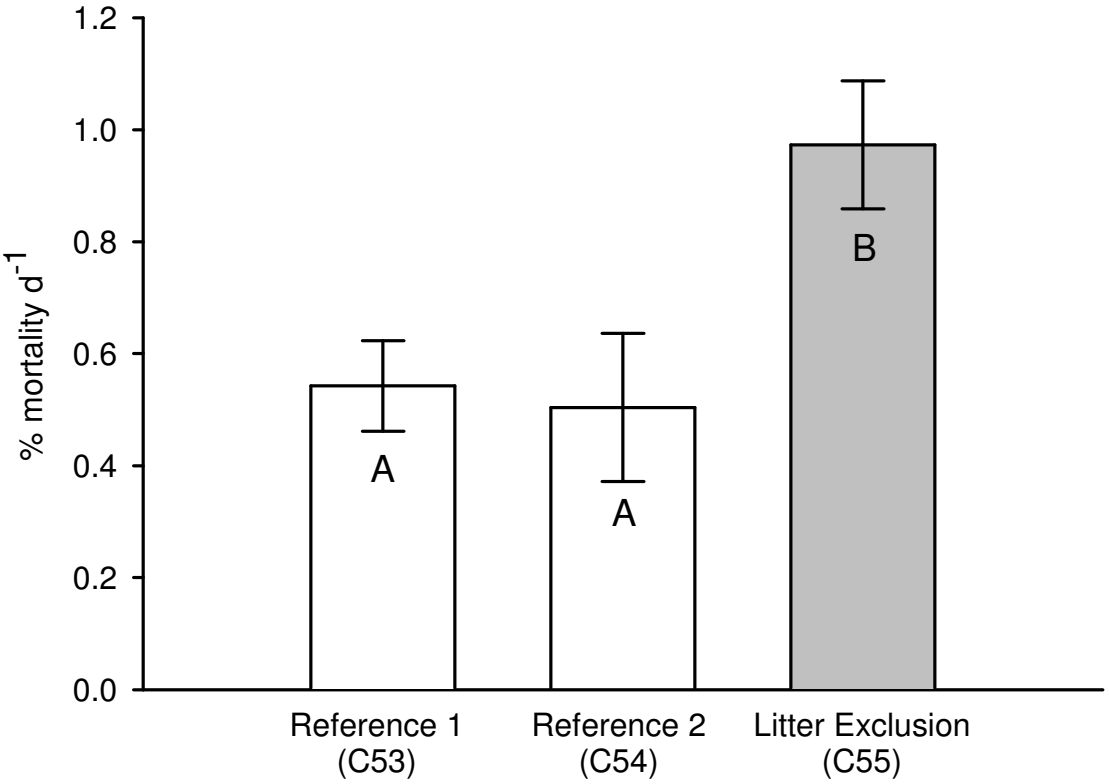
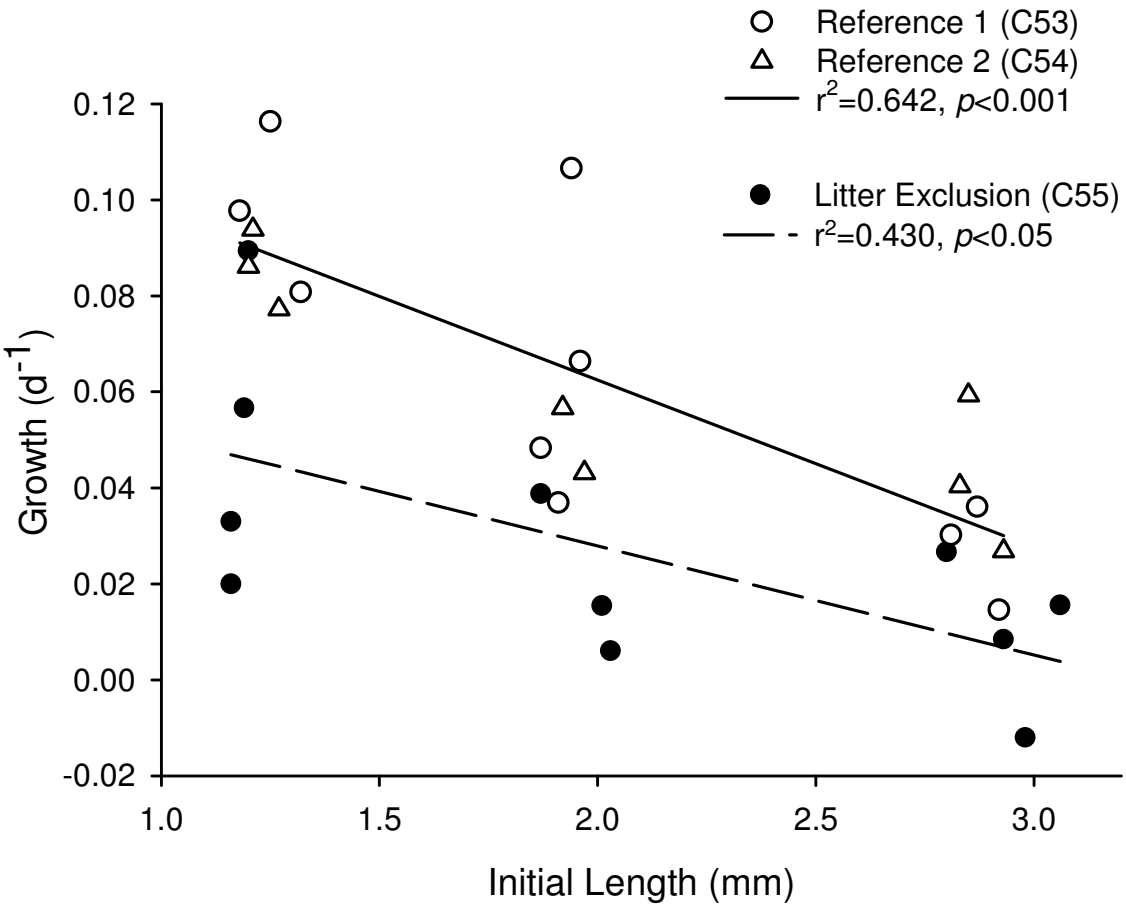

