

UNDERSTANDING THE EFFECTS OF CONSUMERS AND LIGHT ON STREAM
FOOD WEBS USING STABLE ISOTOPE TECHNIQUES

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UNDERSTANDING THE EFFECTS OF CONSUMERS AND LIGHT ON STREAM FOOD WEBS USING STABLE ISOTOPE TECHNIQUES

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In headwater streams that are heavily shaded by surrounding forests, primary production is often low and inputs of particulate and dissolved organic carbon from terrestrial environments are important resources in food webs. Stable isotope methods are useful for quantifying food web fluxes of different carbon sources but data are frequently difficult to interpret. Here, I developed novel stable isotope techniques to study the role of terrestrial energy subsidies in stream food webs in Trinidad and Tobago and the Adirondack region of New York. Specifically, I sought to determine the importance of carbon sources from terrestrial environments versus in-stream primary production in streams with varying light availability and fish communities. I compared natural canopy streams to streams with experimentally thinned canopies using a novel carbon and nitrogen dual isotope tracer technique. Comparing data from ^{13}C -acetate tracers, which are assimilated only by heterotrophic bacteria, and ^{15}N -ammonium tracers, which are assimilated by both heterotrophs and autotrophs, allowed me to evaluate the relative importance of terrestrial carbon inputs and heterotrophic pathways. I also compared food webs in Trinidad and Adirondack streams along a natural gradient of canopy cover using stable isotopes of hydrogen, which indicated how much terrestrial organic matter was assimilated by consumers.

In the Adirondacks, terrestrial carbon subsidies and heterotrophic bacteria were important resources for consumers, but the amount of bacterial carbon assimilated by

invertebrates declined when light availability and primary production were experimentally increased. Fish introductions and light availability both influenced food webs in Trinidad streams. There were increased fluxes of carbon and nitrogen to consumers in streams with high light. Effects of fish introduction varied by consumer taxon, with strong positive effects of fish on filter-feeding invertebrates, and weaker positive effects of fish on grazer invertebrates. In comparative studies across light gradients, most consumer taxa were flexible in the amount of terrestrial carbon they assimilated, with higher reliance on terrestrial subsidies in streams with high canopy cover than in larger streams with less canopy cover. Overall, these results suggest that the light environment can have a strong influence on the role of terrestrial subsidies in streams.

BIOGRAPHICAL SKETCH

Sarah Collins was born on March 7, 1985 in Bellingham, Washington to Michael and Wanda Collins. She grew up in Bellingham, where she learned to love the natural world, especially aquatic habitats. Sarah graduated from Bellingham High School in 2003 and enrolled in Lewis & Clark College in Portland, Oregon to pursue a degree in Biochemistry and Molecular Biology. Her interest in freshwater ecology stemmed from two National Science Foundation Research Experience for Undergraduates internships: one during Summer 2005 at the Flathead Lake Biological Station (University of Montana), and the second during Summer 2006 at Lake Tanganyika, East Africa, through the Nyanza Project. She graduated from Lewis & Clark in May 2007 with a double major in Biology and Biochemistry and Molecular Biology. In August 2007, Sarah enrolled in a PhD program at Cornell University studying Ecology and Evolutionary Biology. On August 18, 2012, she married William Warren Fetzer at the Cornell Biological Field Station.

To my family

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PREFACE

Energy and material subsidies from adjacent environments can comprise substantial food web pathways in unproductive ecosystems (Polis *et al.* 2004). Primary production in many forested headwater streams is strongly limited by light availability, and terrestrial subsidies in the forms of particulate and dissolved organic carbon can be important food sources for organisms at higher trophic levels (reviewed by Doi 2009, Tank *et al.* 2010). While a large body of research has demonstrated that terrestrial subsidies can be key food sources for invertebrates and fishes in streams (reviewed by Webster *et al.* 2009, Tank *et al.* 2010), it is not well understood whether changes in the light environment have a strong influence on the importance of subsidies in food webs. Additionally, little is known about whether energy and material fluxes from heterotrophic bacteria to consumers are important in streams. While bacterial energy is thought to dissipate through trophic transfers in lake food webs (Pace *et al.* 1990, Hairston & Hairston 1993), a small number of studies suggest that it may be important in streams (Meyer 1994, Hall & Meyer 1998).

Methodological problems have been a major hurdle to understanding the importance of bacterial carbon and comparing the roles of terrestrial versus aquatic organic matter in stream food webs. Stable isotopes have emerged as useful tools (reviewed by Fry 2006), but there is often overlap in the natural isotopic signature of different resource compartments, making it difficult to determine which resource pools are important for production at higher trophic levels (Fry 2013). In this dissertation, I developed and applied new isotopic techniques to investigate the role of terrestrial

subsidies and heterotrophic bacteria as resource pools in temperate and tropical streams. I conducted experimental stable isotope tracer studies in two regions: 1) the Adirondack Mountains in New York State, where I examined the effects of light availability on food webs by reducing the riparian canopy around a stream, and 2) in Trinidad and Tobago, where I conducted food web studies in streams where a large group of collaborators had reduced riparian canopies and introduced fish populations. In both Trinidad and the Adirondacks, I also conducted comparative studies in which I used natural abundance hydrogen stable isotopes to compare the role of terrestrial and aquatic resources in streams sites along a natural gradient of canopy cover.

In Chapter 1, I review sources of uncertainty and variation associated with using isotopic analysis to study stream food webs. The review is focused on natural abundance measurements of three elements commonly used in food web studies: carbon (C), nitrogen (N) and hydrogen (H). I discuss new modeling methods and emerging empirical techniques (fatty acid analysis, compound specific stable isotope analysis, and radiocarbon isotope analysis) that may be useful to address problems associated with natural abundance stable isotope measurements.

In Chapter 2, I examine the role of terrestrial subsidies along a stream size and canopy cover gradient. I used natural abundance hydrogen stable isotopes ($\delta^2\text{H}$) as a proxy for allochthonous energy use, which is a technique that has only been widely used in freshwater food web studies over the past few years. I found that some functional groups of invertebrates and fishes are flexible in the type of energy they assimilate, while others are fixed. Specifically, grazers, collectors, and predators assimilated less energy from terrestrial subsidies in larger streams with more open

canopies, while shredders assimilated predominantly terrestrial energy in all sites and did not vary with canopy cover.

In Chapter 3, I developed a dual C and N stable isotope tracer technique to examine the role of bacteria in Adirondack stream food webs. I simultaneously enriched streams with both a ^{13}C -acetate tracer that can be assimilated only by heterotrophic bacteria and a ^{15}N -ammonium tracer that can be assimilated by both heterotrophic and autotrophs. Comparing the two tracers allowed me evaluate the role of bacterial carbon in stream food webs. I compared the percent bacterial carbon use by invertebrate taxa in a stream with natural canopy to a stream where canopy cover had been experimentally reduced, and found that increased light led to a decrease in the amount of bacterial energy assimilated by all consumer taxa.

In Chapter 4, I applied the same dual isotope tracer method in streams in Trinidad to examine the effects of experimental light manipulation and fish introduction. Results indicate that both light availability and fish introduction can have a strong influence on fluxes of heterotrophic and autotrophic resource pools to consumers. Some effects on food webs are straightforward (*e.g.*, increased light leads to increased fluxes to grazer invertebrates) while others are indirect (*e.g.*, fish presence leads to increased fluxes to filter feeding invertebrates, presumably because of increased sediment suspension due to fish feeding activity). Further, relatively few detailed food web studies have been conducted in tropical stream ecosystems. The results of this experiment support the idea that terrestrial subsidies are important food web pathways in headwater streams in tropical regions as well as in temperate ones.

Combined, these results build on existing knowledge of how environmental

context influences food web fluxes in stream ecosystems. While terrestrial subsidies are often important in headwater streams, changes in the light environment can have a strong influence on the relative magnitude of fluxes from different resource pools to consumers. Bacterial carbon can be a substantial energy source for invertebrate consumers, but was less important when light availability and primary production were increased. Results from Chapter 4 also demonstrate that changes in the light environment and fish community can both influence food web architecture, and that the effect sizes of light and fish on food web fluxes were similar in magnitude. The results of this work also suggest that human alterations to streams and watersheds, such as land-use change or species introductions, may have a strong influence on stream food webs.

REFERENCES

- Doi, H. 2009. Spatial patterns of autochthonous and allochthonous resources in aquatic food webs. *Population Ecology* 51: 57-64.
- Fry, B. 2006. *Stable Isotope Ecology*. Springer, New York, NY, USA.
- Fry, B. 2013. Alternative approaches for solving undetermined isotope mixing problems. *Marine Ecology Progress Series* 427: 1-13.
- Hairston, N.G., Jr. and N.G. Hairston, Sr. 1993. Cause-effect relationships in energy flow, trophic structure and interspecific interactions. *American Naturalist* 142(3): 379-411.
- Hall, R.O. and J.L. Meyer. 1998. The trophic significance of bacteria in a detritus-based stream food web. *Ecology* 79: 1995-2012.
- Meyer, J.L. 1994. The microbial loop in flowing waters. *Microbial Ecology* 28: 195-199.
- Nakano, S. and M. Murakami. 2001. Dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences* 98(1): 166-170.
- Pace, M.L., G.B. McManus and S.E.G. Findlay. 1990. Planktonic community structure determines the fate of bacterial production in a temperate lake. *Limnology & Oceanography* 35(4): 795-808.
- Polis, G.A, M.E. Power and G.R. Huxel. 2004. *Food webs at the landscape level*. Chicago, IL: University of Chicago Press.
- Tank, J.L., E.J. Rosi-Marshall, N.A. Griffiths, S.A. Entekin and M.L. Stephen. 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society* 29: 118-146.
- Webster, J.R., E.F. Benfield, T.P. Ehrman, M.A. Schaffer, J.L. Tank, J.J. Hutchens, and D.J. D'Angelo. 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology* 41: 687-705.

CHAPTER 1

VARIABILITY AND UNCERTAINTY IN STABLE ISOTOPE STUDIES OF STREAM FOOD WEBS: CHALLENGES AND NEW DIRECTIONS

Abstract

Stable isotopes are used ubiquitously in studies of stream food webs to determine which sources of organic matter fuel higher trophic levels. Variation in the stable isotope signature among different types of food sources makes it possible to use stable isotope data to describe food web pathways, yet variation in the stable isotope signature within a single food web compartment can be problematic for interpreting data. Many studies have noted high spatial and temporal variation in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^2\text{H}$ within single food web compartments. High variation within compartments relative to between compartments is especially common in $\delta^{13}\text{C}$ measurements, which often leads to inability to resolve food web pathways or distinguish between autochthonous and allochthonous organic matter sources. Here, I summarize drivers of stable isotope variation in stream food web studies and discuss how they affect the interpretation of food web data. I then outline several modeling approaches and newly developed empirical methods to address the problems associated with variation and uncertainty. While stable isotope techniques are powerful tools for characterizing freshwater food web structure, quantifying spatial and temporal variation within food web compartments and accounting for them in models is necessary to correctly describe food webs.

Introduction

Many key studies in ecosystem ecology have aimed to quantify how energy and materials move through food webs and to determine which types of organic matter consumers assimilate. In freshwater systems, a great deal of research has focused on the relative importance of autochthonous sources of carbon, which are fixed through *in situ* primary production, versus allochthonous sources of carbon, which are fixed terrestrially and enter the system as particulate or dissolved organic carbon. Many empirical studies in freshwater ecosystems around the globe have quantified the relative importance of allochthonous and autochthonous organic matter in small streams (reviewed by Doi 2009, Tank *et al.* 2010), large rivers (reviewed by Roach 2013) and lakes (*e.g.*, Cole *et al.* 2011, Wilkinson *et al.* 2013, reviewed by Bartels *et al.* 2012).

Stable isotope ratios are a commonly used tool for evaluating food web connections and identifying which organic matter sources fuel higher trophic levels in food webs. Early studies and reviews noted that stable isotope ratios of C and N ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were useful tools in food web studies (Peterson & Fry 1987). $\delta^{13}\text{C}$ is a good indicator of food source because it often varies among types of organic matter while consumers are not enriched in ^{13}C relative to their diets. $\delta^{15}\text{N}$ is a good indicator of trophic position because fractionation causes predictable enrichment of ^{15}N at higher trophic levels (*e.g.*, DeNiro & Epstein 1978, DeNiro & Epstein 1981, Minagawa & Wada 1984, Peterson & Fry 1987). Measurements of natural abundance $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have been refined and used extensively over the past decade (Post 2002, Fry 2006, Middleburg 2014). More recently, $\delta^2\text{H}$ has been applied in a limited number of

studies and is effective for distinguishing between allochthonous and autochthonous organic matter (Doucett *et al.* 2007). There are many advantages to using stable isotope analysis for food web studies: 1) isotopic techniques can be used in organisms ranging from microbes to large animals (reviewed by Middleburg 2014), 2) there are multiple elemental tracers available to suit different research questions that provide detailed information when they are used in concert (Soto *et al.* 2013), 3) the use of natural abundance $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have become ubiquitous in studies of food web structure, and there are a wealth of data in the literature for comparison across systems (*e.g.*, Vander Zanden & Fetzer 2007), and 4) stable isotope analyses are less time-intensive than gut content analyses and they integrate the diet of an organism over time.

Mixing models are commonly used to resolve food web connections with stable isotope data. A very simple mixing model might consist of two food sources, or end members, that have different $\delta^{13}\text{C}$ signatures, and a consumer that eats a proportion of each organic matter source and has an intermediate $\delta^{13}\text{C}$ signature between the two sources. In real-world food webs with contributions from many sources, data from multiple elemental tracers and complex modeling approaches can make it possible to solve a mixing model, yet it remains somewhat difficult to acquire sufficient information to resolve food web pathways in many ecosystems (reviewed by Fry 2013). Although variation in the stable isotope signature of different food pools is needed to solve a mixing model, variation within a single food pool can lead to problems. For example, variability in the stable isotope signature of a single food web compartment over space or time can lead to overlap in the isotopic signatures of food

resources. In freshwater systems, presumed food pools are often a mixture of allochthonous and autochthonous elements (*e.g.*, biofilms can be composed of algae, bacteria, fungi and detritus). Mixed composition of biofilms can lead to difficulty in characterizing allochthonous and autochthonous end members even though consumer isotope signatures can still be compared to bulk biofilm isotope signatures.

Here, I review sources of variation that may cause problems in interpreting stable isotope food web data in stream ecosystems. I detail sources of variation that affect the usefulness of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in quantifying food web links. These have been widely-documented in both temperate and tropical streams. I also discuss sources of variation in $\delta^2\text{H}$, which have been documented on a less extensive scale because $\delta^2\text{H}$ has only become popular for freshwater food web studies during the past decade. The spatial scale of variation differs among elements, with some drivers of variation acting on a local-scale (*e.g.*, within a stream reach), and others on larger scales (*e.g.*, along a river network, or between biomes). Likewise, the temporal scale of variation also varies widely, ranging from changes that occur over a course of days to changes that are driven by geological processes and operate on extremely long time-scales. Consideration of the spatial and temporal scale of variation may be important in successful design of stable isotope research and incorporating variability into models will be important for achieving unbiased food webs descriptions. Variation can also be partitioned into variation that creates signals that are necessary to discern patterns in nature, and variation that creates noise and obscures meaningful ecological patterns.

Because sources of variability differ among elements, I discuss $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^2\text{H}$ separately and then discuss sources of variability that apply to all elements

(Figure 1.1). I then describe recently developed modeling methods that can account for sources of variability to achieve accurate food web descriptions, and several new empirical methods that may be useful for minimizing problems associated with variability. The scope of this review focuses on comparisons of allochthonous and autochthonous organic matter sources in streams, but many of the techniques are also applicable to comparisons in other aquatic systems and comparisons between other organic matter sources (*e.g.*, littoral versus pelagic carbon sources in lakes).

Sources and patterns of variation in $\delta^{13}\text{C}$

Food webs have been resolved in many streams using $\delta^{13}\text{C}$, which is the stable isotope ratio of $^{13}\text{C}:^{12}\text{C}$ relative to Vienna Pee Dee Belemnite, or VPDB. However, strong documentation of spatial and temporal variation in $\delta^{13}\text{C}$ exists, which can lead to overlap in food source $\delta^{13}\text{C}$ and make it difficult to resolve food web linkages with $\delta^{13}\text{C}$ data in some stream ecosystems (*e.g.*, Herwig *et al.* 2007, Bergfur *et al.* 2009, Hadwen *et al.* 2010). While terrestrial plant $\delta^{13}\text{C}$ is less variable and usually around -28‰ with a typical range from -31‰ to -26‰, $\delta^{13}\text{C}$ of stream algae can vary widely depending on algal species composition, the form and ultimate source of carbon available for photosynthesis (*e.g.*, CO_2 or HCO_3^-), and many other factors, and can range from -19‰ to -45‰ (Fry 2006). High variation in $\delta^{13}\text{C}$ has been acknowledged for decades. France (1995) reviewed published measurements of $\delta^{13}\text{C}$ and concluded that it was impossible to determine what type of organic matter was assimilated by 50% of fishes and 70% of invertebrates, mostly due to wide variation in the $\delta^{13}\text{C}$ of within system primary producers. Twenty years later, the utility of $\delta^{13}\text{C}$ analyses for

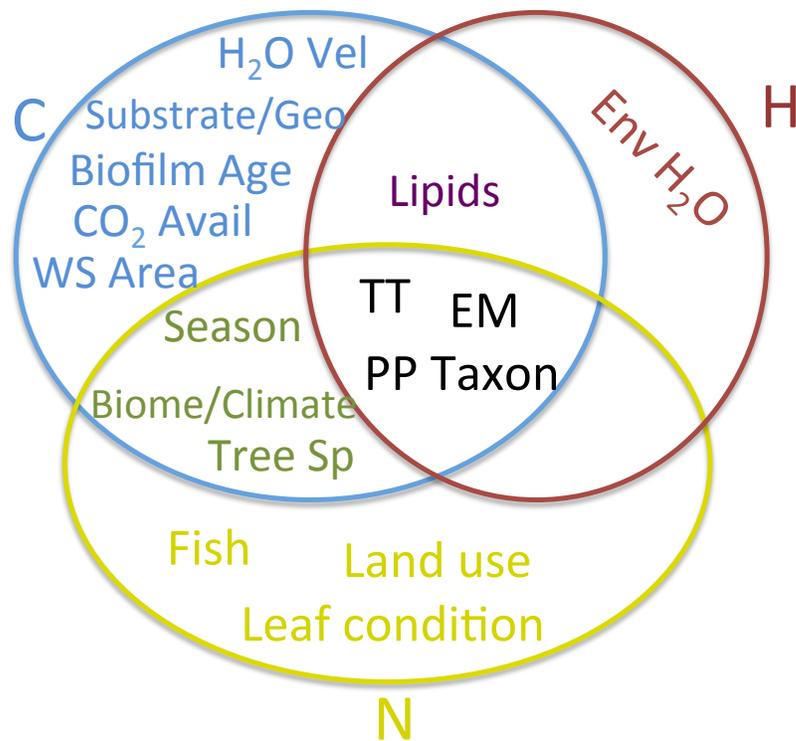


Figure 1.1: Shared and distinct drivers of spatial and temporal variation for C, H and N isotopes. Abbreviations are as follows: WS Area= Watershed area, TT= turnover time, EM= end member uncertainty or overlap, PP taxon= primary producer taxon, H₂O vel = water velocity, Geo= geomorphology.

describing some aquatic food webs still remains questionable: failure to achieve source separation between organic matter pools is a common problem in published studies, and it seems likely that there are also isotope datasets that remain unpublished because of failure to achieve source separation or failure to identify a source that is sufficiently enriched or depleted to explain consumer $\delta^{13}\text{C}$. High variability and uncertainty in $\delta^{13}\text{C}$, as detailed below, suggests that future research should at minimum collect samples to document spatial and temporal variation that exists in the system (Figure 1.2).

Ishikawa *et al.* (2012) summarized controls on periphyton $\delta^{13}\text{C}$ variation in a meta-analysis of 42 published papers, finding that both local and regional drivers of variation could result in a large range of $\delta^{13}\text{C}$ (-47.3 to -9.3‰). Key factors on regional or larger geographic scales included variation among biomes and longitudinal variation along river networks, which correlated with watershed size. On a local scale, canopy cover, water velocity, chlorophyll-*a* abundance, and taxonomic differences among primary producers were important drivers of the $\delta^{13}\text{C}$ of periphyton (Ishikawa *et al.* 2012). Case studies from other systems have identified other drivers of variability (Figure 1.1), many of which likely co-vary with each other and with the drivers identified by Ishikawa *et al.* (2012). Generally, when primary production is higher, there is less discrimination against ^{13}C , and patterns in primary production can explain many patterns in $\delta^{13}\text{C}$ of periphyton. On the watershed scale, primary production is generally higher in streams with larger watersheds, but CO_2 concentrations are not, hence $\delta^{13}\text{C}$ of algae is generally higher because of less discrimination against ^{13}C during primary production (Finlay 2003, Ishikawa *et al.*

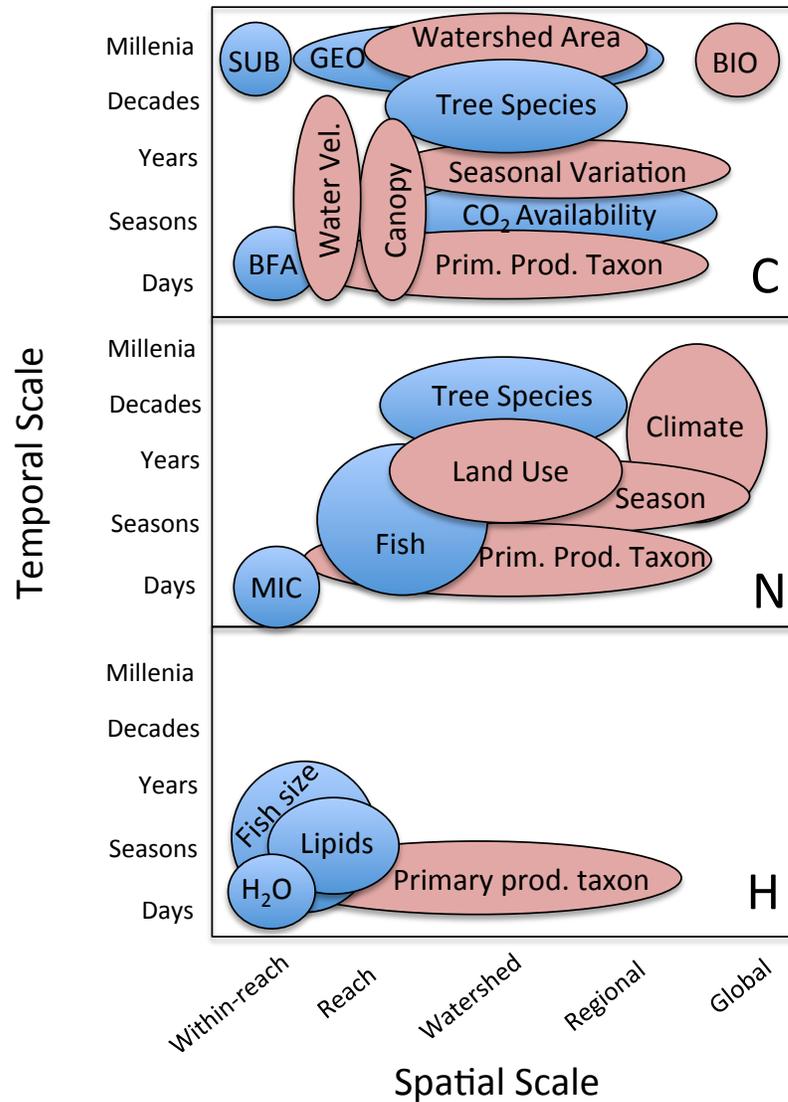


Figure 1.2: Spatial and temporal scales of variation for different drivers of isotopic variability. Abbreviations are as follows: SUB= substrate, GEO=geomorphology, Water vel. = water velocity BIO=biome, BFA=biofilm age, MIC=microbial conditioning, H₂O=environmental water. Drivers in red were identified by meta-analysis as significant predictors across systems (from Ishikawa *et al.* 2012 for C, Peipoch *et al.* 2012 for N, Hondula *et al.* 2014 for H) while drivers in blue were identified as important in one or more single-system studies.

2012). Effects of dissolved inorganic carbon availability along a watershed are likely driven by CO₂, which has been shown to vary in streams of different sizes, and not HCO₃⁻, which does not vary with stream size (Finlay 2003).

At the local scale, some primary producers have highly variable δ¹³C signatures relative to others. Epilithic biofilms are generally more variable than other types of primary producers (*e.g.*, macrophytes, filamentous green algae) and allochthonous carbon pools (*e.g.*, leaf litter). Trudeau and Rasmussen (2003) conducted a mesocosm experiment and found that differences in water velocity led to more δ¹³C fractionation in diatom communities (δ¹³C ranging from -16‰ to -28‰ from low to high water velocity treatments) compared with water velocity effects on filamentous green algae. While differences in boundary layers and CO₂ availability vary depending on the types of primary producers and substrate, different algal species may differ in their use of dissolved organic carbon (CO₂ versus HCO₃⁻), which can have a strong influence on δ¹³C (Trudeau and Rasmussen 2003). In California streams, δ¹³C of green algae (*Cladophora*) varied among sites while cyanobacteria (*Nostoc*) and red algae (*Lemanea*) did not (Finlay 2004), presumably due to differences in how the three taxa acquire carbon. Specifically, *Lemanea* can only utilize CO₂, and cannot use HCO₃⁻, and is therefore not subject to complex mechanisms driving patterns in use of the two DIC compounds by *Cladophora* (Finlay 2004). *Nostoc* actively concentrate DIC, which prevented fractionation and made it highly enriched in δ¹³C (Finlay 2004).

The type of substrate on which a biofilm is growing can also influence variation in δ¹³C. Hladysz *et al.* (2011) found higher δ¹³C variation in biofilms

growing on cobbles (11‰ range) compared with biofilms on leaves (2‰ range), presumably because biofilms on leaves utilize leaf C, which has a consistent $\delta^{13}\text{C}$ signature relative to the carbon available for biofilms on cobbles. Consequently, grazing snails that fed on leaf biofilm had a greater allochthonous $\delta^{13}\text{C}$ signature than snails that fed on cobble biofilm (Hladyz *et al.* 2011). While allochthonous plant material is generally less variable than biofilms, it does sometimes vary among tree species; for example, leaf litter from coniferous and deciduous trees can have different isotopic signatures (Kominoski *et al.* 2012). Generally, there is a difference in fractionation and the $\delta^{13}\text{C}$ of plants with different photosynthetic systems (C3 versus C4), which could lead to differences in $\delta^{13}\text{C}$ of allochthonous plant material depending on the type of plants in a watershed (Fry 2006).

It is not surprising that biofilm $\delta^{13}\text{C}$ would vary more than terrestrial primary producers. Epilithon is a mixture of algae, bacteria and detritus, and is therefore likely composed of both autochthonous and allochthonous carbon in proportions that are driven by local environmental conditions, and the particular combination of taxa and functional groups present. Studies in systems with biofilms that are dominated by primary producers have a higher likelihood of success in separating allochthonous and autochthonous resource pools because biofilms will reflect the isotopic signature of autochthonous production. In systems that are not dominated by primary production, biofilms will be a mixture of autotrophs that assimilate inorganic carbon and heterotrophs that assimilate allochthonous carbon rather than a true autochthonous end member.

Temporal changes in light availability and nutrient availability can both have a strong effect on biofilm $\delta^{13}\text{C}$, which ranged from -14 to -36‰ in laboratory and field studies where light and nutrients were manipulated (Hill *et al.* 2008). With higher light levels and nutrients concentrations, photosynthetic rates increase and lead to less discrimination against $^{13}\text{CO}_2$, making $\delta^{13}\text{C}$ of biofilms higher (Ishikawa *et al.* 2012). The range of $\delta^{13}\text{C}$ in different light and nutrient conditions identified by Hill *et al.* (2008) encompasses the mean values for allochthonous and autochthonous end members in most studies, so they concluded that failure to account for variable light and phosphorus conditions could have a strong effect on results of food web studies. Hence, natural changes in light availability due to canopy changes in deciduous forest are also likely to lead to seasonal variation in biofilm $\delta^{13}\text{C}$.

In addition to $\delta^{13}\text{C}$ variation within resource pools, there can also be high variation within taxa of stream consumers. Lancaster and Waldron (2001) found high within-population variation for many taxa of stream insects for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, which was partially due to measurement error but also due to differences in age, developmental stage and nutritional state within a taxon. Most consumers are also more mobile than their resource pools, with the exception of consumers that utilize dissolved carbon or drifting invertebrates that move through the water column. When there is spatial variation in resource $\delta^{13}\text{C}$ at the stream-reach scale, it may be difficult to reconcile food web position of mobile species. For example, if fishes move among stream reaches that contain organic matter with different $\delta^{13}\text{C}$ signatures, it can be difficult to match them to organic matter sources in the single stream where they are sampled. In Australian rivers with biofilm $\delta^{13}\text{C}$ values that varied among rivers,

invertebrate $\delta^{13}\text{C}$ was highly correlated with that of the biofilm where they lived, suggesting that invertebrates assimilated local resources, but more mobile fishes did not correlate with local biofilm $\delta^{13}\text{C}$, suggesting resource use integrated the carbon signal over a broader scale (Jardine *et al.* 2012). Similarly, spatial variation in $\delta^{13}\text{C}$ (and $\delta^{15}\text{N}$) in streams in the Southwestern USA made it difficult to compare resource use by native and non-native fishes, which presented challenges in managing native fish populations (Gido *et al.* 2006). However, movement of consumers across habitats with different isotopic signatures can also make it possible to identify external food sources, for example, Winemiller and Jepsen (2004) used stable isotope data to determine that food sources from whitewater rivers subsidize predatory fishes from nutrient poor blackwater rivers.

