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Bottom-Up and Top-Down Controls on Food Webs in Headwater Streams

By

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Thesis

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Bottom-Up and Top-down Controls on Food Webs in Headwater Streams

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Headwater streams account for 70% of stream channel length in the USA and are important as hotspots of nutrient uptake and native biodiversity. Biofilm, the mixed auto- and heterotrophic microbial community covering stream substrates, is where the majority of nutrient processing occurs, and forms the base of stream food webs, particularly in heavily shaded, oligotrophic streams. Both bottom-up (e.g., nutrients, light) and top-down (i.e., consumption) processes are known to affect periphyton, the autotrophic component of biofilm, but little is known about what controls the biofilm community as a whole. Top-down effects are common in streams, where fish are often the top predator and can cause cascading effects. However, salamanders – not fish – are the top predators in many small headwater streams, and the top-down effects of salamanders on stream food webs have received much less attention. I used experimental and observational approaches to investigate the role of top-down and bottom-up controls on headwater stream food webs. Specifically, I used stream mesocosms with two salamander species, *Eurycea bislineata* and *Gyrinophilus porphyriticus*, alone or in combination, to test the effects of salamander community composition on benthic and emergent macroinvertebrate density, biomass and community composition. To assess the relative importance of bottom-up and top-down determinants of biofilm biomass, I used a combination of stream surveys and pre-existing stream chemistry data from across the oligotrophic headwater streams of the Hubbard Brook Experimental Forest (HBEF). My experiment showed that stream salamanders alter benthic macroinvertebrate densities, but only when *G. porphyriticus* occurs alone, as it consumes *E. bislineata*, the smaller species, when it is present. This intraguild predation removes the top-down effects of *G. porphyriticus* on benthic macroinvertebrates. In my stream surveys, bottom-up variables (i.e., aspect, canopy cover, nutrients and pH) determined biofilm biomass, not salamander occupancy or benthic macroinvertebrate biomass. However, I did not encounter streams with the specific salamander community (i.e., *G. porphyriticus* present and *E. bislineata* absent) shown to produce top-down effects in my experimental study. My results demonstrate that salamanders can exert top-down control on benthic macroinvertebrate communities. This effect is, however, dependent on the salamander species present, and can be removed by intraguild interactions between salamander species. Furthermore, bottom-up factors, in particular light, appear to play the primary role in determining biofilm biomass. Despite prior evidence for the dominance of heterotrophic microbes in biofilms at the HBEF, the importance of light in controlling biofilm biomass suggests that the autotrophic component of these biofilms may be disproportionately important, and emphasizes the importance of understanding both the auto- and heterotrophic components of stream biofilms. My work links the rich history of research on stream salamander community ecology to broader studies of stream food webs, providing new avenues for future research.

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CHAPTER 1: TOP-DOWN EFFECTS OF SALAMANDERS ON MACROINVERTEBRATES IN FISHLESS HEADWATER STREAMS

ABSTRACT

In streams, fish are often the top predator and their effects can cascade to the base of the food web, but many small headwater streams are fishless and it is not known if these stream communities still experience top-down regulation. Salamanders are the top predators in many fishless headwater streams, and much is known about intraguild interactions among stream salamanders. However, little is known about the top-down effects of salamanders on stream food webs, or how intraguild interactions mediate these effects. To investigate the effects of salamanders on macroinvertebrate communities of headwater streams, I conducted an experiment in stream mesocosms to test for effects of two stream salamander species, *Eurycea bislineata* and *Gyrinophilus porphyriticus*, alone or in combination, on benthic and emergent macroinvertebrate density, biomass and community composition. I also assessed intraguild interactions between these salamander species by comparing *E. bislineata* survival and *G. porphyriticus* growth in single-species v. two-species treatments. *G. porphyriticus* reduced benthic macroinvertebrate densities when alone, but not when co-occurring with *E. bislineata*. There were no effects of salamanders on benthic or emergent macroinvertebrate biomass or community composition. *E. bislineata* survival decreased and *G. porphyriticus* weight increased when they co-occurred, suggesting that intraguild predation was occurring. These results suggest that salamanders can exert top-down control on macroinvertebrate communities in fishless headwater streams, decreasing benthic macroinvertebrate density. This effect is, however, dependent on the salamander species present, and can be removed by intraguild interactions between salamander species.

Keywords: intraguild predation, headwater streams, benthic macroinvertebrates, *Eurycea bislineata*, *Gyrinophilus porphyriticus*

INTRODUCTION

Studies of aquatic food webs have produced dramatic examples of top-down effects. The presence or absence of predators in freshwater and marine systems can have cascading effects that extend to the base of a food web (Power, Matthews & Stewart, 1985; Estes & Duggins, 1995; Carpenter *et al.*, 2001; Zimmer, Hanson & Butler, 2001). In streams, these top predators are often fish, which have strong effects on benthic macroinvertebrates (Diehl, 1992; Ruetz, Newman & Vondracek, 2002; Baxter *et al.*, 2004; Winkelmann, Schneider & Mewes, 2014), as well as on macroinvertebrates emerging from streams (Baxter *et al.*, 2004; Wesner, 2010, 2016). By affecting emergent macroinvertebrates, fish regulate an important subsidy from streams to forests, where birds, spiders, bats and lizards all use emergent macroinvertebrates as a food source (Gray, 1993; Sabo & Power, 2002; Fukui *et al.*, 2006; Marczak & Richardson, 2007). But many headwater streams are fishless (Richardson & Danehy, 2007), and these streams account for 70% of total stream channel length in the United States alone (Leopold, Wolman & Miller, 2012). By testing for top-down effects in fishless headwater streams, we can better understand the ecology of the headwater streams themselves, as well as riparian food webs.

In the absence of fish, salamanders are the top predators in many headwater streams of the eastern and western USA (Murphy & Hall, 1981; Hawkins *et al.*, 1983; Grant, Green & Lowe, 2009; Gould, Cecala & Drukker, 2017). These salamanders may co-occur with fish in the lower reaches of streams, but populations often extend upstream of barriers that prevent fish colonization (Resetarits Jr, 1997; Lowe & Bolger, 2002). Interactions among stream salamanders and between stream salamanders and fish have been the subject of classic studies in community ecology (Hairston, 1987; Resetarits Jr, 1991, 1995). However, few studies have addressed the top-down effects of stream salamanders on other components of headwater communities,

including – most obviously – macroinvertebrates. Reice & Edwards (1986) found no effect of *Eurycea bislineata* on benthic macroinvertebrates, but that experiment lasted only eight days and used adult *E. bislineata* individuals. Keitzer & Goforth (2013a) found that *Eurycea wilderae* and *Desmognathus quadramaculatus* larvae decreased benthic macroinvertebrate abundances only when they co-occurred, but that experiment intentionally reduced the intraguild predation that normally occurs between *D. quadramaculatus* and *E. wilderae* by using individuals of similar size. It is possible, therefore, that these salamanders would have had a different impact on benthic macroinvertebrates if intraguild predation was allowed to occur.

All stream salamanders have aquatic larvae that are restricted to the stream channel, with the length of this larval period varying among species (Petranka, 1998). After metamorphosis, these species exhibit a range of associations with the aquatic stream habitat, but many species remain largely aquatic, and all return to the stream channel to breed (Petranka, 1998). Benthic macroinvertebrates are known to be a significant component of the diet of larval and adult stream salamanders (Burton, 1976; Lowe, Nislow & Likens, 2005; Mondelli, Davenport & Lowe, 2014). From studies in pond and wetland systems, we know that salamanders can decrease benthic macroinvertebrate abundances (Blaustein, Friedman & Fahima, 1996; Benoy, 2008; Reinhardt *et al.*, 2017). A study by Progar and Moldenke (2002) found that temporary streams with neither fish nor salamanders produced a higher biomass of emergent macroinvertebrates than perennial streams, which were assumed to have salamanders and/or fish. In addition, Atlas & Palen (2014) used a model to show that salamander predation can reduce benthic and emergent macroinvertebrate biomass, both when salamanders occur alone and when they co-occur with fish. These studies suggest that salamanders may exert top-down pressure on benthic and emergent macroinvertebrates, with implications for both stream and forest ecosystems.

Furthermore, because multiple salamander species often co-occur in streams, with a wide range of resulting intraguild interactions (Hairston, 1980; Gustafson, 1993; Jaeger, Gabor & Wilbur, 1998; Bruce, 2008), any assessment of top-down effects on macroinvertebrates must account for these intraguild interactions.

From work in other systems, we know that intraguild interactions among predators can determine effects on shared prey. For example, when intraguild competition results in predators using different habitats, this can reduce spatial refugia for shared prey (Van Son & Thiel, 2006; Steinmetz, Soluk & Kohler, 2008). The resulting decrease in prey survival is known as risk enhancement (Sih, Englund & Wooster, 1998). Alternatively, intraguild predation or interference competition can reduce mortality rates of shared prey (Soluk & Collins, 1988; Fauth, 1990; Vance-Chalcraft & Soluk, 2005), a result known as risk reduction (Sih *et al.*, 1998). For example, under intraguild predation, consumption of one predator (i.e., the intraguild prey) by the other predator (i.e., the intraguild predator) releases the shared prey from top-down control by the intraguild prey species (Polis, Myers & Holt, 1989). Many studies have documented intraguild predation in stream salamander communities (Gustafson, 1993; Lowe *et al.*, 2005), suggesting that top-down effects of stream salamanders on benthic macroinvertebrates may be altered by interactions between co-occurring salamander species.

The salamanders *Gyrinophilus porphyriticus* and *Eurycea bislineata* are common throughout the headwater streams of New Hampshire, occurring both together and alone (Burton & Likens, 1975; Barr & Babbitt, 2002; Lowe & Bolger, 2002; Lowe, 2005; Lowe *et al.*, 2012). Both species are confined to the stream channel as larvae, with *G. porphyriticus* having a larval period of 3-4 years and *E. bislineata* having a larval period of 1-2 years (Bruce, 1980, 1985). *G. porphyriticus* adults may remain in the stream channel or forage in the riparian forest at night,

but are found under rocks and wood in and along the stream channel during the day (Greene, Lowe & Likens, 2008). *E. bislineata* individuals move over 100 m into the forest after metamorphosis and return to the stream to breed (MacCulloch & Bider, 1975; Petranka, 1998). In New Hampshire, larvae of both species feed primarily on aquatic macroinvertebrate larvae in the benthos (Burton, 1976). *G. porphyriticus* larvae also prey on *E. bislineata* larvae (Burton, 1976; Petranka, 1998), and the occasional presence of terrestrial macroinvertebrates and winged aquatic macroinvertebrate adults in larval diets suggests that both species also feed at the water's surface (Burton, 1976; Lowe *et al.*, 2005).

G. porphyriticus and *E. bislineata* exhibit intraguild predation when they co-occur, with both larvae and adults of the smaller *E. bislineata*, the intraguild prey, being consumed by larvae and adults of *G. porphyriticus*, the intraguild predator (Burton, 1976; Petranka, 1998; Lowe *et al.*, 2005). In mesocosm experiments, Resetarits (1991) found that *E. bislineata* larvae experienced reduced growth rates in the presence of *G. porphyriticus* larvae, presumably due to altered foraging behavior. Larvae of *E. cirrigera*, a closely related species to *E. bislineata*, also showed reduced nocturnal foraging and decreased survival in the presence of *G. porphyriticus* larvae (Gustafson, 1993; Rudolf, 2006).

Here, my goal was to advance understanding of the role of stream salamanders in headwater streams and surrounding forests by testing for individual and combined effects of *G. porphyriticus* and *E. bislineata* on benthic and emergent macroinvertebrates, while explicitly incorporating intraguild interactions. Specifically, I used a stream mesocosm experiment to test the following predictions: (i) *G. porphyriticus* and *E. bislineata* each reduce benthic macroinvertebrate densities and biomass, and alter community composition through direct, top-down effects, (ii) these direct effects will carry over to emergent macroinvertebrates, and (iii)

intraguild interactions between *G. porphyriticus* and *E. bislineata* will alter their effects on shared macroinvertebrate prey. If prediction (i) was supported, it would indicate that macroinvertebrate communities in fishless headwater streams experience top-down control when salamanders are present, with potential implications for basal components of headwater food webs (e.g., algae, leaf litter, Power, 1990; Townsend, 2003; Baxter *et al.*, 2004; Woodward, Papantoniou & Edwards, 2008) and associated ecosystem processes (e.g., productivity, nutrient retention; Baxter, Fausch & Saunders, 2005; Eby *et al.*, 2006). If prediction (ii) was supported, it would mean that salamanders affect the flow of macroinvertebrate subsidies from streams to forests, thereby potentially affecting terrestrial food webs (Gray, 1993; Nakano & Murakami, 2001; Sabo & Power, 2002; Fukui *et al.*, 2006). Finally, if prediction (iii) was supported, it would indicate that the specific salamander assemblage in a stream must be known to assess top-down effects on aquatic macroinvertebrates, as well as resulting subsidies and ecosystem processes. Importantly, support for prediction (iii) would help connect the influential body of work on stream salamander community ecology (Hairston, 1980; Gustafson, 1993; Jaeger *et al.*, 1998; Bruce, 2008) to the equally influential body of work on headwater ecosystem ecology (Fisher & Likens, 1973; Wallace *et al.*, 1997; Townsend, Scarsbrook & Dolédec, 1997; Gulis & Suberkropp, 2003).

METHODS

Study site

This experiment was conducted within the Hubbard Brook Experimental Forest (HBEF) in the White Mountains Region of central New Hampshire, USA. There are many small, fishless

headwater streams within the 31.6 km² area of the HBEF (Warren *et al.*, 2008). These streams tend to be slightly acidic (pH \approx 5.48), with high dissolved oxygen (80-90%), mild midday summer temperatures (13.0-17.0 C), a base flow rate of 1 L second⁻¹ and low conductivity (mean = 17.4 μ S cm⁻¹, Likens & Buso, 2006; Likens, 2013). The HBEF streams also tend to be heterotrophic and nutrient poor, with primary productivity contributing less than 1% of energy and most carbon entering the system through allochthonous inputs (Fisher & Likens, 1973; Mayer & Likens, 1987).

Experimental design

I used stream mesocosms to test how stream salamanders affect benthic and emergent macroinvertebrate density, biomass, and community composition. The salamander treatment had four levels: *E. bislineata* alone (EBIS), *G. porphyriticus* alone (GPOR), *E. bislineata* and *G. porphyriticus* (BOTH), and no-salamander controls (CONTROL). Each of these treatments was replicated four times for a total of sixteen mesocosms. Comparing EBIS and GPOR to CONTROL allowed assessment of the effects of these salamander species individually on benthic and emergent macroinvertebrates. Including the BOTH treatment allowed assessment of how salamander intraguild interactions affect benthic and emergent macroinvertebrates.

Experimental mesocosms were 189-L polyethylene tubs set along the bank of Zig-Zag Brook in the HBEF and modeled after those of Davenport and Lowe (2015). Mesocosms were 1 m long, 0.54 m wide, and 0.46 m high with a water depth of 19 cm. Substrate mimicked that of the HBEF streams, using a similar approach to Resetarits (1991). Each mesocosm contained 7 L of untreated playground sand, 7 L of pea gravel, 8 L of gravel, 7 small cobble stones measuring 80-100 mm in diameter, and 3 large cobble stones measuring 150-340 mm in diameter. Each

mesocosm also received 600 mL of leaf litter gathered from along the bank of Zig-Zag Brook. Mesocosms were open to colonization by flying aquatic insects and inputs of falling leaf litter and terrestrial insects. To prevent salamanders from climbing out of mesocosms, a line of petroleum jelly was placed in a ring along the top lip of each mesocosm and along the ring of aluminum siding extending 5.5 cm above the top lip of each mesocosm and 7.5 cm down towards the interior of the mesocosm. Water was gravity fed to each mesocosm through two arrays of pipes running from Zig-Zag Brook. Each array delivered water to eight mesocosms and split in a symmetrical branching pattern forming a balanced binary tree. This design was chosen to prevent bias in the distribution of water among mesocosms. The intake for each array was covered with both a PVC filter and a mesh bag to prevent leaf litter or salamanders from entering the mesocosms through these pipes. The intakes were then submerged adjacent to each other in a deep pool upstream of the mesocosms. Covering the intakes did not fully prevent inputs of drifting invertebrates and small *E. bislineata* larvae to the mesocosms, but I expected that the symmetrical branching pattern of the pipes, and the random interspersion of treatments, would prevent any bias in delivery of these organisms. Water drained out of the mesocosms through mesh screens at the downstream end of each mesocosm. Flow rate through the mesocosms was maintained at 2.4 L minute⁻¹.

Salamander treatment densities were within the range of natural densities reported for *G. porphyriticus* (0.16-10 individuals m⁻²) and *E. cirrigera* (23-169 individuals m⁻²), a sister taxa of *E. bislineata* (Resetarits Jr, 1991; Nowakowski & Maerz, 2009; Davenport & Lowe, 2016). The EBIS treatment consisted of 16 larval *E. bislineata* individuals, resulting in a density of 29.6 individuals m⁻². The GPOR treatment consisted of 2 *G. porphyriticus* individuals, resulting in a

density of 3.4 individuals m⁻². The BOTH treatment consisted of 1 *G. porphyriticus* and 8 *E. bislineata*, with a density of 1.9 individuals m⁻² and 14.8 individuals m⁻², respectively.

I did not use a substitutive experimental design to assess the effects of salamander treatments on macroinvertebrate prey (Siddon & Witman, 2004; Griffen, 2006; Carey & Wahl, 2010). Substitutive designs frequently use treatments with one individual of each predator species in their combined treatments (Vance-Chalcraft, Soluk & Ozburn, 2004; Siddon & Witman, 2004; Griffen, 2006), but this would not have resulted in realistic *E. bislineata* densities for my system (i.e., 1.85 individuals m⁻²). Instead, I held biomass of salamanders equivalent across treatments (Carpenter, Kitchell & Hodgson, 1985; Carey & Wahl, 2010). Due to the larger size and lower density of *G. porphyriticus* relative to *E. bislineata* in New Hampshire streams, holding biomass constant across treatments resulted in realistic salamander densities for New Hampshire streams (Resetarits Jr, 1991; Nowakowski & Maerz, 2009; Davenport & Lowe, 2016). *E. bislineata* individuals ranged in wet mass between 0.19 and 0.32 g (mean \pm 1 SE = 0.21 \pm 0.003 g) and *G. porphyriticus* individuals ranged in wet mass from 1.47 to 2.6 g (mean \pm 1 SE = 1.97 \pm 0.10 g). Therefore, I considered eight *E. bislineata* larvae to be roughly equivalent to one *G. porphyriticus* larva. Total salamander biomass across treatments ranged from 3.22 to 4.83 g (mean \pm 1 SE = 3.75 \pm 0.14 g). All salamanders were collected from Bagley Trail Brook in the HBEF and randomly assigned to a treatment and mesocosm. Salamanders were added to their assigned mesocosm on 22 July 2014. The experiment ran for 51 days.

Initial prey density has been shown to alter the effects of multiple predators (Peckarsky, 1991; Griffen, 2006). To account for initial variation among mesocosms in benthic macroinvertebrate communities, I used a randomized complete block design with four levels of starting benthic macroinvertebrates densities. Benthic macroinvertebrate addition occurred on 25

June 2014 and on 30 June 2014 due to time constraints and a high flow event in the collection stream. For each mesocosm, benthic macroinvertebrates were collected from a separate 0.6 m² area of stream run in Zig-Zag Brook, the stream adjacent to the mesocosms. During collection the top inch of stream substrate was disturbed by hand for four minutes and by kicking for one minute in front of a D-frame net of mesh size 800 x 900 μm.