1.2
1.0
0.8
0.6
0.4
0.2
0.0
% mortality d-1
A
A
B
Reference 1
(C53)
Reference 2
(C54)
Litter Exclusion
(C55)

Figure 4.3. Relationship between instantaneous growth rate ($day^{-1}$) and initial length (mm) for non-Tanypodinae Chironomidae in two reference streams (C53 and C54) and the litter exclusion stream (C55). Growth measurements were made seasonally in each stream beginning November 1999 (C53 and C55 through September 2000 [n=4 seasons]; C54 through June 2000 [n=3 seasons]).


Reference 1 (C53)
Reference 2 (C54)
r2=0.642, p<0.001
Litter Exclusion (C55)
r2=0.430, p<0.05
Growth (d-1)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
1.0
1.5
2.0
2.5
3.0
Initial Length (mm)

Appendix A. Size and temperature-specific growth data used to derive A) non-Tanypodinae chironomid and B) *Tallaperla* spp. growth rates and regression equations used in this study. *Li*- Average initial length (mm); *Mi* and *Mf* – average initial and final mass (in µg for chironomids and mg for *Tallaperla* spp.) of individuals used in growth chambers; *ni* and *nf* – initial and final numbers of individuals observed; *t* – number of days incubated; *g* – daily growth rate; *T* – average stream temperature (°C) during incubation; X – no recovery.

*A) Non-Tanypodine Chironomidae*

| Date | Li | Mi | ni | Mf | nf | t | g | T |
|---|---|---|---|---|---|---|---|---|
| | | | | *C53* | | | | |
| Nov 7 99 | 1.25 | 1.18 | 30 | 5.99 | 18 | 14 | 0.116 | 10.9 |
| Nov 7 99 | 1.91 | 3.77 | 50 | 6.32 | 12 | 14 | 0.037 | 10.9 |
| Nov 7 99 | 2.92 | 11.89 | 19 | 14.59 | 4 | 14 | 0.015 | 10.9 |
| Feb 21 00 | 1.18 | 1.00 | 51 | 4.33 | 27 | 14 | 0.098 | 9.6 |
| Feb 21 00 | 1.87 | 3.60 | 94 | 7.43 | 21 | 14 | 0.048 | 9.6 |
| Feb 21 00 | 2.87 | 11.30 | 29 | 19.41 | 8 | 14 | 0.036 | 9.6 |
| May 25 00 | 1.96 | 4.14 | 59 | 9.17 | 16 | 12 | 0.066 | 14.8 |
| May 25 00 | 2.81 | 10.64 | 26 | 15.29 | 15 | 12 | 0.030 | 14.8 |
| Sept 9 00 | 1.32 | 1.32 | 40 | 2.52 | 16 | 8 | 0.081 | 16.3 |
| Sept 9 00 | 1.94 | 3.97 | 82 | 9.32 | 10 | 8 | 0.107 | 16.3 |
| | | | | *C54* | | | | |
| Nov 7 99 | 1.20 | 1.05 | 30 | 3.52 | 13 | 14 | 0.086 | 10.7 |
| Nov 7 99 | 2.83 | 10.99 | 20 | 19.35 | 3 | 14 | 0.040 | 10.7 |
| Feb 20 00 | 1.21 | 1.05 | 53 | 4.31 | 22 | 15 | 0.094 | 9.6 |
| Feb 20 00 | 1.92 | 3.85 | 71 | 9.01 | 31 | 15 | 0.057 | 9.6 |
| Feb 20 00 | 2.93 | 12.03 | 29 | 17.99 | 13 | 15 | 0.027 | 9.6 |
| May 25 00 | 1.27 | 1.20 | 40 | 3.04 | 5 | 12 | 0.077 | 13.9 |
| May 25 00 | 1.97 | 4.16 | 59 | 6.98 | 8 | 12 | 0.043 | 13.9 |
| May 25 00 | 2.85 | 11.14 | 30 | 22.69 | 4 | 12 | 0.059 | 13.9 |
| | | | | *C55* | | | | |
| Nov 7 99 | 1.16 | 0.96 | 30 | 1.52 | 9 | 14 | 0.033 | 10.2 |
| Nov 7 99 | 2.01 | 4.33 | 51 | 5.37 | 8 | 14 | 0.015 | 10.2 |
| Nov 7 99 | 2.98 | 12.61 | 20 | 10.66 | 3 | 14 | -0.012 | 10.2 |
| Feb 21 00 | 1.16 | 0.94 | 36 | 1.27 | 13 | 14 | 0.020 | 8.5 |
| Feb 21 00 | 2.03 | 4.50 | 90 | 4.93 | 29 | 14 | 0.006 | 8.5 |
| Feb 21 00 | 3.06 | 13.58 | 23 | 17.16 | 12 | 14 | 0.016 | 8.5 |
| May 25 00 | 1.19 | 1.02 | 31 | 2.02 | 10 | 12 | 0.057 | 14.5 |
| May 25 00 | 2.80 | 10.53 | 34 | 14.50 | 14 | 12 | 0.027 | 14.5 |
| Sept 9 00 | 1.20 | 1.01 | 28 | 2.08 | 4 | 8 | 0.089 | 16.3 |
| Sept 9 00 | 1.87 | 3.66 | 56 | 5.00 | 20 | 8 | 0.039 | 16.3 |
| Sept 9 00 | 2.93 | 11.97 | 28 | 12.81 | 9 | 8 | 0.008 | 16.3 |