On a larger spatial scale, variation along river networks and watershed size have been associated with variation in $\delta^{13}\text{C}$ in riverine systems. In mountain to middle sections of a Japanese river, $\delta^{13}\text{C}$ increased with stream size and productivity for periphyton and macroinvertebrates, but $\delta^{13}\text{C}$ decreased from middle to lowland, perhaps because lowland streams have more terrestrial detritus and more pool habitat (Kobayshi *et al.* 2011). However, larger patterns along a river network can also correlate with patterns of reach-scale geomorphology, which may also explain variation in $\delta^{13}\text{C}$ along a river network. For example, Walters *et al.* (2007) found that reach-scale geomorphology explained variation in $\delta^{13}\text{C}$ better than longitudinal position in South Carolina Piedmont rivers, where rock bed sites resulted in more enriched $\delta^{13}\text{C}$ than sand bed sites, presumably due to low primary production and high CO_2 concentrations at sand bed sites leading to higher discrimination of ^{13}C .

Associations between isotope signature and geomorphic category were consistent for consumers and biofilms but not for allochthonous particulate organic matter (Walters *et al.* 2007).

Predictable variation along river networks and over seasonal timescales can also be useful in drawing conclusions about food sources for consumers. In glacial streams in the Swiss Alps, both macroinvertebrates and their presumed food source, a chrysophyte alga (*Hydrurus foetidus*), had similar seasonal patterns in $\delta^{13}\text{C}$, which led Zah *et al.* (2001) to conclude that autochthonous production was dominant. Some modeling frameworks (*e.g.*, gradient-based models, Table 1.1, described in detail below) take advantage of longitudinal co-variation between biofilms and consumers in river networks to calculate fluxes from autochthonous pathways to consumers (Rasmussen 2010).

Sources of variation in $\delta^{15}\text{N}$ measurements

$\delta^{15}\text{N}$, or the ratio of $^{15}\text{N}:^{14}\text{N}$ relative to the standard of atmospheric air, is also a useful tracer for food web research in freshwaters. Many studies in freshwater systems have noted a predictable increase in $\delta^{15}\text{N}$ from lower to higher trophic levels, which has made $\delta^{15}\text{N}$ data useful for evaluating trophic position of consumers (Post 2002). Despite decades of study demonstrating differences in $\delta^{15}\text{N}$ among trophic levels, the mechanisms behind this enrichment in $\delta^{15}\text{N}$ at higher trophic levels are just beginning to be understood and can vary depending on protein content of diet, amino acid composition of tissue and other factors (reviewed by Wolf *et al.* 2009). Although increases in $\delta^{15}\text{N}$ of stream consumers relative to their resources are often predictable,

Table 1: Modeling frameworks appropriate for different types of variation or uncertainty in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and/or $\delta^2\text{H}$ data.

Goal/Problem	Modeling approach	Specific application	Reference
Incorporate uncertainty into model	Bayesian mixing model	Incorporate uncertainty about number of food sources and proportions	Parnell <i>et al.</i> 2013 Ward <i>et al.</i> 2011
		Maximize information about organic matter sources through three-isotope Bayesian mixing model	Solomon <i>et al.</i> 2011
Resource compartments are temporally variable	Dynamic baseline framework	Account for temporal variation in baseline food web compartments to estimate fish resource use	Woodland <i>et al.</i> 2012
	Growth-dependent tissue turnover model	Account for more rapid turnover in resources compared to consumers	Perry <i>et al.</i> 2003
Large-scale spatial variation (e.g., within a river network)	Gradient-based model	Utilize spatial co-variation in biofilm and consumers to determine autochthonous contributions along gradient	Rasmussen 2010
Use both C and N isotope data to construct food web model	Concentration-dependent mixing model	Account for different pathways of C and N through food web	Phillips & Koch 2002
	Inverse modeling	Incorporate community and ecosystem measurements in reconstruction of C and N flows through food web, account for differential assimilation of ingested food	Larned <i>et al.</i> 2008

this relationship can vary depending on consumer diet and analysis method (McCutchan *et al.* 2003). Specifically, McCutchan *et al.* (2003) found that trophic shifts in $\delta^{15}\text{N}$ for consumers that were raised on invertebrate diets were 1‰ lower than shifts for consumers raised on algal diets and 2‰ lower than consumers raised on high-protein diets, indicating that diet type should be considered when using $\delta^{15}\text{N}$ data to determine trophic position.

In addition to variation among trophic levels, $\delta^{15}\text{N}$ also varies on larger spatial and temporal scales in different ecosystems (Figure 1.2). A recent meta-analysis revealed that $\delta^{15}\text{N}$ of dissolved inorganic nitrogen (DIN) and basal resources in streams were highly variable among sites, ranging from -8.4 to 19.4‰ for DIN and -4 to 16‰ for basal resources (Peipoch *et al.* 2012). Human land use and season were the most significant factors explaining variation in DIN $\delta^{15}\text{N}$, while land use, season, basal resource type and climate were all significant predictors of basal resource $\delta^{15}\text{N}$ (Peipoch *et al.* 2012). The significant drivers identified by Peipoch *et al.* (2012) span a broad range of spatial and temporal scales: basal resource type can vary across patches within a single stream reach, while climate varies over a large geographic scale.

Other empirical studies indicate that watershed land-use is a very strong predictor of $\delta^{15}\text{N}$ in stream consumers. For example, Winemiller *et al.* (2011) noted that primary consumers in rivers in watersheds impacted by human activity were more $\delta^{15}\text{N}$ enriched than those in pristine watersheds, likely due to high $\delta^{15}\text{N}$ of fertilizers and sewage that are more common in systems with human activity. They suggested that primary consumer $\delta^{15}\text{N}$ could be a marker for determining watershed impact level

(Winemiller *et al.* 2011). However, other work in lakes suggests that attributing $\delta^{15}\text{N}$ to non-point human and watershed sources may be somewhat complex, and that denitrification and stoichiometric constraints are also important considerations (Jankowski *et al.* 2012). Bergfur (2013) also cautioned that generalizing about ecosystems with different perturbations could be misleading: she found variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in two streams in Sweden, but relationships between basal resources and consumers were very site-specific, differing between agricultural and forested streams.

While spatial variation in $\delta^{15}\text{N}$ among rivers is generally very large (Anderson & Cabana 2007), site-specific variation in $\delta^{15}\text{N}$ has actually been helpful in systems where $\delta^{13}\text{C}$ variation confounded food web interpretations. For example, in a study of Australian lowland rivers, $\delta^{15}\text{N}$ in both resources and consumers varied spatially and temporally, with higher variation in autochthonous resources than allochthonous, but depleted $\delta^{15}\text{N}$ in terrestrial sources relative to aquatic sources aided in solving mixing models impeded by variable $\delta^{13}\text{C}$ (Hladyz *et al.* 2012). Similarly, Reid *et al.* (2008), determined that biofilm $\delta^{13}\text{C}$ was variable in Australian streams, but they were able to assign trophic position based on a mixing model because $\delta^{15}\text{N}$ varied among basal resources, presumably because terrestrial and aquatic plants used different N sources.

Sources of variation in $\delta^2\text{H}$ measurements

The stable isotope ratio of deuterium ($\delta^2\text{H}$), or the ratio of $^2\text{H}:^1\text{H}$ measured relative to Vienna Standard Mean Ocean Water (VSMOW), has become increasingly popular as a tracer of allochthonous and autochthonous matter in freshwaters.

Widespread use of deuterium in freshwater food web studies began with a 2007 report that demonstrated excellent source separation between leaves and algae in streams in the Southwest USA, low spatial and temporal variation in $\delta^2\text{H}$ of river water, and better food web resolution than $\delta^{13}\text{C}$ data from the same sites (Doucett *et al.* 2007). Terrestrial plants are more enriched in ^2H than aquatic plants due to water fractionation by terrestrial plants (usually $\sim 100\text{‰}$), which results in much greater differences for $\delta^2\text{H}$ than for $\delta^{13}\text{C}$ between allochthonous and autochthonous organic matter (the $\delta^{13}\text{C}$ difference is usually $\sim 10\text{‰}$ at most). Both field and laboratory studies have begun to address and compare the utility of $\delta^2\text{H}$ and $\delta^{13}\text{C}$ for identifying the organic matter sources supporting higher trophic levels. Spatial and temporal variability in $\delta^2\text{H}$ seems to be small compared with $\delta^{13}\text{C}$ in stream ecosystems (Finlay *et al.* 2010), but other sources of uncertainty must be accounted for in order to interpret $\delta^2\text{H}$ data correctly (Soto *et al.* 2013b). $\delta^2\text{H}$ of terrestrial plant material is relatively constant and lab experiments revealed that it does not change over the course of leaf decomposition (Yang *et al.* 2014). Thus, live leaves are a reasonable proxy for $\delta^2\text{H}$ of allochthonous materials that are available to consumers in aquatic systems (Yang *et al.* 2014).

Organisms incorporate H from both food and water in the synthesis of new tissue, so the proportion of H from each source must be determined and a correction must be made for the influence of dietary water on the $\delta^2\text{H}$ of consumer tissue. In most systems, contributions to consumer tissue from environmental water are substantial, and in some rivers it is even possible to use $\delta^2\text{H}$ of water as a tracer of natal habitat in invertebrates because the $\delta^2\text{H}$ of water is so distinct among rivers due

to evaporative fractionation and flooding patterns (Myers *et al.* 2012). A limited number of laboratory studies have determined the proportion of H of invertebrate and fish tissue that is derived from environmental water and found that it ranges from 6% to 50% depending on consumer taxon (Solomon *et al.* 2009, Soto *et al.* 2013b). The proportion of consumer H from environmental water versus food varies among functional feeding groups within stream macroinvertebrates, lake zooplankton and fishes (Solomon *et al.* 2009), so corrections must be taxon-specific. While some studies have drawn conclusions about allochthonous contributions to food webs without accounting for water contributions to consumer $\delta^2\text{H}$ (*e.g.*, Doucett *et al.* 2007), laboratory tests demonstrate that correction for environmental water is imperative for unbiased results (Soto *et al.* 2013b).

Differences in the isotopic signature among types of consumer body tissues may be particularly important for $\delta^2\text{H}$ analyses. Although, lipids in consumer tissue often have a different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than other tissue types, so differences in lipid content can sometimes bias $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope studies (Post *et al.* 2007), efforts have been made to create standard correction protocols for lipid content in isotope analyses (Smyntek *et al.* 2007, Logan *et al.* 2008). Lipids may also have a strong influence on $\delta^2\text{H}$ analyses and could confound results if they are not removed. For example, lipid extraction to remove those molecules from analyses resulted in $\delta^2\text{H}$ of consumers in streams in Eastern Canada that matched the $\delta^2\text{H}$ of their presumed food sources (Jardine *et al.* 2009). Prior to lipid extraction, there were large differences between the $\delta^2\text{H}$ of food and consumer sources, which may be a reason why over half of the streams in that study had consumers that were outside the range of

autochthonous and allochthonous end members. In laboratory experiments, Soto *et al.* (2013) noted large differences among tissue types and found that lipids introduced variation and that lipid $\delta^2\text{H}$ was highly correlated with the $\delta^2\text{H}$ of water.

A recent meta-analysis by Hondula *et al.* (2014) revealed that differences in $\delta^2\text{H}$ among types of primary producers could confound results of food-web studies in lakes, streams and estuaries. While most primary producers were depleted in $\delta^2\text{H}$ relative to terrestrial plants, some groups of primary producers were difficult to distinguish. For example, algal $\delta^2\text{H}$ was easily distinguishable from terrestrial plants in freshwater systems, but macrophytes were not, hence the use of $\delta^2\text{H}$ to distinguish between allochthonous and autochthonous energy sources might not be effective in macrophyte-dominated systems. However, $\delta^2\text{H}$ could be very effective in distinguishing algal from macrophyte contributions to herbivore diets.

The three major drivers of variation that have been identified for $\delta^2\text{H}$ (*i.e.*, environmental water, lipids, and variation among taxa of primary producers), can all vary on relatively small spatial and short temporal time-scales: lipid content of fishes and community structure of primary producer communities vary seasonally, and taxa within the same stream reach can assimilate varying proportions of environmental water (Figure 1.2). While $\delta^2\text{H}$ of water is known to vary on global and regional scales (Rozanski *et al.* 1993), and larger spatial and temporal patterns are likely to exist for $\delta^2\text{H}$, to date, existing data are much more limited than for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and are insufficient for broader meta-analysis. However, regional studies suggest that within-group variation in $\delta^2\text{H}$ is likely to be lower than that for $\delta^{13}\text{C}$ (Finlay *et al.* 2010).

General sources of uncertainty and variation for all elements

Inability to correctly identify the true isotopic signature of purely allochthonous or autochthonous organic matter often causes problems in the interpretation of stable isotope food web data. Many examples exist of consumer species falling outside the range of presumed end members in streams of different sizes from different biomes. For example, Herwig *et al.* (2007) collected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ food web data in the Mississippi River and many consumers fell outside the resource space defined by mixing models. Hadwen *et al.* (2010) found that temporal variability in $\delta^{13}\text{C}$ of benthic algae led to mixing model scenarios where consumer isotope signature could not be explained by sampled resources in five subtropical Australian streams, and Bergfur *et al.* (2009) could not resolve food webs in Swedish streams because consumers had $\delta^{13}\text{C}$ signatures outside the range of basal resources.

In some cases, additional data or correction factors for end members can address problems of consumers falling outside of the isotopic range of resource pools. Although gut analyses do not integrate resource use over time, they can be helpful in confirming stable isotope results. For example, gut content data made it possible to determine trophic position for fishes in a tropical river when $\delta^{13}\text{C}$ agreed with gut content data but $\delta^{15}\text{N}$ did not (Davis *et al.* 2012). Pure algal samples can be collected during bloom conditions in some systems and used to make a correction factor for autochthonous end members (*e.g.*, in lakes Wilkinson *et al.* 2013), but algal isotope ratios can vary over time so multiple snapshots may lead to more accurate end member characterization (Post 2002). In other systems, autochthonous end members can be estimated by correcting the isotopic signature of seston samples for

allochthonous contamination based on the concentration of non-volatile suspended solids, which correlate with terrestrial particulate organic carbon (Babler *et al.* 2011).

In cases where researchers have been able to separate algal and detrital elements of organic matter pools (*e.g.*, Delong & Thorp 2006), algae and detritus have distinctive $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures. However, separation of biofilm components is often not done because it is time consuming and only effective for biofilms containing particular types of organisms. Since biofilms are a mixture of autotrophs and heterotrophs that have different isotopic signatures, scrapers and other consumers often have an allochthonous isotope signature even though they are known to consume “algal” biofilms. Conversely, shredders sometimes assimilate autochthonous materials even though they are known to consume allochthonous leaf material because leaf biofilms can include algae (Franken *et al.* 2005). Some stream insects selectively assimilate algal elements of biofilms while others assimilate bulk biofilm (McNeely *et al.* 2006), so separating algae from bulk biofilm may be especially important for some invertebrate species.

It is also possible that consumers with stable isotope signatures that fall outside of the range of the presumed resource end members might occur if the incorrect resource pool was sampled. For example, McWilliam-Hughes *et al.* (2009) found that the signature of bryophytes could explain $\delta^{13}\text{C}$ of consumers while other high-quality presumed resources frequently sampled in stream ecosystem studies (*i.e.*, filamentous algae, biofilm, macrophytes, cyanobacteria) could not. Similarly, Watson & Barnuta (2011) confirmed their unexpected isotope result that shredders consume macrophytes through feeding trials. In those cases, gut contents and laboratory studies are helpful

in confirming results from IsoSource (Phillips & Gregg 2003) and other isotope mixing models (Mantel *et al.* 2004).

Temporal variability in basal resources can also contribute to an inability to identify a distinct food source if its isotope signature changes more rapidly than consumers and both are only sampled at one time point. A common method to circumvent baseline variability in stable isotopes is to use a primary consumer as an end member rather than a highly-variable basal resource pool (*e.g.*, Finlay *et al.* 1999, Post 2002, Post & Walters 2008). Primary consumers that eat algae can serve as autochthonous end members because it is logistically complicated to extract single algal cells and analyze their isotopic signature, and they also integrate temporally variable algal signatures over time to reflect the average algal isotope signature. However, additional information is needed to understand whether primary consumers are also temporally variable and whether it is appropriate to use them as a more stable proxy for baseline data (Jardine 2014).

Turnover time of consumer and resource tissue is also an important consideration when there is temporal or spatial variation in isotopic signatures. Consumer tissue generally turns over more slowly than that of algae and bacteria, which may lead to discrepancies in the timescale of isotopic variation. Christian *et al.* (2004) found that freshwater mussels did not vary in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, but their presumed food source, microbes in fine particulate organic matter, did vary seasonally. Conversely, Wilson & Blinn (2007) found no difference in primary producer $\delta^{13}\text{C}$ but did find variation in $\delta^{13}\text{C}$ for pupfish consumers, which may have been a result of seasonal changes in food source, with consumption dominated by cyanobacteria in the

summer but allochthonous resources most important in the winter. Primary consumers in streams have very different turnover times, ranging from a half-life of less than 3 days for some mayflies and blackflies to over 100 days for some mussels (Jardine 2014), so some primary consumers isotopic signatures may integrate variation over a scale of days while others may integrate variation over a scale of several months.

Empirical and modeling approaches to account for variation and uncertainty in stable isotope data

Given the extent of variation and uncertainty in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^2\text{H}$ data, it is nevertheless encouraging that aquatic ecologists have been able to resolve food webs and identify important organic matter sources using stable isotopes as successfully as they have. Methods for data analysis and analytical techniques have advanced substantially over the past decade, which will continue to aid in the interpretation of variable data and provide complementary methods that can eliminate uncertainty when used in tandem with classic techniques. Understanding whether data are spatially variable, temporally variable, variable within a presumably homogenous food source, and what the scale of variation is, will lead to an appropriate modeling approach. Collecting data over spatial and temporal scales relevant to the particular application to capture variation, and using modeling frameworks that incorporate variation into estimates of food web fluxes will lead to the successful construction of food webs. Potential modeling frameworks for incorporating uncertainty and variation are detailed below.

Modeling approaches to account for variation and uncertainty

Stable isotope mixing models have been modified to deal with complex data, especially when they are applied in a Bayesian framework (Table 1.1, *e.g.*, Solomon *et al.* 2011, Parnell *et al.* 2013). For example, sometimes there is uncertainty about how to partition food sources (*e.g.*, should different autochthonous food pools be combined into a single autochthonous source to simplify a model?). Models can be modified to account for uncertainty in source contributions. Ward *et al.* (2011) estimated proportional contributions of food sources to two distinct groups of consumers, rainbow trout and coastal mink, in a Bayesian mixing model framework that allowed them to have an uncertain number of source groups and uncertain group assignment. By estimating posterior probabilities of multiple group configurations with varying complexity, they improved model inference and reduced biases (Ward *et al.* 2011).

A number of recent papers have described modeling techniques that specifically account for spatially or temporally variable isotope data to draw stronger conclusions about food web pathways (Table 1.1). Woodland *et al.* (2012) created a dynamic baseline framework that incorporates temporal variation in $\delta^{13}\text{C}$. They tested their model on nine species of juvenile fish in a fluvial lake, and found that the dynamic model performed better than a static mixing model. Results from the static model were highly variable and ranged from completely benthic to completely pelagic support of fish production, and application of the dynamic model resulted in increased benthic support of all nine species compared with static models (Woodland *et al.* 2012). While many static baseline models assume that consumers and food sources are in equilibrium, the dynamic model also allowed them to eliminate equilibrium

assumptions, which are rarely met in nature. This modeling approach presents a feasible way to eliminate bias in systems with temporally variable baseline $\delta^{13}\text{C}$.

Equilibrium assumptions are often violated when consumers have relatively long turnover times compared with their resources. Perry *et al.* (2003) accounted for a lack of equilibrium between consumers and resources using a growth-dependent tissue turnover model to evaluate autochthonous and allochthonous contributions to Chinook salmon diets. Salmon tissue did not turn over rapidly enough to reach equilibrium with their diet, which made data unsuitable for other modeling frameworks.

Incorporating salmon tissue turnover time allowed Perry *et al.* (2003) to construct hypothetical scenarios based on assimilation of entirely allochthonous or entirely autochthonous resources so they could determine end members for a mixing model that allowed them to compare allochthonous contributions in different streams.

For consumers and resources with spatially variable isotope signatures, gradient-based models can utilize patterns of variation to determine food web fluxes. Rasmussen (2010) developed a gradient-based model for $\delta^{13}\text{C}$ that can determine source partitioning even if basal resource $\delta^{13}\text{C}$ signatures do not differ within a single site. The gradient-based model utilized the pattern that autochthonous signatures are depleted in ^{13}C relative to terrestrial sources in upstream sites but enriched relative to terrestrial sources in downstream sites; however terrestrial $\delta^{13}\text{C}$ did not change along the gradient. The gradient-based mixing model evaluated terrestrial consumption by comparing the slope of autochthonous $\delta^{13}\text{C}$, which becomes more enriched in downstream sites relative to upstream sites, to the slope of consumer $\delta^{13}\text{C}$. Consumer $\delta^{13}\text{C}$ would not change if they assimilated allochthonous resources, but would follow

the upstream to downstream gradient if they assimilated autochthonous resources (Rasmussen 2010). Gradient-based modeling approaches were also effective at evaluating allochthonous contributions along a temperate to tropical gradient of Australian Rivers (Jardine 2014a).

Other modeling approaches acknowledge that C and N might not have the same pathways through food webs and evaluate the two tracers separately to improve food web resolution. For example, algae are relatively more N-rich than terrestrial plant leaves, so consumers might assimilate proportionally more N from algal sources than leaf sources. Concentration-dependent mixing models account for differences in pathways of C and N through food webs (Phillips & Koch 2002). England and Rosemond (2004) applied concentration-dependent mixing models to data from streams in the southeastern United States, and identified that both allochthonous and autochthonous resources were important food sources for crayfish and fish. Concentration-dependent models also allowed them to determine that decreased forest cover can lead to decreased terrestrial support of headwater stream food webs (England and Rosemond 2004).

Inverse models, which provide solutions to systems of linear difference equations that describe flows between food web compartments, are also effective for describing C and N fluxes in streams ecosystems, even when some fluxes or pathways are unknown. Larned *et al.* (2008) demonstrated how inverse models can be applied to food webs in an Hawaiian stream, and determined that future modeling efforts would benefit from concentrated sampling in compartments that have the strongest effect on model output, and limited sampling in those that do not (*e.g.*, in their study

system, fluxes from C4 plants to consumers were minimal, so sampling efforts should have focused on other resource compartments that were more important for consumer diets). Of course, it is difficult to have *a priori* knowledge of which fluxes are most important, but sensitivity analyses and continued work in the same system will permit better descriptions of C and N fluxes and the development of data collection priorities. The results of inverse models presented by Larned *et al.* (2008) produced estimates for ecosystem parameters (gross primary productivity, respiration) that were similar to field data and results demonstrated that the modeling technique has the potential to accurately describe energy and material fluxes through stream ecosystems.

Empirical approaches to account for variation and uncertainty

In addition to developing modeling frameworks that suit the variable and uncertain nature of isotope data sets, strategic sampling and use of new analysis methods may improve the use of isotopic data. If overlap in source $\delta^{13}\text{C}$ seems likely, physical separation of biofilm elements to characterize end members (*e.g.*, Hamilton *et al.* 2001, Delong & Thorp 2006) or use of additional elements besides $\delta^{13}\text{C}$ that can indicate food source can be helpful in distinguishing between allochthonous and autochthonous pathways. Recent research has already demonstrated that the combination of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^2\text{H}$ data can improve accuracy of food web models (*e.g.*, Cole *et al.* 2011, Solomon *et al.* 2011, Cole & Solomon 2012). Below, I detail three other relatively new methods (^{14}C , compound-specific isotope ratios, and fatty acid analysis) that might also improve resolution of food web studies in aquatic systems when used in tandem with standard methods. Although these methods are

likely to be more labor-intensive and expensive than typical natural abundance isotope measurements, they may be worth the additional costs when it is difficult to resolve food webs using more standard methods.

$\Delta^{14}\text{C}$ as an additional tracer of allochthonous material: Aged allochthonous carbon may be an important subsidy to microbes and animals in aquatic systems and can be detected with natural abundance radioisotopes of carbon (^{14}C), which is expressed as $\Delta^{14}\text{C}$ when values are corrected based on a standard of 1950s oxalic acid. $\Delta^{14}\text{C}$ varies in rivers across the United States, and is enriched (*i.e.*, composed of recently-fixed carbon from atmospheric CO_2) in watersheds with high discharge, high vegetation cover and low human activity (Butman *et al.* 2012). $\Delta^{14}\text{C}$ has been analyzed in relatively few food web studies, but, where it has been used, it has provided additional information beyond the scope of information gleaned from $\delta^{13}\text{C}$ data, especially when combined with other isotopes (reviewed by Ishikawa *et al.* 2013). For example, Caraco *et al.* (2010) were able to detect that 21-57% of zooplankton production in the food web of the Hudson River (New York, USA) could be fueled by ancient allochthonous C that has aged within the watershed for an extended period of time. Their results suggest that zooplankton likely consume small particles of aged particulate organic carbon (Caraco *et al.* 2010). In Japanese streams, combining $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ data provided better resolution in distinguishing between autochthonous and allochthonous sources than $\delta^{13}\text{C}$ data alone (Ishikawa *et al.* 2010). Good source separation resulted from differences in $\Delta^{14}\text{C}$ in catchment dissolved organic carbon

(reflected in aquatic production) and atmospheric CO₂ (reflected in terrestrial production), which led to variation among consumer species (Ishikawa et al 2010).

Compound specific stable isotope analyses: Compound-specific stable isotope techniques have been used extensively in hydrology, paleoclimate and geology studies (e.g., Blyth *et al.* 2013, Lutz *et al.* 2013, Wilkie *et al.* 2013, Schmidt *et al.* 2014), and might also be useful for food web studies in freshwater systems. For example, $\delta^{15}\text{N}$ of amino acids (phenylalanine and glutamic acid) can be used to calculate trophic position of stream consumers with less variable results than $\delta^{15}\text{N}$ of bulk tissue (Ishikawa *et al.* 2014). Aquatic and terrestrial primary producers differ in $\delta^{15}\text{N}$ of phenylalanine versus glutamic acid, so compound specific methods may be particularly relevant for studies of allochthony in aquatic systems, but they have been applied in very few instances (Chikaraishi *et al.* 2009).

Combining fatty acid analyses with stable isotope ratios: Fatty acid profiles are also useful for tracking the fate of autochthonous production in aquatic systems, as markers are specific to primary producer groups and can be detected in higher trophic levels. Fatty acid studies of stream food webs have been applied only recently, starting with a seasonal characterization of fatty acid profiles in a small, forested stream that identified unexpectedly high proportions of autochthonous energy for some taxa (Torres-Ruiz *et al.* 2007). Subsequent studies have highlighted autochthonous contributions to tropical forest streams using a combination of fatty acids, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Lau *et al.* 2009), and autochthonous contributions to macroinvertebrates in a

large temperate river (Descroix *et al.* 2010). Some recent work has demonstrated that fatty acids could be useful indicators of allochthonous food sources because terrestrial and aquatic plants differ greatly in their highly unsaturated fatty acid (HUFA) content. There are few or no HUFAs in terrestrial plants (Simopoulos *et al.* 2004), but high amounts of HUFAs in diatoms and other aquatic primary producers (*e.g.*, Ahlgren *et al.* 1992, Hill *et al.* 2011, Volk & Kiffney 2012).

Conclusions

Variability in isotope ratio measurements in aquatic systems is extensive for elements that are commonly used in natural abundance studies, especially for $\delta^{13}\text{C}$. For $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, attributes associated with variation appear on a wide range of spatial and temporal scales. Fewer studies using $\delta^2\text{H}$ exist, so known drivers are local and operate on a shorter timescale, but larger patterns will likely emerge with further research. Ideally, ecologists would account for variability by collecting a large sample size of isotopic measurements for all food web compartments over broad temporal and spatial scales, and use appropriate modeling frameworks to eliminate bias in food web characterization. Such extensive collections are unlikely due to time and funding constraints, but using existing data from hundreds of freshwater systems compared with *a priori* knowledge of a study system may allow researchers to pinpoint sources of variability that are most likely to be important and focus efforts on characterizing them. For example, if a stream reach has high spatial variability in light availability, sampling efforts may be made to characterize variation in primary producers between light and dark patches the reach. In a situation where temporal variation is expected

due to dynamic water velocity, consideration of the turnover times of target consumer groups would influence the time-scale for characterization of baseline variability.

In some systems where variability and uncertainty are especially high, using three elemental tracers ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$) rather than just one or two, or incorporating new methods ($\Delta^{14}\text{C}$, compound-specific isotopes, and fatty acids) may aid in the quantification of food web pathways. Using additional markers to get adequate source separation is especially important in unproductive systems where biofilms are likely to be composed of a mixture of allochthonous and autochthonous organic matter. Autotroph-specific markers like fatty acids or isotopic analysis of biofilms that have been separated into algal and detrital components can help characterize an autotrophic end member when biofilm is a mixture.

Quantifying isotopic variability in freshwater systems and creating analysis frameworks that incorporate variability when describing food web fluxes are challenging but necessary tasks. While problems in resolving food webs are most apparent in studies where consumers fall outside of the isotopic range of presumed food resources, failure to account for variability is likely to bias all studies. Problems with variability and data interpretation are apparent when food webs cannot be resolved, but could also exist even when mixing models produce plausible solutions for food web pathways. Continued synthesis of data across spatial and temporal scales will aid in understanding the extent and cause of isotopic variation and provide information for improving future research.