Hester-Dendy samplers (NKY Environmental Supply) were placed into mesocosms on 30 June 2014 and left in place for two weeks prior to the start of the experiment to assess initial benthic macroinvertebrate communities. Hester-Dendy samplers have been used to sample benthic macroinvertebrates from mesocosms (King & Richardson, 2003; Kaatz *et al.*, 2010), and experimental enclosures (Brazner & Kline, 1990), and were selected for this experiment because they do not disturb the substrate of the mesocosms (Hester & Dendy, 1962). The Hester-Dendys used consisted of eight square plates with a central hole, each 7.62 cm by 7.62 cm, and with a total sampling area of 774.2 cm². Plates were spaced sufficiently far apart to allow salamanders of both species access to benthic macroinvertebrates that had colonized the Hester-Dendys. After calculating the density of benthic macroinvertebrates in each mesocosm, I created four blocks representing categories of initial density: low (13-142 individuals m⁻²), mid-low (220-245 individuals m⁻²), mid-high (245-271 individuals m⁻²), and high (323-491 individuals m⁻²).

Benthic macroinvertebrates

To test for effects of salamander treatments on benthic macroinvertebrates, Hester-Dendys were added to each mesocosm on 18 August 2014 and left in place for two weeks (Hester & Dendy, 1962; Dudgeon, 1996). Hester-Dendys were removed on 2 September 2014, during the last week on the experiment, when benthic macroinvertebrate communities had been

exposed to salamander treatments for 6 weeks. Benthic macroinvertebrates were stored in 75% ethanol for identification.

Benthic macroinvertebrates were identified to the level of family for insects and subclass for all other macroinvertebrates (Merrit & Cummins, 1996; Voshell, 2002). Family was deemed sufficient taxonomic resolution for this study due to the potential for high correlation between species and family diversity (Heino & Soininen, 2007). Invertebrate lengths were measured using a stereoscopic microscope with an ocular micrometer accurate to 0.05 mm. Published length-mass relationships at the level of order or sub-order (for Diptera) were used to calculate biomass (Benke *et al.*, 1999).

Emergent macroinvertebrates

Emergent macroinvertebrate samples were collected over 72 hours using mesh nets suspended above the mesocosms and connected to collection jars (Wesner, 2010; Merten, Snobl & Wellnitz, 2014). Collection took place from 3 September 2014 to 6 September 2014, during the last week of the experiment, when benthic macroinvertebrate communities had been exposed to salamander treatments for six weeks. Macroinvertebrates were removed from collection jars and placed in 75% ethanol. Each net had a skirt of mesh size $750 \mu\text{m}^2$ and an upper portion of net with mesh size $1100 \mu\text{m}$ by $1700 \mu\text{m}$. Nets were connected to a collection jar with an inverted funnel of opening size 10 cm. Emergent macroinvertebrates were killed using a 2.5 cm^2 section of commercially available insect poison hung inside each jar (Prozap, insect guard). Emergent macroinvertebrates were identified to family, except when prohibited due to damage (Merrit & Cummins, 1996). Biomass was measured using published length-mass relationships at

the order or sub-order level (Sabo, Bastow & Power, 2002). Invertebrate lengths were measured using a stereoscopic microscope with an ocular micrometer accurate to 0.05 mm.

Salamander survival and growth

I compared survival of *E. bislineata* in treatments with just *E. bislineata* (EBIS) and with *G. porphyriticus* (BOTH) to determine if intraguild predation occurred between these two species. Proportional survival was quantified as number of *E. bislineata* remaining in a mesocosm on 11 September 2014 divided by the number originally added to that mesocosm on 22 July 2014.

I measured change in mass of *G. porphyriticus* individuals in treatments with and without *E. bislineata* (BOTH v. GPOR) to further assess if intraguild predation was occurring. If intraguild predation occurred, I expected *G. porphyriticus* individuals to gain more mass in the BOTH treatments than in the GPOR treatments, where the only prey resource was benthic macroinvertebrates (Holt & Polis, 1997). To quantify change in mass, each *G. porphyriticus* was weighed prior to addition to mesocosms and at the end of the experiment. To track weight changes in individuals, all *G. porphyriticus* were individually marked using a florescent visible implant elastomer tag injected subcutaneously in the dorsal region (Northwest Marine Technology, Shaw Island, WA, USA).

Statistical analyses

It is possible that sampled benthic and emergent macroinvertebrates did not represent the same communities due to macroinvertebrate phenology (Merritt & Cummins, 1996; Progar & Moldenke, 2002; Macneale, Peckarsky & Likens, 2005) or differences in sampling methods

(Malison, Benjamin & Baxter, 2010). To assess the correspondence of the benthic and emergent macroinvertebrate communities at the time of sampling, I ran a permutation multivariate analysis of variance (MANOVA) on community dissimilarity in the CONTROL treatments. The Bray-Curtis distance metric was used to calculate community dissimilarity. Only the CONTROL treatments were used for the MANOVA to ensure that any differences between benthic and emergent macroinvertebrates communities were due to macroinvertebrate phenology or sampling methods, not predation by salamanders. Specifically, I used the function Adonis in the package Vegan in the program R and performed 999 permutations.

I used analysis of variance (ANOVA) to test the effect of salamander treatment on benthic macroinvertebrate density. I calculated benthic macroinvertebrate density by dividing the total number of individuals collected from each Hester-Dendy sampler by the surface area of the sampler (774.2 cm²). I square-root transformed density data to meet the assumptions of ANOVA. I also used ANOVA to test for effects of salamander treatment on benthic macroinvertebrate biomass. If I found a significant effect of salamander treatment, I assessed multi-predator effects using a two-tailed paired samples t-test to compare expected and observed benthic macroinvertebrate density at each level of starting insect density (Schmitz & Sokol-Hessner, 2002; Siddon & Witman, 2004). Predicted predator effects in the BOTH treatment were calculated using the equation $P_{BOTH} = (O_{EBIS} + O_{GPOR})^{0.5}$, where O_{EBIS} is the observed predator effect in the EBIS treatment, O_{GPOR} is the observed predator effect in the GPOR treatment, and P_{BOTH} is the predicted multi predator effect in the BOTH treatment (Griffen, 2006; Carey & Wahl, 2010).

To test how stream salamanders affected benthic macroinvertebrate community composition, I used both taxonomic richness (S) and the exponential of the Shannon-Wiener

Index (Exp H'). These indices are widely used, statistically robust, and biologically relevant (Gray, 2000; Hubálek, 2000; Jost, 2007). I did not include in these analyses benthic macroinvertebrates that were too damaged to be identified to the level of family (for insects) or subclass (for oligochaetes). I compared results across salamander treatments using ANOVA.

I used ANOVA to test for the effect of salamander treatment on emergent macroinvertebrate density. I obtained density of emerged macroinvertebrates per square meter per day by dividing the number of emergent macroinvertebrates by the surface area of the mesocosm (0.54 m^2) and the number of days the emergence traps were up (3 d). I also used ANOVA to test for the effects of salamander treatment on emergent macroinvertebrate biomass. Biomass of emerged macroinvertebrates was also expressed as $\text{m}^{-2} \text{ d}^{-1}$. Like benthic macroinvertebrates, I used S and Exp H' to test how stream salamanders affected emergent macroinvertebrate taxonomic diversity.

Due to the lack of normality, I used a one-tailed Mann-Whitney-Wilcoxon test to analyze proportional survival of *E. bislineata* in EBIS versus BOTH treatments. I used a one-tailed test based on the *a priori* expectation that *G. porphyriticus* would reduce survival of *E. bislineata* (Burton, 1976; Lowe *et al.*, 2005). I used a two-tailed student's t-test to analyze weight change of *G. porphyriticus* in GPOR versus BOTH treatments. Although I expected the intraguild prey's presence to benefit the intraguild predator, this test was two-tailed because Gustafson (1993) found availability of *E. cirrigera* larvae, a sister species of *E. bislineata*, did not increase larval *G. porphyriticus* growth rates.

RESULTS

Community dissimilarity

The permutational MANOVA showed that benthic and emergent macroinvertebrate communities were significantly different ($F_{1,6} = 7.78$, $P = 0.04$). This indicates that emergent macroinvertebrates represent a subset of the benthic macroinvertebrate community present in each mesocosm, and justified separate analyses of the benthic and emergent macroinvertebrate data.

Benthic macroinvertebrates

There was a significant effect of salamander treatment on benthic macroinvertebrate densities (ANOVA, $F_{3,9} = 4.05$, $P = 0.04$; Figure 1), and a significant non-additive multi-predator effect (two-tailed paired samples t-test, $P = 0.02$). Specifically, mean benthic macroinvertebrate density (individuals $m^{-2} \pm SE$) was significantly lower in the GPOR treatment (16.15 ± 10.3) than the remaining treatments, which were remarkably similar (EBIS: 64.59 ± 19.65 , BOTH: 69.97 ± 19.65 , CONTROL: 64.59 ± 15.22 ; Table 1). Salamander treatment did not affect benthic macroinvertebrate biomass (ANOVA, $F_{3,8} = 1.49$, $P = 0.29$), taxonomic richness (ANOVA, $F_{3,9} = 3.3$, $P = 0.07$), or species diversity (ANOVA, $F_{3,7} = 0.59$, $P = 0.64$). Abundance of benthic macroinvertebrate taxa by treatment is given in Table 2.

Emergent macroinvertebrates

Salamander treatment did not affect the densities of emerged macroinvertebrates (ANOVA, $F_{3,9} = 1.11$, $P = 0.40$) or the biomass of emergent macroinvertebrates (ANOVA, $F_{3,9} = 1.19$, $P = 0.37$). There was also no effect of salamander treatment on the taxonomic richness

(ANOVA, $F_{3,9} = 1.23$, $P = 0.35$) or species diversity (ANOVA, $F_{3,9} = 1.14$, $P = 0.38$) of emergent macroinvertebrates.

Salamander Survival and Growth

Survival of *E. bislineata* was higher in the EBIS treatment than in the BOTH treatment, indicating that the presence of *G. porphyriticus* reduced survival of *E. bislineata* (one-tailed Mann-Whitney-Wilcoxon test, $W = 16$, $P = 0.01$, Fig. 2A). *G. porphyriticus* individuals also gained proportionally more weight in the BOTH treatment than the GPOR treatment (two-tailed student's t-test, $t_{10} = -2.32$, $P = 0.04$; Fig. 2B), further suggesting that *G. porphyriticus* preyed on *E. bislineata* individuals, and supporting the expectation of intraguild interactions between the salamander species. Several *E. bislineata* larvae of a smaller size class than used in the EBIS and BOTH treatments (i.e., shorter snout vent length and lower mass; $n = 7$) or present in a treatment to which no *E. bislineata* were added ($n = 4$), were retrieved from six of the fourteen mesocosms at the end of the experiment. Specifically, two *E. bislineata* were removed from the EBIS treatment, three from the GPOR treatment, two from the BOTH treatment and four from the control treatments. These *E. bislineata* were assumed to have entered the mesocosms through the array of pipes delivering stream water, their small size having allowed them to bypass both the PVC and mesh filters on the intake valves. The small size of these additional *E. bislineata* in the EBIS and BOTH treatments allowed them to be removed from estimates of *E. bislineata* survival.

DISCUSSION

G. porphyriticus decreased the density of benthic macroinvertebrates when it was the only salamander species in stream mesocosms, despite its low (but realistic) density in mesocosms (3.4 individuals m², Resetarits Jr, 1991; Davenport & Lowe, 2016). While fish are known to decrease the densities of both benthic and emergent macroinvertebrates (Baxter *et al.*, 2004; Wesner, 2010, 2013), until now there was little evidence that salamanders could also have this effect (but see Keitzer & Goforth, 2013a). More broadly, my findings indicate that benthic macroinvertebrate communities may experience top-down regulation by predators even in fishless headwater streams (Wipfli & Gregovich, 2002; Richardson & Danehy, 2007).

The negative effect of *G. porphyriticus* on benthic macroinvertebrates densities was removed when its intraguild prey, *E. bislineata*, was present. Thus, the specific composition of the stream salamander community can influence the abundance of shared invertebrate prey. When *G. porphyriticus* and *E. bislineata* were together in mesocosms, *G. porphyriticus* individuals gained more weight and *E. bislineata* experienced reduced survival than when each species occurred alone (Table 1). Those results are consistent with my hypothesis that intraguild predation between *G. porphyriticus* and *E. bislineata* would occur, and match a previous study in which larval *G. porphyriticus* growth increased and survival of their intraguild prey (*E. wilderae*) decreased when they co-occurred (Beachy, 1994). However, that study did not examine effects on shared invertebrate prey.

Intraguild predation by *G. porphyriticus* on *E. bislineata* resulted in risk reduction for the salamanders' shared benthic macroinvertebrate prey – the expected outcome when predators interfere with each other (Sih *et al.*, 1998). My finding adds to a body of literature suggesting that intraguild predation, a type of predator interference, results in risk reduction for shared prey (Huang & Sih, 1991; Crumrine & Crowley, 2003; Griffen & Byers, 2006). These results contrast

with results showing that stream salamanders decreased benthic macroinvertebrate abundance when two species (*Desmognathus quadramaculatus* and *Eurycea wilderae*) were present (Keitzer & Goforth, 2013a). However, Keitzer & Goforth (2013a) sought to limit intraguild predation by using only small *D. quadramaculatus* individuals, which reduced the size difference between the two salamander species. It is likely that this reduction in size variation, and resulting decrease in the intensity of intraguild interactions, led to the observed risk enhancement in the shared macroinvertebrate prey. More generally, the combination of results from my experiment and Keitzer & Goforth (2013a) reinforce a broader conclusion that the composition of the stream salamander community – specifically the interactions among those species – determine top-down effects on stream invertebrates.

Confounding a decrease in intraspecific interactions with an increase in interspecific interactions is a known pitfall of the substitutive design used in this study. Specifically, in my design the BOTH level had half the number of *G. porphyriticus* individuals as the GPOR level. However, the additive design, the common alternative to the substitutive design, confounds changes in predator density with changes in predator diversity (Sih *et al.*, 1998; Griffen, 2006; Schmitz, 2007). I was unable to run both additive and substitutive designs concurrently due to the limited number of stream mesocosms I could create and maintain. Therefore, I cannot fully separate the effects of decreasing *G. porphyriticus* density from the effects of intraguild predation between *G. porphyriticus* and *E. bislineata*. It is possible, then, that release from intraspecific interactions contributed to the increase in *G. porphyriticus* weight in the BOTH versus the GPOR level. It seems unlikely, however, that these intraspecific interactions explain the reduction in *E. bislineata* survival in the BOTH treatment, or the similarity of benthic macroinvertebrate densities in BOTH and CONTROL mesocosms relative to GPOR (Figure 1).

Stream salamanders have been a valuable model system in community ecology, with studies demonstrating a wide range of interspecific interactions and elucidating the mechanisms and consequences of niche partitioning (Hairston, 1980; Gustafson, 1993; Jaeger *et al.*, 1998; Bruce, 2008). This study builds on that strong foundation of research by assessing the role of stream salamanders in affecting the broader stream food web. For example, a classic study by Hairston (1986) showed that stream salamander communities partition the stream-to-forest gradient by size, thereby limiting intraguild predation. This finding was further supported by Grover and Wilbur (2002), who found that the terrestrial salamander *Plethodon cinereus* benefit from artificially created seeps placed close to forested streams, until excluded by the more aquatic and aggressive *Desmognathus fuscus*. My findings suggest that such interspecific interactions, which decrease the spatial overlap of salamander niches, may also increase salamanders' effects on shared invertebrate prey. Consequently, the net effects of salamander assemblages on stream food webs cannot be determined without first understanding these interspecific interactions.

In addition to showing the top-down implications of intraguild predation, the findings of this study imply that the roles of salamanders in stream food webs likely differ among species. Unlike *G. porphyriticus*, larval *E. bislineata* did not affect benthic macroinvertebrate densities when they occurred alone. Benthic macroinvertebrate densities in mesocosms with only *E. bislineata* were not significantly different from densities in predator-free control mesocosms. This is consistent with a previous study showing that adult *E. bislineata* did not affect benthic macroinvertebrate prey (Reice & Edwards, 1986). These results suggest that in streams with only *E. bislineata*, benthic macroinvertebrate are likely regulated primarily by bottom-up, instead of top down, mechanisms. It is also important to acknowledge that several small *E. bislineata*

larvae were introduced to mesocosms through the water pipes. I believe it is unlikely that these individuals altered the outcome of the experiment for two reasons: their small size allowed me to exclude them from calculations of *E. bislineata* survival and the species was not found to alter benthic or emergent macroinvertebrate densities.

The effects of multiple predators on shared prey can depend on starting prey density (Peckarsky, 1991; Griffen, 2006). By seeding the mesocosms with benthic macroinvertebrates two weeks prior to the addition of salamanders, I was able to measure the benthic macroinvertebrate density in each mesocosm just prior to salamander addition and block based on starting density. Salamander treatments were then assigned randomly to mesocosms in each of these blocks. Nevertheless, one of the challenges of working in stream systems is their connected nature, making them open to ongoing colonization. To limit colonization of mesocosms by benthic macroinvertebrates via the incoming stream water, I placed a PVC filter and mesh bag around the inflow valves of the pipes. Despite these efforts, the input of small *E. bislineata* individuals across treatments suggests that these systems were not entirely closed to colonization. However, any additional – though unbiased – variation in invertebrate densities caused by the open nature of the mesocosms would make results of this study conservative (i.e., Fig. 1), in addition to mimicking the open nature of natural stream reaches.

My permutational MANOVA comparing collected benthic and emergent macroinvertebrates from CONTROL treatments indicates that the benthic and emergent macroinvertebrates sampled represented different communities, likely due to the phenology of emergent macroinvertebrates. The emergence of macroinvertebrates from streams is not constant: different taxa emerge at different time periods (Merritt & Cummins, 1996; Progar & Moldenke, 2002; Baxter *et al.*, 2005). In contrast, the benthic macroinvertebrate community is

more consistent and represents a broader range of taxa at any given time (Mackay & Kalff, 1969; Merrit & Cummins, 1996; Macneale *et al.*, 2005). Therefore, the macroinvertebrates emergent in September likely represented a subset of the benthic community. There are two plausible explanations for the finding that *G. porphyriticus* decreased benthic macroinvertebrate densities without affecting the density of emergent macroinvertebrates. First, the taxa *G. porphyriticus* was feeding on may not have emerged in September. Second, the subset of taxa that *G. porphyriticus* consumed and which also emerged in September may have been abundant enough to obscure the effects of predation within samples of all emergent macroinvertebrates. Previous studies provide insight into both possibilities.

From studies on the diet of *G. porphyriticus* larvae, we know they are gape-limited predators that eat a wide range of macroinvertebrates (Burton, 1976; Lowe *et al.*, 2005; Mondelli *et al.*, 2014). Several of the taxa they consume, including Chironomidae and Simuliidae, were represented in the emergent macroinvertebrates sampled in September. Therefore, it is likely that *G. porphyriticus* individuals were consuming at least some taxa included in the emergent macroinvertebrates sampled. However, a previous study has shown that the effects of salamander predation in ponds, while detectable in less abundant taxa, can be obscured in more abundant taxa (Reinhardt *et al.*, 2017). If the subset of benthic macroinvertebrate prey that *G. porphyriticus* were consuming, and which emerge in September, were particularly abundant, then the effects of this predation may have been visible in the benthic samples, but not in the emergent samples. Conversely, it is possible that my experiment did not run for long enough to see an effect of *G. porphyriticus* on emergent macroinvertebrates. My experiment ran for seven weeks, which may have constrained effects of salamanders on benthic and emergent macroinvertebrates, particularly considering the slow metabolism of stream salamanders

(Fitzpatrick, 1973; Feder, 1983) and variation in the timing of emergence among stream macroinvertebrates (Merritt & Cummins, 1996; Progar & Moldenke, 2002; Baxter *et al.*, 2005).