Appendix A (continued)

B) *Tallaperla* spp.

| Date | Li | Mi | ni | Mf | nf | t | g | T |
|---|---|---|---|---|---|---|---|---|
| | | | | *C53* | | | | |
| Nov 3 99 | 4.30 | 1.31 | 30 | 1.62 | 22 | 67 | 0.003 | 9.2 |
| Nov 3 99 | 8.30 | 8.12 | 15 | 8.80 | 9 | 67 | 0.001 | 9.2 |
| Feb 7 00 | 4.50 | 1.45 | 40 | 2.33 | 21 | 55 | 0.008 | 9.8 |
| Feb 7 00 | 6.20 | 3.58 | 27 | 4.74 | 24 | 55 | 0.005 | 9.8 |
| April 23 00 | 3.20 | 0.55 | 36 | 1.00 | 33 | 58 | 0.010 | 14 |
| April 23 00 | 5.30 | 2.25 | 30 | 3.61 | 22 | 58 | 0.008 | 14 |
| July 26 00 | 2.80 | 0.36 | 20 | 0.77 | 7 | 65 | 0.012 | 16.8 |
| July 26 00 | 5.66 | 2.74 | 18 | 3.59 | 7 | 65 | 0.004 | 16.8 |
| July 26 00 | 6.69 | 4.39 | 5 | 4.70 | 4 | 65 | 0.001 | 16.8 |
| Nov 6 00 | 4.72 | 1.63 | 9 | 2.47 | 5 | 65 | 0.006 | 5.7 |
| Nov 6 00 | 6.25 | 3.64 | 35 | 4.27 | 30 | 65 | 0.002 | 5.7 |
| Nov 6 00 | 9.13 | 10.69 | 6 | 11.31 | 2 | 65 | 0.001 | 5.7 |
| Feb 10 01 | 1.95 | 0.14 | 39 | 0.19 | 23 | 61 | 0.005 | 9.2 |
| Feb 10 01 | 5.60 | 2.67 | 25 | 3.71 | 23 | 61 | 0.005 | 9.2 |
| Feb 10 01 | 7.60 | 6.40 | 10 | 6.77 | 7 | 61 | 0.001 | 9.2 |
| | | | | *C54* | | | | |
| Nov 3 99 | 4.30 | 1.23 | 30 | 2.22 | 10 | 67 | 0.009 | 9.3 |
| Nov 3 99 | 8.20 | 7.90 | 17 | 8.02 | 9 | 67 | 0.0002 | 9.3 |
| Feb 7 00 | 4.50 | 1.42 | 40 | 2.26 | 27 | 55 | 0.008 | 9.7 |
| Feb 7 00 | 6.20 | 3.48 | 26 | 5.07 | 21 | 55 | 0.007 | 9.7 |
| April 23 00 | 3.30 | 0.61 | 35 | 0.90 | 33 | 58 | 0.007 | 13.2 |
| April 23 00 | 5.20 | 2.19 | 30 | 3.25 | 25 | 58 | 0.007 | 13.2 |
| | | | | *C55* | | | | |
| Nov 3 99 | 4.40 | 1.35 | 30 | 1.23 | 6 | 67 | -0.001 | 8.2 |
| Nov 3 99 | 8.10 | 7.66 | 15 | 7.53 | 7 | 67 | -0.0002 | 8.2 |
| Feb 7 00 | 4.39 | 1.33 | 42 | 1.52 | 27 | 55 | 0.002 | 8.8 |
| Feb 7 00 | 6.26 | 3.65 | 32 | 4.00 | 27 | 55 | 0.002 | 8.8 |
| April 23 00 | 3.97 | 0.99 | 35 | X | 0 | 58 | X | 13.6 |
| April 23 00 | 6.64 | 4.31 | 30 | X | 0 | 58 | X | 13.6 |
| July 26 00 | 6.45 | 4.04 | 29 | 4.01 | 5 | 65 | -0.0001 | 16.6 |
| Nov 6 00 | 4.59 | 1.52 | 12 | 1.79 | 7 | 65 | 0.003 | 5.0 |
| Nov 6 00 | 6.36 | 3.82 | 37 | 3.59 | 19 | 65 | -0.001 | 5.0 |
| Nov 6 00 | 7.63 | 6.42 | 29 | 5.68 | 19 | 65 | -0.002 | 5.0 |
| Nov 6 00 | 9.23 | 11.04 | 10 | 10.41 | 4 | 65 | -0.001 | 5.0 |
| Feb 10 01 | 1.81 | 0.11 | 51 | 0.23 | 22 | 61 | 0.011 | 8.4 |
| Feb 10 01 | 5.70 | 2.80 | 26 | 3.48 | 5 | 61 | 0.004 | 8.4 |
| Feb 10 01 | 7.69 | 6.56 | 15 | 7.82 | 7 | 61 | 0.003 | 8.4 |

# CHAPTER 5

# THE EFFECTS OF NUTRIENT ENRICHMENT ON LARVAL SALAMANDER GROWTH IN A DETRITUS-BASED STREAM[1]

[1]Johnson, B.R., and J.B. Wallace. To be submitted to *Limnology and Oceanography*.