REFERENCES

- Ahlgren, G., I-B. Gustafsson and M. Boberg. 1992. Fatty acid content and chemical composition of freshwater microalgae. *Journal of Phycology* 28: 37-50.
- Anderson, C. and G. Cabana. 2007. Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. *Journal of the North American Benthological Society* 26(2): 273-285.
- Augspurger, C., G. Gleixner, C. Kramer and K. Kusel. 2008. Tracking carbon flow in a 2-week-old and 6-week-old stream biofilm food web. *Limnology and Oceanography* 53(2): 642-650.
- Babler, A.L., A. Pilati and M.J. Vanni. 2011. Terrestrial support of detritivorous fish populations decreases with watershed size. *Ecosphere* 2(7): article 76.
- Bartels, P., J. Cucherousset, K. Steger, P. Eklov, L.J. Tranvik and H. Hillebrand. 2012. Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. *Ecology* 93(5): 1173-1182.
- Bergfur, J. 2013. Temporal variation in carbon and nitrogen isotope ratios of aquatic biota in two contrasting boreal streams. *Fundamental and Applied Limnology* 182(3): 205-218.
- Bergfur, J., R.K. Johnson, L. Sandin and W. Goedkoop. 2009. Effects of nutrient enrichment on C and N stable isotope ratios of invertebrates, fish and their food resources in boreal streams. *Hydrobiologia* 628: 67-79.
- Blyth, A.J., C.I. Smith and R.N. Drysdale. 2013. A new perspective on the delta 13-C signal preserved in speleotherms using LC-IRMS analysis of bulk organic matter and compound specific stable isotope analysis. *Quaternary Science Reviews* 75: 143-149.
- Butman, D., P.A. Raymond, K. Butler and G. Aiken. 2012. Relationships between $\Delta^{14}\text{C}$ and the molecular quality of dissolved organic carbon in rivers draining to the coast from the coterminous United States. *Global Biogeochemical Cycles* 26: 1-15.
- Caraco, N., J.E. Bauer, J.J. Cole, S. Petsch and P. Raymond. 2010. Millennial-aged organic carbon subsidies to a modern river food web. *Ecology* 91(8): 2385-2393.
- Chikaraishi, Y., N.O. Ogawa, Y. Kashiyama, Y. Takano, H. Suga, A. Tomitani, H. Miyashita, H. Kitazato and N. Ohkouchi. 2009. Determination of aquatic

food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnology and Oceanography Methods* 7: 740-750.

- Christian, A.D., B.N. Smith, D.J. Berg, J.C. Smoot and R.H. Findlay. Trophic position and potential food sources of two species of unionid bivalves. *Journal of the North American Benthological Society* 23(1): 101-113.
- Cole, J.J. and C.T. Solomon. 2012. Terrestrial support of zebra mussels and the Hudson River food web: A multi-isotope, Bayesian analysis. *Limnology and Oceanography* 57(6): 1802-1815.
- Cole, J.J., S.R. Carpenter, J. Kitchell, M.L. Pace, C.T. Solomon and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen and hydrogen. *Proceedings of the National Academy of Sciences* 108(5): 1975-1980.
- Davis, A.M., M.L. Blanchette, B.J. Pusey, T.D. Jardine and R.G. Pearson. 2012. Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. *Freshwater Biology* 57: 2156-2172.
- Delong, M.D. and J.H. Thorp. 2006. Significance of instream autotrophs in trophic dynamics of the Upper Mississippi River. *Oecologia* 147: 76-85.
- DeNiro, M.J. and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42: 495-506.
- DeNiro, M.J. and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45: 341-351
- Descroix, A., A. Bec, G. Bourdier, D. Sargos, J. Sauvanet, B. Mission and C. Desvillettes. 2010. Fatty acids as biomarkers to indicate main carbon sources of four major invertebrate families in a large river (the Allier, France). *Fundamental and Applied Limnology* 177(1): 39-55.
- Doi, H. 2009. Spatial patterns of autochthonous and allochthonous resources in aquatic food webs. *Population Ecology* 51: 57-64.
- Doucett, R.R., D.R. Barton, K.R.A. Guiguer, G. Power and R.J. Drimmie. 1996. Comment: Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 1913-1915.

- Doucett, R.R., J.C. Marks, D.W. Blinn, M. Caron and B.A. Hungate. 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology* 88(6): 1587-1592.
- England, L.E. and A.D. Rosemond. 2004. Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. *Freshwater Biology* 49: 721-734.
- Finlay, J.C. 2001. Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. *Ecology* 82(4): 1052-1064.
- Finlay, J.C. 2003. Controls of streamwater dissolved inorganic carbon dynamics in a forested watershed. *Biogeochemistry* 62: 231-252.
- Finlay, J.C. 2004. Patterns and controls of lotic algal stable carbon isotope ratios. *Limnology and Oceanography* 49(3): 850-861.
- Finlay, J.C., M.E. Power and G. Cabana. 1999. Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. *Limnology and Oceanography* 44(5): 1198-1203.
- Finlay, J.C., R.R. Doucett and C. McNeely. 2010. Tracing energy flow in stream food webs using stable isotopes of hydrogen. *Freshwater Biology* 55: 941-951.
- France, R. 1995. Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 651-656.
- France, R. Carbon-13 conundrums: limitations and cautions in the use of stable isotope analysis in stream ecotonal research. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 1916-1919.
- France, R. and A. Cattaneo. 1998. $\delta^{13}\text{C}$ variability of benthic algae: effects of water colour via modulation by stream current. *Freshwater Biology* 39: 617-622.
- Franken, R.J.M., B. Waluto, E.T.H.M. Peeters, J.J.P. Gardeniers, J.A.J. Beijer and M. Scheffer. 2005. Growth of shredders on leaf litter biofilms: the effect of light intensity. *Freshwater Biology* 50: 459-466.
- Fry, B. 2006. *Stable Isotope Ecology*. Springer, New York, NY, USA.
- Fry, B. 2013. Alternative approaches for solving undetermined isotope mixing problems. *Marine Ecology Progress Series* 427: 1-13.

- Hadwen, W.L., M. Spears and M.J. Kennard. 2010. Temporal variability of benthic algal $\delta^{13}\text{C}$ signatures influences assessments of carbon flows in stream food webs. *Hydrobiologia* 651: 239-251.
- Harding, J.N., J.M.S. Harding and J.D. Reynolds. 2014. Movers and shakers: nutrient subsidies and benthic disturbance predict biofilm biomass and stable isotope signatures in coastal streams. *Freshwater Biology* 59: 1361-1377.
- Herwig, B.R., D.H. Wahl, J.M. Dettmers and D.A. Soluk. 2007. Spatial and temporal patterns in the food web structure of a large floodplain river assessed using stable isotopes. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 495-508.
- Hicks, B.J. 1997. Food webs in forest and pasture streams in the Waikato region, New Zealand: a study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. *New Zealand Journal of Marine and Freshwater Research* 31: 651-664.
- Hill, W.R., J. Rinchard and S. Czesny. 2011. Light, nutrients and the fatty acid composition of stream periphyton. *Freshwater Biology* 56: 1825-1836.
- Hill, W.R., S.E. Fanta and B.J. Roberts. 2008. ^{13}C dynamics in benthic algae: Effects of light phosphorus, and biomass development. *Limnology and Oceanography* 53(4): 1217-1226.
- Hladyz, S. R.A. Cook, R. Petrie and D.L. Nielsen. 2011. Influence of substratum on the variability of benthic biofilm stable isotope signatures: implications of energy flow to a primary consumer. *Hydrobiologia* 664: 135-146.
- Hladyz, S., D.L. Nielsen, P.J. Suter and E.S. Krull. 2012. Temporal variation in organic carbon utilization by consumers in a lowland river. *River Research and Applications* 28: 513-528.
- Hondula, K.L., M.L. Pace, J.J. Cole and R.D. Batt. 2014. Hydrogen isotope discrimination in primary producers: implications for aquatic food web studies. *Aquatic Sciences* 76: 217-229.
- Ishikawa, N.F., F. Hyodo and I. Tayasu. 2013. Use of carbon-13 and carbon-14 natural abundances for stream food web studies. *Ecological Research* 28: 759-769.
- Ishikawa, N.F., H. Doi and J.C. Finlay. 2012. Global meta-analysis for controlling factors on carbon stable isotope ratios of lotic periphyton. *Oecologia* 170: 541-549.

- Ishikawa, N.F., M. Uchida, Y. Shibata and I. Tayasu. 2010. A new application of radiocarbon (^{14}C) concentrations to stream food web analysis. *Nuclear Instruments and Methods in Physics Research B* 268: 1175-1178.
- Jardine, T.D. 2014a. Organic matter sources and size structuring in stream invertebrate food webs across a tropical to temperate gradient. *Freshwater Biology* 59: 1509-1521.
- Jardine, T.D. 2014b. Understanding and overcoming baseline isotopic variability in running waters. *River Research and Applications* 30: 155-165.
- Jardine, T.D., K.A. Kidd and R.A. Cunjak. 2009. An evaluation of deuterium as a food source tracer in temperate streams of eastern Canada. *Journal of the North American Benthological Society* 28(4): 885-893.
- Jardine, T.D., N.E. Pettit, D.M. Warfe, B.J. Pusey, D.P. Ward, M.M. Douglas, P.M. Davies and S.E. Bunn. 2012. Consumer-resource coupling in wet-dry tropical rivers. *Journal of Animal Ecology* 81: 310-322.
- Kobayshi, S., F. Akamatsu, K. Amano, S. Nakanishi and Y. Oshima. 2011. Longitudinal changes in $\delta^{13}\text{C}$ of riffle macroinvertebrates from mountain to lowland sections of a gravel bed river. *Freshwater Biology* 56: 1434-1446.
- Kominoski, J.S., S. Larranga and J.S. Richardson. 2012. Invertebrate feeding and emergence timing vary among streams along a gradient of riparian forest composition. *Freshwater Biology* 57: 759-772.
- Larned, S.T., P.M. Eldridge and R.A. Kinzie III. 2008. Modeling C and N flows through a stream food web: an inverse approach. *Journal of the North American Benthological Society* 27(3): 674-689.
- Lancaster, J. and S. Waldron. 2001. Stable isotope values of lotic invertebrates: Sources of variation, experimental design, and statistical interpretation. *Limnology and Oceanography* 46(3): 723-730.
- Lau, D.C.P., K.M.Y. Leung and D. Dudgeon. 2009. Are autochthonous foods more important than allochthonous resources to benthic consumers in tropical headwater streams? *Journal of the North American Benthological Society* 28(2): 426-439.
- Logan, J.M., T.D. Jardine, T.J. Miller, S.E. Bunn, R.A. Cunjak and M.E. Lutcavage. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modeling methods. *Journal of Animal Ecology* 77: 838-846.

- Lutz, S.R., H.J. van Meerveld, M.J. Waterloo, H.P. Broers and B.M. van Breukelen. 2013. A model-based assessment of the potential use of compound-specific stable isotope analysis in river monitoring of diffuse pesticide pollution. *Hydrology and Earth System Sciences* 17(11): 4505-4524.
- Mantel, S.K., M. Salas and D. Dudgeon. 2004. Foodweb structure in a tropical Asian forest stream. *Journal of the North American Benthological Society* 23(4): 728-755.
- McCutchan, J.H. W.M. Lewis, Jr., C. Kendall and C.C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. *Oikos* 102: 378-390.
- McGoldrick, D.J., D.R. Barton, M. Power, R.W. Scott, and B.J. Butler. 2008. Dynamics of bacteria-substrate stable isotope separation: dependence on substrate availability and implications for aquatic food web studies. *Canadian Journal of Fisheries and Aquatic Sciences* 65: 1983-1990.
- McNeely, C., S.M. Clinton and J.M. Erbe. 2006. *Journal of the North American Benthological Society* 25(4): 787-799.
- McWilliam-Hughes, S.M., T.D. Jardine and R.A. Cunjak. 2009. Instream C sources for primary consumers in two temperate, oligotrophic rivers: possible evidence for bryophytes as a food source. *Journal of the North American Benthological Society* 28(3): 733-743.
- Middleburg, J.J. 2014. Stable isotopes dissect aquatic food webs from the top to the bottom. *Biogeosciences* 11: 2357-2371.
- Minagawa, M. and E.Wada. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48(5): 1135-1140.
- Myers, D.J., G.W. Whitley and M.R. Whiles. 2012. Evaluation of δD and $\delta^{18}\text{O}$ as natural markers of invertebrate source environment and dispersal in the middle Mississippi river-floodplain ecosystem. *River Research and Applications* 28: 135-142.
- Parnell, A.C., D.L. Phillips, S. Bearhop, B.X. Semmens, E.J. Ward, J.W. Moore, A.L. Jackson, J. Grey, D.J. Kelly and R. Inger. 2013. Bayesian stable isotope mixing models. *Environmetrics* 24(6): 387-399.
- Peipoch, M., E. Marti and E. Gacia. 2012. Variability in $\delta^{15}\text{N}$ natural abundance of basal resources in fluvial ecosystems: a meta-analysis. *Freshwater Science* 31(3): 1003-1015.

- Perry, R.W., M.J. Bradford and J.A. Grout. Effects of disturbance on contribution of energy sources to growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) in boreal streams. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 390-400.
- Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology & Systematics* 18: 293-320.
- Phillips, D.L. and J.W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136: 261-269.
- Phillips, D.L. and P.L. Koch. 2002. Incorporating concentration dependence in stable isotope mixing models. *Oecologia* 130: 114-125.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* 83(3): 703-718.
- Post, D.M., C.A. Layman, D.A. Arrington, G. Takimoto, J. Quattrochi and C.G. Montana. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152: 179-189.
- Rasmussen, J.B. 2010. Estimating terrestrial contribution to stream invertebrates and periphyton using a gradient-based mixing model for $\delta^{13}\text{C}$. *Journal of Animal Ecology* 79: 393-402.
- Reid, D.J., G.P. Quinn, P.S. Lake and P. Reich. 2008. Terrestrial detritus supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study. *Freshwater Biology* 53: 2036-2050.
- Roach, K.A. 2013. Environmental factors affecting incorporation of terrestrial material into large river food webs. *Freshwater Science* 32(1): 283-298.
- Rozanski, K., L. Araguas-Araguas and R. Gonfiantini. 1993. Isotopic patterns in modern global precipitation. *Geophysical Monograph* 78: 1-36.
- Schmidt, F., H. Oberhansli and H. Wilkes. 2014. Biocoenosis response to hydrological variability in Southern Africa during the last 84 ka BP: a study of lipid biomarkers and compound-specific stable carbon and hydrogen isotopes from the hypersaline Lake Tswaing. *Global and Planetary Change* 112: 92-104.
- Simopoulos, A.P. 2004. Omega-3 fatty acids and antioxidants in edible plants. *Biological Research* 37: 263-277.

- Smyntek, P.M., M.A. Teece, K.L. Schulz and S.J. Thackeray. 2007. A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models. *Limnology and Oceanography* 52(5): 2135-2146.
- Solomon, C.T., J.J. Cole, R.R. Doucett, M.L. Pace, N.D. Preston, L.E. Smith and B.C. Weidel. 2009. The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers. *Oecologia* 161(2): 313-324.
- Solomon, C.T., S.R. Carpenter, M.K. Clayton, J.J. Cole, J.J. Coloso, M.L. Pace, M.J. Vander Zanden and B.C. Weidel. 2011. Terrestrial, benthic and pelagic resource use in lakes: results from a three isotope Bayesian mixing model. *Ecology* 92: 1115-1125.
- Soto, D.X., E. Gacia and J. Catalan. 2013a. Freshwater food web studies: a plea for multiple tracer approach. *Limnetica* 32(1): 97-106.
- Soto, D.X., L.I. Wassenaar and K.A. Hobson. 2013b. Stable hydrogen and oxygen isotopes in aquatic food web studies are tracers of diet and provenance. *Functional Ecology* 27: 535-543.
- Soto, D.X., L.I. Wassenaar, K.A. Hobson and J. Catalan. 2011. Effects of size and diet on stable hydrogen isotope values (δD) in fish: implications for tracing origins of individuals and their food sources. *Canadian Journal of Fisheries and Aquatic Sciences* 68: 2011-2019.
- Tank, J.L., E.J. Rosi-Marshall, N.A. Griffiths, S.A. Entekin and M.L. Stephen. 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society* 29(1): 118-146.
- Torres-Ruiz, M., J.D. Wehr and A.A. Perrone. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. *Journal of the North American Benthological Society* 26(3): 509-522.
- Trudeau, V. and J.B. Rasmussen. 2003. The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton. *Limnology & Oceanography* 48(6): 2194-2199.
- Volk, C. and P. Kiffney. 2012. Comparison of fatty acids and elemental nutrients in periphyton, invertebrates and cutthroat trout (*Oncorhynchus clarki*) in conifer and alder streams of western Washington state. *Aquatic Ecology* 46: 85-99.
- Walters, A.W. and D.M. Post. 2008. An experimental disturbance alters fish size structure but not food chain length in streams. *Ecology* 89(12): 3261-3267.

- Walters, D.M., K.M. Fritz and D.L. Phillips. 2007. Reach-scale geomorphology affects organic matter and consumer $\delta^{13}\text{C}$ in a forested Piedmont stream. *Freshwater Biology* 52: 1105-1119.
- Ward, E.J., B.X. Semmens, D.L. Phillips, J.W. Moore and N. Bouwes. 2011. A quantitative approach to combine sources in stable isotope mixing models. *Ecosphere* 2(2), Article 19.
- Watson, A. and L.A. Barmuta. 2011. Feeding-preference trials confirm unexpected stable isotope analysis results: freshwater macroinvertebrates do consume macrophytes. *Marine and Freshwater Research* 62: 1248-1257.
- Wilkie, K.M.K., B. Chaplignin, H. Meyer, S. Burns, S. Petsch and J. Brigham-Grette. 2013. Modern isotope hydrology and controls on delta D of plant leaf waxes at Lake El'gygytgyn, NE Russia. *Climate of the Past* 9(1): 335-352.
- Winemiller, K.O. and D.B. Jepsen. 2004. Migratory neotropical fish subsidize food webs of oligotrophic blackwater rivers. Pages 115-132 in: G.A. Polis, M.E. Power and G.R. Huxel, eds. *Food Webs at the Landscape Level*, University of Chicago Press, Chicago.
- Wilkinson, G.M., S.R. Carpenter, J.J. Cole, M.L. Pace and C. Yang. 2013. Terrestrial support of pelagic consumers: patterns and variability revealed by a multilake study. *Freshwater Biology* 58: 2037-2049.
- Wilson, K.P. and D.W. Blinn. 2007. Food web structure, energetics, and importance of allochthonous carbon in a desert cavernous limnocrone: Devils Hole, Nevada. *Western North American Naturalist* 67(2): 185-198.
- Winemiller, K.O., D.J. Hoeninghaus, A.S. Pease, P.C. Esselman, R.L. Honeycutt, D. Gbanaador, E. Carrera and J. Payne. 2011. Stable isotope analysis reveals food web structure and watershed impacts along the fluvial gradient of a Mesoamerican coastal river. *River Research and Applications* 27: 791-803.
- Wolf, N., S.A. Carleton and C. Martinez del Rio. 2009. Ten year of experimental animal isotope ecology. *Functional Ecology* 23: 17-26.
- Woodland, R.J., M.A. Rodriguez, P. Magnan, H. Glemet and G. Cabana. 2012. Incorporating temporally dynamic baselines in isotope mixing models. *Ecology* 93(1): 131-144.
- Yang, C., G.M. Wilkinson, J.J. Cole, S.A. Macko and M.L. Pace. 2014. Assessing hydrogen, carbon, and nitrogen isotope values for phytoplankton and terrestrial detritus in aquatic food web studies. *Inland Waters* 4: 233-242.

CHAPTER 2

THE IMPORTANCE OF TERRESTRIAL SUBSIDIES IN STREAM FOOD WEBS VARIES ALONG A STREAM SIZE GRADIENT

Abstract

Energy and material subsidies can comprise a substantial fraction of food web fluxes in some ecosystems, especially when autochthonous primary production is strongly limited by light or nutrients. We explored whether assimilation of terrestrial energy varied in the same taxon of benthic invertebrates and fish collected from streams of different sizes and resource availabilities. Since headwater streams are often unproductive, we expected that inputs from surrounding terrestrial systems (*i.e.*, leaf litter, terrestrial invertebrates) were likely to be an important food source for consumers, while mid-size rivers typically would have more open canopies and higher amounts of primary production available for consumers. We collected basal resources, invertebrates, and fish along stream size gradients in the Adirondack Mountains (NY) and in Trinidad & Tobago and analyzed each sample for hydrogen isotope ratio, an indicator of material derived from allochthonous versus autochthonous sources. Consistent with expectations, allochthonous energy use was positively correlated with canopy cover in both regions, with individuals from small, shaded streams having a more allochthonous signal than individuals collected from larger streams with more open canopies. Our results demonstrate that the importance of energy from terrestrial subsidies can vary markedly and further show that some taxa

range from being entirely allochthonous to entirely autochthonous, while other taxa are relatively fixed in the source of energy they assimilate.

Introduction

The transfer of energy and materials from one ecosystem to another ecosystem can have strong effects on the food webs and community structure of both donor and recipient systems (Polis et al. 2004). Previous research has demonstrated that allochthonous energy subsidies from riparian habitats into streams can be very important to freshwater food webs, especially in heavily shaded streams where aquatic primary production is strongly limited by light availability (*e.g.*, Webster et al. 1999, Nakano & Murakami 2001) and the supply of external detritus is high. The role of cross-system subsidies likely varies depending on environmental conditions and consumer attributes. Understanding which environmental factors influence subsidy use by consumers will improve our knowledge of under-studied detrital food web pathways (Moore et al. 2004) and our predications about how land use change will alter stream ecosystems (Allan 2004).

Subsidies from terrestrial environments have been recognized for decades as an important energy source in stream food webs (Hynes 1975, reviewed by Tank et al. 2010). Early descriptions of the River Continuum Concept (Vannote et al. 1980) suggested that differences in food availability should dictate aquatic invertebrate community structure, specifically the distribution of functional feeding groups (FFGs) of aquatic invertebrates. For example, headwater streams should contain a high

abundance of leaf-shredding invertebrates, while mid-sized rivers should contain a higher abundance of invertebrates that scrape algal biofilms.

However, more recent studies highlight the observation that “functional” feeding groups may really be quite “flexible”. For example, shredders can eat algae (Dangles 2002) and can sometimes grow better on algae than leaf litter. Likewise, predatory invertebrates can be omnivorous and eat algae (Lancaster *et al.* 2005) and scraper caddisflies preferentially assimilate algal elements of the biofilm while scraper mayflies assimilate bulk biofilm (McNeilly *et al.* 2006). Even in streams where detrital resources are readily available, some invertebrates preferentially assimilate much less abundant but high-quality algal food (McCutchan & Lewis 2002, Lau *et al.* 2009). In summary, studies during the past decade and a half suggest that many stream invertebrates may typically consume whatever type of organic matter is available, rather than what is expected based on functional group classification (Mihuc 1997). Because benthic invertebrate diets have high potential for flexibility, we used stream consumers to explore whether the same individual taxa can utilize both subsidized (allochthonous) organic matter from terrestrial systems and autochthonous primary production, and if so, in what proportions and under what extent of canopy cover.

Evaluating what type of organic matter is ingested and assimilated by consumers can be challenging. Examining gut contents can be effective for quantifying what food was consumed at a single time point, but it does not actually indicate what is assimilated and converted into tissue. Several types of isotopic methods can be used to examine assimilation. Natural abundances of carbon (C) and

nitrogen (N) stable isotopes in the tissue of an individual are effective tracers of diet in some systems, especially lakes (*e.g.* Vander Zanden *et al.* 1999, Post *et al.* 2000, Post 2002). However, leaf litter and epilithon often do not have distinct $\delta^{13}\text{C}$ signatures in stream systems (France 1995), making it difficult to match consumers and food sources with C and N natural abundance data. Another approach is to add an isotopic tracer that labels only the allochthonous or autochthonous pool and subsequently trace them through the food web (*e.g.*, Hall & Meyer 1998, Pace *et al.* 2004). However, tracer studies are time- and cost-intensive, and so are less effective for cross-system comparisons. The amount of tracer that is required to label food web compartments makes it so that tracer studies are only feasible in small streams or lakes.

Recently, hydrogen (H) isotopes have been successfully used to quantify allochthonous contributions in freshwater food webs (Doucett *et al.* 2007, Deines *et al.* 2009, Finlay *et al.* 2010, Babler *et al.* 2011, Cole *et al.* 2011, Dekar *et al.* 2012). Differences in hydrogen fractionation in water by terrestrial and aquatic plants lead to extremely different $\delta^2\text{H}$ signatures ($\sim 100\text{‰}$) in their tissues, which results in allochthonous resources in streams being substantially more enriched in $\delta^2\text{H}$ than autochthonous resources. Methodological issues for H isotopes have been investigated in preliminary laboratory studies (Jardine *et al.* 2009, Solomon *et al.* 2009, Soto *et al.* 2011, Soto *et al.* 2013, Graham *et al.* 2014, Hondula *et al.* 2014), especially concerns about the incorporation of H from both water and diet at varying proportions depending on the taxonomic group of interest. Values for dietary water incorporation from laboratory studies have made it possible to quantify with reasonable reliability allochthonous energy use with H isotope ratios (Solomon *et al.*

2009, Soto *et al.* 2013), and H data have often been more useful than C isotope ratios, or useful as a secondary tracer in conjunction with $\delta^{13}\text{C}$ data.

In the study reported here, we used H isotope ratios to examine within-species variation in allochthonous resource use by invertebrates and fish in streams in the Adirondack Mountains (New York, USA) and in the Northern Range of Trinidad (Trinidad and Tobago). Specifically, we aimed to determine whether allochthonous resource use by consumers (*i.e.*, invertebrates, fishes) is plastic and driven by resource availability, or is relatively fixed for each taxon. If resource use is plastic, we expect to find that individuals within a single taxonomic group assimilate more allochthonous energy in headwater streams with high inputs of leaf litter and low levels of primary production, and assimilate more autochthonous energy from in-stream primary production in larger streams with more open canopies and higher levels of primary production. If resource use is a fixed characteristic of a single species, we expect that individuals from the same taxonomic group would assimilate comparable amounts of allochthonous energy regardless of resource availability at a site.

Methods

Study sites

We conducted our research on the tropical island of Trinidad, Trinidad and Tobago, and in the Adirondack Mountains, New York State, USA. In Trinidad, we collected samples from three river drainages in the Northern Range (Figure 2.1), one on the south side of the mountains (Aripo River), one on the east side (Quare River) and one on the north side (Marianne River). In each drainage, we collected samples in



Figure 2.1: Map of study sites in Trinidad. River drainages are abbreviated as follows: M=Marianne River, A=Aripo River, Q=Quare River.

three types of sites: 1) small, highly-shaded headwater sites that are high in the drainage and contain no fish other than killifish, *Rivulus hartii* (upstream sites), 2) slightly larger, still relatively shaded sites that are typically lower in the drainage and contain both *Rivulus* and guppies (*Poecilia reticulata*) but no other fish species (midstream sites), and 3) larger streams that are lower in the drainage, have relatively open canopies, and contain a diverse fish community including *Rivulus*, *P. reticulata*, and predatory fishes (downstream sites). The differences in light, resource availability and fish community among our sampling sites (as described above) have been characterized in several previous studies (Grether *et al.* 1997, Kohler *et al.* 2012, Zandonia *et al.* 2011). We collected samples in all nine sites during the middle of the dry season (*i.e.*, late February-March) in 2009 and 2013.