My results add to mounting evidence suggests that salamanders are integral components of headwater stream food webs. Specifically, this experiments shows that salamanders can exert strong top-down control on headwater food webs, consistent with model predictions (Atlas & Palen, 2014). But salamanders are also important from a bottom-up perspective, representing significant standing stocks of nitrogen and phosphorus, and capable of meeting up to 30% of stream nitrogen needs pre-leaf fall (Peterman, Crawford & Semlitsch, 2008; Keitzer & Goforth, 2013b; Milanovich, Maerz & Rosemond, 2015). Both these top-down and bottom-up effects may be sensitive to intraguild interactions among stream salamanders and with brook trout, which often co-occur with salamanders in the lower reaches of headwater streams (MacCrimmon & Campbell, 1969; Resetarits Jr, 1991; Warren *et al.*, 2008). There are clearly many opportunities for studies further elucidating the top-down and bottom-up effects of salamanders in fishless and fish-bearing headwater streams.

Although easily overlooked, salamanders can exert top-down pressure on stream food webs, decreasing the densities of benthic macroinvertebrates. Furthermore, my results show that these top-down effects may be regulated by the assemblage of stream salamander species present, with intraguild predation resulting in risk reduction for shared benthic macroinvertebrate prey. As an important model system in community ecology, there is a wealth of knowledge on intraguild interactions among stream salamanders (Hairston, 1980; Gustafson, 1993; Jaeger *et al.*, 1998; Bruce, 2008). By demonstrating that these intraguild interactions are integral to understanding the role of salamanders in stream food webs, I hope this work opens new avenues

of research on the direct and indirect effects of salamanders on community and ecosystem dynamics in headwater streams.

REFERENCES

- American Water Works Association & Water Environment Federation (2005) *Standard Methods for The Examination Of Water And Wastewater*, 21st edn. American Public Health Association, Washington DC.
- Angradi T.R. (1996) Inter-habitat variation in benthic community structure, function, and organic matter storage in 3 Appalachian headwater streams. *Journal of the North American Benthological Society* **15**, 42–63.
- Atlas W.I. & Palen W.J. (2014) Prey vulnerability limits top-down control and alters reciprocal feedbacks in a subsidized model food web. *Plos One* **9**, e85830.
- Banin E., Brady K. & Greenberg E. (2006) Chelator-induced dispersal and killing of *Pseudomonas aeruginosa* cells in a biofilm. *Applied and environmental microbiology* **72**, 2064–2069.
- Barr G.E. & Babbitt K.J. (2002) Effects of biotic and abiotic factors on the distribution and abundance of larval two-lined salamanders (*Eurycea bislineata*) across spatial scales. *Oecologia* **133**, 176–185.
- Battin T., Besemer K., Bengtsson M., Romani A. & Packmann A. (2016) The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology* **14**, 251.
- Battin T., Kaplan L., Newbold J. & Hansen C. (2003) Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* **426**, 439.
- Baxter C., Fausch K. & Saunders W.C. (2005) Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology* **50**, 201–220.
- Baxter C. V, Fausch K.D., Murakami M. & Chapman P.L. (2004) Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology* **85**, 2656–2663.
- Beachy C. (1994) Community ecology in streams: effects of two species of predatory salamanders on a prey species of salamander. *Herpetologica* **50**, 129–136.
- Benke A., Huryn A., Smock L. & Wallace J. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society* **18**, 308–343.
- Benoy G.A. (2008) Tiger salamanders in prairie potholes: A “fish in amphibian’s garments?” *Wetlands* **28**, 464–472.
- Bernhardt E.S. & Likens G.E. (2004) Controls on periphyton biomass in heterotrophic streams. *Freshwater Biology* **49**, 14–27.
- Biggs B.J., Francoeur S.N., Huryn A.D., Young R., Arbuckle C.J. & Townsend C.R. (2000) Trophic cascades in streams: effects of nutrient enrichment on autotrophic and consumer benthic communities under two different fish predation regimes. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1380–1394.
- Blaustein L., Friedman J. & Fahima T. (1996) Larval Salamandra drive temporary pool community dynamics: evidence from an artificial pool experiment. *Oikos* **76**, 392–402.
- Brazner J.C. & Kline E.R. (1990) Effects of chlorpyrifos on the diet and growth of larval fathead minnows, *Pimephales promelas*, in littoral enclosures. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 1157–1165.
- Bruce R. (1980) A model of the larval period of the spring salamander, *Gyrinophilus porphyriticus*, based on size-frequency distributions. *Herpetologica* **36**, 78–86.
- Bruce R.C. (2008) Intraguild interactions and population regulation in Plethodontid salamanders.

- Herpetological Monographs* **22**, 31–53.
- Bruce R.C. (1985) Larval period and metamorphosis in the salamander *Eurycea bislineata*. *Herpetologica* **41**, 19–28.
- Burnham K. & Anderson D. (2002) *Model selection and multimodel inference: a practical information-theoretic approach*, 2nd edn. Springer-Verlag, New York.
- Burton T.M. (1976) An analysis of the feeding ecology of the salamanders (Amphibia, Urodela) of the Hubbard Brook Experimental Forest, New Hampshire. *Journal of Herpetology* **10**, 187–204.
- Burton T.M. & Likens G.E. (1975) Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. *Copeia* **1975**, 541–546.
- Burton T.M., Ulrich K.E. & Haack S.K. (1988) Community dynamics of bacteria, algae, and insects in a first order stream in New Hampshire, U.S.A. *Internationale Vereinigung für theoretische und angewandte Limnologie* **23**, 1125–1134.
- Cantlon J.E. (1953) Vegetation and microclimates on north and south slopes of Cushtunk Mountain, New Jersey. *Ecological Monographs* **23**, 241–270.
- Cardinale B., Palmer M., Swan C., Brooks S. & Poff N L (2002) The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. *Ecology* **83**, 412–422.
- Carey M.P. & Wahl D.H. (2010) Interactions of multiple predators with different foraging modes in an aquatic food web. *Oecologia* **162**, 443–452.
- Carpenter S.R., Cole J.J., Hodgson J.R., Kitchell J.F., Pace M.L., Bade D., *et al.* (2001) Trophic cascades, nutrients, and lake productivity: whole-lake experiments. *Ecological Monographs* **71**, 163–186.
- Carpenter S.R., Kitchell J.F. & Hodgson J.R. (1985) Cascading trophic interactions and lake productivity. *BioScience* **35**, 634–639.
- Carrick H. & Lowe R. (2007) Nutrient limitation of benthic algae in Lake Michigan: the role of silica. *Journal of Phycology* **43**, 228–234.
- Chadwick M.A. & Huryn A.D. (2005) Response of stream macroinvertebrate production to atmospheric nitrogen deposition and channel drying. *Limnology and Oceanography* **50**, 228–236.
- Cole J.J. (1982) Interactions between bacteria and algae in aquatic ecosystems. *Annual Review of Ecology and Systematics* **13**, 291–314.
- Cornwall C.E., Boyd P.W., McGraw C.M., Hepburn C.D., Pilditch C.A., Morris J.N., *et al.* (2014) Diffusion boundary layers ameliorate the negative effects of ocean acidification on the temperate coralline macroalga *Arthrocardia corymbosa*. *PLoS ONE* **9**, e97235.
- Crumrine P.W. & Crowley P.H. (2003) Partitioning components of risk reduction in a dragonfly-fish intraguild predation system. *Ecology* **84**, 1588–1597.
- Cummins K.W. & Klug M.J. (1979) Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* **10**, 147–172.
- Davenport J. & Lowe W. (2016) Does dispersal influence the strength of intraspecific competition in a stream salamander? *Journal of Zoology* **298**, 46–53.
- Diehl S. (1992) Fish predation and benthic community structure: the role of omnivory and habitat complexity. *Ecology* **73**, 1646–1661.
- Dudgeon D. (1996) The influence of refugia on predation impacts in a Hong Kong stream. *Archiv für Hydrobiologie* **138**, 145–159.
- Dufour P. & Torrétion J. (1996) Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical northeastern Atlantic Ocean. *Deep Sea*

- Research Part I: Oceanographic Research Papers* **43**, 1305–1320.
- Eby L., Roach W., Crowder L. & Standord J. (2006) Effects of stocking-up freshwater food webs. *Elsevier* **21**, 576–584.
- Elser J.J. & Goldman C.R. (1991) Zooplankton effects on phytoplankton in lakes of contrasting trophic status. *Limnology and Oceanography* **36**, 64–90.
- Estes J.A. & Duggins D.O. (1995) Sea otters and kelp forests in Alaska: generality and variation in a community ecological paradigm. *Ecological Monographs* **65**, 75–100.
- Fairchild G.W. & Sherman J.W. (1993) Algal periphyton response to acidity and nutrients in softwater lakes: Lake comparison vs. nutrient enrichment approaches. *Journal of the North American Benthological Society* **12**, 157–167.
- Fauth J.E. (1990) Interactive effects of predators and early larval dynamics of the treefrog *Hyla-Chrysoscelis*. *Ecology* **71**, 1609–1616.
- Feder M. (1983) Integrating the ecology and physiology of Plethodontid salamanders. *Herpetologica* **39**, 291–310.
- Feminella J.W. & Hawkins C.P. (1995) Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of the North American Benthological Society* **14**, 465–509.
- Findlay S. & Howe K. (1993) Bacterial algal relationships in streams of the Hubbard Brook Experimental Forest. *Ecology* **74**, 2326–2336.
- Fisher S.G. & Likens G.E. (1973) Energy flow in Bear Brook, New Hampshire - integrative approach to stream ecosystem metabolism. *Ecological Monographs* **43**, 421–439.
- Fitzpatrick L. (1973) Influence of seasonal temperatures on the energy budget and metabolic rates of the northern two-lined salamander *Eurycea bislineata bislineata*. *Comparative Biochemistry and Physiology Part A: Physiology* **45**, 807–818.
- Flemming H. & Wingender J. (2010) The biofilm matrix. *Nature reviews microbiology* **8**, 623.
- Flemming H., Wingender J., Szewzyk U., Steinberg P., Rice S. & Kjelleberg S. (2016) Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology* **14**, 563.
- France R. (2011) Leaves as “crackers”, biofilm as “peanut butter”: exploratory use of stable isotopes as evidence for microbial pathways in detrital food webs. *Oceanological and Hydrobiological studies* **40**, 110–115.
- Francoeur S.N. (2001) Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society* **20**, 358–368.
- Fukui D.A.I., Murakami M., Nakano S. & Aoi T. (2006) Effect of emergent aquatic insects on bat foraging in a riparian forest. *Journal of Animal Ecology* **75**, 1252–1258.
- Fuller R., Kennedy B. & Nielsen C. (2004) Macroinvertebrate responses to algal and bacterial manipulations in streams. *Hydrobiologia* **523**, 113–126.
- Fuss C.B., Driscoll C.T. & Campbell J.L. (2015) Recovery from chronic and snowmelt acidification: Long-term trends in stream and soil water chemistry at the Hubbard Brook Experimental Forest, New Hampshire, USA. *Journal of Geophysical Research: Biogeosciences* **120**, 2360–2374.
- Galicia L., López-Blanco J., Zarco-Arista A., Filips V. & Garcia-Oliva F. (1999) The relationship between solar radiation interception and soil water content in a tropical deciduous forest in Mexico. *Catena* **36**, 153–164.
- Gasol J., Pedrós-Alió C. & Vaqué D. (2002) Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. *Antonie Van*

Leeuwenhoek **81**, 435–452.

- Geesey G.G., Wigglesworth-Cooksey B. & Cooksey K.E. (2000) Influence of calcium and other cations on surface adhesion of bacteria and diatoms: A review. *Biofouling* **15**, 195–205.
- Geiger R., Aron R. & Todhunter P. (2009) *The climate near the ground*. Rowman & Littlefield.
- Gould P., Cecala K. & Drukker S. (2017) Biogeographical factors affecting the distribution of stream salamanders on the Cumberland Plateau, USA. *The Science of the Total Environment* **599–600**, 1622–1629.
- Grady A.E., Scanlon T.M. & Galloway J.N. (2007) Declines in dissolved silica concentrations in western Virginia streams (1988–2003): Gypsy moth defoliation stimulates diatoms? *Journal of Geophysical Research* **112**, G01009.
- Graham M.H. (2003) Confronting multicollinearity in ecological multiple regression. *Ecology* **84**, 2809–2815.
- Grant E., Green L. & Lowe W. (2009) Salamander occupancy in headwater stream networks. *Freshwater Biology* **54**, 1370–1378.
- Gray J.S. (2000) The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and Ecology* **250**, 23–49.
- Gray L. (1993) Response of insectivorous birds to emerging aquatic insects in riparian habitats of a tallgrass prairie stream. *American Midland Naturalist* **129**, 288–300.
- Greene B., Lowe W. & Likens G. (2008) Forest succession and prey availability influence the strength and scale of terrestrial-aquatic linkages in a headwater salamander system. *Freshwater Biology* **53**, 2234–2243.
- Griffen B.D. (2006) Detecting emergent effects of multiple predator species. *Oecologia* **148**, 702–709.
- Griffen B.D. & Byers J.E. (2006) Intraguild predation reduces redundancy of predator species in multiple predator assemblage. *Journal of Animal Ecology* **75**, 959–966.
- Grover M. & Wilbur H. (2002) Ecology of ecotones: interactions between salamanders on a complex environmental gradient. *Ecology* **83**, 2112–2123.
- Gulis V. & Suberkropp K. (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater biology* **48**, 123–134.
- Gustafson M.P. (1993) Intraguild predation among larval Plethodontid salamanders - a field experiment in artificial stream pools. *Oecologia* **96**, 271–275.
- Haack S., Burton T. & Ulrich K. (1988) Effects of whole-tree harvest on epilithic bacterial populations in headwater streams. *Microbial Ecology* **16**, 165–181.
- Hairston N. (1987) *Community ecology and salamander guilds*. Cambridge University Press.
- Hairston N. (1986) Species packing in *Desmognathus* salamanders: experimental demonstration of predation and competition. *The American Naturalist* **127**, 266–291.
- Hairston N. (1980) Species packing in the salamander genus *Desmognathus*: what are the interspecific interactions involved? *The American Naturalist* **115**, 354–366.
- Hall-Stoodley L., Costerton J. & Stoodley P. (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nature reviews microbiology* **2**, 95.
- Hall Jr R. & Meyer J. (1998) The trophic significance of bacteria in a detritus-based stream food web. *Ecology* **79**, 1995–2012.
- Hall Jr R.O., Likens G.E. & Malcom H.M. (2001) Trophic basis of invertebrate production in 2 streams at the Hubbard Brook Experimental Forest. *Journal of the North American Benthological Society* **20**, 432–447.

- Hall R.J., Likens G.E., Fiance S.B. & Hendrey G.R. (1980) Experimental acidification of a stream in the Hubbard Brook experimental forest, New Hampshire. *Ecology*, 976–989.
- Hawkins C., Murphy M., Anderson N.H. & Wilzbach M.A. (1983) Density of fish and salamanders in relation to riparian canopy and physical habitat in streams of the northwestern United States. *Canadian Journal of Fisheries and Aquatic Sciences* **40**, 1173–1185.
- Heino J. & Soininen J. (2007) Are higher taxa adequate surrogates for species-level assemblage patterns and species richness in stream organisms? *Biological Conservation* **137**, 78–89.
- Hester F.E. & Dendy J.S. (1962) A multiple-plate sampler for aquatic macroinvertebrates. *Transactions of the American Fisheries Society* **91**, 420–421.
- Hill B.H. & Webster J.R. (1982) Periphyton production in an Appalachian river. *Hydrobiologia* **97**, 275–280.
- Hillebrand H. (2002) Top-down versus bottom-up control of autotrophic biomass—a meta-analysis on experiments with periphyton. *Journal of the North American Benthological Society* **21**, 349–369.
- Hoagland K.D., Rosowski J.R., Gretz M.R. & Roemer S.C. (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. *Journal of Phycology* **29**, 537–566.
- Holland P. & Steyn D. (1975) Vegetational responses to latitudinal variations in slope angle and aspect. *Journal of Biogeography* **2**, 179–183.
- Holt R.D. & Polis G.A. (1997) A theoretical framework for intraguild predation. *American Naturalist* **149**, 745–764.
- Huang C.F. & Sih A. (1991) Experimental studies on direct and indirect interactions in a 3 trophic-level stream system. *Oecologia* **85**, 530–536.
- Hubálek Z. (2000) Measures of species diversity in ecology: an evaluation. *Folia Zool.* **49**, 241–260.
- Huryn A.D., Butz Huryn V.M., Arbuckle C.J. & Tsomides L. (2002) Catchment land-use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. *Freshwater Biology* **47**, 401–415.
- Jaeger R., Gabor C. & Wilbur H. (1998) An assemblage of salamanders in the southern Appalachian Mountains: competitive and predatory behavior. *Behaviour* **135**, 795–821.
- Johnson J. & Omland K.S. (2004) Model selection in ecology and evolution. *Trends in ecology & evolution* **19**, 101–108.
- Johnson N., Driscoll C., Eaton J., Likens G. & McDowell W. (1981) “Acid rain”, dissolved aluminum and chemical weathering at the Hubbard Brook Experimental Forest, New Hampshire. *Geochimica et Cosmochimica Acta* **45**, 1421–1437.
- Jolliffe I.T. & Cadima J. (2016) Principal component analysis: a review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **374**, 20150202.
- Jost L. (2007) Partitioning diversity into independent alpha and beta components. *Ecology* **88**, 2427–2439.
- Kaatz S.E., Morris J.E., Rudacille J.B. & Clayton R.D. (2010) Origin of *Chironomid* larvae in plastic-lined culture ponds: airborne or water supply? *North American Journal of Aquaculture* **72**, 107–110.
- Kaspari M. & Powers J.S. (2016) Biogeochemistry and geographical ecology: embracing all twenty-five elements required to build organisms. *The American Naturalist* **188**, S62–S73.

- Keitzer S.C. & Goforth R.R. (2013a) Salamander diversity alters stream macroinvertebrate community structure. *Freshwater Biology* **58**, 2114–2125.
- Keitzer S.C. & Goforth R.R. (2013b) Spatial and seasonal variation in the ecological significance of nutrient recycling by larval salamanders in Appalachian headwater streams. *Freshwater Science* **32**, 1136–1147.
- King R.S. & Richardson C.J. (2003) Integrating bioassessment and ecological risk assessment: an approach to developing numerical water-quality criteria. *Environmental Management* **31**, 795–809.
- Lamberti G.A. (1996) The role of periphyton in benthic food webs. In: *Algal Ecology: freshwater benthic ecosystem*, 1st edn. (Eds S. Jan, L. Max & R. Lowe), pp. 533–572. Academic Press, Inc, San Diego, California.
- Lawrence J.R., Scharf B., Packroff G. & Neu T.R. (2002) Microscale evaluation of the effects of grazing by invertebrates with contrasting feeding modes on river biofilm architecture and composition. *Microbial Ecology* **44**, 199–207.
- Lear G., Niyogi D., Harding J., Dong Y. & Lewis G. (2009) Biofilm bacterial community structure in streams affected by acid mine drainage. *Applied and environmental microbiology* **75**, 3455–3460.
- Ledger M. & Hildrew A. (2005) The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution* **137**, 103–118.
- Ledger M.E. & Hildrew A.G. (2001) Growth of an acid-tolerant stonefly on epilithic biofilms from streams of contrasting pH. *Freshwater Biology* **46**, 1457–1470.
- Lemmon P. (1956) A spherical densiometer for estimating forest overstory density. *Forest Science* **2**, 314–320.
- Leopold L., Wolman M. & Miller J. (2012) *Fluvial processes in geomorphology*. Courier Corporation.
- Likens G. (2013) *Biogeochemistry of a Forested Ecosystem*, 3rd edn. New York.
- Likens G. & Buso D. (2006) Variation in streamwater chemistry throughout the Hubbard Brook Valley. *Biogeochemistry* **78**, 1–30.
- Likens G., Driscoll C. & Buso D. (1996) Long-term effects of acid rain: response and recovery of a forest ecosystem. *Science* **272**, 244–246.
- Lowe W. (2005) Factors affecting stage-specific distribution in the stream salamander *Gyrinophilus porphyriticus*. *Herpetologica* **61**, 135–144.
- Lowe W. & Bolger D. (2002) Local and landscape-scale predictors of salamander abundance in New Hampshire headwater streams. *Conservation Biology* **16**, 183–193.
- Lowe W.H. & Likens G.E. (2005) Moving headwater streams to the head of the class. *BioScience* **55**, 196–197.
- Lowe W.H., McPeck M.A., Likens G.E. & Cosentino B.J. (2012) Decoupling of genetic and phenotypic divergence in a headwater landscape. *Molecular Ecology* **21**, 2399–2409.
- Lowe W.H., Nislow K.H. & Likens G.E. (2005) Forest structure and stream salamander diets: Implications for terrestrial-aquatic connectivity. *Verhandlungen des Internationalen Verein Limnologie* **29**, 279–286.
- MacCrimmon H. & Campbell J. (1969) World distribution of brook trout, *Salvelinus fontinalis*. *Journal of the Fisheries Board of Canada* **26**, 1699–1725.
- MacCulloch R. & Bider J. (1975) Phenology, migrations, circadian rhythm and the effect of precipitation on the activity of *Eurycea b. bislineata* in Quebec. *Herpetologica* **31**, 433–439.

