

Short Research Note

## Larval salamander growth responds to enrichment of a nutrient poor headwater stream

Brent R. Johnson<sup>1,3,\*</sup>, J. Bruce Wallace<sup>1,2</sup>, Amy D. Rosemond<sup>2</sup> & Wyatt F. Cross<sup>2</sup>

<sup>1</sup>Department of Entomology, The University of Georgia, Athens, GA 30602, USA

<sup>2</sup>Institute of Ecology, The University of Georgia, Athens, GA 30602, USA

<sup>3</sup>Ecological Exposure Research Division, United States Environmental Protection Agency, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, USA

(\* Author for correspondence: Tel.: +513-569-7335; Fax: +513-569-7609; E-mail: johnson.brent@epa.gov)

Received 18 May 2006; in revised form 15 June 2006; accepted 19 June 2006; published online 31 August 2006

**Key words:** bottom-up, detritus, *Eurycea*, food web, vertebrate, predator

### Abstract

While many studies have measured effects of nutrient enrichment on higher trophic levels in grazing food webs, few such studies exist for detritus-based systems. We measured effects of nitrogen and phosphorus addition on growth of larval *Eurycea wilderae* in a heterotrophic headwater stream using a repeated mark-recapture design. Growth estimates for 208 recaptured larvae (control stream  $n = 92$ ; treatment stream  $n = 116$ ) resulted in a growth rate of  $0.0027 \text{ d}^{-1}$  in each stream prior to enrichment, whereas during enrichment treatment growth rates ( $g = 0.0069 \text{ d}^{-1} [\pm 0.0019, 95\% \text{ C.I.}]$ ) were significantly higher than control ( $g = 0.0043 \text{ d}^{-1} [\pm 0.0007, 95\% \text{ C.I.}]$ ). Results indicate that *E. wilderae* growth is tightly linked to the detrital resource and that growth may be indirectly affected by both quantity and quality of detritus. This study provides some of the first evidence that nutrient enrichment of detritus-based systems can influence multiple trophic levels in ways similar to autotrophic systems.

Numerous ecosystem experiments have artificially enriched autotrophic lakes (reviewed by Elser et al., 1990) and streams (e.g., Hart & Robinson, 1990; Peterson et al., 1993; Rosemond et al., 1993) to evaluate the relative strengths of resources and consumers in structuring aquatic food webs. Such studies have contributed heavily to food web theory for traditional grazing food chains and have often demonstrated that nutrient enrichment can produce strong indirect effects, with multiple trophic levels responding to enrichment. Yet, manipulative studies in detritus-based ecosystems have been fewer in number and less is known about specific effects of nutrient enrichment or influences of top-down and bottom-up forces in these systems (Polis & Strong, 1996).

In detritus-based streams, such as forested Appalachian headwaters, nutrient enrichment may result from atmospheric nitrogen deposition or from treated and untreated (i.e., “straight pipes”, septic leaks) domestic wastewater associated with 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. It has been theorized that omnivory, multiple food web links, and low interaction strengths may be common in detritus-based systems and may prevent the simplified cascading trophic interactions inherent in traditional models for grazing food chains (Strong, 1992; Polis, 1994; Polis & Strong, 1996).

The limited nutrient addition experiments in detritus-based systems have shown increases in microbial production and organic matter decomposition rates (e.g., Suberkropp & Chauvet, 1995; Tank & Webster, 1998; Gulis & Suberkropp, 2003; Gulis et al., 2004), and increases in invertebrate abundance, biomass, production, and growth (Pearson & Connolly, 2000; Robinson & Gessner, 2000; Rosemond et al., 2001; Cross et al., 2005, 2006). However, the effects of nutrient enrichment on vertebrate predators in detritus-based ecosystems remain unknown.

Larval salamanders are the top predators in fishless, high-gradient streams of the southern Appalachians. The Blue Ridge two-lined salamander, *Eurycea wilderae* Dunn, is the most abundant salamander 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 1 or 2 years (Bruce, 1985, 1988; Voss, 1993). The objective of this study was to measure the effects of nutrient enrichment on growth rates of larval *E. wilderae* in a headwater stream. We expected that if *E. wilderae* growth rates were affected, the response would be delayed because enrichment would first have to influence prey growth and production before affecting higher trophic levels.