In the Adirondacks, we collected samples in the Moose River, a 4th order river with an open canopy, in five sites along two tributaries of the Moose River (Otter Brook and Combs Brook), and in Blues Brook, a small tributary with an experimentally thinned canopy that increased light availability (Figure 2.2). In Otter Brook, we sampled a small, highly shaded tributary site in the headwater region (Otter Headwater), and a larger site with a more open canopy near where Otter Brook enters the Moose River (Otter Camp). We sampled three sites on Combs Brook, ranging from a very small headwater site to one near where Combs Brook enters the Moose River (Combs Headwater, Combs Trailcross, Combs Hatchery, respectively). Blues Brook and all of the Otter Brook and Combs Brook sites contained brook trout (*Salvelinus fontinalis*) and no other fish species. We collected samples in the

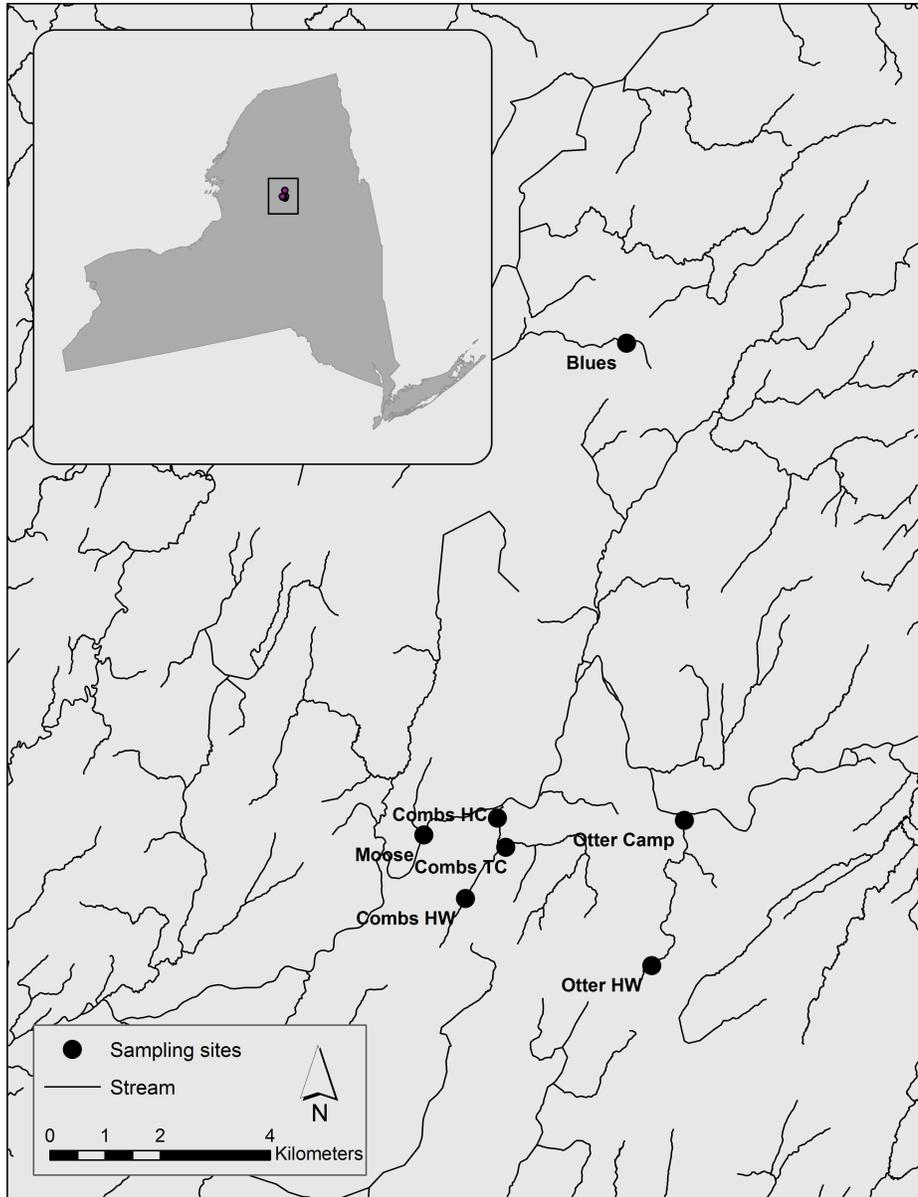


Figure 2.2: Map of study sites in the Adirondack region of New York.

Adirondacks during July and August 2011, which is typically the driest part of the summer with few rain events.

Field collections

At all sites, we quantified canopy cover using a convex densiometer (Ben Meadows, Model A) and quantified standing stocks of basal food sources. The approximate size of each stream was characterized by measuring the width of the channel at 4-5 haphazardly selected points along the reach. Terrestrial leaf matter and wood were quantified at the same locations where we took leaf measurements by collecting all leaf matter along a 20 cm wide transect across the stream at that point. We collected leaf material from 4-5 transects in each site. Samples were dried at 55°C, weighed, homogenized by grinding with a mortar and pestle, and subsampled for isotopic analysis. Algal standing stocks were quantified by scrubbing biofilm from rocks of known surface area and filtering subsamples of epilithon on Whatman (0.7 µm) GFF glass-fiber filters. We used one filter to quantify chlorophyll-*a* and a second to quantify ash-free dry mass (AFDM). To quantify chlorophyll-*a*, we placed filters with epilithon samples in film canisters and extracted chlorophyll-*a* in 15 mL of 90% ethanol for 18-24 hours. After extraction, we quantified chlorophyll-*a* using an Aquaflor handheld flurometer (Turner Designs, Sunnyvale, CA). We measured AFDM by filtering material on a preweighed Whatman GF/F filter (0.7 µm pore size), drying the filter at 55°C for 24 hours, and ashing the filter at 450°C for six hours. We calculated AFDM as the difference between the weight of the filter with the dry sample and the weight of the filter with the ashed sample. We also collected non-

quantitative samples of fine benthic organic matter in all sites using a turkey baster and opportunistically collected filamentous algae or diatom films in sites where they were present either by hand or using a turkey baster.

We collected a range of consumer taxa for isotopic analysis. In Trinidad, we used hand nets to collect guppies (*P. reticulata*) in downstream and midstream sites, and killifish (*Rivulus*) in all sites. We recorded standard length and wet weight and removed all fish guts before drying fish at 55°C and grinding whole fish for isotopic analysis. Most streams in Trinidad contained similar dominant invertebrate taxa, which we collected opportunistically when they were present at a site. Common taxa (and their FFG classifications determined based on Merritt *et al.* 2008) included: *Thraulodes* sp., a mayfly collector-gatherer; *Argia* sp., a predatory damselfly; *Psephenus* sp., a coleopteran scraper; *Anacroneturia* sp., a predatory stonefly; *Euthyplocia* sp., a predatory mayfly; *Smicridea* sp., a caddisfly filter-feeder; *Phylloicus* sp., a caddisfly shredder; *Simulium* sp., a dipteran collector-filterer; and *Petrophila* sp., a lepidopteran scraper. Larger downstream sites with more light availability typically have more diverse and abundant invertebrate faunas (Zandona *et al.* 2011), and we were generally able to collect more taxonomic groups at downstream sites. We removed invertebrate guts manually under a dissecting microscope for taxa that were sufficiently large-bodied (i.e., *Argia*, *Anacroneturia*, *Euthyplocia*, *Smicridea*, *Phylloicus*, *Petrophila*). We kept small-bodied taxa live in plastic containers for at least six hours so they could evacuate their guts as much as possible before analysis.

In the Adirondacks, we also collected invertebrates opportunistically. Common taxa (and their FFG classifications) included: *Heptagenia* sp., *Maccaffertium* sp., *Stenacron* sp., and *Epeorus* sp., all scraper mayflies; *Isoperla* sp., a predatory stonefly, *Pycnopsyche* sp, a caddisfly shredder; *Pteronarcys* sp., a stonefly shredder; *Lanthus* sp., a predatory dragonfly; and *Ephemerella* sp., a collector-gatherer mayfly. We removed guts manually from all invertebrate species. Using a backpack electrofishing unit, we collected brook trout (*Salvelinus fontinalis*) at all collection sites except the Moose River, where they were likely not present because of higher water temperatures (D. Josephson, personal communication). Fish were measured and weighed, guts were removed and a sample of muscle tissue was saved for isotopic analysis.

All isotope samples from both regions were dried at 55°C, ground to a fine powder using a mortar and pestle, and approximately 0.3 mg of dry sample was packaged into silver capsules. All $\delta^2\text{H}$ tissue and water samples were analyzed at the Cornell University Stable Isotope Laboratory (COIL, Ithaca, NY, USA). Tissue samples were analyzed using a Temperature Conversion Elemental Analyzer interfaced to a Thermo Delta V isotope ratio mass spectrometer (both manufactured by Thermo Fisher Scientific, Bremen, Germany) and water samples were analyzed using a Gasbench II (Thermo Fisher Scientific, Bremen, Germany) interfaced to a Thermo Delta V isotope ratio mass spectrometer. Both water and tissue samples are expressed in units of per mil (‰) relative to Vienna Standard Mean Ocean Water.

Dietary water correction

Because animals assimilate H from both food and water, we collected water samples at each site for H isotope analysis to correct consumer $\delta^2\text{H}$ for the contribution of dietary water. Filtered water samples were stored in vials sealed with a rubber gasket with no headspace air until they were analyzed at COIL. We used a simple two-ended mixing model to account for dietary water (Babler *et al.* 2011, Solomon *et al.* 2009):

$$\delta^2 H_{corrected} = \frac{\delta^2 H_{uncorrected} - \omega \delta^2 H_{water}}{1 - \omega}$$

We solved for corrected $\delta^2\text{H}$ of each consumer using $\delta^2\text{H}_{\text{water}}$ of the collection site, uncorrected values for consumer $\delta^2\text{H}$, and published values for ω , which represents the proportion of H derived from water for a given consumer taxon (Solomon *et al.* 2009). We did not account for fractionation because previous laboratory studies have found fractionation to be negligible for deuterium (^2H) in aquatic systems (Solomon *et al.* 2009). Since we did not directly measure ω for the consumers in our study, we explored a range for ω in our analyses based on published values from laboratory experiments (Solomon *et al.* 2009), as described below.

Calculating allochthonous contributions to diets

Because epilithon is a mixture of detritus, heterotrophic bacteria and microconsumers as well as primary producers (algae and cyanobacteria), it is often not representative of an autochthonous autotrophic food source. We corrected epilithon values to estimate an autotrophic autochthonous end member as follows: for sites

where patches of filamentous green algal material were present, we were able to use direct measurements of $\delta^2\text{H}$ for filamentous algae to estimate an autochthonous end member. At sites where we could not directly collect algal material, we estimated $\delta^2\text{H}_{\text{algae}}$ by creating a correction factor based on the ratio of known $\delta^2\text{H}_{\text{algae}}$ to $\delta^2\text{H}_{\text{epilithon}}$ at other sites. We weighted correction factors by the ratio of chlorophyll-*a* to AFDM in epilithon at the site with known $\delta^2\text{H}_{\text{algae}}$ to the site with an unknown $\delta^2\text{H}_{\text{algae}}$ because the amount of chlorophyll relative to total organic matter in the epilithon should drive the difference of $\delta^2\text{H}_{\text{algae}}$ and $\delta^2\text{H}_{\text{epilithon}}$ (detailed in Appendix A). Several published studies have noted that $\delta^2\text{H}_{\text{algae}}$ is equal to $\delta^2\text{H}_{\text{water}}$ plus approximately -150 to -170‰ (Babler *et al.* 2011). In most cases, adding -160‰ to our $\delta^2\text{H}_{\text{water}}$ data gave extremely similar values for $\delta^2\text{H}_{\text{algae}}$ compared with our other correction method, so we used $\delta^2\text{H}_{\text{algae}}$ derived from both correction methods in our simulations to estimate percent allochthonous contributions.

We ran simulations that varied dietary water contributions and $\delta^2\text{H}_{\text{algae}}$ to calculate the percentage of a consumer's tissue that was derived from allochthonous material. We used the following equation to calculate the percent of allochthonous contributions to a consumer's diet:

$$\%_{\text{alloch}} = \frac{\delta^2 H_{\text{consumer}} - \delta^2 H_{\text{algae}}}{\delta^2 H_{\text{alloch}} - \delta^2 H_{\text{algae}}} \times 100$$

For each consumer taxon, we ran 10,000 simulations that randomly selected an individual consumer's uncorrected $\delta^2\text{H}$ (sample sizes ranged from 1 to 4 individuals per taxon per site), a value for ω that was randomly selected from a normal distribution of ω based on published means and standard deviations for each taxon

(Solomon *et al.* 2009), and a value for $\delta^2\text{H}_{\text{algae}}$ that was randomly selected from a uniform distribution of $\delta^2\text{H}_{\text{algae}}$, which was bounded by our independent estimates of the autochthonous end member as described above. In cases where we had at least three direct measurements of $\delta^2\text{H}_{\text{algae}}$ from filaments or diatoms, we randomly selected a value from a normal distribution with mean and standard deviation of the independent estimates of $\delta^2\text{H}_{\text{algae}}$. In cases where we knew the mean and standard deviation of leaf litter $\delta^2\text{H}$ (rather than just a single point estimate), we also sampled from a normal distribution of potential values for $\delta^2\text{H}_{\text{alloch}}$. This simulation approach produced a mean and standard deviation for allochthonous energy use, which allowed us to evaluate how variance in $\delta^2\text{H}$ among individuals, uncertainty of ω and uncertainty of $\delta^2\text{H}_{\text{algae}}$ influenced our estimates of allochthonous energy use.

We used a permutation test to make statistical comparisons of our percent allochthonous energy estimates for individuals among sites. We determined the difference in mean allochthonous energy assimilation between the taxon of interest at two sites and comparing it to a null distribution differences in allochthonous energy use. To create a null distribution of differences in allochthonous energy use for a taxon of interest between two sites, we combined replicate percent allochthonous energy use estimates for the taxon of interest for two sites into a single pool, separated them into two random groups, and calculated difference in means between the two groups. We iterated this procedure 1000 times to create a null distribution of differences between random groups, and then determined where the actual difference between our two groups with known site assignment data fell. If the difference among

means was outside the 95% confidence interval of the distribution, we considered differences among groups significant at the $p=0.05$ level.

We used multiple linear regression to examine how allochthonous energy use varied with canopy cover. Since different sites sometimes contained different taxa of the same presumed functional feeding group or more than one taxon of the same presumed functional feeding group, we created multiple candidate models to evaluate which taxa or functional feeding groups had the same response in allochthonous energy use to canopy cover. We grouped similar taxa and functional groups and evaluated whether species fit the same model using model selection. We selected the best-fitting, most parsimonious model using Akaike's Information Criterion (AIC, Burnham and Anderson 2002). All statistical analyses, including simulations and regressions, were conducted in R (R Core Team 2013).

Results

Standing stocks of allochthonous and autochthonous organic matter varied predictably with stream size and canopy cover, with smaller, more shaded streams generally having lower standing stocks of chlorophyll-*a* and higher standing stocks of leaf litter (Table 2.1). In Trinidad, stream width was negatively correlated with canopy cover (Figure 2.3, linear regression, $R^2=0.67$, $t=-3.8$, $df=7$, $p<0.01$), and canopy cover was negatively correlated with standing stocks of chlorophyll-*a* (Figure 3 linear regression, $R^2=0.71$, $t=-4.12$, $df=7$, $p<0.01$), and positively but insignificantly correlated with standing stocks of leaf litter (Figure 2.3, linear regression, $R^2=0.23$, $t=1.46$, $df=7$, $p=0.19$). In the Adirondacks, stream width was negatively correlated

Table 2.1: Characteristics of sites, including size, canopy cover, and standing stocks of autochthonous and allochthonous resources.

	Stream width (m)	Canopy Cover (% closed)	Epilithon Chla (mg m ⁻²)	Epilithon AFDM (g m ⁻²)	Leaf DM (g m ⁻²)	Wood DM (g m ⁻²)
Trinidad						
Quare DS	12.5	54%	26.2 (15)	5.30 (2.9)	42.8 (54)	0
Quare MD	2.37	95%	7.53 (5.3)	7.40 (5.9)	80.3 (68)	0
Quare US	1.91	93%	12.2 (3.5)	6.33 (5.2)	84.6 (44)	0
Aripo DS	17.4	72%	15.6 (8.0)	3.20 (1.9)	28.3 (31)	2.46 (4.9)
Aripo MD	1.63	89%	19.3 (10)	7.13 (3.9)	58.7 (51)	0
Aripo US	2.52	94%	7.41 (5.1)	6.39 (5.4)	127 (106)	0
Marianne DS	5.55	77%	13.9 (5.3)	2.43 (1.5)	14.0 (16)	0
Marianne MD	3.55	88%	10.8 (6.7)	1.87 (1.1)	11.8 (17)	0
Marianne US	2.12	93%	9.43 (6.2)	2.37 (1.2)	44.6 (15)	0
Adirondacks						
Moose River	36.4	5%	18.6 (6.8)	2.05 (.19)	0.057 (0.09)	0.158 (0.27)
Blues Brook	1.53	49%	9.14 (3.9)	1.31 (.35)	-	-
Combs Headwater	1.73	89%	2.01 (0.36)	1.21 (.41)	31.9 (28)	247 (219)
Combs Trail	2.05	86%	2.82 (1.7)	1.21 (.15)	5.57 (9.6)	74.7 (61)
Combs Hatchery	6.13	83%	7.31 (2.3)	0.979 (.44)	8.26 (9.5)	41.4 (56)
Otter Headwater	2.67	78%	3.94 (1.6)	1.20 (.49)	19.8 (19)	140 (26)
Otter Camp	7.57	70%	-	-	6.38 (6.0)	35.2 (10)

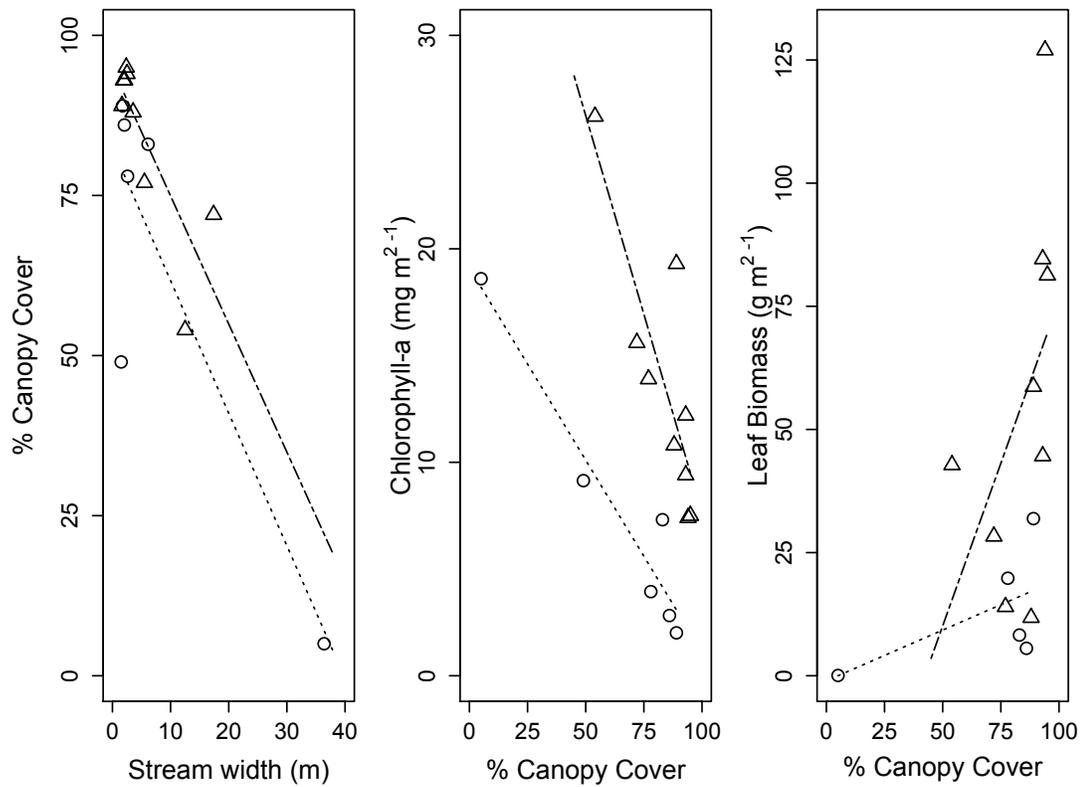


Figure 2.3: In both Trinidad and Adirondack study sites, canopy cover is negatively correlated to stream width, chlorophyll-*a* biomass is negatively correlated to canopy cover, and leaf biomass is positively correlated to canopy cover. Triangle symbols and double dashed lines represent Trinidad data, and circle symbols and dotted lines represent Adirondack data.

with canopy cover (Figure 2.3, linear regression, $R^2=0.76$, $t=-3.6$, $df=4$, $p<0.01$), and canopy cover was negatively correlated with standing stocks of chlorophyll-*a* (Figure 2.3, linear regression, $R^2=0.92$, $t=-7.00$, $df=4$, $p<0.01$), and positively but insignificantly correlated with standing stocks of leaf litter (Figure 2.3, linear regression, $R^2=0.35$, $t=1.28$, $df=3$, $p=0.29$). In the Adirondacks, the Moose River had a far more open canopy (5% cover) than any of the smaller tributaries, followed by the canopy-thinned tributary, Blues Brook (49% cover). In Trinidad, more open downstream sites ranged from 54-77% canopy cover while midstream and upstream sites had more canopy cover, ranging from 88-95% (Table 2.1).

Allochthonous contributions to consumer diets varied between sites and among species in both temperate and tropical streams. In the Adirondacks, all taxa except *Epeorus* had a predominantly allochthonous signal (Figure 2.4). Individuals from the more open sites (Moose River, Blues Brook) were more autochthonous for some taxa (Figure 2.4; heptageniid mayflies and *Ephemerella* in the Moose River, *Epeorus* in Blues Brook), but were not different from sites with more canopy cover for other taxa (Figure 2.4; *Pycnopsyche*, *Isoperla*, *S. fontinalis*). No apparent patterns existed within the smaller, more shaded sites on Combs Brook and Otter Brook.

In Trinidad, trends differed among the three river drainages (Figures 2.5-2.7), with more flexibility in energy use in the Quare River drainage, less flexibility in the Aripo River drainage, and no differences in the Marianne River drainage. Sites in the Quare drainage showed stark within-taxon differences in allochthonous energy use among site types, with downstream sites, which have low canopy cover, consistently more autochthonous than midstream or upstream sites for many taxa (Figure 2.5).

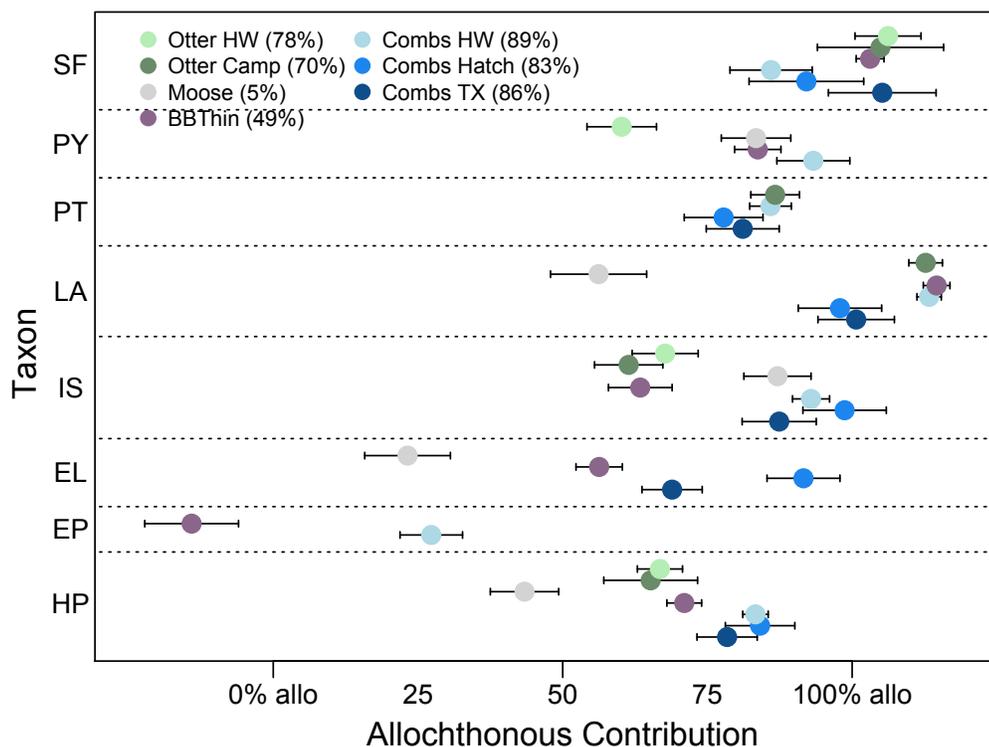


Figure 2.4: Allochthonous energy assimilated by invertebrates and fish in Adirondack stream sites as determined by $\delta^2\text{H}$ method. Taxon codes for consumers on the y-axis are as follows: SF= *S. fontinalis*, PY= *Pycnopsyche*, PT= *Pteronarcis*, LA= *Lanthus*, IS= *Isoperla*, EL= *Ephemerella*, EP= *Epeorus*, HP= *Heptagenia* or *Mccaffertium*. Percent canopy cover for each site is indicated next to the site name in the figure. Site abbreviations are as follows: HW=headwater, TX=trailcross, Hatch=Hatchery, BBThin=Blues Brook. Error bars indicate one standard deviation that was generated from the bootstrapping procedure to estimate % allochthony.

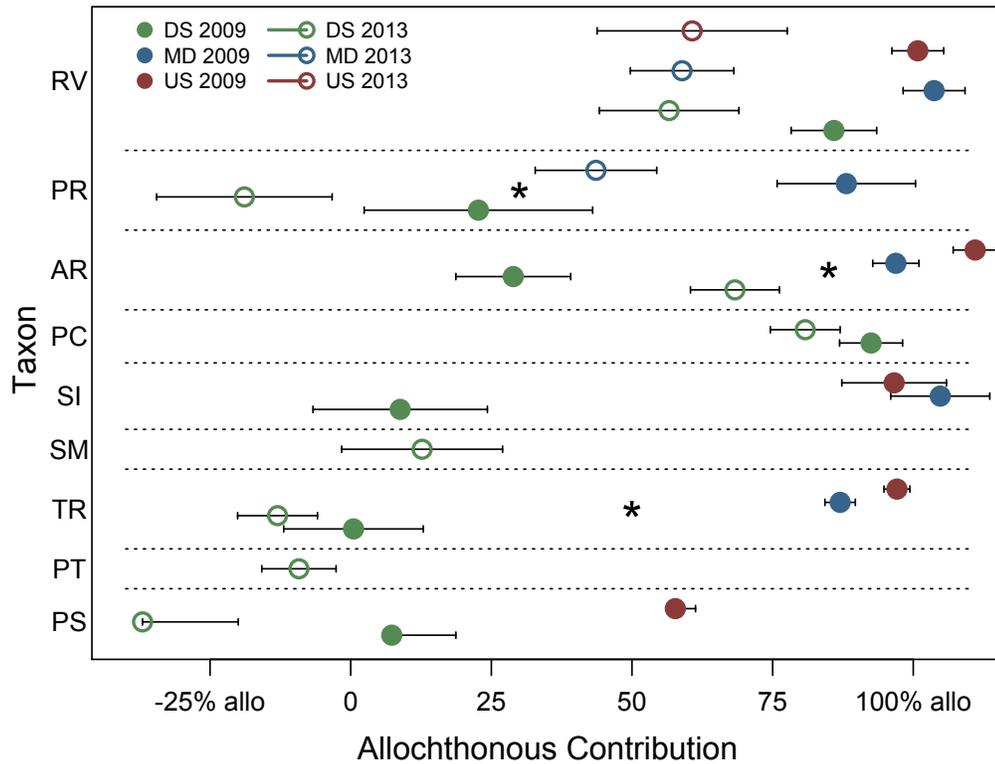


Figure 2.5: Allochthonous energy assimilated by invertebrates and fishes in Quare River (Trinidad) stream sites as determined by $\delta^2\text{H}$ method. Sites are color coded by downstream (DN), midstream (MD) and upstream (UP) sampling locations. Taxon codes for consumers on the y-axis are as follows: RV= *Rivulus*, PR= *P. reticulata*, AR= *Argia*, SM= *Smicridea*, PC= *Phylloicus*, PT= *Petrophila*, SI= *Simulium*, TR= *Tricorythodes*, PS= *Psephenus*. Canopy cover for each site is indicated next to the site name in the figure. Error bars indicate one standard deviation that was generated from the bootstrapping procedure to estimate % allochthony. Asterisks (*) show contrasts where sites differed at the $p < 0.05$ level.

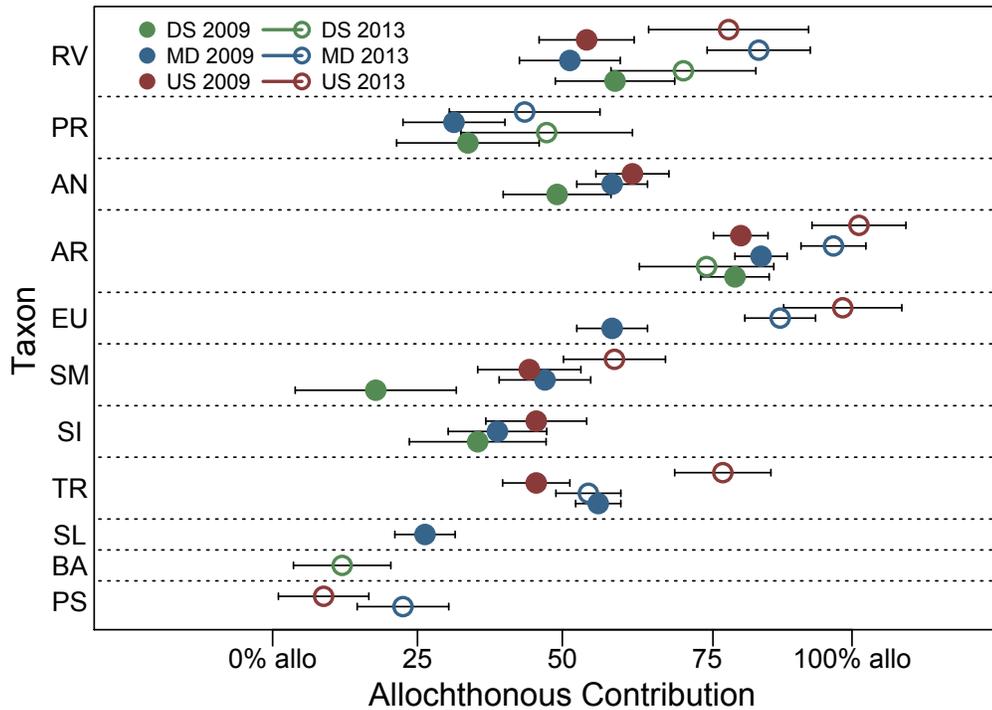


Figure 2.6: Allochthonous energy assimilated by invertebrates and fishes in Marianne River (Trinidad) stream sites as determined by $\delta^2\text{H}$ method. Sites are color coded by downstream (DN), midstream (MD) and upstream (UP) sampling locations. Taxon codes for consumers on the y-axis are as follows: RV= *Rivulus*, PR= *P. reticulata*, AN= *Anacroneturia*, AR= *Argia*, EU = *Euthyplocia*, SM= *Smicridea*, SI= *Simulium*, TR= *Tricorythodes*, SL= unknown snail, BA = *Baetodes*, PS= *Psephenus*. Canopy cover for each site is indicated next to the site name in the figure. Error bars indicate one standard deviation that was generated from the bootstrapping procedure to estimate % allochthony.