ABSTRACT

Though many studies have measured the effects of nutrient enrichment on higher trophic levels in grazing food webs, few such studies exist for detritus-based systems. We measured the effects of N+ P addition on growth of larval Blue-Ridge two-lined salamander, *Eurycea wilderae*, in a detritus-based headwater stream. A repeated mark-recapture study was conducted in two streams draining catchments (C) 53 and 54 at the Coweeta Hydrologic Laboratory, North Carolina, USA. Salamander larvae were collected by hand from each stream reach monthly from July 1998 through March 2001. N and P were added to C54 via a continuous flow pump beginning June 2000. Growth estimates based on 208 recaptured larvae (C53 n=92; C54 n=116) resulted in a growth rate of 0.0027 $d^{-1}$ in each stream during pre-treatment. Growth rates were significantly higher in both streams during treatment, indicating inter-annual growth variation. Growth rates in the treatment stream (C54 g= 0.0069 $d^{-1}$ [± 0.0019, 95% C.I.]) were significantly higher than in the reference stream during enrichment (C53 g= 0.0043 $d^{-1}$ [± 0.0007, 95% C.I.]). Interannual growth variation may result from reduced stream discharge and increased organic matter standing crop during treatment. These results indicate that *E. wilderae* growth is tightly linked to detritus in these streams and that growth may be indirectly affected by both quantity and quality of the resource. This study provides some of the first evidence that nutrient enrichment of detrital systems can influence higher trophic levels in ways similar to autotrophic systems.

Human activities have altered the availability of nitrogen and phosphorus on a global scale (Vitousek 1994, Vitousek et al. 1997, Carpenter et al. 1998). Fertilizer production, fossil fuel combustion, and domestic sewage outflows are but a few of the factors that ultimately result in increased quantities of these potentially limiting nutrients in aquatic systems. Nutrient enrichment is widely recognized as one of the primary threats to aquatic ecosystems. Increased nutrients can lead to uncontrolled and noxious algal growths and, subsequently, reduced dissolved oxygen concentrations that can stress aquatic fauna.

Numerous studies have investigated the effects of nutrients in autotrophic streams (e.g., Peterson et al. 1985, 1993, Hart and Robinson 1990, Rosemond et al. 1993, Harvery et al. 1998, Biggs 2000) and lakes (see Elser et al. 1990 for review). In general, nutrient increases result in increased primary production (e.g., Peterson et al. 1985, Hart and Robinson 1990, Rosemond et al. 1993, Biggs 2000) that can potentially increase production at higher trophic levels (e.g., Johnston et al. 1990, Deegan and Peterson 1992, Peterson et al. 1993, Harvey et al. 1998). Far less is known, however, about the effects of nutrient enrichment in detritus-based ecosystems.

In detrital systems, such as forested headwater streams, nutrient enrichment may result from atmospheric nitrogen deposition or from domestic wastewater that results from the increasing expansion of the human population into mountainous regions. In these streams, production of microbes at the base of the food web is dependent on availability of both nutrients and organic carbon. Short-term nutrient addition experiments have shown increases in microbial production (e.g., Suberkropp and Chauvet

1995, Suberkropp 1998, Tank and Webster 1998), increased organic matter decomposition rates (e.g., Elwood et al. 1981, Meyer and Johnson 1983, Robinson and Gessner 2000), and increases in invertebrate abundance and biomass (Pearson and Connolly 2000, Robinson and Gessner 2000). Addition of salmon carcasses to detritus-based streams in Alaska has also shown to increase productivity of fish and invertebrates (Bilby et al. 1996, Wipfli et al. 1998, Wipfli et al. 1999). However, the effects of nutrient enrichment on vertebrate predators in detritus-based ecosystems remain largely unknown.

Larval salamanders are the top predators in high-gradient streams of the southern Appalachians. The Blue Ridge two-lined salamander, *Eurycea wilderae* Dunn, is the most abundant larva in headwaters of this region (Bruce 1985) and is the focus of this study. In these streams, *E. wilderae* hatchlings appear in April and May, and the larval stage lasts one or two years (Bruce 1982, 1985, 1988). The objective of this study was to determine the effects of nutrient enrichment on larval *E. wilderae* growth in a headwater stream. We expected that if *E. wilderae* growth rates were affected, it would be a delayed response because nutrient addition would first have to influence prey growth and production before potentially affecting higher trophic levels.

This study was conducted in two first order streams draining catchments (C) 53 (reference) and 54 (treatment) at the Coweeta Hydrologic Laboratory, Macon County, North Carolina. Vegetation consists of mixed hardwoods and the headwaters are shaded throughout the year by a dense riparian understory of rhododendron (*Rhododendron maximum* L.). Detailed descriptions of the Coweeta basin are provided by Swank and Crossley (1988). The two streams compared in this study have similar physical

characteristics, including catchment size, substrate composition, discharge, and thermal regime (Table 5.1).

Nutrient enrichment of C54 began in June 2000. Both nitrogen and phosphorus were added continually (as $NH^4NO_3$ and $H_3PO_4$, respectively) to the stream using a continuous flow pump powered by solar panels. Flow rate of the nutrient solution was adjusted according to stream discharge on a weekly basis to elevate inorganic nitrogen (NO3 + NO2 + NH4-N) and soluble reactive phosphorus (SRP) concentrations from ca. 5 to 500 µg/l and from ca. 1 to 100 µg/l, respectively. Water samples were collected every two weeks for nutrient analysis.