This study was conducted in two first order streams draining catchments C53 (control) and C54 (treatment) at the Coweeta Hydrologic Laboratory, Macon County, North Carolina. Vegetation consists of mixed hardwoods and a dense understory of rhododendron (*Rhododendron maximum* L.) that shades the streams throughout the year. Heavy shading generally limits primary production to <1% of energetic inputs (Webster et al., 1983). Detailed descriptions of the Coweeta basin are provided by Swank & Crossley (1988). The two streams compared in this study have naturally low nutrient concentrations and have similar physical characteristics, including catchment size, discharge, substrate composition, and thermal regime (e.g., Lugthart & Wallace, 1992).

Nutrient enrichment of the treatment stream began in July 2000. Both nitrogen (N) and phosphorus (P) (as  $\text{NH}_4\text{NO}_3$  and  $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ , respectively) were added continuously along the entire study reach using a solar powered flow pump that adjusted to stream discharge. The

nutrient solution (N:P = 11:1) was dripped from an irrigation line that ran the length of the reach and had ports located approximately every 10 m. Water samples were collected biweekly for laboratory analysis. Enrichment elevated stream N ( $\text{NO}_3\text{-N} + \text{NO}_2\text{-N} + \text{NH}_4\text{-N}$ ) and P (soluble reactive phosphorus) concentrations from ca. 30–400  $\mu\text{g/l}$  and from ca. 8–50  $\mu\text{g/l}$ , respectively (Cross et al., 2003; Gulis et al., 2004). Nutrient concentrations in the enriched stream remained within the natural range for streams of the region with varying land use (Scott et al., 2002). Temperature data were recorded continuously in each stream beginning in April 1999 using Optic StowAway temperature probes.

*E. wilderae* sampling began 2 years prior to treatment (July 1998) and continued through March 2001. Larvae in the treatment stream were exposed to nutrient enrichment for approximately 8 months of the study (July 2000 through March 2001), a time period that covers the majority of the 1 year larval stage for most individuals (Bruce, 1985, 1988). Larvae were collected approximately every month by sampling the entire wetted area of the study reaches (control = 100 m; treatment = 145 m). Larvae were collected at night by turning only loose cover objects (e.g., cobble, wood, leaves) to minimize disturbance to the stream.

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 (12 × magnification) and Vernier calipers. Anesthetized larvae were then given a unique mark by injecting acrylic polymers into the tail (Johnson & 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 (Johnson & Wallace, 2005) and has no adverse effects on growth or survival (Johnson & 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 undisturbed

Coweeta 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:  $g = (\ln M_2 - \ln M_1)/t$ , where  $M_1$  = initial larval mass,  $M_2$  = final larval mass, and  $t$  = time interval in days (Romanovsky & Polischuk, 1982). Only initial and final weights were used for those larvae that were recaptured on multiple sample dates. 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). *E. wilderae* larvae in these streams exhibit near linear growth over the year (Lugthart, 1991; Johnson & Wallace, 2002, 2005), so there was no need for seasonal growth corrections.

A total of 767 *E. wilderae* larvae were captured during the study (control = 342; treatment = 425). Growth estimates were based on 208 recaptured larvae (C53 pre-enrichment,  $n = 73$ ; C53 during enrichment,  $n = 19$ ; C54 pre-enrichment,  $n = 96$ ; and C54 during enrichment,  $n = 20$ ), and recapture rates were ca. 30% for each stream/treatment period. Mean daily *E. wilderae* growth rates in control and treatment streams during the pre-enrichment period were nearly identical ( $g = 0.0027 \text{ d}^{-1}$  [ $\pm 0.0004$  for C53;  $\pm 0.0005$  for C54, 95% C.I.]) (Fig. 1) and agreed closely with