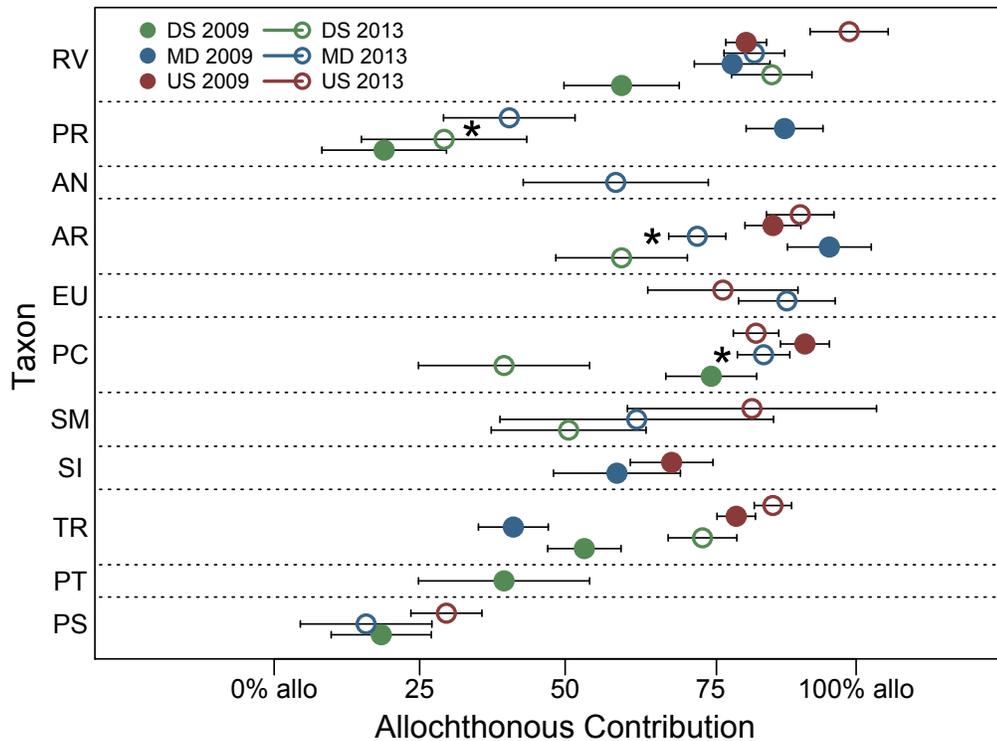


Figure 2.7: Allochthonous energy assimilated by invertebrates and fishes in Aripo River (Trinidad) stream sites as determined by $\delta^2\text{H}$ method. Sites are color coded by downstream (DN), midstream (MD) and upstream (UP) sampling locations. Taxon codes for consumers on the y-axis are as follows: RV= *Rivulus*, PR= *P. reticulata*, AN= *Anacroneuria*, AR= *Argia*, EU = *Euthyplocia*, PC= *Phylloicus*, SI= *Simulium*, SM= *Smicridea*, TR= *Tricorythodes*, PT= *Petrophila*, PS= *Psephenus*. Canopy cover for each site is indicated next to the site name in the figure. Error bars indicate one standard deviation that was generated from the bootstrapping procedure to estimate % allochthony. Asterisks (*) show contrasts where sites differed at the $p < 0.01$ level.

Differences among sites were significant for *P. reticulata* ($p=0.01$), *Argia* ($p=0.03$), and *Tricorythodes* ($p=0.05$) based on permutation tests. Sample sizes of fewer than 3 individuals for some sites made it so differences were not statistically significant (for *Simulium* and *Psephenus*) despite being substantial. In contrast, no significant differences among site types existed in the Marianne drainage (Figure 2.6). The Aripo drainage was intermediate, with some taxa (*Argia*, *Phylloicus*, *P. reticulata*) that were significantly more autochthonous in downstream sites ($p<0.01$ for all three taxa, Figure 2.7). The difference in canopy cover between site types is also greatest for the Quare, intermediate for the Aripo and smallest for the Marianne drainage (Table 2.1).

Allochthonous energy use was significantly related to canopy cover for most taxonomic groups (Table 2.2). In Trinidad, there were significant negative relationships with canopy cover for all fish and invertebrate taxa except shredders (Figure 2.8, Table 2.2), but the slope of the regression was much greater for scrapers and collector-gatherers/collector-filterers than it was for predators (Figure 2.8, Table 2.2). Canopy cover was also significant in the model for scraper and collector-gatherers in the Adirondacks, but not for predators or shredders (Figure 2.9, Table 2.2). Candidate models that included taxon as a predictor were less parsimonious than models that included only canopy cover, indicating that the taxonomic groups that we included in each regression followed the same trends in allochthonous energy use as a function of canopy cover (Table 2.2).

Table 2.2: Multiple linear regression model selection and coefficients. AIC_c , Akaike weights (w_i) and evidence ratios included for models with evidence ratios <10 . All models with interactions between canopy and taxon exceeded 10 and are not shown. The most parsimonious models (lowest AIC_c) are italicized and any significant model coefficients are bolded. Canopy was significant in all models except Adirondack predator-shredders, and models with only canopy were always more parsimonious than models with both canopy and species. Taxonomic abbreviation for consumers are as follows: SI = *Simulium*, TR = *Tricorythodes*, AR = *Argia*, EU = *Euthyplocia*, PC = *Phylloicia*, RV = *Rivulus*, HP =

Model	Intercept	Canopy	Model Coefficients				Delta AIC_c	w_i	w_i/w_1
<i>Trinidad scraper-grazer</i> <i>Canopy</i>	74.7	-23.8					0.000	1.000	1.000
	Intercept	Canopy	SI	TR					
<i>Trinidad collector-gatherer</i> <i>Canopy</i>	134.2	-32.3					0.000	0.877	1.000
Canopy + taxon	127.1	-31.4	5.3	8.9			4.030	0.117	7.501
	Intercept	Canopy	AR	EU	RV				
<i>Trinidad predator</i> <i>Canopy</i>	103.7	-10.6					0.000	0.771	1.000
Canopy + taxon	85.3	-11.3	27.1	22.3	19.9		2.430	0.229	3.370
	Intercept	Canopy							
<i>Trinidad shredder</i> <i>Canopy</i>	67.1	0.15							
	Intercept	Canopy	HP						
<i>Adirondack scraper-collector</i> <i>Canopy</i>	133.6	-20.2					0.000	0.880	1.000
Canopy + taxon	127.6	-19.7	7.1				4.080	0.114	7.691
	Intercept	Canopy	IS	LA					
<i>Adirondack predator</i> <i>Canopy</i>	74.2	0.26					0.000	0.511	1.000
Canopy + taxon	81.1	0.24	-17.3	2.6			0.090	0.489	1.046
	Intercept	Canopy	PY						
<i>Adirondack shredder</i> <i>Canopy</i>	82.7	-0.01					0.000	0.989	1.000
Canopy + taxon	87.4	-0.1	-4.2				9.000	0.011	90.017

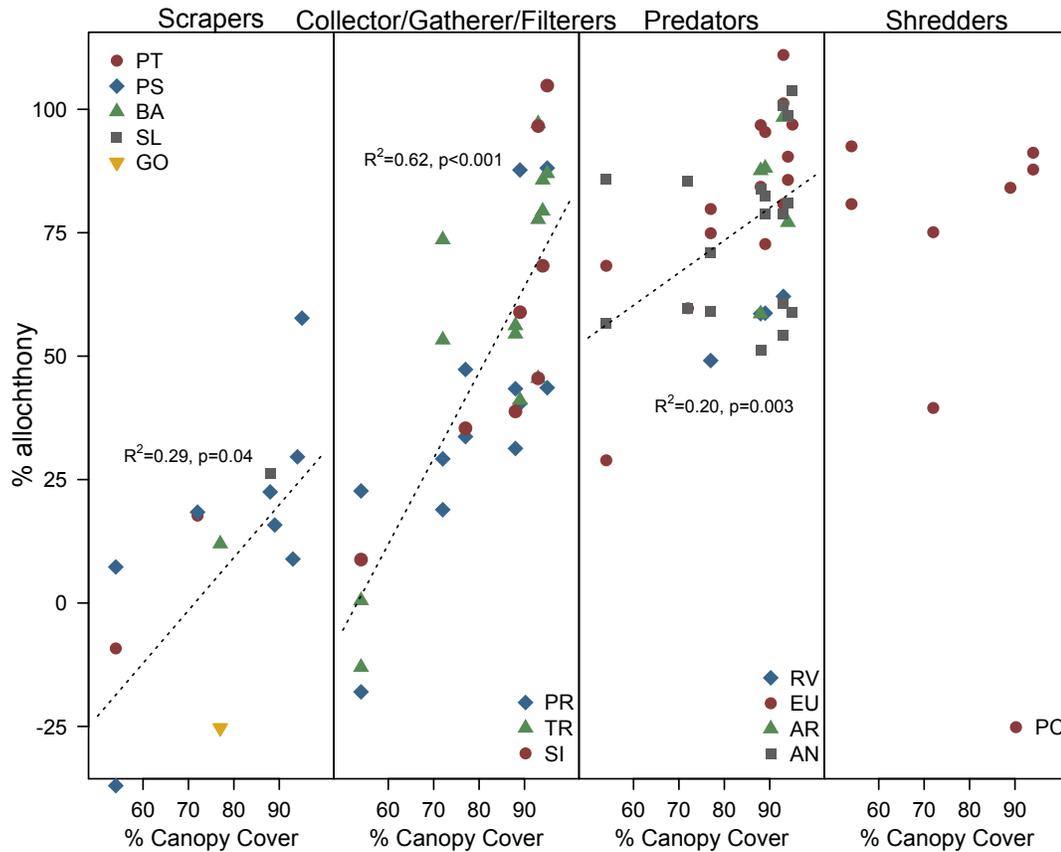


Figure 2.8: The amount of allochthonous energy assimilated by consumers is positively related to canopy cover in Trinidad streams. Figures include data from all three river drainages. Species codes are the same as in Figures 5-7. Regression lines, R^2 values and p values are shown for significant relationships, and regression coefficients and statistics are detailed in Table 2.

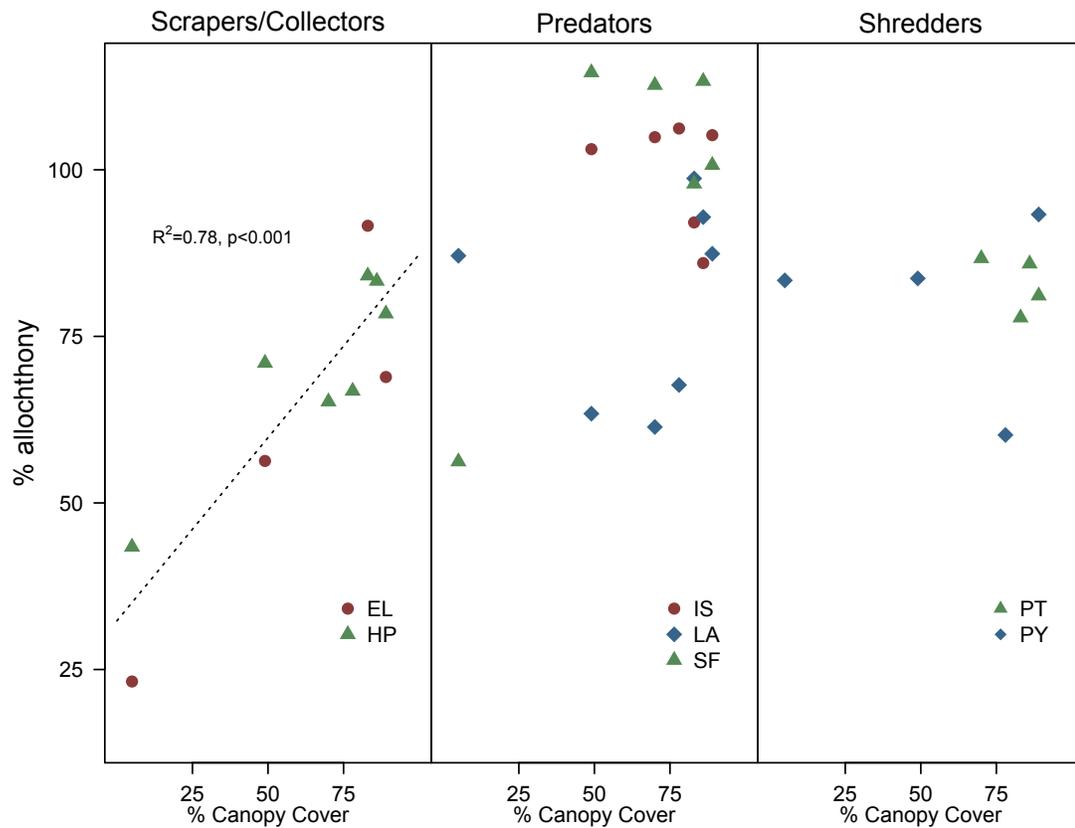


Figure 2.9: Allochthonous energy assimilated by scrapers in Adirondack stream sites is positively related with canopy cover but energy use by shredders and predators is not related to canopy cover. Species codes for consumers are as follows: HP= *Heptagenia*, EL= *Ephemerella*, PT= *Pteronarcis*, IS= *Isoperla*, LA= *Lanthus*, PY= *Pycnopsyche*, SF= *S. fontinalis*. Regression lines, R² values and p values are shown for significant relationships, and regression coefficients and statistics are detailed in Table 2.

Discussion

Our results show that the fraction of energy assimilated by stream insects and fishes varies markedly both between and within taxa and functional feeding groups. In both temperate and tropical systems, invertebrate consumers of epilithon, fine benthic organic matter, and suspended organic matter had extremely flexible diets and ranged from entirely allochthonous to entirely autochthonous (Figures 2.8, 2.9, Table 2.2). Invertebrate shredders and predators were mostly allochthonous in all site types, diets were much more fixed than for other functional groups. Canopy cover still had a significant effect on how much allochthonous energy predators consumed in the Trinidad sites (Figure 2.8, Table 2.2), but did not influence predators and shredders in the Adirondacks or shredders in Trinidad.

Regressions between canopy cover and allochthonous energy use suggest that increased availability of autochthonous algal food in larger streams with more open canopies has a strong influence on the type of energy assimilated by consumers. Our finding of flexibility in the type of resource assimilated by invertebrates agrees with previous studies that have noted high variation in feeding by stream invertebrates (*e.g.*, Mihuc 1997, Friberg & Jacobsen 1999, Lancaster *et al.* 2005). Although identifying taxa by functional feeding groups may accurately describe their feeding mode, our results provide further evidence that FFGs are not effective at characterizing resource use by a community of stream invertebrates.

Despite high variance in the type of energy assimilated, we nevertheless found identifiable differences in allochthonous energy use among functional feeding groups. For example, there was only one instance where scrapers exceeded 50% allochthonous

energy assimilation in Trinidad sites, while shredders and predators almost always assimilated more than 50% allochthonous energy (Figure 2.8). Some taxa, especially shredders and predators, were almost entirely allochthonous in most sites, regardless of stream size or canopy cover (Figures 2.4-2.7). Although we expected that shredders would be allochthonous because they consume terrestrial leaf litter, it was unexpected that allochthonous contributions to predators did not vary across sites. One explanation for the highly allochthonous energy use by predators is that they are buffered from basal resource availability by prey, and that they prey on invertebrate taxa that are allochthonous in all sites. Overall, our results suggest that the presence of a functional feeding group does not necessarily indicate whether a stream is fueled by allochthonous or autochthonous energy: shredders can assimilate partially autochthonous energy and grazers can be mostly allochthonous depending on the size of the stream and resource base available for consumption.

Allochthonous energy use by fishes

Flexibility in allochthonous energy use differed among fish species. Two of the three fish species we collected, *Rivulus* and *S. fontinalis*, were relatively fixed in the type of energy they assimilated, which was mostly allochthonous in all site types (Figures 2.4-2.7). Diet observations indicate that both species consume high proportions of terrestrial invertebrates (Courtwright & May 2013; B. Lamphere, unpublished), so it is not surprising that they have mostly allochthonous signals. Although *Rivulus* were mostly allochthonous, they did occasionally approach 50% allochthony in some sites (Figures 2.5-2.7).

Guppies (*P. reticulata*) are generalists that eat aquatic invertebrates, algae and fine detritus, and their allochthonous energy use differed between larger downstream and smaller midstream sites. Variation in guppy diet among sites is consistent with results from previous studies of gut contents (Zandona *et al.* 2011). However, stream size co-varies with predator presence in Trinidad, with low predation pressure at midstream sites, and high predation pressure in downstream sites (Magurran 2005). Hence, differences in guppy energy use among sites may also be due to changes in feeding behavior that are induced by predation. A large body of literature has documented genetically-based adaptive differences between downstream and midstream guppies for life history and morphological traits (Magurran 2005). Controlled laboratory experiments have revealed differences in guppy feeding mode between downstream and midstream populations that are due to predator presence (C. Dalton, unpublished). Hence, predator presence and genetic differences among populations are also likely to play a role in the differences in allochthonous energy use observed between by downstream and midstream guppies.

Habitat selection and behavior may also be a driver of allochthonous energy use for fishes. For example, at larger downstream sites in Trinidad, predators can have a strong influence on whether guppies feed in the water column or on the streambed (Zandona *et al.* 2011), making effects of predators and resource availability on guppy phenotypes difficult to disentangle (Grether *et al.* 1997). *Rivulus* habitat selection also may drive patterns of consistent allochthonous energy use. At downstream sites, we captured most *Rivulus* from the edges of backwaters of the main channel, but they were abundant in the center of the channel in smaller midstream and upstream sites.

Channel edges in downstream sites were more shaded, often included high amounts of allochthonous organic matter, and were likely to receive more terrestrial invertebrate inputs than mid-channel habitats that were not covered by forest.

Comparison of patterns in the Adirondacks vs. Trinidad

Previous studies have found that algae are a dominant food source for invertebrates in headwater streams despite being much less abundant than terrestrial organic matter in both tropical (March & Pringle 2003, Lau *et al* 2009) and temperate (Torres-Ruiz *et al.* 2007) environments. Our results indicate that patterns between Trinidad and the Adirondacks are similar and that most invertebrate and fish taxa in sites with high canopy cover are primarily fueled by allochthonous energy in both systems. For relatively flexible taxa (grazers, collector-gatherers, collector-filterers), consumers in smaller streams with closed canopies are generally very allochthonous and consumers in larger streams with more open canopies are more autochthonous. However, the extent of canopy cover in our larger tropical stream sites was higher (~77%, 72% and 54% for the three DS sites, Table 2.1) compared with our more open temperate sites (5% for the Moose River and 49% for Blues Brook, Table 2.1), suggesting that Trinidad streams sites may switch to autochthonous energy pathways at a lower canopy cover threshold than Adirondack sites.

Results from temperate systems indicate that some scraper taxa are more selective toward algal portions of biofilms than others; for example, McNeely *et al.* (2006) found that *Glossosoma* caddisflies fed selectively on algae while heptageniid mayflies assimilated bulk biofilm. Hence, it is not surprising that the heptageniid

mayflies at our Adirondack sites became more autochthonous as bulk epilithon became more autochthonous in the open canopy sites. We collected scrapers opportunistically in Trinidad streams, where *Psephenus* was the most common taxon and *Petrophila* and *Baetodes* occurred at a more limited number of sites. Based on our results, those taxa do not appear to feed selectively on algae in headwater sites (Figures 2.4-2.6). Expanding our scraper collections to include a wider taxonomic breadth might have identified other taxa that are more selective toward algae even at the smaller stream sites with limited algal availability.

Using δ^2H as an indicator of allochthony

We found that hydrogen isotope ratios provided sufficient source separation to calculate the proportion of allochthonous and autochthonous contributions for both our tropical and temperate stream sites. We were able to resolve a reasonable allochthonous contribution (*i.e.*, between 0% and 100% allochthony) for most individuals in our study. The exceptions were a small number of individuals for which we calculated less than 0% allochthonous energy assimilation in Trinidad (Figures 2.5, 2.6, 2.7) and a small number of individuals for which we calculated over 100% allochthonous energy assimilation in the Adirondacks (Figure 2.4). Results outside the 0-100% allochthony range could have resulted from a variety of methodological factors, including incorrectly estimated end member isotope ratios, incorrect assumptions about dietary water contributions, spatial or temporal variability in the isotope ratio of food sources. Nevertheless, the simulation approach we used to

account for variability in these factors worked well for most taxa in our two study systems.

The sources of uncertainty of the type that we incorporated into our simulations have led in previous studies to estimates of allochthony that are either less than 0% or over 100% (*e.g.*, Jardine *et al.* 2009). Dietary water contributions to consumer H are likely to vary depending on taxon, food source, and other environmental conditions. Several studies (Solomon *et al.* 2009, Soto *et al.* 2013, Graham *et al.* 2014) have conducted laboratory experiments to determine the proportion of dietary water assimilated by different species of consumers, but additional work is needed to account for water contributions accurately. Differences in H isotope content among body tissues may also confound H isotope results; for example, Jardine *et al.* (2009) hypothesized that the reason for many consumers being outside of the range of end members may be due to fat content of fish. Variation in primary producer $\delta^2\text{H}$ could also confound results (Hondula *et al.* 2014). We used the simulation approach to account for the potential for error from end member estimation, dietary water contributions, and variation among primary producers and to understand the extent to which uncertainty might have influenced our estimates of allochthonous energy use.

Hydrogen isotope ratios have also been used to determine allochthonous energy assimilation by consumers in studies of Arizona and California streams (Doucett *et al.* 2007, Finlay *et al.* 2010), but were less effective in Canadian streams, where consumers in 12 of 16 streams had $\delta^2\text{H}$ signatures that were outside of the range 0-100% allochthony (Jardine *et al.* 2009). Several studies in lakes and reservoirs have

successfully used $\delta^2\text{H}$ as an indicator of allochthony. However, most studies have had to employ some sort of a correction factor (Babler *et al.* 2011) or laboratory data (Wilkinson *et al.* 2013) to determine an autochthonous end member because bulk seston is generally not composed of pure algae. In those studies, the estimated autochthonous end member was consistently -150‰ to -170‰ different from the $\delta^2\text{H}$ of water in the same site, which also is consistent with our results in both temperate and tropical streams. However, Hondula *et al.* (2014) found that discrimination between $\delta^2\text{H}$ of water and $\delta^2\text{H}$ of some types of primary producers can be highly variable (*e.g.*, marine macroalgae) while discrimination between water and other types of primary producers is more consistent (*e.g.* microalgae). Hence, what broad taxonomic group of primary producer is present should be considered when making assumptions about discrimination between water and primary producers, and systems dominated by macrophytes or marine macroalgae might not be suitable for $\delta^2\text{H}$ analysis.

Difficulty in resolving allochthonous or autochthonous end members is not limited to H isotope studies. For example, both C and N isotope tracer studies in streams have often obtained values implying that consumers are more enriched than their food sources, presumably because consumers are selective about which components of biofilm they consume or assimilate (*e.g.* Hall & Meyer 1998, Dodds *et al.* in press). Combining C, H and N isotope data in a multi-element approach may improve resolution of the results in this study (see Deines *et al.* 2009, Finlay *et al.* 2009 for comparison of C and H isotope data in streams, and Cole *et al.* 2011, Cole & Solomon 2012 for CHN multi-isotope analyses).

In our study sites in Trinidad, food web analysis using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ natural abundance data were much less effective for distinguishing different organic matter sources than our $\delta^2\text{H}$ isotope data (E. Zandonà, unpublished data). Unfortunately, we cannot directly compare our $\delta^2\text{H}$ isotope data from Trinidad streams with unpublished $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data because we collected H isotope samples during the dry season while C and N data were from the wet season. Physical and chemical conditions and biofilms are different between the two seasons (Kohler *et al.* 2012), so the use of different elements would be confounded with the existence of different seasonal conditions.

Our results highlight that invertebrate and fish taxa can assimilate very different proportions of terrestrial subsidies in streams with differing resource availabilities, and that some consumer taxa are relatively fixed in the type of energy consumed but others are highly flexible. In both Trinidad and Adirondack streams, consumption of allochthonous material increased as function of canopy cover for invertebrate and fish taxa that eat biofilm and fine organic matter, suggesting that grazer, collector-gatherer and collector-filterer consumer taxa can be very flexible in the type of organic matter they consume. Both predatory and shredder consumers had much less flexibility in the type of organic matter consumed, with weak or no relationship with canopy cover and mostly allochthonous energy use. Hydrogen isotopes were mostly effective for distinguishing allochthonous and autochthonous contributions to consumer diets. Our results complement previous studies that have demonstrated high levels of plasticity in stream invertebrate diets (Mihuc 1997) and

suggest that the role of terrestrial subsidies in freshwater food webs can vary widely among systems.

While we refer to terrestrial resource inputs as subsidies, recent papers (Jones *et al.* 2013, Kelly *et al.* 2014) have pointed out that terrestrial organic matter might not actually subsidize freshwater systems despite high contributions of allochthonous energy to consumer diets in many lakes and streams. As defined by Polis *et al.* (1997) subsidies should increase population productivity of consumers in the recipient ecosystem. Terrestrial organic matter typically has a much larger C:N ratio, lower food quality, and lower fatty acid content (Simopoulos 2004) than *in situ* primary production. Meta-analysis and modeling studies in lakes suggest that terrestrial inputs can have negative impacts on primary production and zooplankton production (Jones *et al.* 2013, Kelly *et al.* 2014). However, in many of the smaller, closed-canopy streams in our system, algal growth is extremely low and shredders and other consumers are adapted to eating terrestrial resources, so terrestrial inputs may still subsidize consumer growth in streams despite contrary findings in lake ecosystems. Our results also suggest that whether terrestrial resources are actually a subsidy for stream consumers may vary among taxa and among streams with different levels of canopy cover.

REFERENCES

- Allan, J.D. 2004. Landscapes and Riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology & Systematics* 35: 257-284.
- Babler, A.L., A. Pilati and M.J. Vanni. 2011. Terrestrial support of detritivorous fish populations decreases with population size. *Ecosphere* 2(7):art76.
- Cole, J.J. and C.T. Solomon. 2012. Terrestrial support of zebra mussels and the Hudson River food web: A multi-isotope, Bayesian analysis. *Limnology and Oceanography* 57(6): 1802-1815.
- Cole, J.J., S.R. Carpenter, J. Kitchell, M.L. Pace, C.T. Solomon and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen and hydrogen. *Proceedings of the National Academy of Sciences* 108(5): 1975-1980.
- Courtwright, J. and C.L. May. 2013. Importance of terrestrial subsidies for native brook trout in Appalachian intermittent streams. *Freshwater Biology* 58(11): 2423-2438.
- Dangles, O. 2002. Functional plasticity of benthic macroinvertebrates: implications for trophic dynamics in acid streams. *Canadian Journal of Fisheries and Aquatic Sciences*. 59(9): 1563-1573.
- Deines, P., M.J. Wooller, and J. Grey. 2009. Unravelling complexities in benthic food webs using a dual stable isotope (hydrogen and carbon) approach. *Freshwater Biology* 54: 2243-2251.
- Dekar, M.P., R.S. King, J.A. Back, D.F. Wingham and C.M. Walker. 2012. Allochthonous inputs from grass-dominated wetlands support juvenile salmonids in headwater streams: evidence from stable isotopes of carbon, hydrogen and nitrogen. *Freshwater Science* 31(1): 121-132.
- Doucett, R.R., J.C. Marks, D.W. Blinn, M. Caron and B.A. Hungate. 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology* 88(6):1587-1592.
- Downes, B.J., J. Lancaster, R. Hale, A. Glaister and W.D. Bovill. 2011. Plastic and unpredictable responses of stream invertebrates to leaf pack patches across sandy-bottomed streams. 2011. *Marine and Freshwater Research* 62: 394-403.
- Finlay, J.C., R.R. Doucett and C. McNeely. 2010. Tracing energy flow in stream food webs using stable isotopes of hydrogen. *Freshwater Biology* 55: 941-951.

- France, R.L. 1995. Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystem. *Canadian Journal of Fisheries and Aquatic Sciences*. 52: 651-656.
- Friberg, N. and D. Jacobsen. 1999. Variation in growth of the detritivore-shredder *Sericostoma personatum* (Trichoptera). *Freshwater Biology* 42: 625-635.
- Graham, C.T., S.S.C. Harrison and C. Harrod. 2014. Differences in the contributions of dietary water in the hydrogen stable isotope ratios of cultured Atlantic salmon and Arctic charr tissues. *Hydrobiologia* 721: 45-55.
- Greathouse, E.A. and C.M. Pringle. 2006. Does the river continuum concept apply on a tropical island? Longitudinal variation in a Puerto Rican stream. *Canadian Journal of Fisheries and Aquatic Sciences* 63(1): 134-152.
- Grether, G.F., D.F. Millie, M.J. Bryant, D.N. Reznick and W. Mayea. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology* 82(6): 1546-1559.
- Grubaugh, J.W., J.B. Wallace and E.S. Houston. 1997. Production of benthic macroinvertebrate communities along a southern Appalachian river continuum. *Freshwater Biology* 37: 581-596.
- Hill, W.R., J.G. Smith and A.J. Stewart. 2010. Light, nutrients and herbivore growth in oligotrophic streams. *Ecology* 91(2): 518-527.
- Hill, W.R., M.G. Ryon and E.M. Schilling. 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. *Ecology* 76: 1297-1309.
- Hill, W.R., P.J. Mulholland and E.R. Marzolf. 2001. Stream ecosystem response to forest leaf emergence in spring. *Ecology* 82(8): 2306-2319.
- Hondula, K.L., M.L. Pace, J.J. Cole and R.D. Batt. 2014. Hydrogen isotope discrimination in aquatic primary producers: implications for aquatic food web studies. *Aquatic Sciences* 76: 217-229.
- Hynes, H.B.N. 1975. The stream and its valley. *Verh. Int. Ver. Theor. Ang. Limnol.* 19: 1-15.
- Jones, S.E., C.T. Solomon and B. Weidel. 2012. Subsidy or subtraction: how do terrestrial inputs influence consumer production in lakes? *Freshwater Reviews* 5: 21-35.