- Mackay R.J. & Kalff J. (1969) Seasonal variation in standing crop and species diversity of insect communities in a small Quebec stream. *Ecology* **50**, 101–109.
- Macneale K.H., Peckarsky B.L. & Likens G.E. (2005) Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. *Freshwater Biology* **50**, 1117–1130.
- Malison R.L., Benjamin J.R. & Baxter C. V. (2010) Measuring adult insect emergence from streams: the influence of trap placement and a comparison with benthic sampling. *Journal of the North American Benthological Society* **29**, 647–656.
- Marczak L.B. & Richardson J.S. (2007) Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. *Journal of Animal Ecology* **76**, 687–694.
- Martínez A., Kominoski J. & Larrañaga A. (2017) Leaf-litter leachate concentration promotes heterotrophy in freshwater biofilms: understanding consequences of water scarcity. *Science of the Total Environment* **599**, 1677–1684.
- Mayer M. & Likens G. (1987) The importance of algae in a shaded headwater stream as food for an abundant caddisfly (Trichoptera). *Journal of the North American Benthological Society* **6**, 262–269.
- McNeely C., Clinton S. & Erbe J. (2006) Landscape variation in C sources of scraping primary consumers in streams. *Journal of the North American Benthological Society* **25**, 787–799.
- Merrit R. & Cummins K. (1996) *An Introduction to the Aquatic Insects of North America*. 3 ed. Kendall Hunt.
- Merten E., Snobl Z. & Wellnitz T. (2014) Microhabitat influences on stream insect emergence. *Aquatic sciences* **76**, 165–172.
- Milanovich J., Maerz J. & Rosemond A. (2015) Stoichiometry and estimates of nutrient standing stocks of larval salamanders in Appalachian headwater streams. *Freshwater biology* **60**, 1340–1353.
- Mondelli M., Davenport J.M. & Lowe W.H. (2014) *Gyrinophilus porphyriticus* diet. *Herpetological Review* **45**, 109–110.
- Montgomery D. & Buffington J. (1998) Channel processes, classification, and response. In: *River Ecology and Management: Lessons from the Pacific Coastal Ecoregion*. (Eds R. Naiman & R. Bilby), pp. 13–42. Springer-Verlag, New York.
- Mulholland P.J., Steinman A.D., Marzolf E.R., Hart D.R. & DeAngelis D.L. (1994) Effect of periphyton biomass on hydraulic characteristics and nutrient cycling in streams. *Oecologia* **98**, 40–47.
- Murphy M. & Hall J. (1981) Vaired effects of clear-cut logging on predators and their habitat in small streams of the Cascade Mountains, Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 137–145.
- Nakano S. & Murakami M. (2001) Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences* **98**, 166–170.
- Noel D.S., Martin C.W. & Federer C.A. (1986) Effects of forest clearcutting in New England on stream macroinvertebrates and periphyton. *Environmental Management* **10**, 661–670.
- Nowakowski A.J. & Maerz J.C. (2009) Estimation of larval stream salamander densities in three proximate streams in the Georgia Piedmont. *Journal of Herpetology* **43**, 503–509.
- Pace M., Cole J., Carpenter S. & Kitchell J. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in ecology & evolution* **14**, 483–488.
- Peckarsky B.L. (1991) Mechanisms of intra-and interspecific interference between larval stoneflies. *Oecologia* **85**, 521–529.

- Peterman W.E., Crawford J.A. & Semlitsch R.D. (2008) Productivity and significance of headwater streams: population structure and biomass of the black-bellied salamander (*Desmognathus quadramaculatus*). *Freshwater Biology* **53**, 347–357.
- Peterson B., Wollheim W., Mulholland P., Webster J., Meyer J., Tank J., *et al.* (2001) Control of nitrogen export from watersheds by headwater streams. *Science* **292**, 86–90.
- Petranka J.W. (1998) *Salamanders of the US and Canada*. Smithsonian Institution Press, Washington, DC.
- Plotnikoff R. & Wiseman C. (2001) Benthic macroinvertebrate biological monitoring protocols for rivers and streams.
- Polis G.A., Myers C.A. & Holt R.D. (1989) The ecology and evolution of intraguild predation - potential competitors that eat each other. *Annual Review of Ecology and Systematics* **20**, 297–330.
- Power M. (1990) Effects of fish in river food webs. *Science* **250**, 811–814.
- Power M., Matthews W. & Stewart A. (1985) Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. *Ecology* **66**, 1448–1456.
- Progar R. & Moldenke A. (2002) Insect production from temporary and perennially flowing headwater streams in western Oregon. *Journal of Freshwater Ecology* **17**, 391–407.
- R Core Team (2017) R: a language and environment for statistical computing.
- Reice S. & Edwards R. (1986) The effect of vertebrate predation on lotic macroinvertebrate communities in Quebec, Canada. *Canadian Journal of Zoology* **64**, 1930–1936.
- Reinhardt T., Brauns M., Steinfartz S. & Weitere M. (2017) Effects of salamander larvae on food webs in highly subsidised ephemeral ponds. *Hydrobiologia* **799**, 37–48.
- Resetarits Jr W.J. (1995) Competitive asymmetry and coexistence in size-structured populations of brook trout and spring salamanders. *Oikos*, 188–198.
- Resetarits Jr W.J. (1997) Differences in an ensemble of streamside salamanders (*Plethodontidae*) above and below a barrier to brook trout. *Amphibia-Reptilia* **18**, 15–25.
- Resetarits Jr W.J. (1991) Ecological interactions among predators in experimental stream communities. *Ecology* **72**, 1782–1793.
- Richardson J. & Danehy R. (2007) A synthesis of the ecology of headwater streams and their riparian zones in temperate forests. *Forest Science* **53**, 131–147.
- Roche K.R., Drummond J.D., Boano F., Packman A.I., Battin T.J. & Hunter W.R. (2017) Benthic biofilm controls on fine particle dynamics in streams. *Water Resources Research* **53**, 222–236.
- Romani A. & Sabater S. (1999) Effect of primary producers on the heterotrophic metabolism of a stream biofilm. *Freshwater Biology* **41**, 729–736.
- Romani A.M., Guasch H., Munoz I., Ruana J., Vilalta E., Schwartz T., *et al.* (2004) Biofilm structure and function and possible implications for riverine DOC dynamics. *Microbial Ecology* **47**, 316–328.
- Rudolf V. (2006) The influence of size-specific indirect interactions in predator-prey systems. *Ecology* **87**, 362–371.
- Ruetz C., Newman R. & Vondracek B. (2002) Top-down control in a detritus-based food web: fish, shredders, and leaf breakdown. *Oecologia* **132**, 307–315.
- Sabo J., Bastow J. & Power M. (2002) Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. *Journal of the North American Benthological Society* **21**, 336–343.
- Sabo J. & Power M. (2002) River–watershed exchange: effects of riverine subsidies on riparian

- lizards and their terrestrial prey. *Ecology* **83**, 1860–1869.
- Schiller D. V., Martí E., Riera J.L. & Sabater F. (2007) Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Freshwater Biology* **52**, 891–906.
- Schmitz O.J. (2007) Predator diversity and trophic interactions. *Ecology* **88**, 2415–2426.
- Schmitz O.J. & Sokol-Hessner L. (2002) Linearity in the aggregate effects of multiple predators in a food web. *Ecology Letters* **5**, 168–172.
- Siddon C.E. & Witman J.D. (2004) Behavioral indirect interactions: multiple predator effects and prey switching in the rocky subtidal. *Ecology* **85**, 2938–2945.
- Sih A., Englund G. & Wooster D. (1998) Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution* **13**, 350–355.
- Soluk D. & Collins N. (1988) Synergistic interactions between fish and stoneflies: facilitation and interference among stream predators. *Oikos* **52**, 94–100.
- Van Son T.C. & Thiel M. (2006) Multiple predator effects in an intertidal food web. *Journal of Animal Ecology* **75**, 25–32.
- Song B. & Leff L. (2006) Influence of magnesium ions on biofilm formation by *Pseudomonas fluorescens*. *Microbiological Research* **161**, 355–361.
- Steinmetz J., Soluk D.A. & Kohler S.L. (2008) Facilitation between herons and smallmouth bass foraging on common prey. *Environmental Biology of Fishes* **81**, 51–61.
- Swift Jr L.W. & Messer J.B. (1971) Forest cuttings raise temperatures of small streams in the southern Appalachians. *Journal of soil and water conservation* **26**, 111–116.
- Thelaus J., Haecky P., Forsman M. & Andersson A. (2008) Predation pressure on bacteria increases along aquatic productivity gradients. *Aquatic Microbial Ecology* **52**, 45–55.
- Townsend C.R. (2003) Individual, population, community, and ecosystem consequences of a fish invader in New Zealand streams. *Conservation Biology* **17**, 38–47.
- Townsend C.R., Scarsbrook M.R. & Dolédec S. (1997) The intermediate disturbance hypothesis, refugia, and biodiversity in streams. *Limnology and Oceanography* **42**, 938–949.
- Ulrich K.E., Burton T.M. & Oemke M.P. (1993) Effects of whole-tree harvest on epilithic algal communities in headwater streams. *Journal of Freshwater Ecology* **8**, 83–92.
- Vance-Chalcraft H. & Soluk D. (2005) Multiple predator effects result in risk reduction for prey across multiple prey densities. *Oecologia* **144**, 472–480.
- Vance-Chalcraft H.D., Soluk D.A. & Ozburn N. (2004) Is prey predation risk influenced more by increasing predator density or predator species richness in stream enclosures? *Oecologia* **139**, 117–122.
- Vinebrooke R.D. (1996) Abiotic and biotic regulation of periphyton in recovering acidified lakes. *Journal of the North American Benthological Society* **15**, 318–331.
- Voshell R. (2002) *A guide to common freshwater invertebrates of North America*. (6 ed.). The University of Chicago Press Blacksburg.
- Vroom J., Grauw K., Gerritsen H., Bradshaw D., Marsh P., Watson G., *et al.* (1999) Depth penetration and detection of pH gradients in biofilms by two-photon excitation microscopy. *Applied and environmental microbiology* **65**, 3502–3511.
- Wallace J.B., Eggert S.L., Meyer J.L. & Webster J.R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**, 102–104.
- Warren D.R., Likens G.E., Buso D.C. & Kraft C.E. (2008) Status and distribution of fish in an acid-impacted watershed of the northeastern United States (Hubbard Brook, NH). *Northeastern Naturalist* **15**, 375–390.

- Webster J.R., Mulholland P.J., Tank J.L., Valett H.M., Dodds W.K., Peterson B.J., *et al.* (2003) Factors affecting ammonium uptake in streams - an inter-biome perspective. *Freshwater Biology* **48**, 1329–1352.
- Wesner J. (2010) Aquatic predation alters a terrestrial prey subsidy. *Ecology* **91**, 1435–1444.
- Wesner J. (2016) Contrasting effects of fish predation on benthic versus emerging prey: a meta-analysis. *Oecologia* **180**, 1205–1211.
- Wesner J. (2013) Fish predation alters benthic, but not emerging, insects across whole pools of an intermittent stream. **32**, 438–449.
- Wilhelm L., Singer G., Fasching C., Battin T. & Besemer K. (2013) Microbial biodiversity in glacier-fed streams. *The ISME journal* **7**, 1651.
- Wilkerson E., Hagan J.M. & Whitman A.A. (2006) The effectiveness of different buffer widths for protecting water quality and macroinvertebrate and periphyton assemblages of headwater streams in Maine, USA. *Forest Science* **52**, 221–231.
- Williamson T.J., Cross W.F., Benstead J.P., Gíslason G.M., Hood J.M., Huryn A.D., *et al.* (2016) Warming alters coupled carbon and nutrient cycles in experimental streams. *Global Change Biology* **22**, 2152–2164.
- Winkelmann C., Schneider J. & Mewes D. (2014) Top-down and bottom-up control of periphyton by benthivorous fish and light supply in two streams. *Freshwater* **59**, 803–818.
- Wipfli M. & Gregovich D. (2002) Export of invertebrates and detritus from fishless headwater streams in southeastern Alaska: implications for downstream salmonid production. *Freshwater Biology* **47**, 957–969.
- Wolman M. (1954) A method of sampling coarse river-bed material. *EOS, Transactions American Geophysical Union*.
- Woodward G., Papanтониou G. & Edwards F. (2008) Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. *Oikos* **117**, 683–92.
- Yan E., Wang X. & Huang J. (2005) Concept and classification of coarse woody debris in forest ecosystems. *Acta Ecologica Sinica*.
- Ylla I., Borrego C., Romaní A. & Sabater S. (2009) Availability of glucose and light modulates the structure and function of a microbial biofilm. *FEMS microbiology ecology* **69**, 27–42.
- Zimmer K., Hanson M. & Butler M. (2001) Effects of fathead minnow colonization and removal on a prairie wetland ecosystem. *Ecosystems* **4**, 346–357.

Table 1 Summary of density, biomass, taxonomic richness (S), and exponential of the Shannon-Wiener Index (Exp H') by treatment for benthic and emergent macroinvertebrates in stream mesocosms beside Zig-Zag Brook at the Hubbard Brook Experimental Forest, NH, USA. The experiment ran from 22 July to 10 September 2014. Values are means \pm SE. Salamander treatments had for levels: *Eurycea bislineata* alone (EBIS), *Gyrinophilus porphyriticus* alone (GPOR), *E. bislineata* and *G. porphyriticus* (BOTH), and no-salamander controls (CONTROL). Each treatment was replicated four times; however, the biomass of benthic macroinvertebrates in the EBIS treatment could only be calculated for three replicates. The Exp H' of the GPOR treatment could only be calculated for two replicates out of four as there were no benthic macroinvertebrates on the samplers collected from two of the GPOR treatments.

Treatment	Benthic Macroinvertebrates				Emergent Macroinvertebrates			
	Density (individuals m ⁻²)	Biomass (mg m ⁻²)	S	Exp H'	Density (individuals m ⁻² d ⁻¹)	Biomass (mg m ⁻² d ⁻¹)	S	Exp H'
EBIS	39 \pm 12	4 \pm 2	1.75 \pm 0.48	1.57 \pm 0.34	10 \pm 2	3.57 \pm 0.78	2.25 \pm 0.25	1.63 \pm 0.14
GPOR	10 \pm 6	9 \pm 8	0.5 \pm 0.29	1 \pm 0	20 \pm 5	7.14 \pm 1.58	2.5 \pm 0.50	1.33 \pm 0.13
BOTH	42 \pm 13	59 \pm 39	1.75 \pm 0.48	1.69 \pm 0.45	19 \pm 3	7.00 \pm 0.79	2.25 \pm 0.25	1.36 \pm 0.15
CONTROL	39 \pm 9	20 \pm 6	2 \pm 0.41	1.78 \pm 0.39	21 \pm 6	7.63 \pm 2.31	3.25 \pm 0.48	1.53 \pm 0.14

Table 2 Summary of benthic macroinvertebrate taxa by treatment in an experiment testing for the effect of salamander community composition on benthic and emergent macroinvertebrates. Experimental mesocosms were placed along Zig-Zag Brook at the Hubbard Brook Experimental Forest, NH, USA. The experiment ran from 22 July to 10 September 2014. Salamander treatments were: *Eurycea bislineata* alone (E), *Gyrinophilus porphyriticus* alone (G), *E. bislineata* and *G. porphyriticus* (B), and no-salamander controls (C). Each treatment was replicated four times. Different columns represent replicates of each treatment.

Taxa		Treatments															
Order or Subclass	Family	E	E	E	E	G	G	G	G	B	B	B	B	C	C	C	C
Oligochaeta	NA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Diptera	Chironomidae	0	0	4	1	1	0	0	0	2	2	1	0	2	0	2	2
Ephemeroptera	Ephemerellidae	4	0	1	0	0	0	2	0	0	2	0	3	1	1	0	2
Plecoptera	Chloroperlidae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Plecoptera	Leuctridae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Trichoptera	Phryganeidae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Trichoptera	Polycentropodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

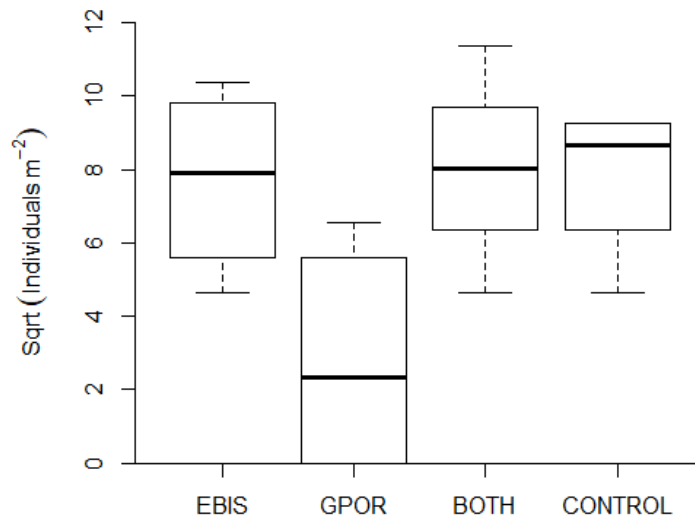


Fig. 1 Benthic macroinvertebrate densities (square root individuals m^{-2}) in mesocosms with treatments consisting of *Eurycea bislineata* alone (EBIS), *Gyrinophilus porphyriticus* alone (GPOR), both salamander species (BOTH), and neither species (CONTROL). Experimental mesocosms were placed along Zig-Zag Brook at the Hubbard Brook Experimental Forest, NH, USA. The experiment ran from 22 July to 10 September 2014. The center line within each box represents the median value, the upper line represents the upper quartile, and the lower line represents the lower quartile. The upper whisker represents the maximum value of the data set and the lower whisker represents the minimum value. When a whisker is not present it indicates that the minimum or maximum value of the dataset is equivalent to the upper or lower quartile.