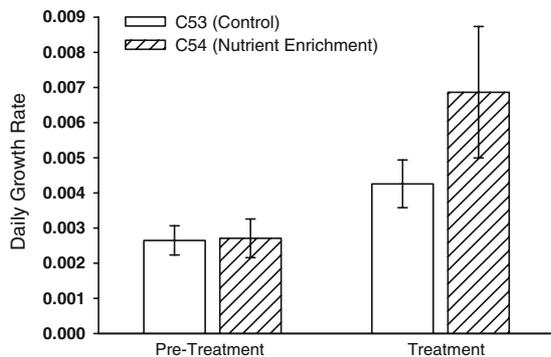


Figure 1. Mean individual daily growth rates of all recaptured *Eurycea wilderae* larvae in C53 (control) (pre-enrichment  $n = 73$ ; enrichment  $n = 19$ ) and C54 (treatment) (pre-enrichment  $n = 96$ ; enrichment  $n = 20$ ) streams for 24 months of pre-treatment (July 1998–June 2000) and 9 months of nutrient addition treatment (July 2000–March 2001). Error bars are mean  $\pm$  95% C.I.

previous *E. wilderae* growth estimates from undisturbed Coweeta streams (Johnson & Wallace, 2002, 2005). Pre-enrichment growth rates also agreed with estimates of Lugthart (1991), who found that *E. wilderae* growth averaged  $0.003 \text{ d}^{-1}$  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. 1). Mean daily stream discharge ( $Q$ ) was significantly higher in the treatment stream over entire study period (Wilcoxon signed rank test,  $p < 0.001$ ), and discharge of each stream was significantly lower during the 9 months treatment period than during pre-treatment (Mann-Whitney rank sum test,  $p < 0.001$ ). The reduction in discharge during treatment was proportionately similar between streams (Fig. 2). While temperature has little effect on *E. wilderae* growth (Lugthart, 1991; Johnson & Wallace, 2002), the observed growth variation between time periods may result from the reduced  $Q$  during treatment. In the reference stream, average  $Q$  decreased from 0.37 L/s (range: 0.02–3.84 L/s) prior to enrichment to 0.15 L/s (range: 0.01–1.36 L/s) during the treatment period. Average  $Q$  in the treatment stream during enrichment (0.34 L/s; range: 0.06–3.76 L/s) was also significantly less than during pre-treatment (beginning 12 May 1999, 0.59 L/s; range: 0.06–4.80) (Fig. 2) and was less than 25% of a 7 y average for the stream (1.45 L/s, 1985–1992, J.B. Wallace, unpublished data). 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 cause greater litter accumulation and increased prey production per unit wetted area. Increased prey availability may then result in increased predator growth rates.

Average daily growth rate in the treatment stream ( $g = 0.0069 \text{ d}^{-1}$  [ $\pm 0.0019$ , 95% C.I.]) was significantly higher than in the control stream ( $g = 0.0043 \text{ d}^{-1}$  [ $\pm 0.0007$ , 95% C.I.]) during the enrichment period (Fig. 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 enrichment. If reduced  $Q$  can cause increased larval growth, the growth differential observed

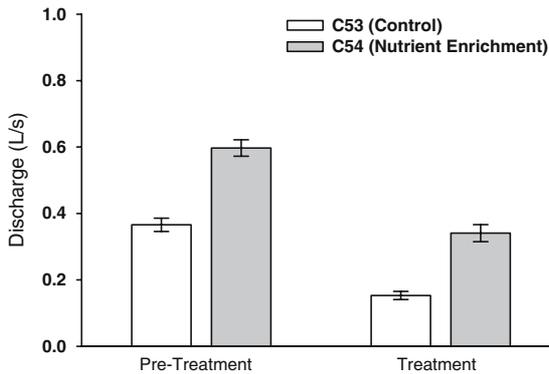


Figure 2. Mean daily stream discharge ( $\pm 1$  S.E.) for C53 (control) and C54 (treatment) for pre-treatment (May 1999–June 2000) and treatment (July 2000–March 2001) periods at the Coweeta Hydrologic Laboratory, Macon Co., North Carolina, USA.

between streams during the treatment period is conservative because of increased  $Q$  in the treatment stream. Moreover, despite  $Q$  differences between streams, growth rates were nearly identical between streams for the ca. 2 y pre-treatment period (Fig. 2). The increased growth response observed following enrichment most certainly then resulted from stimulation of the detrital food web because heavy shading limits primary production and enrichment had little effect on periphyton biomass in the treatment stream (Greenwood & Rosemond, 2005). It has also been demonstrated that nutrient addition increased the quality of stream resources, as evidenced by increased %P and reduced N:P and C:P ratios (Cross et al., 2003), and increased microbial activity and organic matter decomposition rates (Gulis & Suberkropp, 2003; Gulis et al., 2004).