*E. wilderae* sampling began two years prior to treatment (July 1998) and continued through March 2001. Larvae in C54 were exposed to nutrient enrichment for approximately nine months of the study (June 2000 through March 2001), a time period that covers the majority of the larval stage (Bruce 1982, 1985, 1988). Larvae were collected approximately every month by sampling the entire wetted area of the study reaches (C53 =100 m; C54 =150 m). Larvae were collected at night by turning only loose cover objects (e.g. cobble, wood, leaves) that minimized disturbance to the stream. Captured larvae were placed in individual 20-ml plastic vials filled with stream water.

In an on-site laboratory, each larva was anesthetized in Petri dishes containing 0.1% tricaine methylsulfonate (MS-222) (Beachy 1994). Snout-vent length (SVL) was measured to the posterior margin of the vent to the nearest 0.5 mm using a dissecting microscope (12X magnification) and Vernier calipers. Anesthetized larvae were then given a unique mark by injecting acrylic polymers into the tail (Johnson and Wallace 2002). Marks were inserted under the skin of the tail immediately behind the legs. This

insertion point left the mark visible in the event of subsequent tail loss. This marking method has proven effective for long-term marking of *E. wilderae* larvae and has no adverse effects on growth or survival (Johnson and Wallace 2002). After marking, larvae were revived in stream water and released at the point of capture the following morning or evening.

To measure growth, SVL was first converted to ash-free dry mass (AFDM) using a length-mass regression derived for *E. wilderae* in the study streams:

$$M = 0.0023\ L^{3.09}\ (r^2=0.96,\ p<0.001,\ n=22)$$

where M is larval mass (mg AFDM) and L is SVL (mm) (Lugthart 1991). Individual daily growth rate (g) was then calculated as:

$$g=\ln(M_2/M_1)/t$$

where $M_1$= initial larval mass, $M_2$ = final larval mass, and t = time interval in days (Romanovsky and Polischuk 1982). Because we lacked whole-stream replication, average daily growth rates for each stream during pre-treatment and treatment were compared using 95% confidence intervals, a conservative test for differences (Zar 1996, Johnson 1999). *E. wilderae* larvae in these streams exhibit near linear growth over the year (Lugthart 1991, Beachy 1997), so there was no need for seasonal growth corrections.

Total inorganic nitrogen concentration in C54 over the treatment period was 443 µg (± 104 µg S.E.) and was within the target range. SRP concentration during treatment was elevated to 54 µg (± 11 µg S.E.), ten times higher than pre-treatment and reference conditions (Table 5.1).

A total of 767 *E. wilderae* larvae were captured during the study (C53 = 342; C54 =425). Growth estimates were based on 208 recaptured larvae (C53 pre-treatment= 73,

C53 treatment= 19, C54 pre-treatment = 96, and C54 treatment = 20), and recapture rates were ca. 30% for each stream/treatment period. Mean daily *E. wilderae* growth rates in C53 and C54 during the pre-treatment period were nearly identical ($g = 0.0027\ d^{-1}$ [± 0.0004 for C53; ± 0.0005 for C54, 95% C.I.]) (Fig. 5.1). Pre-treatment growth rates also agreed closely with estimates of Lugthart (1991), who found that *E. wilderae* growth averaged $0.003^{-d}$ in growth chambers placed in a nearby headwater stream.

Interannual growth variation resulted in significantly higher growth rates in both streams during the treatment period (Fig. 5.1). Temperature data were not available for C54, but data from C53 indicated no obvious differences among years (Fig. 5.2). Interannual variation in growth may be due to reduced stream discharge during much of the study period (Fig. 5.2). Leaf litter standing crop is strongly correlated with stream discharge (Wallace et al. 1995) and invertebrate production (Wallace et al. 1997, 1999) in Coweeta streams. Reduced stream flows can therefore result in greater litter accumulation and increased prey production that may subsequently affect predator growth rates (Wallace et al. 1999).

Average daily growth rate in C54 ($g= 0.0069\ d^{-1}$ [± 0.0019, 95% C.I.]) was significantly higher than in C53 ($g= 0.0043\ d^{-1}$ [± 0.0007, 95% C.I.]) during the treatment period (Figure 5.1). The significant growth increases during treatment were unexpected given the relatively short treatment period and the fact that *E. wilderae* larvae are predators that would not directly respond to nutrient addition. Wallace et al. (1999) demonstrated that invertebrate predator production closely tracks that of their prey in Coweeta headwater streams. If *E. wilderae* larvae are food limited in the study streams, increases in prey production could result in higher larval growth rates. *E. wilderae* feeds

predominantly on chironomids and copepods in these streams (Lugthart 1991, B. Johnson, unpublished data). Both of these prey groups have high growth rates and rapid turnover ratios (Wallace et al. 1999), and would potentially be the first invertebrates to respond to the organic matter standing crop and to nutrient enrichment. Preliminary insect growth results indicate that chironomid growth rates increased shortly after treatment began (W. Cross, Univ. of Georgia, unpublished data). Evidence also suggests that copepod consumption in particular, can influence growth rates of *E. wilderae* larvae. Lugthart (1991) found that *E. wilderae* growth rates were significantly higher after insecticide treatment in a Coweeta stream resulted in increased copepod consumption. In contrast, larval growth rates were significantly lower after long-term litter exclusion treatment caused a dietary shift that included fewer copepods and more chironomids (B. Johnson, unpublished data). Unfortunately, very little is known about assimilation efficiencies for stream-dwelling copepods.