- Karlsson, J., M. Berggren, J. Ask, P. Bystrom, A. Jonsson, H. Laudon and M. Jansson. 2012. Terrestrial organic matter support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers. *Limnology and Oceanography* 57(4): 1042-1048.
- Kelly, P.T., C.T. Solmon, B.C. Weidel and S.E. Jones. 2014. Terrestrial carbon is a resource, but not a subsidy, for lake zooplankton. *Ecology* 95(5): 1236-1242.
- Kohler, T.J., T.N. Heatherly III, R.W. El-Sabaawi, E. Zandona, M.C. Marshall, A.S. Flecker, C.M. Pringle, D.N. Reznick and S.A. Thomas. 2012. Flow, nutrients and light availability influence Neotropical epilithon biomass and stoichiometry. *Freshwater Science* 31(4): 1019-1034.
- Lancaster, J., D.C. Bradley, A. Hogan and S. Waldron. 2005. Intraguild omnivory in predatory stream insects. *Journal of Animal Ecology* 74: 619-629.
- Lau, D.C.P., K.M.Y. Leung and D. Dudgeon. 2008. Experimental dietary manipulations and concurrent use of assimilation-based analyses for elucidation of consumer-resource relationships in tropical streams. *Marine and Freshwater Research* 59: 963-970.
- Lau, D.C.P., K.M.Y. Leung and D. Dudgeon. 2009. Evidence of rapid shifts in the trophic base of lotic predators using experimental dietary manipulations and assimilation-based analyses. *Oecologia* 159: 767-776.
- MacNeil, C., J.T.A. Dick and R.W. Elwood. 1997. The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): Problems and perspectives concerning the functional feeding group concept. *Biological Reviews* 72: 349-364.
- Magurran, A.E. 2005. *Evolutionary Ecology: The Trinidadian Guppy*. Oxford Series in Ecology and Evolution. Oxford University Press, UK.
- McCutchan Jr., J.H. and W.M. Lewis, Jr. 2002. Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography* 47(3): 742-752.
- McNeily, C., S.M. Clinton and J.M. Erbe. 2006. Landscape variation in C sources of scraping primary consumers in streams. *Journal of the North American Benthological Society* 25(4): 787-799.
- Merritt, R.W., K.W. Cummins and M.B. Berg. 2008. *An Introduction to the Aquatic Insects on North America*. Kendall Hunt Publishing, Dubuque, Iowa.

- Mihuc, T.B. 1997. The functional trophic role of lotic primary consumers: generalist versus specialist strategies. *Freshwater Biology* 37: 455-462.
- Mihuc, T.B. and G.W. Minshall. 1995. Trophic generalists vs. trophic specialists-implications for food web dynamics in postfire streams. *Ecology* 76(8): 2361-2372.
- Moore, J.C. E.L. Berlow, D.C. Coleman, P.C. de Ruiter, Q. Dong, A. Hastings, N. Collins Johnson, K.S. McCann, K. Melville, P.J. Morin, K. Nadelhoffer, A.D. Rosemond, D.M. Post, J.L. Sabo, K.M. Scow, M.J. Vanni and D.H. Wall. 2004. Detritus, trophic dynamics and biodiversity. *Ecology Letters* 7:584-600.
- Nakano, S. and M. Murakami. 2001. Dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences* 98(1): 166-170.
- Pace, M.L., J.J. Cole, S.R. Carpenter, J.F. Kitchell, J.R. Hodgson, M.C. Van de Bogert, D.L. Bade, E.S. Kritzbert & D. Bastviken. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature* 427 240-243.
- Polis, G.A, M.E. Power and G.R. Huxel. 2004. *Food webs at the landscape level*. Chicago, IL: University of Chicago Press.
- Polis, G.A., W.B. Anderson and R.D. Holt. 1997. Toward and integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* 28: 289-316.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* 83: 703-718.
- Post, D.M., M.L. Pace and N.G. Hairston, Jr. 2000. Ecosystem size determines food-chain length in lakes. *Nature* 405: 1047-1049.
- R Core Team. 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rawer-Jost, C., J. Bohmer, J. Blank and H. Rahmann. 2000. Macroinvertebrate functional feeding group methods in ecological assessment. *Hydrobiologia* 423: 225-232.
- Rosi-Marshall, E.J. and J.B. Wallace. 2002. Invertebrate food webs along a stream resource gradient. *Freshwater Biology* 47: 129-141.

- Simopoulos, A.P. 2004. Omega-3 fatty acids and antioxidants in edible plants. *Biological Research* 37: 263-277.
- Solomon, C.T., J.J. Cole, R.R. Doucett, M.L. Pace, N.D. Preston, L.E. Smith and B.C. Weidel. 2009. The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers. *Oecologia* 161:313-324.
- Soto, D.X., L.I. Wassenaar and K.A. Hobson. 2013. Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. *Functional Ecology* 27: 535-543.
- Soto, D.X., L.I. Wassenaar, K.A. Hobson and J. Catalan. 2011. Effects of size and diet on stable hydrogen isotope values (δD) in fish: implications for tracing origins of individuals and their food sources. *Canadian Journal of Fisheries and Aquatic Sciences* 68: 2011-2019.
- Sweeney, B.W., J.M. Battle, J.K. Jackson and T. Dapkey. 2011. Can DNA barcodes of stream macroinvertebrates improve descriptions of community structure and water quality? *Journal of the North American Benthological Society* 30(1): 195-216.
- Tank, J.L., E.J. Rosi-Marshall, N.A. Griffiths, S.A. Entekin and M.L. Stephen. 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society* 29: 118-146.
- Tomanova, S., E. Goitia and J. Helesic. 2006. Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. *Hydrobiologia* 556: 251-264.
- Tomanova, S., P.A. Tedesco, M. Campero, P.A. Van Damme, N. Moya and T. Oberdorff. 2007. Longitudinal and altitudinal changes of macroinvertebrate functional feeding groups in neotropical streams: a test of the River Continuum Concept. *Archiv fur Hydrobiologie* 170(3): 233-241.
- Torres-Ruiz, M., J.D. Wehr and A.A. Perrone. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. *Journal of the North American Benthological Society* 26(3): 509-522.
- Vander Zanden, M.J., J.M. Casselman and J.B. Rasmussen. 1999. Stable isotope evidence for food web shifts following species invasions of lakes. *Nature* 401: 464-467.
- Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 130-137.

- Webb, J.M. and W.P. McCafferty. 2008. Heptageniidae of the world. Part II: Key to the Genera. *Canadian Journal of Arthropod Identification* 7: 1-55.
- Webster, J.R., E.F. Benfield, T.P. Ehrman, M.A. Schaffer, J.L. Tank, J.J. Hutchens, and D.J. D'Angelo. 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology* 41: 687-705.
- Whiting, D.P., M.R. Whiles and M.L. Stone. 2011. Patterns of macroinvertebrate production, trophic structure, and energy flow using a tallgrass prairie stream continuum. *Limnology and Oceanography* 56(3): 887-898.
- Wilkinson, G.M., M.L. Pace, and J.J. Cole. 2013. Terrestrial dominance of organic matter in north temperate lakes. *Global Biogeochemical Cycles* 27: 1-9.
- Zandona, E., S.K. Auer, S.S. Kilham, J.L. Howard, A. Lopez-Sepulcre, M.P. O'Connor, R.D. Bassar, A. Osorio, C.M. Pringle, and D.N. Reznick. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Functional Ecology* 25: 964-973.

CHAPTER 3

LIGHT AVAILABILITY ALTERS THE IMPORTANCE OF BACTERIAL CARBON IN HEADWATER STREAM FOOD WEBS

Abstract

Many ecosystems rely on subsidies of carbon and nutrients from surrounding environments. In headwater streams that are heavily shaded by riparian forests, allochthonous inputs from terrestrial systems often comprise a major part of the organic matter budget. Bacteria play a key role in processing leaf litter inputs and assimilating dissolved organic carbon, but there is limited evidence about how much bacterial carbon is actually assimilated by invertebrate and fish consumers, and how bacterial carbon assimilation varies among streams. We conducted stable isotope tracer additions of ^{13}C -acetate, which is assimilated only by bacteria, and ^{15}N -ammonium, which is assimilated by both bacteria and algae, in two small, shaded streams in the Adirondack region of New York State. Our goal was to determine whether there is an important trophic linkage between bacteria and macroconsumers, and whether the linkage changes when the light environment is experimentally altered. After evaluating bacterial carbon use in both streams with natural canopy cover in 2009 using 10-day isotope tracer releases, we thinned the canopy of one stream to increase light availability and primary production and repeated our tracer releases in 2010. As part of the tracer experiments, we developed a respiration assay technique to measure the $\delta^{13}\text{C}$ content of live bacteria, which provided critical information for

determining how much of the carbon assimilated by invertebrate consumers is from bacterial sources. Some invertebrate taxa, including scraper mayflies (*Heptagenia* spp.) that feed largely on biofilms assimilated over 66% of their carbon from bacterial sources, while shredder caddisflies (*Pycnopsyche* spp.) that feed on decomposing leaves assimilated less than 1% of their carbon from bacteria. The light environment had a strong influence on the magnitude of bacterial carbon fluxes to different consumers, suggesting that bacterial energy assimilation differs not only among consumer taxa, but also within the same consumer taxa in streams with different resource bases. Our results indicate that different invertebrate taxa differentially assimilate labile parts of basal resource pools, while demonstrating that, in general, fluxes of carbon from heterotrophic bacteria to higher trophic levels can be substantial in stream food webs.

Introduction

Detritus and dissolved organic carbon as external subsidies can be much more important than local primary production in freshwater food-web pathways, yet detrital food-web linkages often receive less attention than linkages between primary producers and consumers (Moore *et al.* 2004). Research in unproductive systems like forested streams has highlighted the need for viewing food web pathways in a landscape context. Streams are particularly tightly connected to bordering terrestrial environments by transfers of particulate and dissolved organic matter and nutrients (Hynes 1975, Vannote *et al.* 1980) and subsidies from riparian forests can make up a substantial portion of the energy budget in headwater streams and vice versa (*e.g.* Nakano & Murakami 2001, Baxter *et al.* 2005, Sato *et al.* 2011, Bartels *et al.* 2012).

Microbes play an important role in the assimilation and degradation of allochthonous carbon in streams, through both uptake of dissolved organic carbon (DOC) and breakdown of particulate organic matter such as leaf litter (Suberkropp & Chauvet 1995, Webster & Benfield 1986, Webster *et al.* 1999, Wiegner *et al.* 2005). In planktonic systems, the transfer of allochthonous energy from microbes to higher trophic levels, or the “microbial loop”, is considered “closed” because bacterial energy is dissipated in trophic transfers and has little effect on higher trophic levels (*e.g.*, Pace *et al.* 1990, Pozzato *et al.* 2013, reviewed by Hairston & Hairston 1993). Because larger macroinvertebrate consumers in streams often directly eat organic matter that includes bacteria, it is thought that allochthonous carbon from heterotrophic bacteria pathways comprises a substantial portion of the energy used by higher trophic levels (Cummins 1974, Meyer 1994). Nevertheless, quantifying fluxes from bacteria to

higher trophic levels in stream food webs has remained a challenge, and little work has been done in natural streams to elucidate controls on the importance of bacterial carbon.

Previous research also indicates that changes in the environment, such as urban light pollution (Meyer & Sullivan 2013), fish invasion (Baxter *et al.* 2004) and riparian forest species composition (Kominoski *et al.* 2012) can alter the role of subsidies in stream food webs. Many headwater streams are light limited, thus increases in light availability are known to enhance primary production, the relative biomass of autochthonous versus allochthonous carbon sources, and autochthonous carbon assimilated by consumers (Hill *et al.* 1995). Understanding how changes in the light environment affect stream food webs is also relevant to understanding how streams respond to watershed land-use changes, which often increase light and nutrient inputs to streams and in turn often results in increased primary production and reduced input of terrestrial leaf litter (Allan 2004).

Previous research suggests that heterotrophic bacteria are likely a significant source of carbon to higher trophic levels, but additional study is needed to generalize about the role of bacteria in stream food webs and how it varies among systems (Findlay 2010). Several studies have shown that single species of stream invertebrates can consume high proportions of bacteria relative to other food sources (*e.g.*, Edwards & Meyer 1987, Edwards & Meyer 1990), while other species assimilate very little bacterial carbon (Findlay *et al.* 1986). Few studies have investigated bacterial energy fluxes in whole stream systems (Hall & Meyer 1998, Simon *et al.* 2003). Hall & Meyer (1998) found that many invertebrate taxa assimilate high proportions of carbon

from heterotrophic bacteria, but results are difficult to interpret because methodological challenges led to some implausible results (*i.e.*, >100% bacterial contributions to diets). Simon *et al.* (2003) also found high contributions of heterotrophic bacterial carbon to higher trophic levels, but their study was conducted in cave streams where no light was available for autotrophic production.

Isotope tracer injections are one effective way to trace energy and material flows through food webs. Many studies, most notably the Lotic Intersite Nitrogen Experiment (LINX), have conducted N tracer additions in streams across a range of biomes (*e.g.*, Ashkenas *et al.* 2004, Peterson *et al.* 2001). Because all microbes (*i.e.*, bacteria, fungi and algae) assimilate dissolved inorganic nitrogen (*e.g.*, ammonium, nitrate), these experiments have allowed investigators to trace the movement of N from the water column to basal resources (*i.e.*, microbes in biofilms, fine organic sediment and leaf litter) and higher trophic levels in the food web. Carbon isotope tracers have been used in few stream studies but provide a powerful method to examine linkages between bacteria and consumers. Bacteria and algae are closely associated in the biofilm of stream sediments, so it is difficult or impossible to separate them physically. However, labile organic compounds are selectively taken up by bacteria (Wright & Hobbie 1966) while photosynthesizing algae fix CO₂. Hence, the addition of isotopically-labeled DOC/DIC makes it possible to differentially track the flow of carbon from algae and bacteria to other compartments of the food web. In this study, we sought to examine the role of heterotrophic bacteria as a resource in stream food webs, and to determine how changes in the light environment influence the amount of bacterial carbon assimilated by consumers. We conducted a dual-

isotope tracer injection, simultaneously enriching a stream with ^{13}C -acetate, which is primarily assimilated by heterotrophic bacteria and ^{15}N - NH_4 , which is assimilated by bacteria, fungi and algae. Our objectives were: first, to determine the magnitude of bacterial carbon fluxes to several taxa on higher trophic levels in headwater stream food webs, and second, to determine whether the role of bacterial carbon differs in streams with dense intact riparian forests compared with streams where riparian cover has been experimentally reduced. In accordance with previous studies (Edwards & Meyer 1987, Hall & Meyer 1998), we predicted that bacteria would comprise a high proportion of the diets of some invertebrate functional feeding groups, especially scrapers. Even though they feed on decomposing organic matter that includes bacteria, based on results of previous studies (Findlay *et al.* 1986) we predicted that shredders would assimilate low amounts of bacterial carbon. We predicted that canopy thinning would lead to a shift in the resource base from allochthonous to autochthonous production, which would lead to a subsequent shift in the source of organic matter assimilated by consumer taxa. Understanding how the role of heterotrophic bacteria in food webs varies in different light environments will reveal a potential indirect influence on the importance of bacteria as a trophic link in streams, and will also provide information on how stream food webs respond to changes in watershed land use.

Methods

Study Site

In July-August 2009 and July-August 2010, we conducted stable isotope tracer studies in two small, headwater streams located near Old Forge, NY in the Adirondack Mountains. Both were small, groundwater-fed, headwater streams (discharge approximately 5 L s^{-1}) with low concentrations of dissolved inorganic nitrogen ($\text{NH}_4 < 2 \text{ } \mu\text{g L}^{-1}$, $\text{NO}_3 < 100 \text{ } \mu\text{g L}^{-1}$) and phosphorus ($\text{SRP} < 10 \text{ } \mu\text{g L}^{-1}$), and moderate concentrations of dissolved organic carbon ($\text{DOC} < 2.5 \text{ mg L}^{-1}$), and were surrounded by second-growth forests dominated by American beech (*Fagus grandifolia*). Our reference stream, Combs Brook Tributary, had a natural canopy cover during both study years. We thinned the canopy of the treatment stream, Blues Brook, at the end of the first year in September 2009 to increase light availability for the second year.

Benthic macroinvertebrate communities in both streams were depauperate and dominated by a suite of large-bodied invertebrate genera that spanned multiple functional feeding groups (classified according to Barbour *et al.* 1999). Common taxa included: *Heptagenia* spp. (mayfly scraper), *Ephemerella* spp. (mayfly collector-gatherer), *Lanthus* sp. (predatory dragonfly), *Remenus* sp. (predatory stonefly), and *Pycnopsyche* spp. (caddisfly shredder). The only fish species observed during electrofishing surveys in each stream was brook trout (*Salvelinus fontinalis*). Algal standing stocks increased in Blues Brook after canopy thinning but did not change in Combs Brook and standing stocks of leaf litter declined in both streams in 2010, but were highly variable (Table 3.1).

Table 3.1: Characteristics of the two study streams in 2009 and 2010. Biomasses of epilithon and leaves are means with standard deviations in parentheses.

	Stream width (m)	Discharge (L sec⁻¹)	Epilithon Chl-a (mg m⁻²)	EpilithonAFDM (g m⁻²)	Leaf DM (g m⁻²)
Combs Brook					
Natural canopy- 2009	1.7	4.8	4.22 (2.1)	2.03 (1.3)	92.4 (106)
Natural canopy- 2010	1.7	4.0	3.94 (1.6)	1.20 (0.49)	19.8 (19)
Blues Brook					
Natural canopy- 2009	1.5	5.2	0.896 (0.71)	2.50 (1.6)	74.5 (62)
Thinned canopy - 2010	1.5	4.3	9.14 (3.9)	1.31 (0.35)	23.8 (26)

¹³C-acetate and ¹⁵N-ammonium addition

We added ¹³C labeled acetate (as sodium acetate-2-¹³C) at and ¹⁵N labeled ammonium (as ¹⁵NH₄Cl) to both streams using a continuous drip with an injection rate of 10 mL min⁻¹ over a 10-day period. The injection increased the concentration of ¹³C in streamwater by 1.25 µg L⁻¹, which elevated the δ¹³C of the DOC pool to approximately 100‰. The ammonium injection elevated δ¹⁵N of dissolved ammonium to approximately 15,000‰. Despite the disparity in enrichment between the C and N tracers, uptake compartments became comparably enriched in both ¹³C and ¹⁵N because acetate is a highly labile compound and is taken up rapidly relative to bulk DOC. The target enrichments were not intended to fertilize the system, and the concentration of ¹⁵N added was <5% of ambient NH₄ and the concentration of ¹³C added was <1% of ambient DOC but added acetate tracer was more labile than the bulk DOC pool. We also added sodium chloride as a conservative tracer for the entire 10-day drip, which we used to correct for dilution along the study reach. Algal uptake of the ¹³C tracer should have been minimal relative to bacterial uptake (Wright & Hobbie 1966) and fungal uptake would have been negligible because the half saturation constants for fungal uptake are several orders of magnitude higher than the concentration at which we added acetate to the streams (Newell 1984, Hall & Meyer 1998, Simon *et al.* 2003).

Food web sampling

We collected samples of food web compartments at three sites: 10, 30 and 60 m downstream from the point of isotope addition. Distances were based on a pilot

study in Combs Brook Tributary, which demonstrated we would be able to detect both C and N isotope tracer in all food web compartments. We collected samples during the week before isotope addition (to record background isotopic levels and standing stocks of basal resources), on three dates during the 10-day tracer release period, and on three dates during the 10 days after the end of tracer addition. In 2010, we also collected samples approximately one month after the tracer addition began. All samples were dried at 50°C, ground, loaded into tin capsules, and analyzed on a Thermo Finnigan isotope ratio mass spectrometer coupled to a Carlo Erba elemental analyzer with a Conflow II open split at the Cornell Isotope Laboratory.

Food web compartments in our collections included: epilithon, surface fine benthic organic matter (FBOM), coarse benthic organic matter (CBOM), four abundant macroinvertebrate taxa representing different functional groups (shredder, scraper, collector/gatherer, predator), and brook trout. We conducted quantitative food web sampling of organic matter compartments before and after our study at three replicate sampling stations (10, 30 and 60 m downstream of the isotope injection) in each stream. During the isotope drip and during the post-drip sampling, we carried out non-quantitative sampling of organic matter compartments to avoid substrate disruptions.

Because both streams contained many large rocks that could not be removed from the stream, we sampled epilithon with Loeb samplers with a sampling area of 12 cm² (Loeb 1981). Seven Loeb samples per transect were combined into a single sample for analysis. Epilithon samples were filtered through glass fiber filters (Whatman GFF) to analyze chlorophyll *a* and ash free dry mass. We extracted

chlorophyll *a* by incubating filters in film canisters in the dark in 15 mL of 90% buffered ethanol for 18-22 hours. Immediately after extraction, we analyzed chlorophyll extractions using an Aquaflor handheld fluorometer (Turner Designs, Sunnyvale, CA).

Fine benthic organic matter (FBOM) was sampled by sinking a plastic cylinder with a diameter of 13 cm into an area of soft sediment, measuring the water depth in the cylinder, suspending the surface layer of organic matter into the water, and removing a known quantity of slurry from the bucket. We allowed FBOM to settle, removed excess water, and dried remaining organic material. Coarse benthic organic matter (CBOM) was sampled by haphazardly selecting a point on the stream and removing all leaf litter and woody material in a transect that stretched across the width of the stream at that point. This protocol for sampling CBOM can lead to results with high variation because leaf aggregations are not spatially evenly distributed in streams, but we could not do more intensive CBOM biomass sampling because we needed to avoid major disturbance to the streambed for other sampling efforts. For all organic matter compartments, we sampled at points near to our sampling stations (10, 30 and 60 m downstream from the point of isotope injection) but did not sample at exact sampling stations so we did not disturb the substrate. We dried epilithon, seston, FBOM and CBOM samples at 50°C until they reached a constant mass and recorded all biomasses. After measuring dry mass of epilithon filters, we burned them at 450°C for six hours to quantify ash free dry mass.

Invertebrates were sampled by hand to avoid disturbing the substrate, either by turning over rocks or by sorting through leaf packs in a plastic tray before returning

them to the stream. We targeted abundant large-bodied invertebrate groups and kept them alive in 15 mL plastic tubes filled with stream water until they could be returned to the laboratory for identification under a dissecting microscope. We removed the guts from all invertebrates manually before drying their tissue for stable isotope analysis. Brook trout populations were small, so they were only sampled on the last day of the isotope release and 10 days after the isotope release. We used a backpack electrofishing unit to capture brook trout and euthanized them immediately. We removed a sample of muscle tissue from each fish for isotopic analysis.

Ecosystem response to canopy thinning

We monitored other ecosystem attributes to assess responses to canopy thinning. We recorded light levels every 10 minutes throughout the summer and fall seasons of 2009 and 2010 with HOBO pendant temperature and light loggers (Onset Computer Corporation, Part UA-002-64) and converted data to units of photosynthetically active radiation (PAR) using a correction factor from Thimijan & Heins (1983). In each stream, light loggers were attached approximately 25 cm above the water level to six stakes of construction rebar spaced evenly along our study reaches. In July 2010, we deployed substrates in each stream to determine how canopy thinning affected algal accrual. We put 7 replicate glass crucible covers (19.6 cm² area) in three locations: approximately 20 meters upstream of the canopy thinning in Blues Brook, in the thinned canopy area of Blues Brook, and in our reference stream, Combs Brook. We allowed algae to accrue on the substrates for 18 days before

removing them, placing them in film canisters, and extracting and analyzing chlorophyll using the same methods as our epilithon analyses.

We used our ^{15}N tracer injection to measure N uptake during each isotope tracer release. We measured $\delta^{15}\text{N}$ of the ammonium tracer in water at each downstream sampling station using a filter pack diffusion method (Holmes *et al.* 1998). Briefly, we collected 0.95 L of filtered stream water in 1.0 L bottles and added 50 g of salt, 5.0 g magnesium oxide and a 1.0 cm filter sealed in Teflon tape to each sample. Because stream water ammonium concentrations were low ($< 2 \mu\text{g L}^{-1}$), we also added a 50 μg ammonium spike to each incubation to provide sufficient N on the filter for analysis on a mass spectrometer. After one month of incubation at room temperature with occasional shaking (approximately once every 3-4 days), we removed filters and dried them in a desiccator for isotopic analysis. We corrected data to account for the 50 μg ammonium spike and to account for dilution along the reach using our conservative tracer, and created a regression of decline in $^{15}\text{N-NH}_4$ as a function of distance downstream from the point of tracer addition. We used the negative slope of the regression (k) to calculate uptake length and uptake velocity of N (Tank *et al.* 2007). Uptake length (S_w), or the distance it takes a molecule of nitrogen to be immobilized from its dissolved form, is equal to the inverse of k (k^{-1}). Uptake lengths often differ among streams with different discharge, so we also calculated uptake velocity (V_f):

$$V_f = Qk w^{-1}$$

where Q is stream discharge and w is the average wetted width of the stream (2.6 m for Combs Brook and 1.5 m for Blues Brook).

We compared chlorophyll accrual and N uptake length among streams and canopy thinning treatments using fixed effects linear models in R (R Core Team 2013). For chlorophyll data, we compared algal accrual rates among three groups: blues thinned canopy, blues unthinned canopy and combs, and for N uptake, we compared uptake length or uptake velocity rates among four groups: Blues 2009, Combs 2009, Blues 2010, and Combs 2010.

Measuring $\delta^{13}\text{C}$ of active bacteria using respiration incubations

One challenge in conducting bulk collections of food web compartments is that invertebrates often selectively ingest or selectively assimilate live parts of organic matter pools (Dodds *et al.* in press). Although it is possible in some cases to physically separate the components of epilithon (*i.e.*, algae, bacteria, detritus) using centrifugation gradients (Hamilton 2001), the process is time intensive and does not work in many systems nor with all types of organic matter. We developed a respiration chamber method to measure the $\delta^{13}\text{C}$ of active bacterial biomass by examining the isotope ratio of respired CO_2 in field incubations. We conducted field incubations during our isotope tracer addition by filling 475 mL glass incubation chambers with 150 mL of stream water containing either coarse organic matter or fine organic matter. It was not possible to incubate rocks with epilithon because all rocks in the study sites were much larger than our chambers. Incubations were sealed and headspace was scrubbed for CO_2 by circulating air from the headspace through a container of soda lime using a vacuum pump. We used a syringe and needle to collect 12 mL samples of headspace air through a rubber septum on the top of the incubation

chamber. Samples were collected at 1, 4 and 7 hour time periods after the incubation began and stored in evacuated vials for $^{13}\text{CO}_2$ analysis at the Cornell Isotope Laboratory. We also killed all live bacteria with HgCl_2 in control treatments to establish that enriched CO_2 in the headspace was actually a product of bacterial respiration.

A high proportion of the CO_2 released into the respiration chamber headspace was from inorganic diffusion from the water, so we used a two-ended mixing model to calculate the $\delta^{13}\text{C}$ of CO_2 in respiration chambers due to organic respiration and so reflective of the $\delta^{13}\text{C}$ of live bacteria. We used the following simple model, where f_{inorg} and f_{org} are the relative fractions of headspace CO_2 that are derived from inorganic diffusion and respiration, respectively and always sum to 1, and δ is the $\delta^{13}\text{C}$ of CO_2 from either the bulk headspace air (“total”), organic component from respiration (“org”), or inorganic component from diffusion (“inorg”).

$$\delta_{\text{total}} = \delta_{\text{org}} \times f_{\text{org}} + \delta_{\text{inorg}} \times f_{\text{inorg}}$$

We used this equation to solve for δ_{org} , which we used to calculate $\delta^{13}\text{C}$ of bacteria under the assumption that bacteria respire CO_2 with an isotopic ratio that reflects their tissue isotopic ratio. We calculated f_{org} through comparisons of the amount of CO_2 diffused in natural respiration assays versus chambers where all bacteria had been killed with sodium azide, and used f_{org} data to calculate f_{inorg} by difference ($f_{\text{org}} + f_{\text{inorg}} = 1$). Although we used HgCl_2 to kill bacteria during field experiments, we found that HgCl_2 increased inorganic diffusion of CO_2 and confounded results, but sodium azide did not. Hence, we used sodium azide to determine f_{org} . Estimated values for all

parameters and a detailed description of field experiments to obtain them are in Appendix C.

Since fungi also respire and greatly outweigh bacteria on leaf detritus (*e.g.* Das *et al.* 2006, Mille-Lindblom & Tranvik 2003, Weyers & Suberkropp 1996), we corrected for dilution due to fungal respiration using a 1:10 ratio of fungal to bacterial respiration for coarse organic matter (leaves) and a 1:1 ratio for fine organic matter (sediment). We used those ratios as a conservative estimates to account for dilution due to fungal respiration based on values from the literature, which demonstrates that fungal and bacterial respiration can vary depending on substrate and stream (*e.g.* Findlay *et al.* 2002). We picked conservative values because underestimation of fungal production relative to bacterial production on leaves essentially leads to underestimation of f_{inorg} because fungal respiration should not be enriched and would hence dilute bacterial respiration in the same way that inorganic diffusion of CO_2 does. Hence, incorporating more respiration due to fungi would lead to lower bacterial $\delta^{13}\text{C}$ estimates, and higher estimates of bacterial carbon assimilation by invertebrates based on the calculations below.