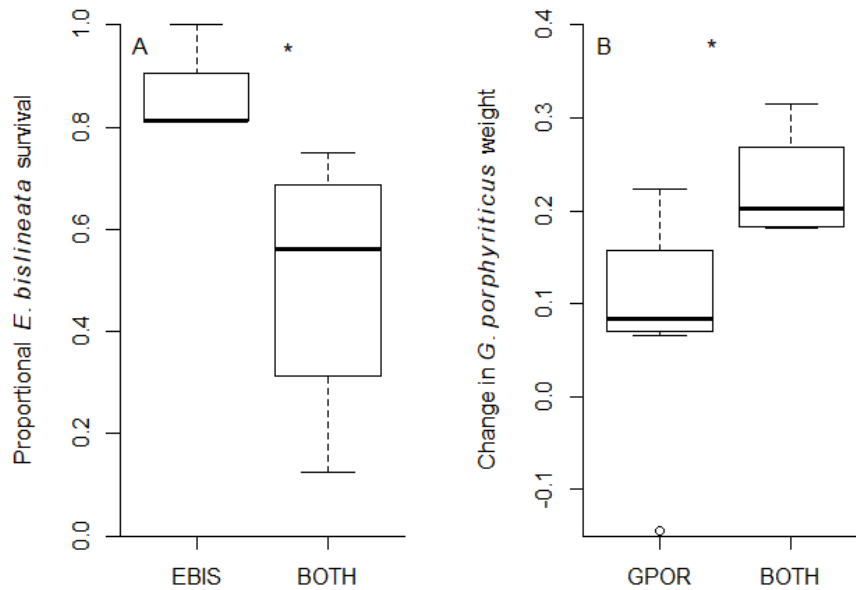


Fig. 2 Box plot of proportional *Eurycea bislineata* survival (A) and change in *Gyrinophilus porphyriticus* weight (B) by salamander treatment. Experimental mesocosms were placed along Zig-Zag Brook at the Hubbard Brook Experimental Forest, NH, USA. The experiment ran from 22 July to 10 September 2014. Treatments were *E. bislineata* alone (EBIS), *G. porphyriticus* alone (GPOR) and *E. bislineata* and *G. porphyriticus* (BOTH). Asterisks indicate significant differences between treatment levels at $P < 0.05$. The center line within each box represents the median value, the upper line represents the upper quartile, and the lower line represents the lower quartile. The upper whisker represents the maximum value of the data set, excluding outliers, and the lower whisker represents the minimum value, excluding outliers. When a whisker is not present it indicates that the minimum or maximum value of the dataset is equivalent to the upper or lower quartile. Outliers are shown as open circles.

CHAPTER 2: BIOFILM BIOMASS IN OLIGOTROPHIC HEADWATER STREAMS: TOP-DOWN OR BOTTOM-UP CONTROL?

ABSTRACT

Biofilm, the mixed auto- and heterotrophic microbial community covering stream substrates, forms the base of food webs as an *in situ* resource in nutrient poor, heavily shaded headwater streams, and is important in nutrient uptake, transformation and retention. However, we know little about the relative importance of bottom-up and top-down controls on the biomass of these mixed microbial communities. I used a combination of stream surveys and pre-existing stream chemistry data to assess the relative importance of bottom-up and top-down determinants of biofilm biomass in the oligotrophic headwater streams of the Hubbard Brook Experimental Forest (HBEF), New Hampshire, USA. Potential bottom-up controls were light (e.g., canopy cover, aspect), nutrient resources (e.g., nitrogen, phosphorus), physical habitat variables (e.g., stream width, substrate) and chemical parameters (e.g., stream conductivity, pH). Potential top-down drivers, those controlling biofilm biomass by direct and indirect consumption, were benthic macroinvertebrates and stream salamanders. I used stepwise multiple linear regression to assess the relative importance of these bottom-up and top-down controls, with all models ranked by AIC_c score. The top-ranked model predicting biofilm biomass included aspect, canopy cover, nutrients and pH. Specifically, I found that biofilm biomass increased with light availability, nutrient availability, and lower acidity. No top-down drivers affected biofilm biomass. The importance of light in controlling biofilm biomass, despite prior evidence for numerical and metabolic dominance of heterotrophic microbes in biofilms at the HBEF, suggests that the autotrophic component of these biofilms may be disproportionately important, perhaps by providing increased surface area for colonization or labile carbon to the heterotrophic components. My results show the importance of looking at both the auto- and heterotrophic components of stream biofilms when considering the potential determinants of biofilm biomass.

Key Words: biofilm, periphyton, Hubbard Brook Experimental Forest, headwater streams, benthic macroinvertebrates

INTRODUCTION

Biofilm, the microbial community covering stream substrates, is an integral part of stream ecosystems, forming the base of stream food webs and controlling nutrient cycling (Mulholland *et al.*, 1994; Battin *et al.*, 2003, 2016). Many taxa consume stream biofilms, making it an essential source of secondary productivity (Feminella & Hawkins, 1995; Merrit & Cummins, 1996), even in forested headwater streams with high inputs of terrestrially derived organic matter (Hall Jr & Meyer, 1998; Hall Jr, Likens & Malcom, 2001; McNeely, Clinton & Erbe, 2006). Biofilms are also important sites of nutrient uptake and transformation (Mulholland *et al.*, 1994; Battin *et al.*, 2003, 2016). This is particularly true for headwaters streams, which are often the first sites of terrestrial derived nutrient inputs (Peterson *et al.*, 2001; Lowe & Likens, 2005).

Historically, studies of stream biofilms have focused on periphyton, the autotrophic component of biofilms (i.e., green algae, diatoms, cyanobacteria, Battin *et al.*, 2016). However, the autotrophic and heterotrophic components (i.e., bacteria, fungi, protozoans) generally co-occur in nature in a matrix of extracellular polymeric substances (EPS) that they produce (Hoagland *et al.*, 1993; Flemming & Wingender, 2010; Battin *et al.*, 2016; Flemming *et al.*, 2016). The resulting physical structure, with its increased sorption ability and retention of extracellular enzymes, can alter the availability of water, light, and oxygen experienced by its members, as well as increasing metabolic efficiency, nutrient uptake, and resistance to both desiccation and high flow events (Hall-Stoodley, Costerton & Stoodley, 2004; Battin *et al.*, 2016; Flemming *et al.*, 2016; Roche *et al.*, 2017). The EPS matrix can make up the majority of biofilm mass and is consumed along with heterotrophic and autotrophic components when taxa feed on biofilms (Hall Jr & Meyer, 1998; Lawrence *et al.*, 2002; Flemming & Wingender, 2010).

Given its integral role in stream food webs and nutrient processing, it is important to understand what controls biofilm biomass in stream ecosystems. Forested headwater streams are often heavily shaded, limiting photosynthesis and resulting in food webs reliant on processing terrestrially derived nutrients (Fisher & Likens, 1973; Richardson & Danehy, 2007). Much of this processing occurs in stream biofilms, where heterotrophic biofilm components transform terrestrially derived nutrients and carbon into a form which larger organisms, such as benthic macroinvertebrates, can eat (Hall Jr & Meyer, 1998; France, 2011). In these systems, we know little about the relative importance of bottom-up versus top-down controls on the mixed microbial community constituting its base.

Our extensive understanding of stream periphyton, and emerging understanding of the combined auto- and heterotrophic components of biofilm, suggests that there are several factors that could influence stream biofilm biomass, including light, nutrient availability, physical habitat structure, and stream water chemistry. Light is important for periphyton (Schiller *et al.*, 2007; Ylla *et al.*, 2009), and periphyton is often limited, or co-limited, by nitrogen and phosphorus (Reviewed in Lamberti, 1996; Francoeur, 2001; Hillebrand, 2002). Calcium and magnesium are important for biofilm attachment, although their role in limiting biofilm production is less studied than that of nitrogen and phosphorus (Geesey, Wigglesworth-Cooksey & Cooksey, 2000; Song & Leff, 2006; Flemming *et al.*, 2016). Additional categories of bottom-up variables important for stream biofilms are physical habitat (e.g., substrate; Cardinale *et al.*, 2002) and stream chemistry (e.g., pH, Ledger & Hildrew, 2001; Lear *et al.*, 2009).

Top-down control of biofilm biomass can result from consumption of biofilm by omnivorous benthic macroinvertebrates that consume bacterial, fungal and EPS components, in addition to the periphyton component of biofilms (Cummins & Klug, 1979; Feminella &

Hawkins, 1995; Hall Jr & Meyer, 1998). There is some evidence that in oligotrophic systems, where lower productivity supports lower numbers of primary consumers, the importance of these top-down effects may be reduced relative to bottom-up effects (Elser & Goldman, 1991; Dufour & Torr ton, 1996; Pace *et al.*, 1999; Thelaus *et al.*, 2008). However, this is not always the case (Gasol, Pedr s-Ali  & Vaqu , 2002), and the strength of bottom-up versus top-down effects in lotic systems have focused almost exclusively on periphyton (reviewed in Hillebrand, 2002). Top-down effect can also be indirect, such as through the consumption of benthic macroinvertebrates by fish (Lamberti, 1996; Biggs *et al.*, 2000; Winkelmann *et al.*, 2014).

Here I use a combination of stream surveys and pre-existing stream chemistry data to quantify the relative importance of bottom-up and top-down drivers of stream biofilm biomass at the Hubbard Brook Experimental Forest (HBEF). The HBEF is an oligotrophic watershed located in the White Mountains Region of central New Hampshire, USA. The HBEF streams are nutrient poor and heavily shaded, making biofilms an important *in situ* resource. Biofilms in headwater stream at the HBEF are dominated by heterotrophic, non-periphyton members, and this dominance is reflected in measures of P:R ratio, cell counts, and contribution to benthic macroinvertebrate diets (Burton, Ulrich & Haack, 1988; Hall Jr *et al.*, 2001; Webster *et al.*, 2003). However, previous work at the HBEF has focused almost entirely on bottom-up controls of periphyton, the autotrophic component of biofilm, and has usually taken place in only one or two focal streams. These studies have found conflicting effects on periphyton of both nutrients (i.e., nitrogen, phosphorus; Ulrich, Burton & Oemke, 1993; Bernhardt & Likens, 2004; Chadwick & Hurn, 2005) and light (Findlay & Howe, 1993; Ulrich *et al.*, 1993; Fuller, Kennedy & Nielsen, 2004). In these streams, pH has been shown to increase periphyton and decrease fungi (Hall *et al.*, 1980). However, studies on biofilm as a whole at the HBEF are

lacking, as are studies on top-down drivers (but see Bernhardt & Likens, 2004). Top-down control of biofilm biomass at the HBEF may occur through its primary consumers, benthic macroinvertebrates (Hall Jr *et al.*, 2001), and their consumers, stream salamanders (Burton, 1976). Fish are not present in the upper reaches of the headwater streams at the HBEF (Warren *et al.*, 2008), where I focused my sampling, and where stream salamanders are the top predators.

METHODS

Study Area and Reach Selection

To assess top-down versus bottom-up drivers of biofilm biomass, I sampled 20 fishless study reaches throughout the stream network at the Hubbard Brook Experimental Forest (HBEF). In the fishless headwater reaches where I focused my sampling, the top predators are two stream salamanders, *Gyrinophilus porphyriticus* and *Eurycea bislineata*. Both salamanders have aquatic larvae that prey on benthic macroinvertebrates, and *G. porphyriticus* larvae and adults also consume *E. bislineata* and terrestrial insects that fall on the stream's surface (Burton, 1976; Petranka, 1998; Lowe *et al.*, 2005). Field sampling took place between 1 July and 10 September, 2016. The HBEF is part of the Long-Term Ecological Research Network (LTER). Many small, fishless headwater streams run within the 31.6 km² area of the HBEF (Warren *et al.*, 2008). The forest at the HBEF is dominated by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*) and yellow birch (*Betula alleghaniensis*, Likens, 2013). The HBEF streams also tend to be heterotrophic, with most carbon entering the streams through allochthonous inputs (Fisher & Likens, 1973; Mayer & Likens, 1987). These streams are nutrient poor, with

average fall concentrations of ammonium, nitrate and phosphate values of 0.01, 0.03 and 0.003 mg/L respectively (Likens & Buso, 2006).

Each study reach was 10 m long and randomly selected from a list of stream monitoring sites across the entire stream network at the HBEF (Likens & Buso, 2006). I used a vector file of Hubbard Brook hydrography (<http://data.hubbardbrook.org/gis/>, accessed 24 August 2014) to determine the distance between selected study reaches. These distances were calculated in QGIS 2.14.3-Essen (QGIS Development Team 2016, <http://www.qgis.org/>). I rejected study reaches that were less than 200 m from all nearest study reaches, and those that were within designated experimental watersheds or occupied by fish. I randomly chose replacement sites until 20 study reaches were selected (Figure 1). Fishless status of study reaches was based on Warren *et al.* (2008). I confirmed fish absence from study reaches by placing three minnow traps (model 0822711271, Frabill, 2.54 cm opening) in study reaches for 24 hours prior to conducting surveys.

Several of the randomly selected study reaches ($n = 11$) were located along the same stream channel. To ensure that data from these study reaches were independent, I ran t-tests comparing the pH, conductivity, and nutrient data between two study reaches located on the same stream channel and two randomly chosen study reaches. I conducted these comparisons for all 11 study reaches of interest. All t-test were not significant ($p > 0.20$), indicating that paired study reaches were not more similar than non-paired study reaches.

Biofilm Sampling

At each study reach I collected biofilm from three cobbles at each of three distances from the downstream end of the site: 0, 5 and 10 m. At some sites three cobbles were not available at each distance, so I collected biofilm from all available cobble (total cobble scraped per study

reach \pm SE = 8 ± 0.5). Only cobbles that were submerged in the stream and free of moss were selected, and individual rocks were chosen haphazardly. On each cobble I scraped an area of 23.8 cm² using a razorblade. I placed the collected biofilm on ice in the dark and froze it within five hours (Kilroy *et al.* 2013). Samples were kept frozen until they were ashed and weighed using Standard Method 2540E (American Water Works Association & Water Environment Federation, 2005). I calculated biomass of biofilm at each site as the average of values at 0, 5 and 10 m divided by the total area sampled at a study reach to give biomass of biofilm in grams per m².

Bottom-Up Drivers of Biofilm

I predicted the bottom-up variables controlling biofilm biomass to be light, nutrients, physical habitat structure and stream chemistry. I used aspect and canopy cover to assess light availability. Light is necessary for periphyton, but can also increase the growth, density, and enzymatic activity of heterotrophic microbes co-occurring with autotrophs (Romani & Sabater, 1999; Ylla *et al.*, 2009). Aspect is known to be an important factor regulating growth in both terrestrial and aquatic plants due to its effects on light, transpiration and temperature (Cantlon, 1953). Specifically, in the Northern hemisphere a southern aspect is known to provide more light, and induce more growth than a northern aspect when water is not limiting (Holland & Steyn, 1975). I determined aspect (southern or northern) in relation to the Hubbard Brook mainstem, which flows roughly east to west through the center of the HBEF (Figure 1). I measured canopy cover using a spherical convex densitometer (Forestry Suppliers Inc., Jackson, MS, Lemmon, 1956). I took six measurements at 0, 5 and 10 m from the downstream end of each study reach. I took the first four of these measurements in the center of the stream facing

upstream, downstream, the right bank, and the left bank. I took the last two measurements while standing on the right and left bank. I used the average of the eighteen canopy cover measurements from a study reach in my analysis (Plotnikoff & Wiseman, 2001, Table 1).

The specific nutrients I predicted would affect stream biofilm biomass were nitrate (NO_3^-), phosphate (PO_4^{3-}), calcium (Ca^{2+}), magnesium (Mg^{2+}), dissolved inorganic carbon (DIC), silicon dioxide (SiO_2) and dissolved organic carbon (DOC). I obtained values for each of these nutrients, in milligrams per liter, from Likens and Buso (2006, Table 1). There has been a steady decline in stream water concentrations of NO_3^- , PO_4^{3-} , Ca^{2+} , Mg^{2+} , and a concurrent increase in stream pH, across the HBEF since the 1960's (Likens, Driscoll & Buso, 1996; Fuss, Driscoll & Campbell, 2015). However, these changes have been gradual, and it is unlikely that a significant change in stream chemistry occurred between 2001, when the stream chemistry data were collected, and 2016, when I conducted this sampling.

Nitrogen and phosphorus are known to be important for periphyton (reviewed in Feminella & Hawkins, 1995; Lamberti, 1996; Hillebrand, 2002). Magnesium and calcium are important for the attachment of biofilms, perhaps by strengthening the biofilm's EPS matrix (Geesey *et al.*, 2000; Song & Leff, 2006; Flemming *et al.*, 2016). DIC can affect periphyton growth (Fairchild & Sherman, 1993; Vinebrooke, 1996), and silica is important for diatom production (Hill & Webster, 1982; Carrick & Lowe, 2007; Grady, Scanlon & Galloway, 2007). The heterotrophic component of stream biofilms (i.e., heterotrophic bacteria, fungi, protozoans) use DOC as a food source, obtained from stream water or from their autotrophic neighbors (Cole, 1982; Romani & Sabater, 1999; Romani *et al.*, 2004).

I expected my nutrient variables to be autocorrelated, which is known to lead to spurious results in regression analyses (Graham, 2003). Therefore I used principal component analysis to

reduce the dimensionality of my stream nutrients data to one independent variable (Nutrient PC 1). I then utilized Nutrient PC1 in my analyses (Table 2). In principal components analysis the original variables are replaced by an equal number of principal components, which are each a linear combination of the original variables (Jolliffe & Cadima, 2016). Each principal component is uncorrelated with any other principle component and the components are ordered by the amount of variation in the data they explain. Therefore, the effect of individual nutrients on biofilm biomass is obscured by PCA. However, we may associate variables and principal components, provided the component loading's (CL) magnitude is relatively large (Huryn et al., 2002). Nutrient PC 1 explained 43.5% of the variation in stream nutrients. The component loadings for Nutrient PC 1 were Ca^{2+} (CL, 0.50), DIC^{-1} (CL, -0.45), Mg^{2+} (CL, 0.43), SiO_2 (CL, 0.22), $(\text{NO}_3^-)^{-1}$ (CL, -0.05), DOC^{-1} (CL, 0.38) and PO_4^{3-} (CL, -0.41). Therefore Nutrient PC 1 was primarily positively associated with Ca^{2+} , DIC and Mg^{2+} , and negatively associated with PO_4^{3-} .

The physical habitat variables I predicted would determine biofilm biomass were elevation (m), stream width (m), percent total wood, mesohabitat and substrate. I determined the elevation of each of my study reaches using data from Likens and Buso (2006). I measured stream width as the bankfull stream width at a study reach. I visually estimated the percent of stream bed covered by coarse woody debris and the percent of each stream mesohabitat (i.e., pools, rifles, runs or cascades) at each study reach (Montgomery & Buffington, 1998, Table1). I also conducted a Wolman Pebble Count to characterize stream substrate at each study reach (Wolman, 1954; Yan, Wang & Huang, 2005). Briefly, for the Wolman Pebble Count I recorded substrate type (i.e., sand, gravel, cobble, boulder, bedrock) every meter in transects perpendicular to stream flow which covered the bankfull width of the stream. Moving upstream, I repeated this

process until I recorded 100 measurements or had traversed the entity of the study reach. For each study reach I then calculated the percent of each substrate type (Table 1).

As with nutrients, I expected the percent of each mesohabitat type and percent of each stream substrate type at a study reach to be autocorrelated and used principal component analysis to reduce these data sets to single variables (Mesohabitat PC 1, Substrate PC 1). I then utilized Mesohabitat PC 1 and Substrate PC 1 in my analyses (Table 2). Mesohabitat PC 1 explained 48% of the variation in percent of stream mesohabitat composed of cascades, riffles, pools and runs among study reaches. The component loadings for Mesohabitat PC 1 were percent cascade (CL, 0.48), percent riffle (CL, 0.45), percent pool (CL, 0.26) and percent run (-0.70). Substrate PC 1 explained 48% of the variation in percent of stream substrate composed of sand, gravel, cobble, boulder or bedrock among study reaches. The component loadings for Substrate PC 1 were percent gravel (CL, 0.60), percent sand (CL, 0.44), percent cobble (CL, 0.36), percent bedrock (CL, -0.14) and percent boulder (CL, -0.54).

The stream chemistry variables I predicted would determine biofilm biomass were stream conductivity and pH. Both stream conductivity and pH can alter community composition of biofilms (Ledger & Hildrew, 2001; Lear *et al.*, 2009; Wilhelm *et al.*, 2013), and pH also alters the community composition of benthic macroinvertebrate consumers (Ledger & Hildrew, 2005). As with nutrients, I obtained stream chemistry data for each of my stream reaches from Likens and Buso (2006).