Our results indicate that *E. wilderae* growth is tightly linked to the detrital resource in these streams. While increases in organic matter *quantity* may be responsible for the observed inter-annual growth variation, increased detritus *quality* resulted in higher growth rates in the nutrient enrichment stream. More comprehensive studies that include gut content analyses and prey assimilation efficiencies are required to document full effects of enrichment on *E. wilderae* growth.

Other studies have shown that nutrient enrichment can stimulate vertebrate growth and production in autotrophic systems (e.g., Harvey et al. 1982, Johnston et al. 1990, Deegan and Peterson 1992, Peterson et al. 1993). Marine-derived nutrients from salmon carcasses have also shown to increase growth of juvenile salmonids and to

increase ecosystem productivity in detritus-based Alaskan watersheds (Bilby et al. 1996, Wipfli et al. 1998, 1999). This study however, provides some of the first evidence indicating that similar relationships may exist in other detritus-based streams. Following an obligate aquatic larval stage, salamanders metamorphose and switch to a largely terrestrial existence. Given the abundance and diversity of salamanders in the southern Appalachians, this transfer of energy from the stream to the surrounding landscape may be significant. The influence of resources on larval salamander guilds may therefore affect ecosystem processes at the landscape scale. However, further studies are needed to measure the strength and prevalence of the indirect effects of resources on higher trophic levels in detrital ecosystems.

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Table 5.1. Physical parameters of streams draining catchments 53 (reference) and 54 (nutrient enrichment) at the Coweeta Hydrologic Laboratory before nutrient treatment. Elevations were measured at the gauging flumes. Nutrient data are from 36 sampling times during period 1985-1990.

| Variable | C53 | C 54 |
|---|---|---|
| Catchment | | |
| Area (ha) | 5.2 | 5.5 |
| Elevation (m asl) | 829 | 841 |
| Channel | | |
| Length (m) | 145 | 282 |
| Bankful Area ($m^2$) | 327 | 443 |
| Discharge (L/s) | | |
| Average * | 1.19 | 1.45 |
| Maximum * | 47.2 | 35.5 |
| Temperature (°C) | | |
| Maximum † | 20.3 | 19.5 |
| Minimum † | 0.7 | 1.1 |
| Annual degree-days † | 4485 | 4440 |
| Water Chemistry | | |
| pH | 6.8 | 6.8 |
| NO3-N (µg/L) | 2.5 | 7.0 |
| NH4-N (µg/L) | 2.0 | 2.5 |
| P (µg/L) | 0.8 | 1.0 |

* C53 averages from 12 years (1984-1996), C54 averages from 7 years (1985-1992)

†C53 averages from 12 years (1985 -1997), C54 averages from 8 years (1985-1992)

Figure 5.1. Mean individual daily growth rates of all recaptured *Eurycea wilderae* larvae in C53 (pre-treatment n= 73; treatment n= 19) and C54 (pre-treatment n= 96; treatment n= 20) for 23 months of pre-treatment and 9 months of nutrient addition treatment. Error bars are mean +/- 95% C.I.