Calculating percent bacterial carbon used by invertebrates

We estimated the proportion of total consumer carbon that was derived from bacteria using a simple mixing model (Hall & Meyer 1998):

$$\text{Fraction bacterial C} = (\delta\text{I}_{\text{labeled}} - \delta\text{I}_{\text{background}}) / (\delta\text{B}_{\text{labeled}} - \delta\text{B}_{\text{background}})$$

Invertebrates are represented by I and bacteria by B, and “labeled” samples represent each compartment at maximum isotope enrichment. For bacteria, maximum

enrichment typically occurred on the final day of the 10-day release because rapid turnover rates caused bacterial signatures to decline quickly after isotope addition ceased. Invertebrates often continued to become more enriched after the addition ended because they continued to feed on enriched food sources and their turnover rates are much slower, but they eventually began to decline during the 20 days following the end of the isotope release. “Background” samples are the natural abundance isotope ratio of either compartment before the release began. The mixing model to calculate percent bacterial contributions assumes that both invertebrates and bacteria reach equilibrium with their carbon source during the course of the experiment. Failing this assumption means this model underestimates the percent of invertebrate carbon that is derived from bacteria.

We calculated turnover rates of invertebrates to estimate the degree to which our assumption of equilibrium was violated. We used ^{15}N tracer data in a dynamic compartment model that has been used to analyze data from several dozen ^{15}N tracer studies (*e.g.*, Dodds *et al.* 2000, Dodds *et al.* in press, Hall *et al.* 1998, Whiles *et al.* 2013). Briefly, we modeled each compartment separately and used the Solver function in Microsoft Excel to minimize the sum square error by altering the uptake and loss rates of ^{15}N from the food source. If a consumer became more enriched than its presumed bulk food source, we also solved for a multiplier that elevated the level of the food source by a consistent ratio. The multiplier was constrained by the amount of ^{15}N in the water column (maximum enrichment of pure algae or bacteria). Detailed modeling methods are described in Dodds *et al.* (in press) and in Appendix D.

We used N tracer data instead of C tracer data to make our turnover time calculations because it was easier to detect patterns in N tracer over a time-series in basal resource compartments. For example, in resource compartments with high C:N ratios (*e.g.*, CBOM), N tracer accumulated during the isotope release and declined following, but C tracer showed much less accumulation and decline over time, most likely due to dilution by the high amounts of “inert” C in dead leaf tissue. The lack of appreciable accumulation and decline of C tracer made it so it was not possible to calculate turnover accurately, but N tracer data were appropriate for calculating turnover times.

Results

Tracer addition resulted in high labeling of all food web compartments with both ^{13}C and ^{15}N tracers (C tracer data shown in Figure 3.1, N tracer data shown in Figure 3.2). Most invertebrate groups, especially scraper mayflies (*Heptagenia* spp.), became more enriched than any presumed bulk food sources (*i.e.*, epilithon, CBOM, FBOM), but $\delta^{13}\text{C}$ of active bacteria measured through the incubation chamber method exceeded the label found in any invertebrate group (Figure 3.1). The active bacterial isotope signature was the only measured food source that always exceeded the isotope label found in primary consumers.

Bacterial carbon use varied by invertebrate taxon (Table 3.2), with extremely high (>80%) assimilation of bacterial carbon observed for mayfly scrapers (*Heptagenia* spp.), moderate (20-47%) assimilation of bacterial carbon by predatory stoneflies (*Remenus* spp.), low (3.25%) assimilation of bacterial carbon by collector-

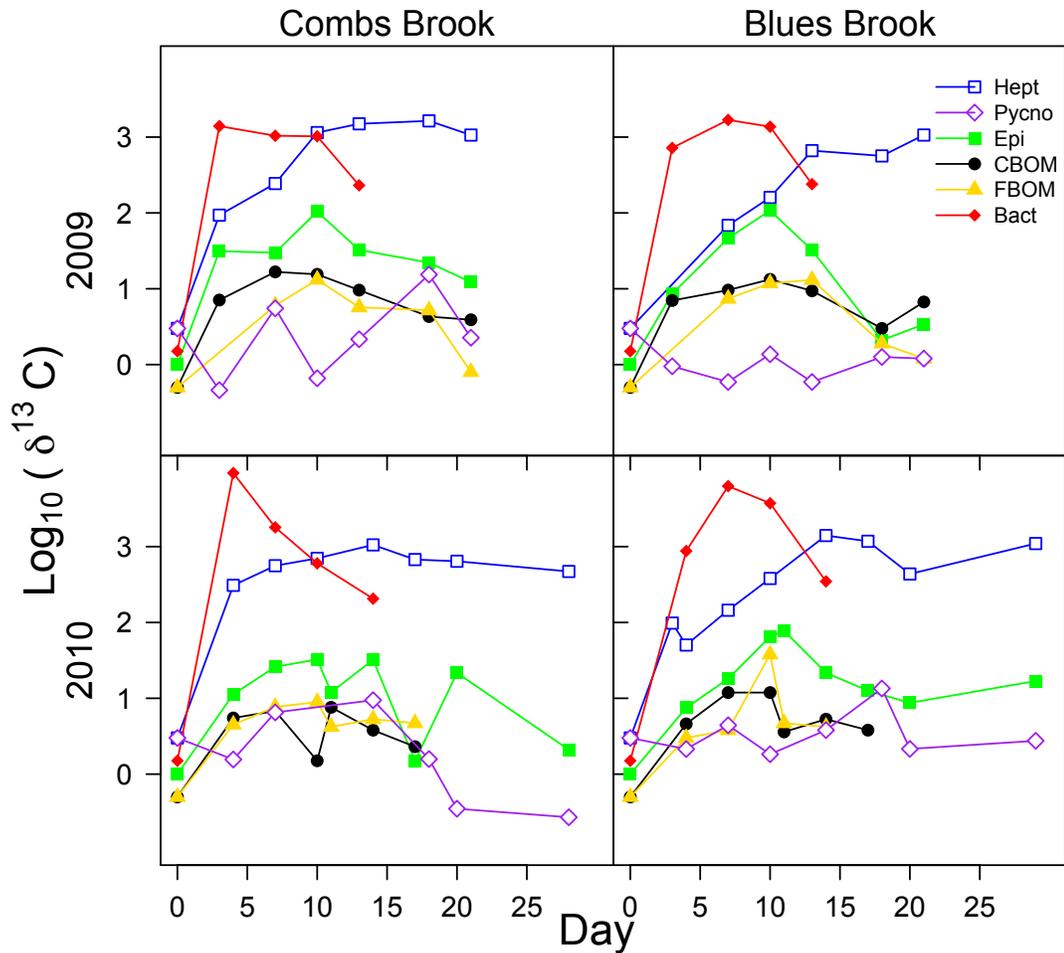


Figure 3.1: Carbon-13 isotope tracer found in different food web compartments (*Heptagenia* mayflies, *Pycnopsyche* caddisflies, bulk epilithon, CBOM, FBOM, and live bacteria) as a function of days since the start of C-13 addition. *Heptagenia* mayflies are shown because they were the most enriched of all invertebrate taxa, with $\delta^{13}\text{C}$ values that consistently exceeded the $\delta^{13}\text{C}$ of bulk basal resource pools. *Pycnopsyche* caddisflies are shown because they were the least enriched of all invertebrate taxa. Other invertebrate taxa were intermediate. Bacteria data are derived from respiration assays and represent the $\delta^{13}\text{C}$ of actively respiring bacteria. Note that data are graphed on a log scale because of substantial differences between food web compartments.

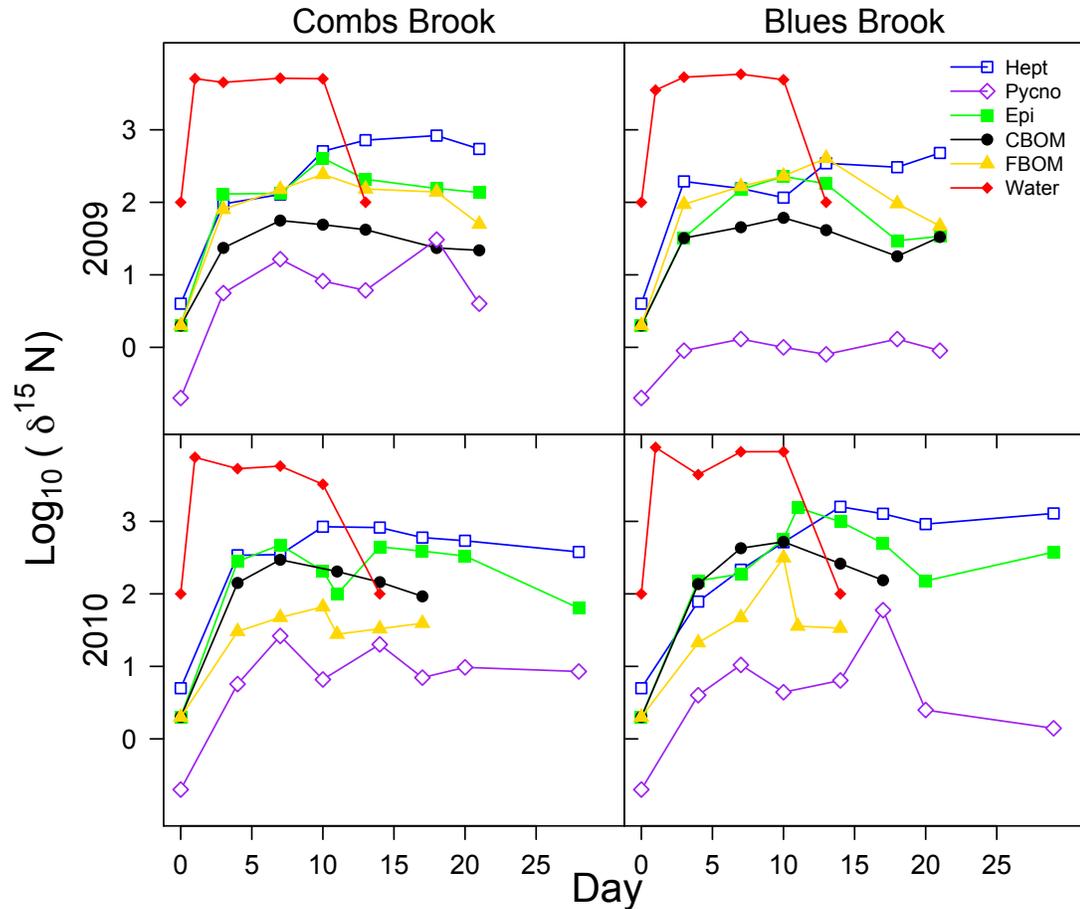


Figure 3.2: Nitrogen-15 isotope tracer found in different food web compartments (*Heptagenia* mayflies, *Pycnopsyche* caddisflies, bulk epilithon, CBOM, FBOM, and water column ammonium) as a function of days since the start of N-15 addition. *Heptagenia* mayflies are shown because they were the most enriched of all invertebrate taxa, with $\delta^{15}\text{N}$ values that consistently exceeded the $\delta^{15}\text{N}$ of bulk basal resource pools. *Pycnopsyche* caddisflies are shown because they were the least enriched of all invertebrate taxa. Other invertebrate taxa were intermediate. Note that data are graphed on a log scale because of substantial differences between food web compartments.

Table 3.2: Percent bacterial carbon assimilation by invertebrate taxon, stream and year. Percent change from 2009-2010 is noted in the right column. *Ephemerella* were only abundant enough for collection in Blues Brook in 2010. We thinned the canopy of Blues Brook after the 2009 field season. Percent changes are not noted for *Pycnopsyche* because all estimates of bacterial carbon use are very small (<0.1%).

		2009	2010	% Change
<i>Heptagenia</i>	Combs	78%	75%	-4%
<i>Heptagenia</i>	Blues	65%	44%	-32%
<i>Remenus</i>	Combs	14%	18%	+29%
<i>Remenus</i>	Blues	10%	0.9%	-91%
<i>Ephemerella</i>	Combs	No data	No data	
<i>Ephemerella</i>	Blues	No data	4.0%	
<i>Pycnopsyche</i>	Combs	0.03%	0.09%	
<i>Pycnopsyche</i>	Blues	0.06%	0.05%	

gatherer mayflies (*Ephemerella* spp.) and extremely low (<1%) assimilation of bacterial carbon in shredder caddisflies (*Pycnopsyche* spp.). These differences among taxa were consistent across years and in both natural and canopy thinned conditions. Brook trout became enriched in both ^{15}N and ^{13}C , but did not follow typical spatial patterns observed in other food web compartments (*i.e.*, enrichment did not decline from upstream to downstream sampling stations like it did for all other food web compartments so we could not determine how much bacterial carbon was assimilated).

Laboratory and field respiration assays allowed for successful measurement of ^{13}C of live bacterial biomass. Control respiration incubation treatments in which all live organisms were killed with HgCl_2 produced the same amount of CO_2 as live treatments, but showed no enrichment beyond background, indicating that the $^{13}\text{CO}_2$ in the headspace of HgCl_2 treatments was a product of bacterial respiration, not inorganic diffusion (Figure 3.3). The fraction of CO_2 in incubation headspace that was derived from organic sources (F_{org}) ranged from 7%-11% depending on stream and type of organic matter (FBOM vs CBOM).

The canopy thinning was successful in increasing light availability, which had the predicted effect of increased algal accrual in the thinned reach of Blues Brook (Figure 3.4). The thinned reach of Blues Brook had significantly higher chlorophyll accrual rates than Combs Brook or the unthinned reach of Blues Brook (Figure 3.4, fixed effects linear model, $F=68.6$, $df=2$, $p<0.001$, no significant contrasts except for Blues Brook Thinned, $t=9.9$, $df=2$, $p<0.001$). Differences in N uptake length were not significant in the study reaches (Figure 3.5, fixed effects linear model, $F=2.027$, $df=3$, $p=0.16$), but a significant increase in N uptake velocity (Figure 3.5, fixed effects linear

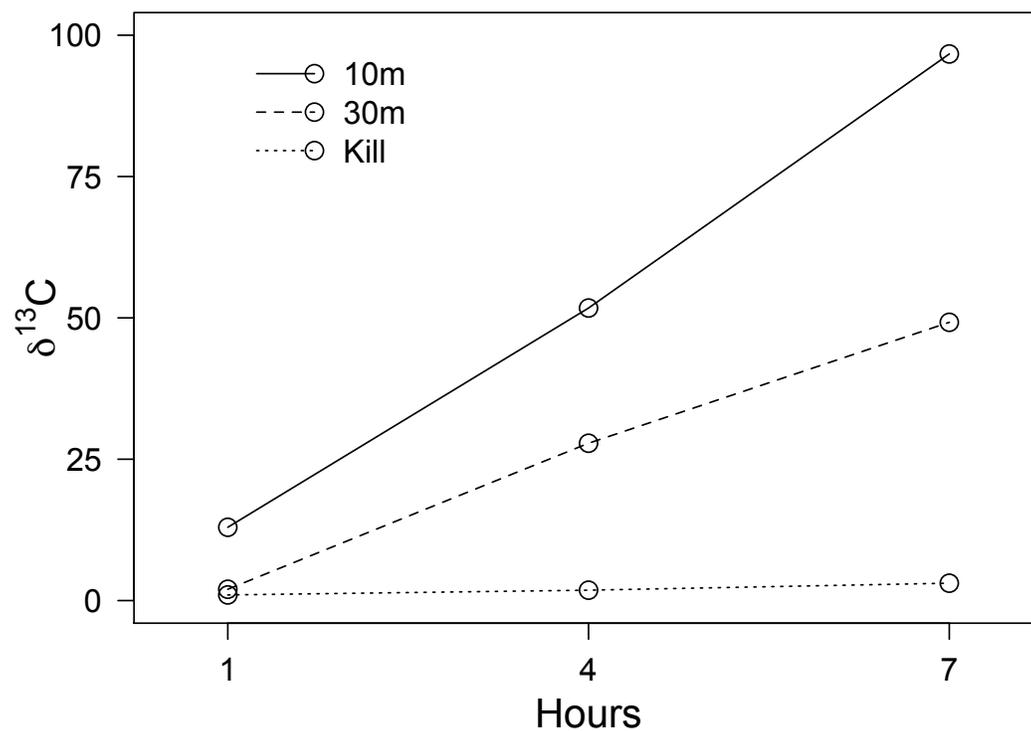


Figure 3.3: Live versus killed CO_2 respiration treatments from the 2009 tracer release in Combs Brook. Live treatments from 10 m downstream of the point of isotope addition (most enriched sampling site) and 30 m downstream of the point of isotope addition (moderately enriched sampling site) are shown with data from incubations in which microbes were killed with HgCl_2 . HgCl_2 treatments produced as much CO_2 as live treatments, but never showed any evidence of isotopic enrichment, demonstrating that labeled CO_2 in the headspace is a product of respiration.

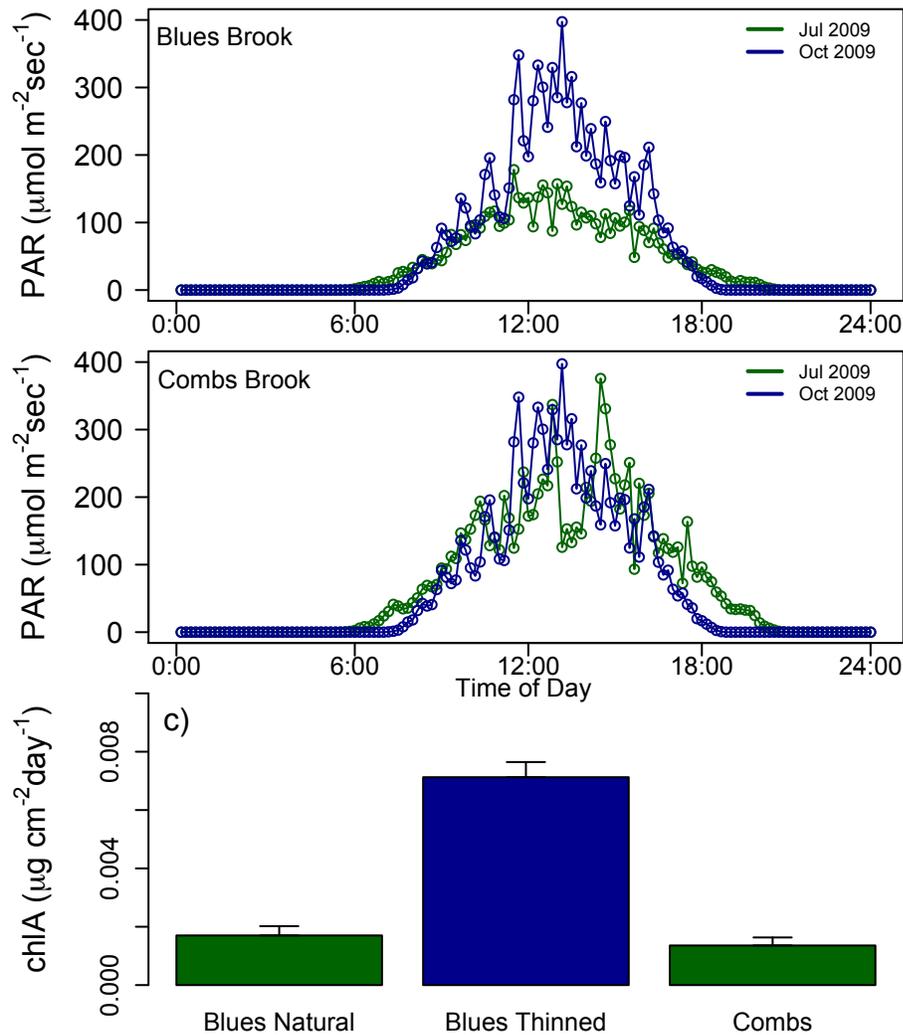


Figure 3.4: Canopy thinning increased light availability and chlorophyll accrual in Blues Brook. Light measurements from Blues Brook (top panel) show increase in light from before (July 2009) to after (October 2009) canopy thinning. There was no change in light availability between the same time periods for the unmanipulated stream, Combs Brook (Panel B). Each point on the light curves is the average photosynthetically active radiation (PAR) based on a week of measurements. Chlorophyll data (Panel C) show differences in chlorophyll-a accrual between the thinned canopy reach of Blues Brook (blue bar) relative to an unthinned reach of Blues Brook and Combs Brook (the reference stream). Error bars represent one standard error. All chlorophyll-a data are from algal accrual measurements in July 2010.

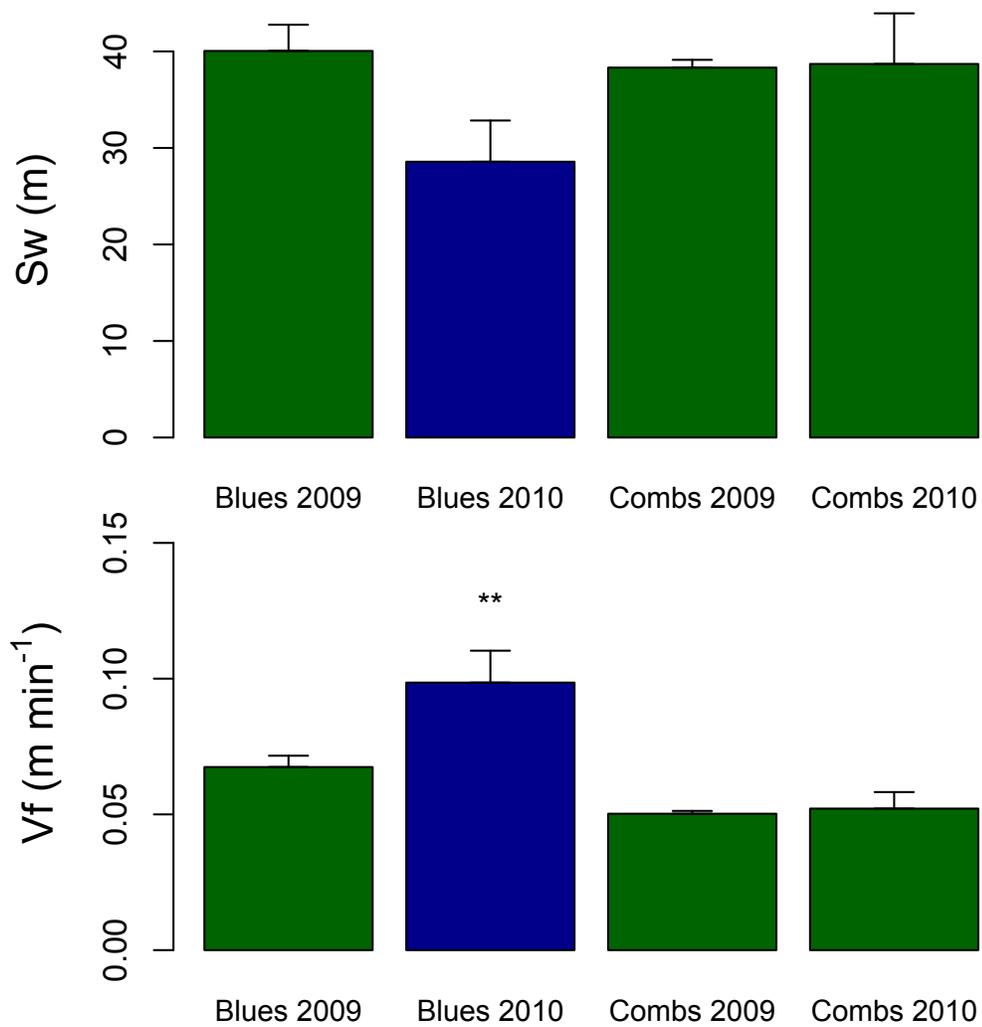


Figure 3.5: Canopy thinning resulted in decreased N uptake length (S_w) but differences were not significant due to high variance, and significantly increased N uptake velocity (V_f) in Blues Brook in 2010, which differed from other groups at the $p < 0.01$ level (denoted by **). Blue bars show the canopy thinned reach and green bars have natural canopy cover.

model, $F=10.3$, $df=3$, $p=0.001$, no significant contrasts except for Blues Brook 2010, $t=3.16$, $df=3$, $p=0.01$). Changes in the resource base associated with canopy thinning in Blues Brook reduced the proportion of bacterial carbon use for all invertebrate taxa, but bacterial carbon use for all invertebrate taxa in Combs Brook Tributary remained similar or increased in 2010 (Table 3.2). We were not able to calculate changes in bacterial carbon use by *Ephemerella* spp. because they were not sufficiently abundant for collection in either stream in 2009 or in Combs Brook Tributary in 2010.

Turnover time of invertebrates calculated using the dynamic compartment model varied by taxon and year, ranging from 4 days to 120 days depending on taxon, stream and year (Figure 3.6). Turnover times were consistently longest for *Pycnopsyche* compared to other invertebrate taxa and shortest for *Remenus* (Figure 3.6). Nearly all invertebrate taxa had longer turnover times than the duration of the isotope tracer release (10 days).

Discussion

Our results indicate that stream invertebrates can derive a high proportion of their carbon from heterotrophic bacteria, but that the proportion depends on functional feeding group and light regime. Scraper mayflies (*Heptagenia*) assimilated nearly all of their carbon from bacteria (70% or more), while shredder caddisflies (*Pycnopsyche*) assimilated almost no carbon from bacterial sources (Table 3.2). Our results are consistent with previous studies that found high bacterial carbon use (50% or more) by filterer black flies and scraper mayflies (Edwards & Meyer 1987, Edwards & Meyer

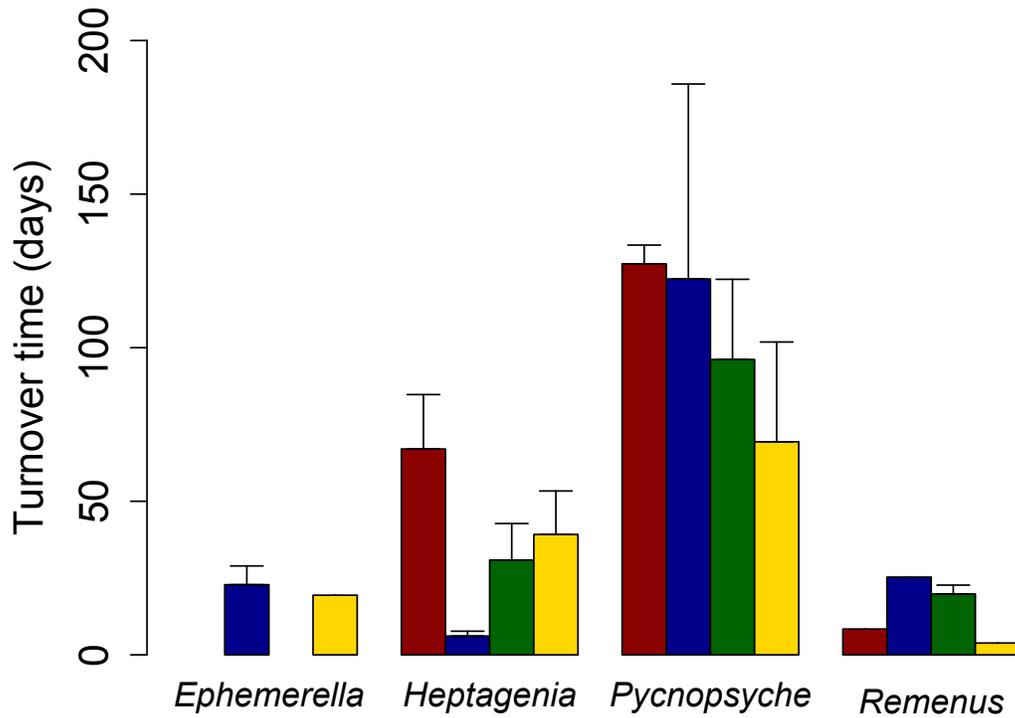


Figure 3.6: Turnover times, calculated with the dynamic compartment model using ^{15}N tracer data, for invertebrates in two study streams in 2009 and 2010 (BB = Blues Brook and CBT = Combs Brook). Error bars represent one standard deviation based on variation among sampling stations within a reach.

1990) and extremely low bacterial carbon use (<1%) by shredder invertebrates (Findlay *et al.* 1986). Our estimates of bacterial carbon use are also comparable to estimates from a previous whole-stream ^{13}C tracer addition study by Hall & Meyer (1998), except that the absolute value of our estimates were generally lower. For example, Hall & Meyer also found that scraper heptageniid mayflies assimilated very high fractions of bacterial carbon relative to other invertebrate taxa, but methodological issues led to values in excess of 100% that are difficult to compare to estimates from our system.

The respiration method we developed to measure $\delta^{13}\text{C}$ of bacteria allowed us to identify a highly enriched source that could account for the isotope label of invertebrate consumers. Actively respiring bacteria were several orders of magnitude more enriched than $\delta^{13}\text{C}$ of bulk epilithon, FBOM or CBOM, and far exceeded the most enriched invertebrates (scraper mayflies in the genus *Heptagenia*). These results suggest that invertebrates selectively feed on or assimilate carbon from components of bulk resource pools, such as live bacteria. Hall & Meyer (1998) suggested that direct consumption of DOC or consumption of highly enriched exopolymers might account for the high ^{13}C label of consumers, but our respiration results suggest that measuring the isotopic label of live components of the biofilm can account for the label found in consumers.

Identifying a carbon source (*i.e.* live bacteria) that was more enriched than consumers greatly improves the plausibility of estimates of bacterial carbon consumed by invertebrates. Previous ^{13}C tracer studies (Hall & Meyer 1998, Simon *et al.* 2003, Parkyn *et al.* 2005) have had difficulty calculating how much carbon invertebrates

derive from food sources because they found that bulk food pools were often less enriched than invertebrate primary consumers, making it impossible to identify the source of isotope label found in invertebrates or to estimate how much carbon invertebrates derive from bacterial sources accurately. Our results indicate that actively respiring components of biofilms are sufficiently enriched to account for the isotope label found in invertebrates. We did not measure extracellular polymers so cannot determine whether they also contribute to the ^{13}C label in invertebrate consumers, but they are unlikely to be as enriched as respired C.