Top-Down Drivers of Biofilm

Variables predicted to drive biofilm biomass from the top-down were benthic macroinvertebrate biomass and salamander occupancy. To measure benthic macroinvertebrate

biomass, I obtained samples from 0, 5 and 10 m at each study reach. I obtained samples by disturbing substrate within a 0.09 m² quadrat in riffle habitats for two minutes and collecting benthic macroinvertebrates in a 800 µm × 900 µm mesh D-frame net placed immediately downstream of the sampling quadrat. When a riffle was not present, I used a run. I filtered samples through two nested sieves of mesh size 10 mm² and 1 mm² and placed all benthic macroinvertebrates in the 1 mm sieve on ice in the field (Angradi, 1996). Upon return to the lab, I froze macroinvertebrates within 48 hours. After thawing, I dried samples at 65°C for 72 hours, cooled them in a desiccator, then weighed them to the nearest 0.0001 gram. I recorded biomass of benthic macroinvertebrates at a study reach as the average of total biomass at 0, 5 and 10 m.

To determine salamander occupancy at a site, I used both cover-controlled and area-constrained survey methods. I modified cover controlled active survey methods from Lowe and Bolger (2002). Specifically, I flipped three cobbles (64 - 256 mm in length by the longest dimension), located either within the stream or along the bank, every meter for a total of thirty rocks per site. I collected all *G. porphyriticus* individuals revealed or flushed by the current in an aquarium dip-net. I conducted one area-constrained survey at 0, 5 and 10 m. I placed a 0.38 m² quadrat on the stream bed, removed all cobble within the quadrat, and collected all *G. porphyriticus* and *E. bislineata* individuals. I constrained area-controlled searches to sixty minutes and released salamanders at their collection site. I considered a site occupied by *G. porphyriticus* or *E. bislineata* if one or more individuals of that species were detected using these survey methods. Due to the occupancy of *E. bislineata* in all but 2 of my study reaches, I was not able to use *E. bislineata* occupancy in my analysis.

Statistical analyses

To assess the effects of bottom-up and top-down variables on biofilm biomass at the HBEF, I used multiple linear regression with full stepwise model selection based on the Akaike information criterion modified for small sample sizes. The bottom-up variables included in this analysis were aspect, canopy cover, Nutrient PC 1, elevation (m), stream width (m), percent total wood, Mesohabitat PC 1, Substrate PC 1, pH and conductivity (Table 2). The top-down variables included in this analysis were benthic macroinvertebrate biomass and occupancy of *G. porphyriticus* (Table 2). I included date as an additional variable in the model selection process to account for possible variation over the sampling period. When necessary, independent variables were transformed to meet the assumption of normality (Tables 1 and 2). I tested for autocorrelation of independent variables using Pearson's correlation analysis prior to running the multiple stepwise linear regression. Explanatory variables were not autocorrelated ($r < 0.7$).

The full stepwise procedure utilized both forwards selection and backwards elimination. The Akaike information criterion modified for small sample sizes (AIC_c) was used to select the most parsimonious model (i.e., that with the lowest AIC_c score) from all candidate models (Burnham & Anderson, 2002). I then calculated the difference in AIC_c scores for each of the top models, as well as their likelihoods and Akaike weights (Table 3). If the top two models differed in AIC_c weight by two or less, I used a likelihood ratio test to identify the most parsimonious model (Burnham & Anderson, 2002; Johnson & Omland, 2004, Table 3). I include P-values for each of the variables in the top model to provide information on relative importance, but recognize that P-values are not, strictly speaking, applicable with models chosen using an information-theoretic approach (Burnham & Anderson, 2002). I also examined univariate regressions of each of the explanatory variables in the top model against biofilm biomass to assess the strength and directionality individual effects.

My data set also allowed me to assess the effects of bottom-up and top-down determinants of benthic macroinvertebrate biomass at the HBEF with the same methods used for biofilm biomass (Table 5). This analyses allowed me to assess if top-down drivers (i.e., salamander occupancy) influenced the immediate consumers of biofilm (i.e., benthic macroinvertebrates). Alternatively, if biofilm biomass were found to be a strong predictor of benthic macroinvertebrate biomass, it would suggest that direct top-down control on biofilm biomass by benthic macroinvertebrates is occurring. All statistical analysis were conducted in R version 3.4.3 (R Core Team, 2017).

RESULTS

Predictors of Biofilm Biomass

The top two models predicting biofilm biomass at the HBEF selected by stepwise multiple linear regression had similar AIC_c weights ($\Delta\text{AIC}_c < 2.0$; Table 3). A likelihood ratio test comparing these two models was not significant ($P = 0.06$), indicating that a model with fewer parameters was most parsimonious. This model included aspect, canopy cover, Nutrient PC 1, and pH as explanatory variables. The top model explained 46% of the total variation in biofilm biomass (Table 4). Aspect, canopy cover, Nutrient PC 1, and pH were also included in the three next highest-ranked models (Table 3). Of these, aspect, canopy cover and pH had partial P-values less than 0.05 (Table 4). Biofilm biomass was negatively associated with canopy cover, indicating that more light was correlated with increased biofilm biomass. Streams with a southern aspect also tended to have more biofilm, further emphasizing the importance of light for biofilms at the HBEF. The positive correlation of biofilm biomass and Nutrient PC 1, given the

weighting of Nutrient PC 1, indicates that biofilm biomass is positively associated with Ca^{2+} , DIC and Mg^{2+} and negatively associated with PO_4^{3-} . Biofilm biomass was negatively correlated with stream pH, indicating that biofilm biomass increased as streams became more acidic. The two potential top-down drivers of biofilm, benthic macroinvertebrate biomass and *G. porphyriticus* occupancy, were not included in any of the top models, indicating strong bottom-up control of biofilm biomass. Again, because *E. bislineata* was detected at all but two of my study reaches, I was not able to use *E. bislineata* occupancy in this analysis.

None of the univariate regressions of the predictor variables in the top model for biofilm biomass were significant at $P < 0.05$. However, the univariate regression of canopy cover was nearly significant ($P = 0.052$) and explained 15% of the total variation in biofilm biomass (Table 4, Figure 2).

Predictors of Benthic Macroinvertebrate Biomass

The top two models predicting benthic macroinvertebrate biomass selected by stepwise multiple linear regression had AIC_c weights less than two apart (Table 6). A likelihood ratio test comparing these top two models was not significant ($P = 0.08$), indicating that the model with fewer parameters was most supported. That model included date and Substrate PC 1, and explained 36% of the variation in benthic macroinvertebrate biomass (Table 5). Date had a P -value less than 0.05, but substrate did not (Table 6). Benthic macroinvertebrate biomass decreased with increasing date (from 1 July to 10 September, 2016) and with increasing Substrate PC 1 (i.e., with more gravel and fewer boulders).

DISCUSSION

Despite the dominance of heterotrophic microorganisms in biofilms of the heavily shaded headwater streams of the HBEF (Burton *et al.*, 1988; Webster *et al.*, 2003), light availability was a major determiner of biofilm biomass. Specifically, I interpreted the presence of both aspect and canopy cover in my top model, and four sequential models, as reflecting the importance of light to biofilm biomass (Table 3). In the northern hemisphere, southern aspect is associated with greater light availability, and was correlated with higher biofilm biomass in my top model (Galicia *et al.*, 1999; Geiger, Aron & Todhunter, 2009). Canopy cover ranged from just 88.8 to 96.8% (mean 92.6 ± 0.5 , Table 2), yet had a strong relationship with biofilm biomass, and was the only variable from the top model to be nearly significant in explaining biofilm biomass in univariate regression ($P = 0.052$). It is also possible that canopy cover and aspect affected biofilm biomass through changes in stream temperature (Swift Jr & Messer, 1971; Wilkerson, Hagan & Whitman, 2006; Williamson *et al.*, 2016) or minor variation in leaf litter inputs to streams (Martínez, Kominoski & Larrañaga, 2017).

Given that previous studies showed that biofilms in the HBEF are dominated by heterotrophic, non-periphyton members (Webster *et al.*, 2003), the disproportionate effect of light in controlling biofilm biomass may indicate that heterotrophic bacteria and fungi are benefiting from algal carbon fixation (Romani & Sabater, 1999; Ylla *et al.*, 2009), or from the structural scaffolding that algae – and diatoms in particular – bring to stream biofilms (Romani *et al.*, 2004; Battin *et al.*, 2016). This interpretation is supported by evidence that bacterial counts increase seasonally with increasing algal and cyanobacteria counts in the HBEF (Burton *et al.*, 1988). The importance of light for stream biofilm biomass is also consistent with previous

studies at the HBEF that found algae increased in streams after clearcutting (Noel, Martin & Federer, 1986; Haack, Burton & Ulrich, 1988), that the main Hubbard Brook has higher algal concentrations than its shadier counterpart, Bear Brook (Hall Jr *et al.*, 2001), and that shading can reduce periphyton (Findlay & Howe, 1993; Ulrich *et al.*, 1993; Fuller *et al.*, 2004).

The positive correlation of biofilm biomass with Nutrient PC 1 suggests that nutrients used for structural support may be more important to biofilm production at the HBEF than nitrogen and phosphorus. Ca^{2+} , DIC and Mg^{2+} were most positively associated with Nutrient PC 1 (component loadings for Ca^{2+} , $(\text{DIC})^{-1}$, and Mg^{2+} were 0.50, -0.45, and 0.43, respectively). Calcium and magnesium are important for biofilm adhesion and the stability of the EPS matrix, which makes up the scaffolding of biofilm (Geesey *et al.*, 2000; Song & Leff, 2006; Flemming *et al.*, 2016). Removal of these cations results in dissolution of biofilms (Banin, Brady & Greenberg, 2006). DIC can be important for the autotrophic components of biofilm, particularly in acidic conditions (Fairchild & Sherman, 1993; Vinebrooke, 1996). PO_4^{3-} had the strongest negative association with Nutrient PC1, while nitrogen had a slightly positive association (component loadings for PO_4^{3-} and $(\text{NO}_3^-)^{-1}$ were -0.41 and -0.05, respectively). This is consistent with previous studies at the HBEF which found that nitrogen and phosphorus had neutral or inhibitory effects on periphyton biomass (Ulrich *et al.*, 1993; Bernhardt & Likens, 2004). These findings emphasize the importance of considering a wide range of nutrients when assessing controls on biofilm growth (Kaspari & Powers, 2016).

Biofilm biomass was negatively correlated with pH (Table 4), even though its autotrophic and heterotrophic components respond to pH differently, raising questions about the emergent properties of biofilms at the HBEF. The streams I sampled were acidic (mean 5.67 ± 0.11 , Table 2), due to the legacy of acid rain (Johnson *et al.*, 1981; Likens *et al.*, 1996). However, the lowest

pH I encountered (4.66) was higher than an acid addition treatment at the HBEF (pH = 4.3) that had no effect on periphyton chlorophyll-*a* concentrations (Ulrich *et al.*, 1993). Additionally, periphyton biomass at the HBEF increased when a stream was artificially maintained at a pH of 4.0 (Hall *et al.*, 1980). This may be a result of the dominance of acid tolerant diatom and algal species (Ulrich *et al.*, 1993) or the ability of biofilms to alter internal pH gradients, both through excretions and by the creation of a diffusion boundary layer (Vroom *et al.*, 1999; Cornwall *et al.*, 2014). However, if biofilm is buffering against pH gradients, the heterotrophic components of biofilm do not seem to benefit from this buffering: experimental reductions of pH in streams at the HBEF resulted in lower biofilm bacterial and fungal densities (Hall *et al.*, 1980; Haack *et al.*, 1988). Overall, these findings suggest that the negative correlation between biofilm biomass and pH I observed resulted from positive effects of low pH on periphyton biomass, which outweighed negative effects on heterotrophic microbes.

Top-down pressure (i.e., consumption) did not appear in any of my top models (Table 3), despite evidence of top-down controls on periphyton biomass in other contexts (reviewed in Feminella & Hawkins, 1995; Lamberti, 1996; Hillebrand, 2002). In fishless streams at the HBEF, salamanders must affect benthic macroinvertebrate density or behavior to exert indirect, top-down effects on biofilm biomass. Specifically, because I collected biofilm from cobble, salamanders would have had to alter the behavior or density of benthic macroinvertebrates in the scraper functional feeding group before a change in biofilm biomass would have occurred (Merrit & Cummins, 1996). However, scrapers make up from just 1% to 13% of the benthic macroinvertebrate assemblage of streams at the HBEF (Hall Jr *et al.*, 2001; Chadwick & Huryn, 2005), which may make these indirect top-down effects difficult to detect. Future studies should look at biofilm biomass on both cobbles and leaves, allowing for salamander effects on both

scraping and shredding invertebrates to be observed. Shredders feed on biofilm attached to leaves and make up 34% to 50% of the benthic macroinvertebrate assemblage in streams at the HBEF (Hall Jr *et al.*, 2001; Chadwick & Huryn, 2005). Alternatively, it is possible that benthic macroinvertebrates densities were simply too low to depress biofilm biomass. The HBEF's nutrient poor streams and low levels of primary and secondary production (Chadwick & Huryn, 2005; Likens, 2013) may not support the densities of benthic macroinvertebrates needed to exert top-down pressure on biofilms, a theory supported by long-term declines in emergent stream insects at the HBEF (Rodenhouse, unpubl. data).

The community composition of salamanders in streams at the HBEF may also explain why I did not see top-down effects of salamanders on biofilm biomass. There is evidence that salamanders can decrease abundances of stream macroinvertebrates (Progar & Moldenke, 2002; Keitzer & Goforth, 2013a; Atlas & Palen, 2014), but salamander occupancy was not present in my top models of benthic macroinvertebrate biomass (Table 5) or biofilm biomass (Table 3). Importantly, my models included only occupancy of *G. porphyriticus* because the smaller salamander species, *E. bislineata* ($n = 2$), was only absent from two study reaches. A separate experimental study examining top-down effects of *G. porphyriticus* and *E. bislineata* on benthic macroinvertebrates, both alone and together, found that only *G. porphyriticus* affected benthic macroinvertebrate abundances, and only when it did not co-occur with *E. bislineata* (Bayer, unpubl. data). This experiment indicated that when the two species occur together, as was the case at all reaches where I detected *G. porphyriticus*, *G. porphyriticus* feeds primarily on *E. bislineata*, not benthic macroinvertebrates.

My findings suggest that light, nutrients, and pH regulate biofilm biomass in streams at the HBEF, that the auto- and heterotrophic components of biofilm may respond to these variable

differently, and that emergent properties of biofilms, such as structural stability or pH tolerance, determine biomass accrual. Similarly, the lack of significance of univariate regression analyses suggests that multiple bottom-up drivers of biofilm biomass must be considered simultaneously for the contribution of any one variable to be evident. Although top-down controls on biofilm have been shown to be important in other systems (Feminella & Hawkins, 1995; Hillebrand, 2002; Winkelmann *et al.*, 2014), their absence in my models suggests that the drivers of stream biofilm biomass at the HBEF are primarily bottom-up. Biofilms of headwater streams are frequently the first sites of terrestrial nutrient uptake and transformation, determining both the local availability and downstream export of these nutrients, while also providing an important source of *in situ* productivity supporting stream food webs. To fully understand the role of stream biofilms in nutrient cycling and stream food webs, I recommend that future studies isolate bottom-up and top-down effects on autotrophic and heterotrophic components of biofilm separately.

REFERENCES

- American Water Works Association & Water Environment Federation (2005) *Standard Methods for The Examination Of Water And Wastewater*, 21st edn. American Public Health Association, Washington DC.
- Angradi T.R. (1996) Inter-habitat variation in benthic community structure, function, and organic matter storage in 3 Appalachian headwater streams. *Journal of the North American Benthological Society* **15**, 42–63.
- Atlas W.I. & Palen W.J. (2014) Prey vulnerability limits top-down control and alters reciprocal feedbacks in a subsidized model food web. *Plos One* **9**, e85830.
- Banin E., Brady K. & Greenberg E. (2006) Chelator-induced dispersal and killing of *Pseudomonas aeruginosa* cells in a biofilm. *Applied and environmental microbiology* **72**, 2064–2069.
- Barr G.E. & Babbitt K.J. (2002) Effects of biotic and abiotic factors on the distribution and abundance of larval two-lined salamanders (*Eurycea bislineata*) across spatial scales. *Oecologia* **133**, 176–185.
- Battin T., Besemer K., Bengtsson M., Romani A. & Packmann A. (2016) The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology* **14**, 251.
- Battin T., Kaplan L., Newbold J. & Hansen C. (2003) Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* **426**, 439.
- Baxter C., Fausch K. & Saunders W.C. (2005) Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology* **50**, 201–220.
- Baxter C. V, Fausch K.D., Murakami M. & Chapman P.L. (2004) Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology* **85**, 2656–2663.
- Beachy C. (1994) Community ecology in streams: effects of two species of predatory salamanders on a prey species of salamander. *Herpetologica* **50**, 129–136.
- Benke A., Huryn A., Smock L. & Wallace J. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society* **18**, 308–343.
- Benoy G.A. (2008) Tiger salamanders in prairie potholes: A “fish in amphibian’s garments?” *Wetlands* **28**, 464–472.
- Bernhardt E.S. & Likens G.E. (2004) Controls on periphyton biomass in heterotrophic streams. *Freshwater Biology* **49**, 14–27.
- Biggs B.J., Francoeur S.N., Huryn A.D., Young R., Arbuckle C.J. & Townsend C.R. (2000) Trophic cascades in streams: effects of nutrient enrichment on autotrophic and consumer benthic communities under two different fish predation regimes. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1380–1394.

- Blaustein L., Friedman J. & Fahima T. (1996) Larval Salamandra drive temporary pool community dynamics: evidence from an artificial pool experiment. *Oikos* **76**, 392–402.
- Brazner J.C. & Kline E.R. (1990) Effects of chlorpyrifos on the diet and growth of larval fathead minnows, *Pimephales promelas*, in littoral enclosures. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 1157–1165.
- Bruce R. (1980) A model of the larval period of the spring salamander, *Gyrinophilus porphyriticus*, based on size-frequency distributions. *Herpetologica* **36**, 78–86.
- Bruce R.C. (2008) Intraguild interactions and population regulation in Plethodontid salamanders. *Herpetological Monographs* **22**, 31–53.
- Bruce R.C. (1985) Larval period and metamorphosis in the salamander *Eurycea bislineata*. *Herpetologica* **41**, 19–28.
- Burnham K. & Anderson D. (2002) *Model selection and multimodel inference: a practical information-theoretic approach*, 2nd edn. Springer-Verlag, New York.
- Burton T.M. (1976) An analysis of the feeding ecology of the salamanders (Amphibia, Urodela) of the Hubbard Brook Experimental Forest, New Hampshire. *Journal of Herpetology* **10**, 187–204.
- Burton T.M. & Likens G.E. (1975) Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. *Copeia* **1975**, 541–546.
- Burton T.M., Ulrich K.E. & Haack S.K. (1988) Community dynamics of bacteria, algae, and insects in a first order stream in New Hampshire, U.S.A. *Internationale Vereinigung für theoretische und angewandte Limnologie* **23**, 1125–1134.
- Cantlon J.E. (1953) Vegetation and microclimates on north and south slopes of Cushtunk Mountain, New Jersey. *Ecological Monographs* **23**, 241–270.
- Cardinale B., Palmer M., Swan C., Brooks S. & Poff N L (2002) The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. *Ecology* **83**, 412–422.
- Carey M.P. & Wahl D.H. (2010) Interactions of multiple predators with different foraging modes in an aquatic food web. *Oecologia* **162**, 443–452.
- Carpenter S.R., Cole J.J., Hodgson J.R., Kitchell J.F., Pace M.L., Bade D., *et al.* (2001) Trophic cascades, nutrients, and lake productivity: whole-lake experiments. *Ecological Monographs* **71**, 163–186.
- Carpenter S.R., Kitchell J.F. & Hodgson J.R. (1985) Cascading trophic interactions and lake productivity. *BioScience* **35**, 634–639.
- Carrick H. & Lowe R. (2007) Nutrient limitation of benthic algae in Lake Michigan: the role of silica. *Journal of Phycology* **43**, 228–234.
- Chadwick M.A. & Huryn A.D. (2005) Response of stream macroinvertebrate production to atmospheric nitrogen deposition and channel drying. *Limnology and Oceanography* **50**, 228–236.