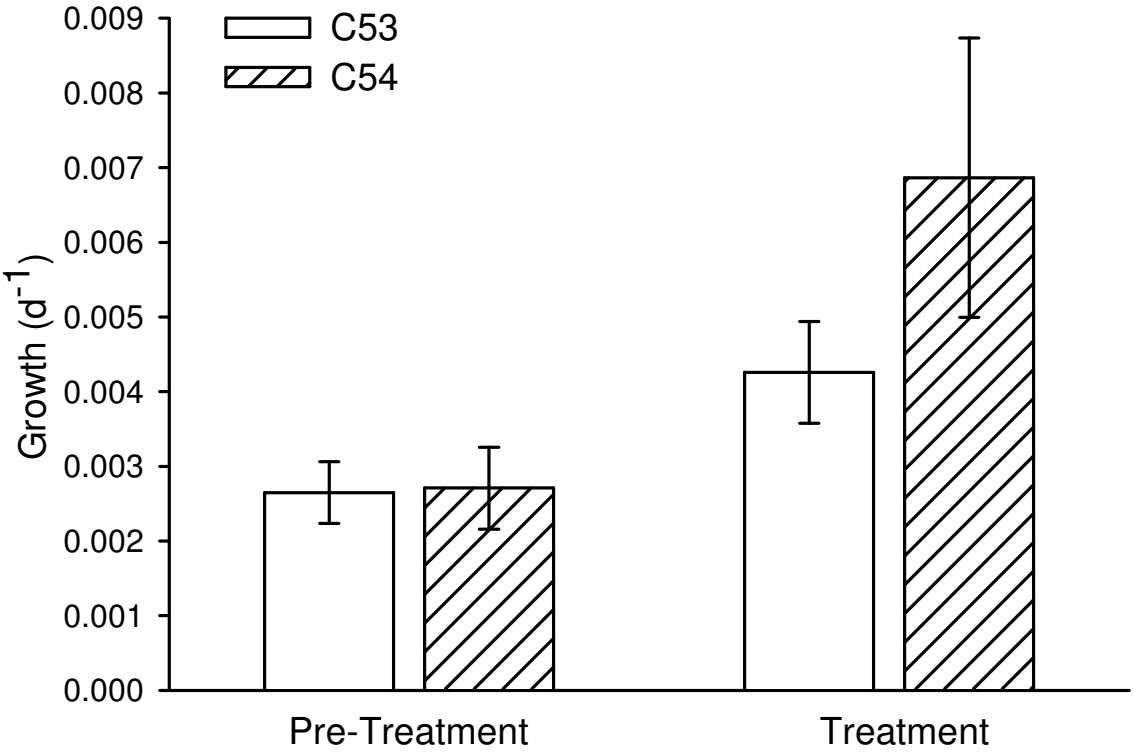
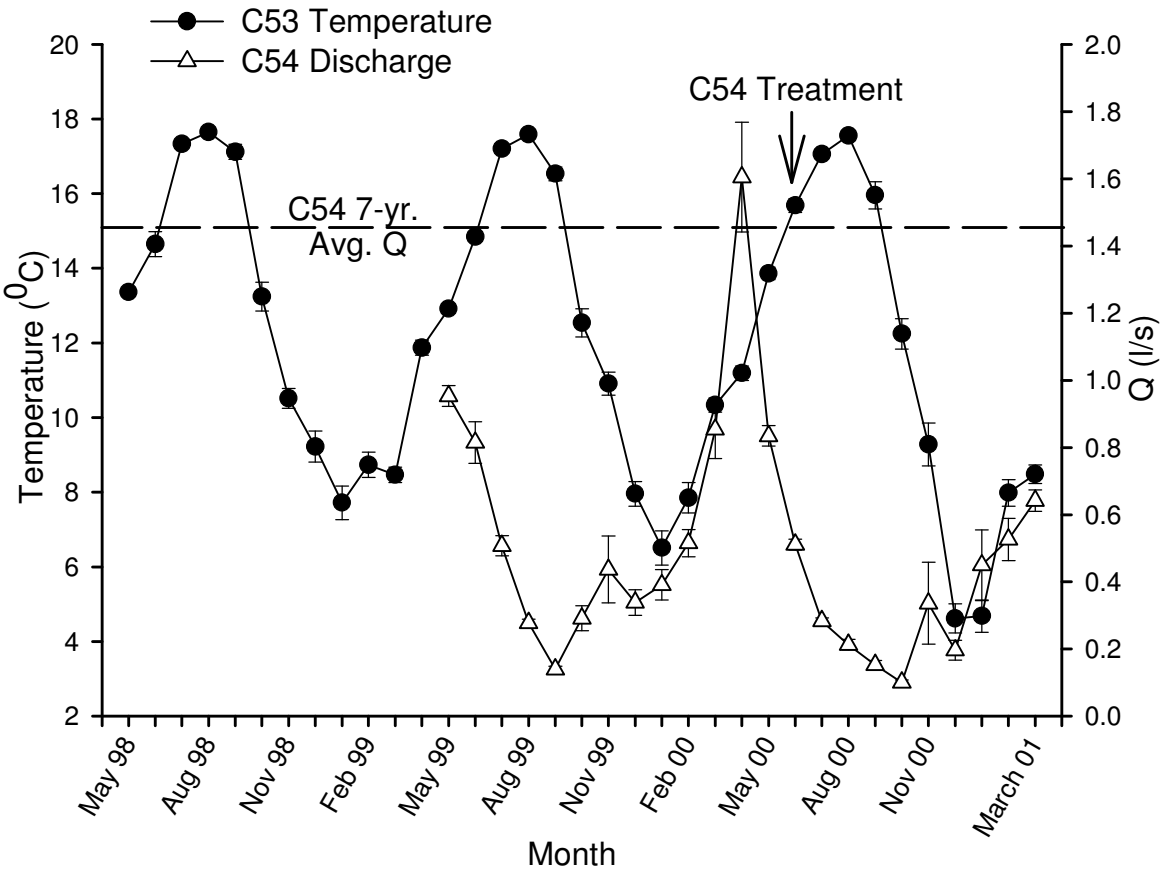

C53
C54
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
Growth (d-1)
Pre-Treatment
Treatment

Figure 5.2. Stream temperature (± S.E.) for C53 and monthly stream discharge (± S.E.) in C54 during nutrient addition treatment and for 13 months of pretreatment (*Eurycea wilderae* sampling began in July 1998). Long-term average discharge in C54 is from 1985-1992.


C53 Temperature
C54 Discharge
C54 Treatment
C54 7-yr.
Avg. Q
Temperature (0C)
Q (l/s)
Month
May 98
Aug 98
Nov 98
Feb 99
May 99
Aug 99
Nov 99
Feb 00
May 00
Aug 00
Nov 00
March 01

# CHAPTER 6

# GENERAL CONCLUSIONS

Results of this research provide additional insight and support for the previous litter exclusion findings of Wallace et al. (1997, 1999). The accuracy of previous benthic sampling estimates of salamander population parameters was in doubt because of the highly mobile and secretive nature of salamander larvae. Mark-recapture results have shown those previous estimates of larval salamander abundance, biomass, and production to be reliable. Furthermore, growth studies for two dominant insect detritivore groups have shown that reduced growth is one mechanism responsible for the significant decline in invertebrate production following litter exclusion and that community level production is even lower than previously reported.

Subcutaneous polymer injection proved to be an effective method for long-term monitoring of larval salamanders and allowed for the first comprehensive study of larval salamander growth rates, density, production, and movement in these streams. The primary benefits of this method are that it is easy to apply, does not affect survival, and allows individual larvae to be followed through time in-situ. As expected for a vertebrate poikilotherm, growth rates of *Eurycea wilderae* larvae were low in the reference stream and were similar to those from growth chamber studies at Coweeta by Lugthart (1991). Recapture frequencies were used to estimate survivorship, and results indicated a type III survivorship curve (Deevey 1947) that has been previously documented for *E. wilderae* (Bruce 1988, Beachy 1997).