Wallace et al. (1997, 1999) showed that 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* larvae feed predominantly on non-Tanypodinae chironomids and copepods (Caldwell & Houtcooper, 1973; Burton, 1976; Lugthart, 1991; Johnson & Wallace, 2005). When combined, these two groups accounted for >84% of all prey items in guts of *E. wilderae* larvae collected from the reference

stream (Johnson & Wallace, 2005). Both copepods and chironomids have high growth rates and rapid turnover ratios (Wallace et al., 1999), and would be among the first invertebrates to respond to organic matter standing crop and to nutrient enrichment. Indeed, benthic data and *in situ* growth measurements confirm that non-Tanypodinae chironomid growth, abundance, biomass, and secondary production all increased significantly following treatment (Cross et al., 2005). Following enrichment, average habitat-weighted chironomid production increased by 183% compared to pre-treatment values. Cross et al. (2005) attributed the positive chironomid response to increased food quality and high P body demand.

Copepods showed a similar positive response to nutrient enrichment. In the treatment stream, average annual copepod abundance, biomass, and production nearly doubled from pre-treatment values during the first year of enrichment (Cross et al., 2006). Annual habitat-weighted copepod production in the treatment stream increased from an average of 360 mg AFDM  $m^{-2} y^{-1}$  before enrichment to 701 mg AFDM  $m^{-2} y^{-1}$  during the first year of enrichment. Evidence 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 *E. wilderae* growth rates were significantly lower after long-term litter exclusion treatment caused a dietary shift that included fewer copepods (Johnson & Wallace, 2005). Coweeta headwaters are soft-water streams and Burton & Likens (1975) further speculated that microcrustaceans high in Ca content may be important dietary components for salamanders because they aid skeletal formation. Unfortunately, very little is known about elemental composition of stream-dwelling copepods or how efficiently they may be assimilated by *E. wilderae* larvae. It is also important to note that growth differences observed in this study could have important implications for *E. wilderae* population growth because larval growth rate affects timing and size at metamorphosis as well as adult fecundity (Bruce, 1988).

Other studies have shown that nutrient enrichment can stimulate vertebrate growth and production in autotrophic streams (e.g., Johnston et al., 1990; Deegan & Peterson, 1992). This study however, provides the first such evidence from a detritus-based ecosystem and indicates that enrichment can affect multiple trophic levels in ways similar to living plant-based systems. Our findings also support those of previous studies (Lugthart, 1991; Johnson & Wallace, 2005) that have demonstrated strong bottom-up control of *E. wilderae* larvae in these headwater streams and that both quantity and quality of the detrital resource can ultimately influence larval *E. wilderae*, a top vertebrate predator. These ecosystem-level manipulations have thus provided strong evidence of the tight linkages that exist between detritus standing crop, stream detritivores, and a vertebrate predator.

Coweeta streams are naturally nutrient poor. Though enrichment resulted in increased *E. wilderae* growth, this study covered only a short period of treatment and was not intended to measure long-term effects. Nutrient addition has accelerated organic matter breakdown rates in the treatment stream (Gulis & Suberkropp, 2003; Gulis et al., 2004). With continued enrichment, the rapid loss of stream organic matter could ultimately result in carbon limitation and shifts in consumer food resources and community production. The faster depletion of leaf litter and woody debris also reduces habitat heterogeneity and retention time. Any short-term gains from enrichment may thus be outweighed by long-term adverse effects of greater organic matter loss from this detritus-based system. These long-term changes potentially alter the natural structure and function of the animal assemblage, including the type of prey available to higher trophic levels.

### Acknowledgements

We thank Darold Batzer, Judy Meyer, Cathy Pringle, Wayne Berisford, Jennifer Greenwood, and Ken Fritz for providing valuable comments on earlier versions of this manuscript. Funding for the nutrient enrichment experiment was provided by National Science Foundation Grant DEB

9806610 to A.D. Rosemond, J.B. Wallace, K. Suberkropp and P.J. Mulholland.

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