- Cole J.J. (1982) Interactions between bacteria and algae in aquatic ecosystems. *Annual Review of Ecology and Systematics* **13**, 291–314.
- Cornwall C.E., Boyd P.W., McGraw C.M., Hepburn C.D., Pilditch C.A., Morris J.N., *et al.* (2014) Diffusion boundary layers ameliorate the negative effects of ocean acidification on the temperate coralline macroalga *Arthrocardia corymbosa*. *PLoS ONE* **9**, e97235.
- Crumrine P.W. & Crowley P.H. (2003) Partitioning components of risk reduction in a dragonfly-fish intraguild predation system. *Ecology* **84**, 1588–1597.
- Cummins K.W. & Klug M.J. (1979) Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* **10**, 147–172.
- Davenport J. & Lowe W. (2016) Does dispersal influence the strength of intraspecific competition in a stream salamander? *Journal of Zoology* **298**, 46–53.
- Diehl S. (1992) Fish predation and benthic community structure: the role of omnivory and habitat complexity. *Ecology* **73**, 1646–1661.
- Dudgeon D. (1996) The influence of refugia on predation impacts in a Hong Kong stream. *Archiv für Hydrobiologie* **138**, 145–159.
- Dufour P. & Torrétion J. (1996) Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical northeastern Atlantic Ocean. *Deep Sea Research Part I: Oceanographic Research Papers* **43**, 1305–1320.
- Eby L., Roach W., Crowder L. & Standord J. (2006) Effects of stocking-up freshwater food webs. *Elsevier* **21**, 576–584.
- Elser J.J. & Goldman C.R. (1991) Zooplankton effects on phytoplankton in lakes of contrasting trophic status. *Limnology and Oceanography* **36**, 64–90.
- Estes J.A. & Duggins D.O. (1995) Sea otters and kelp forests in Alaska: generality and variation in a community ecological paradigm. *Ecological Monographs* **65**, 75–100.
- Fairchild G.W. & Sherman J.W. (1993) Algal periphyton response to acidity and nutrients in softwater lakes: Lake comparison vs. nutrient enrichment approaches. *Journal of the North American Benthological Society* **12**, 157–167.
- Fauth J.E. (1990) Interactive effects of predators and early larval dynamics of the treefrog *Hyla-Chrysoscelis*. *Ecology* **71**, 1609–1616.
- Feder M. (1983) Integrating the ecology and physiology of Plethodontid salamanders. *Herpetologica* **39**, 291–310.
- Feminella J.W. & Hawkins C.P. (1995) Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of the North American Benthological Society* **14**, 465–509.
- Findlay S. & Howe K. (1993) Bacterial algal relationships in streams of the Hubbard Brook Experimental Forest. *Ecology* **74**, 2326–2336.
- Fisher S.G. & Likens G.E. (1973) Energy flow in Bear Brook, New Hampshire - integrative

- approach to stream ecosystem metabolism. *Ecological Monographs* **43**, 421–439.
- Fitzpatrick L. (1973) Influence of seasonal temperatures on the energy budget and metabolic rates of the northern two-lined salamander *Eurycea bislineata bislineata*. *Comparative Biochemistry and Physiology Part A: Physiology* **45**, 807–818.
- Flemming H. & Wingender J. (2010) The biofilm matrix. *Nature reviews microbiology* **8**, 623.
- Flemming H., Wingender J., Szewzyk U., Steinberg P., Rice S. & Kjelleberg S. (2016) Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology* **14**, 563.
- France R. (2011) Leaves as “crackers”, biofilm as “peanut butter”: exploratory use of stable isotopes as evidence for microbial pathways in detrital food webs. *Oceanological and Hydrobiological studies* **40**, 110–115.
- Francoeur S.N. (2001) Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society* **20**, 358–368.
- Fukui D.A.I., Murakami M., Nakano S. & Aoi T. (2006) Effect of emergent aquatic insects on bat foraging in a riparian forest. *Journal of Animal Ecology* **75**, 1252–1258.
- Fuller R., Kennedy B. & Nielsen C. (2004) Macroinvertebrate responses to algal and bacterial manipulations in streams. *Hydrobiologia* **523**, 113–126.
- Fuss C.B., Driscoll C.T. & Campbell J.L. (2015) Recovery from chronic and snowmelt acidification: Long-term trends in stream and soil water chemistry at the Hubbard Brook Experimental Forest, New Hampshire, USA. *Journal of Geophysical Research: Biogeosciences* **120**, 2360–2374.
- Galicia L., López-Blanco J., Zarco-Arista A., Filips V. & Garcia-Oliva F. (1999) The relationship between solar radiation interception and soil water content in a tropical deciduous forest in Mexico. *Catena* **36**, 153–164.
- Gasol J., Pedrós-Alió C. & Vaqué D. (2002) Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. *Antonie Van Leeuwenhoek* **81**, 435–452.
- Geesey G.G., Wigglesworth-Cooksey B. & Cooksey K.E. (2000) Influence of calcium and other cations on surface adhesion of bacteria and diatoms: A review. *Biofouling* **15**, 195–205.
- Geiger R., Aron R. & Todhunter P. (2009) *The climate near the ground*. Rowman & Littlefield.
- Gould P., Cecala K. & Drukker S. (2017) Biogeographical factors affecting the distribution of stream salamanders on the Cumberland Plateau, USA. *The Science of the Total Environment* **599–600**, 1622–1629.
- Grady A.E., Scanlon T.M. & Galloway J.N. (2007) Declines in dissolved silica concentrations in western Virginia streams (1988–2003): Gypsy moth defoliation stimulates diatoms? *Journal of Geophysical Research* **112**, G01009.
- Graham M.H. (2003) Confronting multicollinearity in ecological multiple regression. *Ecology* **84**, 2809–2815.

- Grant E., Green L. & Lowe W. (2009) Salamander occupancy in headwater stream networks. *Freshwater Biology* **54**, 1370–1378.
- Gray J.S. (2000) The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and Ecology* **250**, 23–49.
- Gray L. (1993) Response of insectivorous birds to emerging aquatic insects in riparian habitats of a tallgrass prairie stream. *American Midland Naturalist* **129**, 288–300.
- Greene B., Lowe W. & Likens G. (2008) Forest succession and prey availability influence the strength and scale of terrestrial-aquatic linkages in a headwater salamander system. *Freshwater Biology* **53**, 2234–2243.
- Griffen B.D. (2006) Detecting emergent effects of multiple predator species. *Oecologia* **148**, 702–709.
- Griffen B.D. & Byers J.E. (2006) Intraguild predation reduces redundancy of predator species in multiple predator assemblage. *Journal of Animal Ecology* **75**, 959–966.
- Grover M. & Wilbur H. (2002) Ecology of ecotones: interactions between salamanders on a complex environmental gradient. *Ecology* **83**, 2112–2123.
- Gulis V. & Suberkropp K. (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater biology* **48**, 123–134.
- Gustafson M.P. (1993) Intraguild predation among larval Plethodontid salamanders - a field experiment in artificial stream pools. *Oecologia* **96**, 271–275.
- Haack S., Burton T. & Ulrich K. (1988) Effects of whole-tree harvest on epilithic bacterial populations in headwater streams. *Microbial Ecology* **16**, 165–181.
- Hairston N. (1987) *Community ecology and salamander guilds*. Cambridge University Press.
- Hairston N. (1986) Species packing in *Desmognathus* salamanders: experimental demonstration of predation and competition. *The American Naturalist* **127**, 266–291.
- Hairston N. (1980) Species packing in the salamander genus *Desmognathus*: what are the interspecific interactions involved? *The American Naturalist* **115**, 354–366.
- Hall-Stoodley L., Costerton J. & Stoodley P. (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nature reviews microbiology* **2**, 95.
- Hall Jr R. & Meyer J. (1998) The trophic significance of bacteria in a detritus-based stream food web. *Ecology* **79**, 1995–2012.
- Hall Jr R.O., Likens G.E. & Malcom H.M. (2001) Trophic basis of invertebrate production in 2 streams at the Hubbard Brook Experimental Forest. *Journal of the North American Benthological Society* **20**, 432–447.
- Hall R.J., Likens G.E., Fiance S.B. & Hendrey G.R. (1980) Experimental acidification of a stream in the Hubbard Brook experimental forest, New Hampshire. *Ecology*, 976–989.
- Hawkins C., Murphy M., Anderson N.H. & Wilzbach M.A. (1983) Density of fish and

- salamanders in relation to riparian canopy and physical habitat in streams of the northwestern United States. *Canadian Journal of Fisheries and Aquatic Sciences* **40**, 1173–1185.
- Heino J. & Soininen J. (2007) Are higher taxa adequate surrogates for species-level assemblage patterns and species richness in stream organisms? *Biological Conservation* **137**, 78–89.
- Hester F.E. & Dendy J.S. (1962) A multiple-plate sampler for aquatic macroinvertebrates. *Transactions of the American Fisheries Society* **91**, 420–421.
- Hill B.H. & Webster J.R. (1982) Periphyton production in an Appalachian river. *Hydrobiologia* **97**, 275–280.
- Hillebrand H. (2002) Top-down versus bottom-up control of autotrophic biomass—a meta-analysis on experiments with periphyton. *Journal of the North American Benthological Society* **21**, 349–369.
- Hoagland K.D., Rosowski J.R., Gretz M.R. & Roemer S.C. (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. *Journal of Phycology* **29**, 537–566.
- Holland P. & Steyn D. (1975) Vegetational responses to latitudinal variations in slope angle and aspect. *Journal of Biogeography* **2**, 179–183.
- Holt R.D. & Polis G.A. (1997) A theoretical framework for intraguild predation. *American Naturalist* **149**, 745–764.
- Huang C.F. & Sih A. (1991) Experimental studies on direct and indirect interactions in a 3 trophic-level stream system. *Oecologia* **85**, 530–536.
- Hubálek Z. (2000) Measures of species diversity in ecology: an evaluation. *Folia Zool.* **49**, 241–260.
- Huryn A.D., Butz Huryn V.M., Arbuttle C.J. & Tsomides L. (2002) Catchment land-use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. *Freshwater Biology* **47**, 401–415.
- Jaeger R., Gabor C. & Wilbur H. (1998) An assemblage of salamanders in the southern Appalachian Mountains: competitive and predatory behavior. *Behaviour* **135**, 795–821.
- Johnson J. & Omland K.S. (2004) Model selection in ecology and evolution. *Trends in ecology & evolution* **19**, 101–108.
- Johnson N., Driscoll C., Eaton J., Likens G. & McDowell W. (1981) “Acid rain”, dissolved aluminum and chemical weathering at the Hubbard Brook Experimental Forest, New Hampshire. *Geochimica et Cosmochimica Acta* **45**, 1421–1437.
- Jolliffe I.T. & Cadima J. (2016) Principal component analysis: a review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **374**, 20150202.
- Jost L. (2007) Partitioning diversity into independent alpha and beta components. *Ecology* **88**, 2427–2439.

- Kaatz S.E., Morris J.E., Rudacille J.B. & Clayton R.D. (2010) Origin of *Chironomid* larvae in plastic-lined culture ponds: airborne or water supply? *North American Journal of Aquaculture* **72**, 107–110.
- Kaspari M. & Powers J.S. (2016) Biogeochemistry and geographical ecology: embracing all twenty-five elements required to build organisms. *The American Naturalist* **188**, S62–S73.
- Keitzer S.C. & Goforth R.R. (2013a) Salamander diversity alters stream macroinvertebrate community structure. *Freshwater Biology* **58**, 2114–2125.
- Keitzer S.C. & Goforth R.R. (2013b) Spatial and seasonal variation in the ecological significance of nutrient recycling by larval salamanders in Appalachian headwater streams. *Freshwater Science* **32**, 1136–1147.
- King R.S. & Richardson C.J. (2003) Integrating bioassessment and ecological risk assessment: an approach to developing numerical water-quality criteria. *Environmental Management* **31**, 795–809.
- Lamberti G.A. (1996) The role of periphyton in benthic food webs. In: *Algal Ecology: freshwater benthic ecosystem*, 1st edn. (Eds S. Jan, L. Max & R. Lowe), pp. 533–572. Academic Press, Inc, San Diego, California.
- Lawrence J.R., Scharf B., Packroff G. & Neu T.R. (2002) Microscale evaluation of the effects of grazing by invertebrates with contrasting feeding modes on river biofilm architecture and composition. *Microbial Ecology* **44**, 199–207.
- Lear G., Niyogi D., Harding J., Dong Y. & Lewis G. (2009) Biofilm bacterial community structure in streams affected by acid mine drainage. *Applied and environmental microbiology* **75**, 3455–3460.
- Ledger M. & Hildrew A. (2005) The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution* **137**, 103–118.
- Ledger M.E. & Hildrew A.G. (2001) Growth of an acid-tolerant stonefly on epilithic biofilms from streams of contrasting pH. *Freshwater Biology* **46**, 1457–1470.
- Lemmon P. (1956) A spherical densiometer for estimating forest overstory density. *Forest Science* **2**, 314–320.
- Leopold L., Wolman M. & Miller J. (2012) *Fluvial processes in geomorphology*. Courier Corporation.
- Likens G. (2013) *Biogeochemistry of a Forested Ecosystem*, 3rd edn. New York.
- Likens G. & Buso D. (2006) Variation in streamwater chemistry throughout the Hubbard Brook Valley. *Biogeochemistry* **78**, 1–30.
- Likens G., Driscoll C. & Buso D. (1996) Long-term effects of acid rain: response and recovery of a forest ecosystem. *Science* **272**, 244–246.
- Lowe W. (2005) Factors affecting stage-specific distribution in the stream salamander *Gyrinophilus porphyriticus*. *Herpetologica* **61**, 135–144.

- Lowe W. & Bolger D. (2002) Local and landscape-scale predictors of salamander abundance in New Hampshire headwater streams. *Conservation Biology* **16**, 183–193.
- Lowe W.H. & Likens G.E. (2005) Moving headwater streams to the head of the class. *BioScience* **55**, 196–197.
- Lowe W.H., McPeck M.A., Likens G.E. & Cosentino B.J. (2012) Decoupling of genetic and phenotypic divergence in a headwater landscape. *Molecular Ecology* **21**, 2399–2409.
- Lowe W.H., Nislow K.H. & Likens G.E. (2005) Forest structure and stream salamander diets: Implications for terrestrial-aquatic connectivity. *Verhandlungen des Internationalen Verein Limnologie* **29**, 279–286.
- MacCrimmon H. & Campbell J. (1969) World distribution of brook trout, *Salvelinus fontinalis*. *Journal of the Fisheries Board of Canada* **26**, 1699–1725.
- MacCulloch R. & Bider J. (1975) Phenology, migrations, circadian rhythm and the effect of precipitation on the activity of *Eurycea b. bislineata* in Quebec. *Herpetologica* **31**, 433–439.
- Mackay R.J. & Kalff J. (1969) Seasonal variation in standing crop and species diversity of insect communities in a small Quebec stream. *Ecology* **50**, 101–109.
- Macneale K.H., Peckarsky B.L. & Likens G.E. (2005) Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. *Freshwater Biology* **50**, 1117–1130.
- Malison R.L., Benjamin J.R. & Baxter C. V. (2010) Measuring adult insect emergence from streams: the influence of trap placement and a comparison with benthic sampling. *Journal of the North American Benthological Society* **29**, 647–656.
- Marczak L.B. & Richardson J.S. (2007) Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. *Journal of Animal Ecology* **76**, 687–694.
- Martínez A., Kominoski J. & Larrañaga A. (2017) Leaf-litter leachate concentration promotes heterotrophy in freshwater biofilms: understanding consequences of water scarcity. *Science of the Total Environment* **599**, 1677–1684.
- Mayer M. & Likens G. (1987) The importance of algae in a shaded headwater stream as food for an abundant caddisfly (Trichoptera). *Journal of the North American Benthological Society* **6**, 262–269.
- McNeely C., Clinton S. & Erbe J. (2006) Landscape variation in C sources of scraping primary consumers in streams. *Journal of the North American Benthological Society* **25**, 787–799.
- Merrit R. & Cummins K. (1996) *An Introduction to the Aquatic Insects of North America*. 3 ed. Kendall Hunt.
- Merten E., Snobl Z. & Wellnitz T. (2014) Microhabitat influences on stream insect emergence. *Aquatic sciences* **76**, 165–172.
- Milanovich J., Maerz J. & Rosemond A. (2015) Stoichiometry and estimates of nutrient standing stocks of larval salamanders in Appalachian headwater streams. *Freshwater biology* **60**, 1340–1353.

- Mondelli M., Davenport J.M. & Lowe W.H. (2014) *Gyrinophilus porphytricus* diet. *Herpetological Review* **45**, 109–110.
- Montgomery D. & Buffington J. (1998) Channel processes, classification, and response. In: *River Ecology and Management: Lessons from the Pacific Coastal Ecoregion*. (Eds R. Naiman & R. Bilby), pp. 13–42. Springer-Verlag, New York.
- Mulholland P.J., Steinman A.D., Marzolf E.R., Hart D.R. & DeAngelis D.L. (1994) Effect of periphyton biomass on hydraulic characteristics and nutrient cycling in streams. *Oecologia* **98**, 40–47.
- Murphy M. & Hall J. (1981) Vaired effects of clear-cut logging on predators and their habitat in small streams of the Cascade Mountains, Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 137–145.
- Nakano S. & Murakami M. (2001) Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences* **98**, 166–170.
- Noel D.S., Martin C.W. & Federer C.A. (1986) Effects of forest clearcutting in New England on stream macroinvertebrates and periphyton. *Environmental Management* **10**, 661–670.
- Nowakowski A.J. & Maerz J.C. (2009) Estimation of larval stream salamander densities in three proximate streams in the Georgia Piedmont. *Journal of Herpetology* **43**, 503–509.
- Pace M., Cole J., Carpenter S. & Kitchell J. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in ecology & evolution* **14**, 483–488.
- Peckarsky B.L. (1991) Mechanisms of intra-and interspecific interference between larval stoneflies. *Oecologia* **85**, 521–529.
- Peterman W.E., Crawford J.A. & Semlitsch R.D. (2008) Productivity and significance of headwater streams: population structure and biomass of the black-bellied salamander (*Desmognathus quadramaculatus*). *Freshwater Biology* **53**, 347–357.
- Peterson B., Wollheim W., Mulholland P., Webster J., Meyer J., Tank J., *et al.* (2001) Control of nitrogen export from watersheds by headwater streams. *Science* **292**, 86–90.
- Petranka J.W. (1998) *Salamanders of the US and Canada*. Smithsonian Institution Press, Washington, DC.
- Plotnikoff R. & Wiseman C. (2001) Benthic macroinvertebrate biological monitoring protocols for rivers and streams.
- Polis G.A., Myers C.A. & Holt R.D. (1989) The ecology and evolution of intraguild predation - potential competitors that eat each other. *Annual Review of Ecology and Systematics* **20**, 297–330.
- Power M. (1990) Effects of fish in river food webs. *Science* **250**, 811–814.
- Power M., Matthews W. & Stewart A. (1985) Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. *Ecology* **66**, 1448–1456.