Long-term litter exclusion had pronounced effects on the *E. wilderae* population, indicating strong bottom-up regulation. This is one of the first demonstrations of resource limitation affecting a vertebrate predator in a detritus based ecosystem. Larval growth, density, and production were all significantly reduced as a result of treatment. While the specific mechanisms behind these reductions remain somewhat uncertain, the declines generally result from the dramatic alteration of the benthic community, and possibly the lack of available refugia, following treatment (Wallace et al. 1997, 1999). Reduced density may result from excessive drift of newly hatched larvae in spring. Young larvae prey heavily on copepods (pers. obs.) which declined precipitously since litter exclusion treatment began (Wallace et al. 1999). Larvae that remained in the study reach were surprisingly sedentary, often remaining in the same depositional areas for much of the larval stage. There were no differences in individual movement among streams.

Gut content analyses did indicate that treatment caused a shift to a diet that included fewer copepods and more chironomids and non-insect taxa. Larvae in the litter exclusion stream also ate more terrestrial insects, but they were still only a minor part of the diet. The dietary shift along with a lower number of prey items per gut suggest that larvae had difficulty finding food and that prey items comprising the diet were not sufficient to sustain typical growth and production. *E. wilderae* production is low in Coweeta headwaters as a result of slow growth rates and low densities. Yet production in the reference stream was still more than three times higher than in the litter exclusion stream. Midges alone accounted for more than half of *E. wilderae* production in the treatment stream whereas production in reference and recovery streams was more evenly

distributed among prey groups. *E. wilderae* also consumed very little prey biomass in accounting for larval production. This provides further evidence that invertebrate predators may exert more top-down pressure than vertebrates in stream systems (Allan 1982, Culp 1986, Wooster 1994). Interestingly, the downstream recovery reach was intermediate for many of the measured parameters (density, growth, biomass, P/B, producton, and prey abundance), suggesting that upstream litter exclusion is still affecting salamander larvae several meters downstream.

Growth studies on representative shredders (*Tallaperla* spp.) and collector-gatherers (non-Tanypodinae chironomids) showed that both of these invertebrate groups had reduced growth rates during litter exclusion treatment. Reduced growth is therefore one mechanism responsible for observed declines in secondary production in the treatment stream (Wallace et al. 1997, 1999). Lack of CPOM resulted in reduced growth for later instar *Tallaperla* spp., but growth rates for the smallest size class were similar between streams. This suggests an ontogenetic shift where early instars may utilize FPOM as a food resource. Previous studies had suggested that although FPOM quantity had been reduced in the treatment stream, quality might be increased because of reduced microbial competition for nutrients and DOC (Tank and Webster 1998). The fact that average chironomid growth rates were also reduced indicates that any increase in quality was not enough to sustain normal growth. Previous estimates of chironomid production in the treatment stream (Wallace et al. 1997, 1999) were based on the instantaneous growth method using normal growth rates derived for undisturbed Coweeta streams (Huryn and Wallace 1986, Huryn 1990). The fact that our results show reduced growth

rates means that previous estimates of chironomid production were overestimated and that benthic production is even lower than previously reported.

While many experiments have shown that nutrient enrichment can increase density, growth, and production of higher trophic levels in autotrophic systems (e.g., Johnston et al. 1990, Deegan and Peterson 1992, Peterson et al. 1993, Harvey et al. 1998), similar experiments in detritus based systems are generally lacking. Nutrients from salmon carcasses have shown to increase juvenile salmonid growth in detritus-based Alaskan streams (Bilby et al. 1996), but indirect effects of enrichment in detritus-based systems remain poorly understood. Preliminary observations indicate that nitrogen and phosphorus enrichment led to increased growth rates of larval *E. wilderae*. This result was unexpected given the complex nature of detritus systems, the relatively short duration of treatment, and the fact that larvae are predators that would not respond directly to nutrients. Even though sample size was small because of low stream discharge during the treatment period, average growth rates in the treatment stream were more than double reference and pre-treatment growth rates. Diet composition could be one explanation for observed growth differences. *E. wilderae* larvae prey heavily on copepods and chironomids (Chapter 2, Lugthart 1991). These two groups have fast growth rates, high P/B ratios, and would be some of the first groups to respond to nutrient enrichment. Evidence does suggest that chironomid growth rates (W. Cross, unpublished data) and fungal production (K. Suberkropp, unpublished data) increased soon after initiation of treatment. Copepod consumption in particular seems to be closely linked with *E. wilderae* growth rates (Chapter 2 & 4, Lugthart 1991). More comprehensive

studies on growth, dietary composition, and prey assimilation efficiencies are needed to verify the effects of nutrient enrichment on *E. wilderae* larvae.

These studies provide further evidence of strong bottom-up regulation of benthic communities in detritus based streams and that manipulation of the resource can affect higher trophic levels (Wallace et al. 1997, 1999). Detritus limitation and nutrient enrichment treatments both affected consumers in ways that would be considered typical of autotrophic systems, despite the fact that detrital ecosystems are considered complex, often having multiple food web links, low interaction strength, and high rates of omnivory (Polis 1994, Polis and Strong 1996). Reduction of detritus caused reductions in abundance, biomass, and production of both primary and secondary consumers, while nutrient enrichment appears to have caused a proliferation of biofilms and meiofauna that may have been responsible for the observed growth increases by larval *E. wildearae*. Our findings thus largely conform to conventional food web theory that was developed for living-plant based systems. However, further research is needed to gain a better understanding of the strength and prevalence of indirect effects and cascading interactions in detritus based ecosystems.

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