- Progar R. & Moldenke A. (2002) Insect production from temporary and perennially flowing headwater streams in western Oregon. *Journal of Freshwater Ecology* **17**, 391–407.
- R Core Team (2017) R: a language and environment for statistical computing.
- Reice S. & Edwards R. (1986) The effect of vertebrate predation on lotic macroinvertebrate communities in Quebec, Canada. *Canadian Journal of Zoology* **64**, 1930–1936.
- Reinhardt T., Brauns M., Steinfartz S. & Weitere M. (2017) Effects of salamander larvae on food webs in highly subsidised ephemeral ponds. *Hydrobiologia* **799**, 37–48.
- Resetarits Jr W.J. (1995) Competitive asymmetry and coexistence in size-structured populations of brook trout and spring salamanders. *Oikos*, 188–198.
- Resetarits Jr W.J. (1997) Differences in an ensemble of streamside salamanders (*Plethodontidae*) above and below a barrier to brook trout. *Amphibia-Reptilia* **18**, 15–25.
- Resetarits Jr W.J. (1991) Ecological interactions among predators in experimental stream communities. *Ecology* **72**, 1782–1793.
- Richardson J. & Danehy R. (2007) A synthesis of the ecology of headwater streams and their riparian zones in temperate forests. *Forest Science* **53**, 131–147.
- Roche K.R., Drummond J.D., Boano F., Packman A.I., Battin T.J. & Hunter W.R. (2017) Benthic biofilm controls on fine particle dynamics in streams. *Water Resources Research* **53**, 222–236.
- Romani A. & Sabater S. (1999) Effect of primary producers on the heterotrophic metabolism of a stream biofilm. *Freshwater Biology* **41**, 729–736.
- Romani A.M., Guasch H., Munoz I., Ruana J., Vilalta E., Schwartz T., *et al.* (2004) Biofilm structure and function and possible implications for riverine DOC dynamics. *Microbial Ecology* **47**, 316–328.
- Rudolf V. (2006) The influence of size-specific indirect interactions in predator-prey systems. *Ecology* **87**, 362–371.
- Ruetz C., Newman R. & Vondracek B. (2002) Top-down control in a detritus-based food web: fish, shredders, and leaf breakdown. *Oecologia* **132**, 307–315.
- Sabo J., Bastow J. & Power M. (2002) Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. *Journal of the North American Benthological Society* **21**, 336–343.
- Sabo J. & Power M. (2002) River–watershed exchange: effects of riverine subsidies on riparian lizards and their terrestrial prey. *Ecology* **83**, 1860–1869.
- Schiller D. V, Martí E., Riera J.L. & Sabater F. (2007) Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Freshwater Biology* **52**, 891–906.
- Schmitz O.J. (2007) Predator diversity and trophic interactions. *Ecology* **88**, 2415–2426.
- Schmitz O.J. & Sokol-Hessner L. (2002) Linearity in the aggregate effects of multiple predators

- in a food web. *Ecology Letters* **5**, 168–172.
- Siddon C.E. & Witman J.D. (2004) Behavioral indirect interactions: multiple predator effects and prey switching in the rocky subtidal. *Ecology* **85**, 2938–2945.
- Sih A., Englund G. & Wooster D. (1998) Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution* **13**, 350–355.
- Soluk D. & Collins N. (1988) Synergistic interactions between fish and stoneflies: facilitation and interference among stream predators. *Oikos* **52**, 94–100.
- Van Son T.C. & Thiel M. (2006) Multiple predator effects in an intertidal food web. *Journal of Animal Ecology* **75**, 25–32.
- Song B. & Leff L. (2006) Influence of magnesium ions on biofilm formation by *Pseudomonas fluorescens*. *Microbiological Research* **161**, 355–361.
- Steinmetz J., Soluk D.A. & Kohler S.L. (2008) Facilitation between herons and smallmouth bass foraging on common prey. *Environmental Biology of Fishes* **81**, 51–61.
- Swift Jr L.W. & Messer J.B. (1971) Forest cuttings raise temperatures of small streams in the southern Appalachians. *Journal of soil and water conservation* **26**, 111–116.
- Thelaus J., Haecky P., Forsman M. & Andersson A. (2008) Predation pressure on bacteria increases along aquatic productivity gradients. *Aquatic Microbial Ecology* **52**, 45–55.
- Townsend C.R. (2003) Individual, population, community, and ecosystem consequences of a fish invader in New Zealand streams. *Conservation Biology* **17**, 38–47.
- Townsend C.R., Scarsbrook M.R. & Dolédec S. (1997) The intermediate disturbance hypothesis, refugia, and biodiversity in streams. *Limnology and Oceanography* **42**, 938–949.
- Ulrich K.E., Burton T.M. & Oemke M.P. (1993) Effects of whole-tree harvest on epilithic algal communities in headwater streams. *Journal of Freshwater Ecology* **8**, 83–92.
- Vance-Chalcraft H. & Soluk D. (2005) Multiple predator effects result in risk reduction for prey across multiple prey densities. *Oecologia* **144**, 472–480.
- Vance-Chalcraft H.D., Soluk D.A. & Ozburn N. (2004) Is prey predation risk influenced more by increasing predator density or predator species richness in stream enclosures? *Oecologia* **139**, 117–122.
- Vinebrooke R.D. (1996) Abiotic and biotic regulation of periphyton in recovering acidified lakes. *Journal of the North American Benthological Society* **15**, 318–331.
- Voshell R. (2002) *A guide to common freshwater invertebrates of North America*. (6 ed.). The University of Chicago Press Blacksburg.
- Vroom J., Grauw K., Gerritsen H., Bradshaw D., Marsh P., Watson G., *et al.* (1999) Depth penetration and detection of pH gradients in biofilms by two-photon excitation microscopy. *Applied and environmental microbiology* **65**, 3502–3511.
- Wallace J.B., Eggert S.L., Meyer J.L. & Webster J.R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**, 102–104.

- Warren D.R., Likens G.E., Buso D.C. & Kraft C.E. (2008) Status and distribution of fish in an acid-impacted watershed of the northeastern United States (Hubbard Brook, NH). *Northeastern Naturalist* **15**, 375–390.
- Webster J.R., Mulholland P.J., Tank J.L., Valett H.M., Dodds W.K., Peterson B.J., *et al.* (2003) Factors affecting ammonium uptake in streams - an inter-biome perspective. *Freshwater Biology* **48**, 1329–1352.
- Wesner J. (2010) Aquatic predation alters a terrestrial prey subsidy. *Ecology* **91**, 1435–1444.
- Wesner J. (2016) Contrasting effects of fish predation on benthic versus emerging prey: a meta-analysis. *Oecologia* **180**, 1205–1211.
- Wesner J. (2013) Fish predation alters benthic, but not emerging, insects across whole pools of an intermittent stream. **32**, 438–449.
- Wilhelm L., Singer G., Fasching C., Battin T. & Besemer K. (2013) Microbial biodiversity in glacier-fed streams. *The ISME journal* **7**, 1651.
- Wilkerson E., Hagan J.M. & Whitman A.A. (2006) The effectiveness of different buffer widths for protecting water quality and macroinvertebrate and periphyton assemblages of headwater streams in Maine, USA. *Forest Science* **52**, 221–231.
- Williamson T.J., Cross W.F., Benstead J.P., Gíslason G.M., Hood J.M., Huryn A.D., *et al.* (2016) Warming alters coupled carbon and nutrient cycles in experimental streams. *Global Change Biology* **22**, 2152–2164.
- Winkelmann C., Schneider J. & Mewes D. (2014) Top-down and bottom-up control of periphyton by benthivorous fish and light supply in two streams. *Freshwater* **59**, 803–818.
- Wipfli M. & Gregovich D. (2002) Export of invertebrates and detritus from fishless headwater streams in southeastern Alaska: implications for downstream salmonid production. *Freshwater Biology* **47**, 957–969.
- Wolman M. (1954) A method of sampling coarse river-bed material. *EOS, Transactions American Geophysical Union*.
- Woodward G., Papanoniu G. & Edwards F. (2008) Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. *Oikos* **117**, 683–92.
- Yan E., Wang X. & Huang J. (2005) Concept and classification of coarse woody debris in forest ecosystems. *Acta Ecologica Sinica*.
- Ylla I., Borrego C., Román A. & Sabater S. (2009) Availability of glucose and light modulates the structure and function of a microbial biofilm. *FEMS microbiology ecology* **69**, 27–42.
- Zimmer K., Hanson M. & Butler M. (2001) Effects of fathead minnow colonization and removal on a prairie wetland ecosystem. *Ecosystems* **4**, 346–357.

Table 1 Range, mean, and stand errors for variables used in principle component analysis (PCA) to assess the relative importance of bottom-up versus top-down controls on biofilm in 20 headwater streams of the Hubbard Brook Experimental Forest, NH, USA. Three separate PCA analyses were run one each that summarized nutrients, substrate features, and the stream mesohabitat.

Variable	PCA	Range	Mean	SE
DOC (mgL ⁻¹) †	Nutrients	1.28-7.54	2.94	0.35
DIC (mgL ⁻¹) †	Nutrients	30-222	64.7	9.53
Ca ²⁺ (mgL ⁻¹)	Nutrients	0.5-1.42	1	0.05
NO ₃ ⁻ (mgL ⁻¹) †	Nutrients	0.01-0.23	0.04	0.01
PO ₄ ³⁻ (mgL ⁻¹)	Nutrients	0.004- 0.0005	0.0012	0.0002
Mg ²⁺ (mgL ⁻¹)	Nutrients	0.13-0.61	0.34	0.02
SiO ₂ (mgL ⁻¹)	Nutrients	3.2-7.8	5.89	0.029
Per. Sand*	Substrate	0-48	5.5	2.36
Per. Gravel	Substrate	2-26	11.05	1.46
Per. Cobble	Substrate	2-32	17.85	1.56
Per. Boulder	Substrate	11-88	54.05	3.87
Per. Bedrock*	Substrate	0-51	11.5	2.74
Per. Riffle	Mesohabitat	0-65	30.25	4.34
Per. Run	Mesohabitat	10-85	38.25	4.91
Per. Pool	Mesohabitat	5-60	25	3.26
Per. Cascade	Mesohabitat	0-20	6.5	1.26

*variable was log₁₀ transformed to meet the assumption of normality.

†inverse was taken to meet the assumption of normality.

DOC:dissolved organic carbon.

DIC: dissolved inorganic carbon.

Table 2 Range, mean and standard error of variables used in stepwise multiple linear regression to assess the relative importance of bottom-up versus top-down controls on biofilm in 20 headwater streams of the Hubbard Brook Experimental Forest, NH, USA.

Variable	Range	Mean	SE
Biofilm (gm ⁻²)	0.88-2.82	1.9	0.13
Benthic Macroinvertebrates (gm ⁻²)*	0.04-0.44	0.18	0.03
Date, Julian	183-254	215.1	5.44
pH	4.66-6.68	5.67	0.11
Conductivity (uScm ⁻¹)	12.9-20.1	15.75	0.53
Canopy Cover	88.77-96.77	92.64	0.5
Elevation (m)	402-703	581.4	14.9
Bankfull Width (m)	0.8-6.7	3.62	0.3
Per. Total Wood*	2.5-35	13.38	2.32
Nutrient PC 1	-3.45 - 3.54	0.00	0.39
Substrate PC 1	-2.89 - 3.43	0.00	0.35
Mesohabitat PC 1	-2.57 - 2.33	0.00	0.31

*variable was log₁₀ transformed to meet the assumption of normality.

Table 3 Summary of multiple regression models for biofilm biomass in 20 headwater streams of the Hubbard Brook Experimental Forest, NH, USA selected using stepwise regression based on AIC_c scores to determine model rank. Δ_i are the AIC_c differences and w_i are the AIC_c weights. Due to a small AIC_c difference between the top two models, a likelihood ratio test was used to determine the most parsimonious model. The top model as determined by the likelihood ratio test is designated by an asterisk (*).

Rank	Model	AIC _c	Δ_i	Likelihood [L(g _i x)]	w_i	P- value	Adjusted R ²
1*	pH, Nutr, Canopy, Aspect	36.59	0	1	0.607	0.009	0.46
2	pH, Nutr, Canopy, Aspect, Meso	37.82	1.23	0.54	0.328	0.008	0.51
3	pH, Nutr, Canopy, Aspect, Meso, Cond	41.21	4.62	0.10	0.060	0.010	0.54
4	pH, Nutr, Canopy, Aspect, Meso, Cond, Elevation	46.36	9.77	0.01	0.005	0.010	0.54
5	pH, Nutr, Canopy, Aspect, Meso, Cond, Elevation, Bankfull	50.53	13.9	0.00	0.001	0.010	0.59

Nutr: PCA 1 nutrients

Canopy: canopy cover

Meso: PCA 1 of mesohabitat

Cond: conductivity

Bankfull: bankfull width.

Table 4 Biofilm biomass model summary for final model selected by stepwise AIC_c and the likelihood ratio test. β are the coefficients of each variable. Study was conducted in 20 headwater streams of the Hubbard Brook Experimental Forest NH, USA.

Biofilm model summary				
Variable	β	SE	<i>t</i>	<i>P</i>
(Intercept)	26.19	5.70	5.60	0.000
pH	-0.82	0.28	-2.91	0.011
Nutr	0.14	0.76	1.89	0.078
Canopy	-0.21	0.05	-4.07	0.001
Aspect (South)	0.49	0.22	2.21	0.044

Nutr: Nutrient PC 1

Canopy: canopy cover.

Table 5 Summary of multiple regression models for benthic macroinvertebrates selected using stepwise regression based on AIC_c scores. Δ_i are the AIC_c differences and w_i are the AIC_c weights. Due to a small AIC_c difference between the top two models, a likelihood ratio test was used to determine the best supported model. The top model as determined by the likelihood ratio test is designated by an asterisk (*). Study was conducted in 20 headwater streams of the Hubbard Brook Experimental Forest, NH, USA.

Rank	Model	AIC _c	Δ_i	Likelihood [L(g _i x)]	w_i	P-value	Adjusted R ²
1	Sub, Date*	7.22	0	1	0.501	0.008	0.36
2	Sub, Date, Canopy	7.67	0.45	0.80	0.400	0.008	0.42
3	Sub, Date, Canopy, Cond	10.65	3.43	0.18	0.090	0.014	0.42
4	Sub, Date, Canopy, Cond, Aspect	15.31	8.09	0.02	0.009	0.033	0.38
5	Sub, Date, Canopy, Cond, Aspect, Bankfull	20.5	13.28	0.00	0.001	0.060	0.36

Sub: Substrate PC 1

Canopy: canopy cover

Cond: conductivity

Bankfull: bankfull width.

Table 6 Benthic macroinvertebrate model summary for final model selected by stepwise AIC_c and the likelihood ratio test. Study was conducted in 20 headwater streams of the Hubbard Brook experimental Forest, NH, USA.

Benthic Macroinvertebrate model summary				
Variable	β	SE	t	P
(Intercept)	0.40	0.51	0.77	0.450
Sub	0.07	0.04	1.84	0.083
Date	-0.01	0.00	-2.41	0.027

Sub is Substrate PC 1.

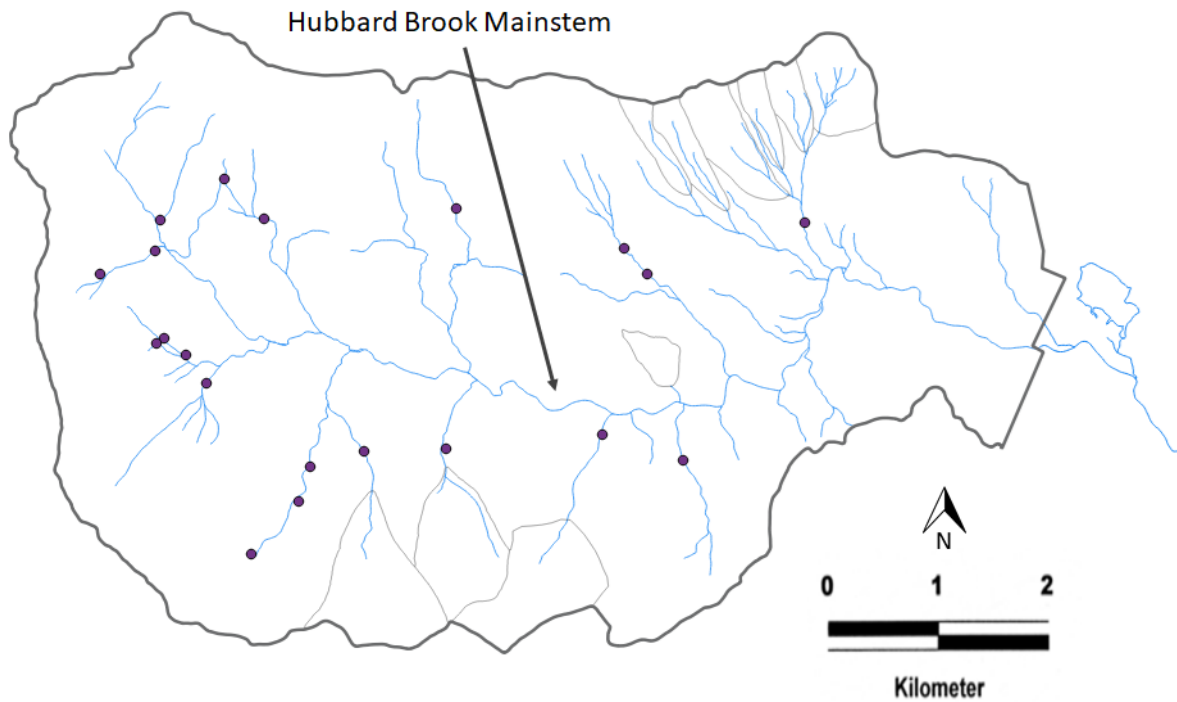


Fig. 1 Map of sites at which biofilm was collected at the Hubbard Brook Experimental Forest, NH, USA (n = 20). Sites were visited between 1 July 2016 and 10 September 2016. Grey outlines denote experimental watersheds.

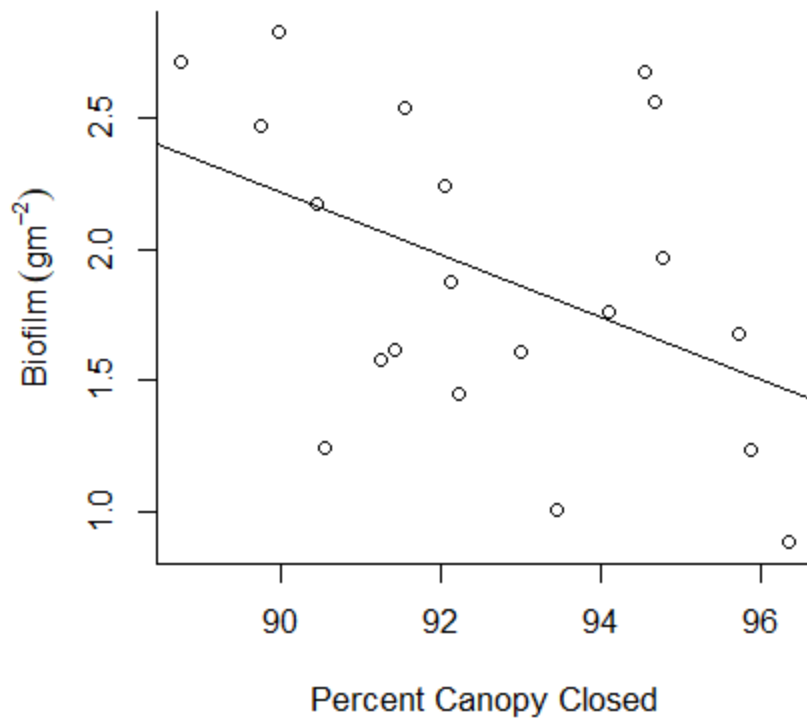


Fig. 2 Biofilm (grams AFDM per meter squared) versus percent canopy closed for all sites (n = 20). A linear regression found this relationship to be slightly significant ($P = 0.052$) and explain 15% of the variation in biofilm biomass between sites at the Hubbard Brook Experimental Forest, NH USA.