Similarly, the problem of “over-enrichment” of consumers is also very common in ^{15}N tracer studies. Dodds *et al.* (in press) synthesized results from 19 ^{15}N isotope tracer studies in streams across North America, Europe, and the Neotropics, and found that 41 of 90 consumer taxa studied became more enriched than their presumed food sources, possibly due to consumers selectively assimilating live elements of the biofilm that took up labeled compounds. Respiration assays would not be applicable to N tracer studies, but physical separation of biofilm components has been effective. For example, Whiles *et al.* (2013) placed tiles in a stream during an isotope tracer study to measure recently accrued biofilm, and found that it was sufficiently enriched to account for the label in invertebrates, and Hamilton *et al.* (2001) separated elements of the biofilm using centrifugation gradients of colloidal silica.

Our data are unlikely to meet the assumption that invertebrates reached an equilibrium isotope content, especially for taxa with slow turnover rates. Our estimates of turnover calculated using the dynamic compartment model indicated that

turnover time of scraper mayflies (*Heptagenia*) was much faster than shredder caddisflies (*Pycnopsyche*). The average turnover time was 97 days for *Pycnopsyche* and 45 days for *Heptagenia*, but the mixing model to determine bacterial carbon use assumes that invertebrates reach equilibrium during the course of our 10-day tracer release. Hence, the model produces a much more severe underestimate of bacterial carbon used by *Pycnopsyche* than by *Heptagenia*. The duration of our study was limited to the dry, late-summer season to avoid flood events, but longer release times would improve future studies by allowing shredder invertebrates to reach equilibrium with enriched food sources.

Although we suspect that the extremely low percent bacterial carbon use by *Pycnopsyche* is partially an artifact of the duration of the tracer release, it is consistent with previous laboratory studies (Findlay *et al.* 1986) that found very low (<1%) bacterial carbon assimilation relative to respired C by shredders. These results suggest that it may be more efficient for *Pycnopsyche* to directly assimilate leaf carbon instead of microbial pools, or at least that they may not preferentially assimilate microbial carbon over leaf carbon. Studies multiple shredder species in other systems indicate that there are differences between *Pycnopsyche* and other shredders (*e.g.* tipulid crane-fly larvae, *Pteronarcys* stoneflies, and *Gammarus* amphipods) in their preference for microbially-conditioned leaf food (Arsuffi & Suberkropp 1989, Motomori *et al.* 2001, Rong *et al.* 2005) and tendency to aggregate and feed in areas with large accumulations of leaves (Tiegs *et al.* 2008). Hence, examination of more than one shredder species would be useful for determining whether these results are consistent

across species given their differences in morphology, feeding behavior and food preference, and our results for *Pycnopsyche* might not apply to all shredder species.

The light environment had a strong influence on how much of the carbon assimilated by higher trophic levels was derived from bacterial sources. Experimental canopy thinning led to increases in light availability and algal accrual that increased the algal resource base available to invertebrate consumers in Blues Brook. In some freshwater systems where autochthonous carbon is important substrate for bacterial production, increases in light and autotrophic production can lead to concurrent increases in bacterial production (*e.g.*, Findlay *et al.* 1986, Cole *et al.* 1988), but in other systems, changes in primary production or algal abundance are not coupled to bacterial production (*e.g.*, Findlay *et al.* 1991, Findlay *et al.* 1998). In our study system, increased light actually led to declines in the bacterial portion of total carbon assimilated by consumers, suggesting that even if an increase in bacterial production had occurred that it was not tracked closely by invertebrate consumers. Either the increase in autochthonous food was large enough to outweigh any increase in bacteria, or consumers preferentially fed on the enhanced algal resource. While we cannot distinguish among mechanisms, it seems clear that for some macro-consumers in detrital-based streams, greater autochthonous production led to a shift in the resource base.

We conducted the canopy thinning experiment in September 2009 and undertook post-manipulation studies during the following summer (2010). The 10-month treatment period was sufficient to see effects on algal accrual, nutrient uptake and bacterial carbon assimilation, and the increases in algal biomass relative to other

streams in the area were substantial. For example, most small streams with heavy canopy shading in the area had chlorophyll standing stocks of less than 4.0 mg m^{-2} , while a mid-order river with an open canopy (Moose River) had chlorophyll standing stocks of 18 mg m^{-2} (S. Collins, unpublished data). Hence, chlorophyll standing stock in Blues Brook after canopy thinning (9.1 mg m^{-2}) was at least twice as much as other small streams, but only about half as high as standing stock in a river with a completely open canopy. Changes in chlorophyll accrual on artificial substrates indicate that the increase in light led to accrual rates that were approximately five times higher in the thinned canopy section of Blues Brook relative to an un-thinned upstream reach (Figure 3.4). While the treatment period was long enough to see effects of light increases on the ecosystem, a longer treatment period might have demonstrated more complex food-web effects. For example, previous studies of nutrient addition in streams found complex effects on predator-prey relationships which developed over a five-year period, with production of all primary consumers increasing over the first two years, but only large-bodies species that were resistant to predation increasing in later years (Davis *et al.* 2010).

Studies of how riparian canopy cover and light influence in-stream processes have implications for how watershed land use change affects stream ecosystem function. While it has been recognized for decades that deforestation of entire watersheds can have profound influences on streams (*e.g.*, Likens *et al.* 1970, Findlay *et al.* 1993), our results indicate that stream food webs are also sensitive to tree removal that is extremely localized around a stream. Results from other systems also indicate that streams are sensitive to small changes in light availability can have a

strong influence on basal resources and consumers in streams. For example, very subtle differences in catchment forests and shading can result in changes in how consumers utilize terrestrial subsidies (England & Rosemond 2004, Giling *et al.* 2009) and changes in leaf breakdown rates and the palatability of leaves to consumers (Lagrue *et al.* 2011). Management practices designed to maintain natural light environments, such as riparian buffers zones around streams, can result in the maintenance of aquatic insect diversity that similar insect diversity to forested streams, while streams in pastures with no buffer had lower insect taxa richness (Lorion & Kennedy 2009).

Overall, our results demonstrate that fluxes of bacterial carbon to invertebrates can be significant in stream food webs, yet they differ among consumer species and are strongly influenced by the light environment. An increase in light availability is one of many changes that may influence in-stream processes when watersheds are deforested, and our results demonstrate that light changes can have substantial effects on food web fluxes, specifically with respect to the type of organic matter that is assimilated by invertebrate consumers. Our data show that in highly-subsidized streams with natural canopy cover, low light, and low primary production, the magnitude of carbon fluxes from heterotrophic bacteria to consumers can be very substantial, but that bacterial carbon is a relatively less important energy source in open-canopy streams with higher rates of primary production.

REFERENCES

- Allan, J.D. 2004. Landscapes and Riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology & Systematics* 35: 257-284.
- Arsuffi, T.L. and K. Suberkropp. 1989. Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. *Oecologia* 79: 30-37.
- Ashkenas, L.R., S.L. Johnson, S.V. Gregory, J.L. Tank and W.M. Wollheim. 2004. A stable isotope tracer study of nitrogen uptake and transformation in an old-growth forest stream. *Ecology* 85(6): 1725-1739.
- Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.
- Bartels, P., J. Cucherousset, K. Steger, P. Eklov, L.J. Tranvik and H. Hillebrand. 2012. Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. *Ecology* 93(5): 1173-1182.
- Baxter, C.V., K.D. Fausch, M. Murakami, and P.L. Chapman. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology* 85(10): 2656-2663.
- Baxter, C.V., K.D. Fausch and W.C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology* 50(2): 201-220.
- Cole, J.J., S. Findlay and M.L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Marine Ecology Progress Series* 43: 1-10.
- Cummins, K.W. 1974. Structure and function of stream ecosystems. *Bioscience* 24: 631-641.
- Das, M., T.V. Royer, and L.G. Leff. 2007. Diversity of fungi, bacteria and actinomycetes on leaves decomposing in a stream. *Applied Environmental Microbiology* 73(3): 756-767.
- Davis, J.M., A.D. Rosemond, S.L. Eggert, W.F. Cross and J.B. Wallace. 2010. Long-term nutrient enrichment decouples predator and prey production. *Proceedings of the National Academy of Sciences* 107: 121-126.

- Dodds, W.K., M.A. Evans-White, N.M. Gerlanc, L. Gray, D.A. Gudder, M.J. Kemp, A.L. Lopez, D. Stagliano, E.A. Strauss, J.L. Tank, M.R. Whiles and W.M. Wollheim. 2000. Quantification of the nitrogen cycle in a prairie stream. *Ecosystems* 3: 574-589.
- Dodds, W.K., S.M. Collins, S.K. Hamilton, J.L. Tank, S. Johnson, J.R. Webster, K.S. Simon, M.R. Whiles, H.M. Rantala, W.H. McDowell, S.D. Peterson, T. Riis, C.L. Crenshaw, S.A. Thomas, P.B. Kristensen, B.M. Cheever, A.S. Flecker, N.A. Griffiths, T. Crowl, E.J. Rosi-Marshall, R. El-Sabaawi and E. Marti. In press. You are not always what we think you eat: selective assimilation across multiple whole-stream isotopic tracer studies. *Ecology*.
- Edwards, R.T. and J.L. Meyer. 1987. Bacteria as a food source for black fly larvae in a blackwater river. *Journal of the North American Benthological Society* 6: 241-250.
- Edwards, R.T. and J.L. Meyer. 1990. Bacterivory by deposit-feeding mayfly larvae (*Stenonema* spp.). *Freshwater Biology* 24: 453-462.
- England, L.E. and A.D. Rosemond. 2004. Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater systems. *Freshwater Biology* 49: 721-734.
- Findlay, S. 2010. Stream microbial ecology. *Journal of the North American Benthological Society* 29(1): 170-181.
- Findlay, S., J.L. Meyer and P.J. Smith. 1986. Incorporation of microbial biomass by *Peltoperla* sp. (Plecoptera) and *Tipula* sp. (Diptera). *Journal of the North American Benthological Society* 5(4): 306-310.
- Findlay, S., J. Tank, S. Dye, H.M. Valett, P.J. Mulholland, W.H. McDowell, S.L. Johnson, S.K. Hamilton, J. Edmonds, W.K. Dodds and W.B. Bowden. 2002. A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecology* 43: 55-66.
- Findlay, S., K. Howe and D. Fontvieille. 1993. Bacterial-algal relationships in streams of the Hubbard Brook Experimental Forest. *Ecology* 74(8): 2326-2336.
- Findlay, S., M.L. Pace and D.T. Fischer. 1998. Response of heterotrophic planktonic bacteria to the zebra mussel invasion of the tidal freshwater Hudson river. *Microbial Ecology* 36: 131-140.

- Findlay, S., M.L. Pace, D. Lints, J.J. Cole, N.F. Caraco and B. Peierls. 1991. Weak coupling of bacterial and algal production in a heterotrophic ecosystem: the Hudson River estuary. *Limnology and Oceanograph* 36(2): 268-278.
- Giling, D., P. Reich and R.M. Thompson. 2009. Loss of riparian vegetation alters the ecosystem role of a freshwater crayfish (*Cherax destructor*) in an Australian intermittent lowland stream. *Journal of the North American Benthological Society* 28: 626-637.
- Hairston, N.G., Jr. and N.G. Hairston, Sr. 1993. Cause-effect relationships in energy flow, trophic structure and interspecific interactions. *American Naturalist* 142(3): 379-411.
- Hall, R.O. and J.L. Meyer. 1998. The trophic significance of bacteria in a detritus-based stream food web. *Ecology* 79: 1995-2012.
- Hall, R.O., B.J. Peterson and J.L. Meyer. 1998. Testing a nitrogen-cycling model of a forest stream by using a nitrogen-15 tracer addition. *Ecosystems* 1:283-298.
- Hall, R.O., J.B. Wallace, and S.L. Eggert. 2000. Organic matter flow in stream food webs with reduced detrital resource base. *Ecology* 81: 3445-3463.
- Hamilton, S.K., J.L. Tank, D.F. Raikow, D.F. Raikow, W.M. Wollheim, B.J. Peterson and J.R. Webster. 2001. Nitrogen uptake and transformation in a Midwestern U.S. stream: a stable isotope enrichment study. *Biogeochemistry* 54: 297-340.
- Hill, W.R., M.G. Ryon and E.M. Schilling. 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. *Ecology* 76(4): 1297-1309.
- Holmes, R.M., J.W. McClelland, D.M. Sigman, B. Fry and B.J. Peterson. 1999. Measuring N-15-NH₄⁺ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. *Marine Chemistry* 60: 235-243.
- Hynes, H.B.N. 1975. The stream and its valley. *Internationale Vereinigung für theoretische und angewandte Limnologie, Verhandlungen* 19: 1-15.
- Kominoski, J.S., S. Larranaga and J.S. Richardson. 2012. Invertebrate feeding and emergence timing vary among streams along a gradient of riparian forest composition. *Freshwater Biology* 57(4): 759-772.
- Laguer, C., J.S. Kominoski, M. Danger, J. Baudoin, S. Lamothe, D. Lambrigtot and A. Lecerf. 2011. Experimental shading alters leaf litter breakdown in streams of contrasting riparian canopy cover. *Freshwater Biology* 56: 2059-2069.

- Likens, G.E., F.H. Bormann, N.M. Johnson, D.W. Fisher and R.S. Pierce. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. *Ecological Monographs* 40(1): 23-47.
- Loeb, S.L. 1981. An *in situ* method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. *Limnology and Oceanography* 26: 394-399.
- Lorion, C.M. and B.P. Kennedy. 2009. Relationships between deforestation, riparian forest buffers and benthic macroinvertebrates in neotropical headwater streams. *Freshwater Biology* 54: 165-180.
- Meyer, J.L. 1994. The microbial loop in flowing waters. *Microbial Ecology* 28: 195-199.
- Meyer, L.A and S.M.P. Sullivan. 2013. Bright lights, big city: influences of ecological light pollution on reciprocal stream-riparian invertebrate fluxes. *Ecological Applications* 23: 1322-1330.
- Mille-Lindblom, C. and L.J. Tranvik. 2003. Antagonism between bacteria and fungi on decomposing aquatic plant litter. *Microbial Ecology* 45(2): 173-182.
- Moore, J.C., E.L. Berlow, D.C. Coleman, P.C. de Ruiter, Q. Dong, A. Hastings, N.C. Johnson, K.S. McCann, K. Melville, P.J. Morin, K. Nadelhoffer, A.D. Rosemond, D.M. Post, J.L. Sabo, K.M. Scow, M.J. Vanni and D.H. Wall. 2004. Detritus, trophic dynamics and biodiversity. *Ecology Letters* 7: 584-600.
- Motomori, K, H. Mitsuhashi and S. Nakano. 2001. Influence of leaf litter quality on the colonization and consumption of stream invertebrate shredders. *Ecological Research* 16: 173-182.
- Newell, S.Y. 1984. Bacterial and fungal productivity in the marine environment: a contrastive overview. *Colloques Internationaux du Centre National de la Recherche Scientifique (Marseilles)*: 331: 133-139.
- Nakano, S. and M. Murakami. 2001. Dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences* 98(1): 166-170.
- Parkyn, S.M., J.M. Quinn, T.J. Cox and N. Broekhuizen. 2005. Pathways of N and C uptake and transfer in stream food webs: an isotope enrichment experiment. *Journal of the North American Benthological Society* 24(4): 955-975.

- Pace, M.L., G.B. McManus and S.E.G. Findlay. 1990. Planktonic community structure determines the fate of bacterial production in a temperate lake. *Limnology & Oceanography* 35(4): 795-808.
- Peterson, B.J. and 14 others. 2001. Control of nitrogen export from watersheds by headwater streams. *Science* 292: 86-90.
- Pozzato, L., D. Van Oevelen, L. Moodley, K. Soetaert, and J.J. Middleburg. 2013. Sink or link? The bacterial role in benthic carbon cycling in the Arabian Sea's oxygen minimum zone. *Biogeosciences* 10: 6879-6891.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rong, Q., K.R. Sridhar and F. Barlocher. 1995. Food selection in three leaf-shredding stream invertebrates. *Hydrobiologia* 316: 173-171.
- Sato, T., K. Watanabe, M. Kanaiwa, Y. Niizuma, Y. Harada and K.D. Lafferty. 2011. Nematomorph parasites drive energy flow through a riparian ecosystem. *Ecology* 92(1): 201-207.
- Simon, K.S., E.F. Benfield and S.A. Macko. 2003. Food web structure of epilithic biofilms in cave streams. *Ecology* 84(9): 2395-2406.
- Suberkropp, K. and E. Chauvet. 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. *Ecology* 76(5): 1433-1445.
- Tank, J.L., M.J. Bernot, and E.J. Rosi-Marshall. 2007. Nitrogen limitation and uptake. Pages 213-238 in *Methods in Stream Ecology*, eds. F.R. Hauer and G.R. Lamberti. San Diego: Academic Press.
- Thimijan, R.W. and R.D. Heins. 1983. Photometric, radiometric and quantum light units of measure: a review of procedures for interconversion. *Horticultural Science* 18: 818-822.
- Tiegs, S.D., F.D. Peter, C.T. Robinson, U. Uehlinger and M.O. Gessner. 2008. Leaf decomposition and invertebrate colonization responses to manipulated litter quality in streams. *Journal of the North American Benthological Society* 27(2): 321-331.
- Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 130-137.

- Webster, J.R. and E.F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* 17: 567-594.
- Webster, J.R., E.F. Benfield, T.P. Ehrman, M.A. Schaffer, J.L. Tank, J.J. Hutchens, and D.J. D'Angelo. 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology* 41: 687-705.
- Weyers, H.S. and K. Suberkropp. 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in two streams. *Journal of the North American Benthological Society* 15(4): 408-420.
- Whiles, M.R., R.O. Hall Jr., W.K. Dodds, P. Verburg, A.D. Huryn, C.M. Pringle, K.R. Lips, S.S. Kilham, C. Colon-Gaud, A.T. Rugenski, S. Peterson and S. Connelly. 2013. Disease-driven amphibian declines alter ecosystem processes in a tropical stream. *Ecosystems* 16: 146-157.
- Wiegner, T.N., L.A. Kaplan, J.D. Newbold, and P.H. Ostrom. 2005. Contribution of dissolved organic C to stream metabolism: a mesocosm study using ¹³C-enriched tree-tissue leachate. *Journal of the North American Benthological Society* 24: 48-67.
- Wright, R.T. and J.E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* 47: 447-464.

CHAPTER 4

FISH INTRODUCTIONS AND LIGHT AVAILABILITY MODULATE FOOD WEB FLUXES IN TROPICAL STREAMS

Abstract

Decades of ecological study have identified top-down and bottom-up controls on food web structure in a diverse set of ecosystems, but relatively little is known about detritus-based ecosystems in the tropics. We conducted an experiment to investigate how fish introductions and light availability influence energy and material fluxes in Trinidadian stream food webs, where the evolution of guppies (*Poecilia reticulata*) has been studied for decades and guppy populations can be manipulated in whole-ecosystem experiments. We introduced guppies to two headwater streams and compared guppy-introduction reaches to upstream (no guppy) reference reaches to evaluate the effects of guppy introduction on food webs. We thinned the canopy of one of the two study streams (Upper LaLaja, UPL) to increase light availability and compared it to the other guppy introduction stream, which had a natural canopy (Lower LaLaja, LOL) to determine how light availability affects food web linkages. A dual carbon (C) and nitrogen (N) stable isotope tracer approach allowed us to compare food web fluxes of N from both algal and bacterial resources with fluxes of C from only heterotrophic bacterial resources, and determine how they differed in response to light availability and guppy introduction. Primary production was higher in the canopy-thinned stream (UPL) compared with the natural canopy stream (LOL),

which led to increased fluxes of N to invertebrate grazers, collector-gatherers and filterers. Heterotrophic pathways were also enhanced by increased light availability, resulting in increased fluxes of bacterial C to invertebrate grazers and collector-gatherers in the canopy-thinned stream. Primary production was also higher in guppy introduction reaches, which resulted in increased magnitude of N fluxes to invertebrate grazers and filter-feeders. The magnitude of N fluxes to invertebrate collector-gatherers decreased in guppy introduction reaches, possibly due to predation, resource competition, or behavioral responses of invertebrates. N fluxes to shredder invertebrates, predatory invertebrates and killifish did not differ across light or guppy treatments. Effect size of guppy and canopy treatments on N flux to consumers revealed that they have similar effects on most taxa, but guppies have a very strong effect on filter feeding invertebrates and canopy had a very strong effect on collector-gatherer invertebrates. Combined, these results indicate that light availability and fish introduction can both influence food web linkages in detritus-based tropical streams by increasing autotrophic production, while detritus-based links remain relatively stable.

Introduction

Quantifying fluxes of nutrients and organic matter in food webs is a key aspect of characterizing food web and ecosystem dynamics. Changes in environmental context, including top-down drivers such as predator introductions, and bottom-up drivers such as changes in light or nutrient availability, can have a strong influence on primary producer biomass and the strength of trophic linkages. The relative importance of top-down and bottom-up effects have been studied, debated, and reviewed extensively in the ecological literature (*e.g.*, reviews by Power *et al.* 1992, Hairston & Hairston 1993, Polis & Strong 1996, Persson 1999, Gruner *et al.* 2008). Many seminal studies of top down and bottom up drivers have been conducted in freshwater systems, where investigators have documented clear effects of predatory or planktivorous fishes (Carpenter & Kitchell 1988, Power 1990, Flecker & Townsend 1994, Hambright 1994), nutrient loading (Schindler 1977, Hambright *et al.* 2007, Davis *et al.* 2010a, Davis *et al.* 2010 b), and light availability (Hansson 1992, Hill *et al.* 1995, Ask *et al.* 2009, De Nadai-Monoury *et al.* 2014) on primary production and food web linkages. Much of this research has been conducted in temperate systems that are driven by autotrophic production while relatively little is known about influences on freshwater ecosystems that are fueled by external inputs of detrital carbon (Johnson & Wallace 2005), especially in tropical regions (Boyero *et al.* 2008).

Syntheses of tropical headwater stream ecosystems demonstrate high variation in food web structure, organic matter sources and invertebrate communities, and indicate that general food web models developed for temperate systems might not be applicable to tropical streams (reviewed by Boyero *et al.* 2009, Dudgeon *et al.* 2010).

In some forested tropical streams, invertebrates rely on high-quality algal food resources despite low light availability and limited primary production (March & Pringle 2003, Li & Dudgeon 2008, Lau *et al.* 2009), while in others detritus and terrestrial energy inputs dominate and leaf-shredding invertebrates are highly abundant and diverse (Yule 1996, Cheshire *et al.* 2005, Coat *et al.* 2009). Top-down effects due to fish presence can have strong effects on some tropical stream food webs (Moulton *et al.* 2010), but might be mediated by a behavioral response of invertebrates in some detritus-based streams (Boyero *et al.* 2008). Tropical streams also often contain a high proportion of omnivorous or generalist species (Cheshire *et al.* 2005, Li & Dudgeon 2008, Frauendorf *et al.* 2013), which makes it difficult to characterize functional feeding groups, trophic levels, and trophic interactions. Differences in rainfall during wet and dry seasons in tropical regions can also have a strong influence on stream food webs (Heatherly 2012, Kohler *et al.* 2012).

Here, we evaluate the effects of increased light availability and fish introductions on tropical stream food webs in a whole ecosystem experiment. We compared the effects of light and fish introductions in headwater streams in the Northern Range of Trinidad (Trinidad and Tobago). Guppies (*Poecilia reticulata*), a common fish species of the region, are well suited for field introduction experiments (*e.g.*, Reznick *et al.* 1990), and the evolution of guppy life history, morphology and diet has been well characterized (reviewed by Magurran 2005). Recent research in mesocosms suggests that not only can the presence of guppies influence ecosystem processes, but also that evolved differences between guppies from streams with high versus low predation pressure can differentially influence the food web (Palkovacs *et*

al. 2009, Bassar *et al.* 2010, Ellner *et al.* 2011). The effect of guppies on natural stream ecosystems is not as well understood, but field observations indicate that guppy feeding behavior varies across sites with different light and resource availability (Zandona *et al.* 2011), and that guppy presence can have a strong effect on other fish species (Walsh *et al.* 2011). Consumer exclusion experiments in Trinidadian streams suggest that guppies can affect rates of biofilm accrual and leaf decomposition (Marshall *et al.* 2012), but survey data from streams across Trinidad indicate that the quantity and quality of biofilms is controlled by a complex set of variables, including light availability, flow and consumers (Kohler *et al.* 2012). Light and fish assemblages co-vary in Trinidad streams, making it difficult to disentangle their effects in comparative studies (Grether *et al.* 2001).

We used novel dual ^{13}C and ^{15}N short-term stable isotope tracer additions to characterize food webs. Adding a nitrogen (^{15}N) or carbon (^{13}C) stable isotope tracer to a stream is more labor intensive than collecting natural abundance isotope data but allows for more detailed characterization of food web linkages. Sites around North America in the Lotic Intersite Nitrogen eXperiment (LINX), and a number of other sites across the world have used ^{15}N tracers to characterize stream food webs (*e.g.* Dodds *et al.* 2000, Mulholland *et al.* 2000, Ashkenas *et al.* 2004, Hamilton *et al.* 2004, Whiles *et al.* 2013). While most isotope tracer studies use ^{15}N tracers to characterize food web fluxes, ^{13}C isotope tracers are especially useful in detritus-based systems because some compounds (*e.g.*, acetate) can be used to label only heterotrophic bacteria and others (*e.g.*, bicarbonate) to label only algae, so ^{13}C tracers can therefore be used to trace either bacterial or algal energy from basal resource pools to

consumers. However, ^{13}C isotope tracers have been used in very few studies (Hall & Meyer 1998, Simon *et al.* 2003, Hotchkiss & Hall in press) and we know of no published studies that have used both C and N tracers in tandem in a field experiment. We conducted whole-ecosystem experiments in a 2×2 factorial design in two tropical streams on the island of Trinidad and quantified food web fluxes using a dual ^{15}N and ^{13}C isotope tracer approach. We manipulated two adjacent streams to evaluate light and fish effects: 1) we compared downstream reaches with introduced guppy populations to upstream reference reaches that do not have guppies present, and 2) we compared a stream with a natural riparian canopy to a second stream with a canopy that had been thinned to increase light availability. Nutrient limitation assays suggest that our study streams are limited by light, not nutrients (Heatherly 2012), so we predicted that increased light availability would lead to increased primary production and subsequently to an increase in fluxes of energy and materials from algal biofilms to grazers. We hypothesized that increased primary production would be accompanied by concurrent increases in heterotrophic bacterial production based on syntheses from other aquatic systems (Cole *et al.* 1988), but it is also possible for increased primary production to lead to declines in heterotrophic bacterial production in some stream ecosystems (*e.g.*, Findlay *et al.* 1993). If canopy thinning stimulated heterotrophic bacterial production as well as primary production, we expected to see similar patterns in the ^{15}N tracer data (which represents both algal and bacterial pathways) and ^{13}C tracer data (which only represents fluxes from heterotrophic bacteria) across the two light environments (*i.e.*, similar effects of light on fluxes of N and fluxes of bacterial C), but if canopy thinning suppressed heterotrophic bacterial

production relative to algal production, we expected to see increased fluxes of N tracer to consumers in the light stream, but decreased fluxes of bacterial C. Previous mesocosm experiments demonstrated that guppy presence leads to lower chlorophyll biomass, higher biomass-specific primary production, and lower areal primary production rates (Bassar *et al.* 2010), so we expected that guppy presence would have an influence primary production and the magnitude of fluxes from biofilms to invertebrate consumers. Since guppies are benthic feeders, we expected that guppy feeding activity would also lead to increased suspension of benthic organic matter in the water column, and lead to increased fluxes to filter feeders. Our study design and hypotheses are summarized in Figure 4.1.

Methods

Study site and ecosystem manipulation

We conducted our field research in two streams in the Guanapo Valley in the Northern Range of Trinidad during the 2010 dry season (March-May) using a 2×2 factorial design where guppy presence and light availability were manipulated (Figure 4.1). Our two streams are parallel tributaries of the Guanapo River and are less than one kilometer apart (Figure 4.2). The area is remote with streams that are relatively pristine with moderate nutrient concentrations (Table 4.1). Prior to manipulation, both streams were heavily shaded by riparian canopy, and killifish (*Rivulus hartii*) were the only fish present. Other fish species are prevented from upstream movement to the study region by barrier waterfalls. In 2007, we increased light availability by thinning the canopy (*i.e.*, removing all trees within 5 m) of one stream, Upper LaLaja (UPL, or

	Canopy Thinned (+Light)	Natural Canopy (-Light)
No-Guppy Reference (-Guppy)	N Tracer only Increased 1° Production compared to natural canopy Higher N fluxes to consumers than natural canopy, no guppy	N Tracer only Low 1° Production Lower N fluxes to consumers than thinned canopy, or guppy intro
Guppy Introduction (+Guppy)	N&C Tracers Increased 1° Production compared to natural canopy Higher N fluxes to consumers than other treatments Higher bacterial C fluxes to consumers than natural canopy Higher N and C fluxes to filter-feeders than no guppy reaches	N&C Tracers Lower 1° Production than thinned canopy reaches Higher N fluxes to consumers than natural canopy no guppy reach Lower bacterial C fluxes to consumers than thinned canopy guppy reach Higher N and C fluxes to filter-feeders than no guppy reaches

Figure 4.1: Study design and hypotheses.

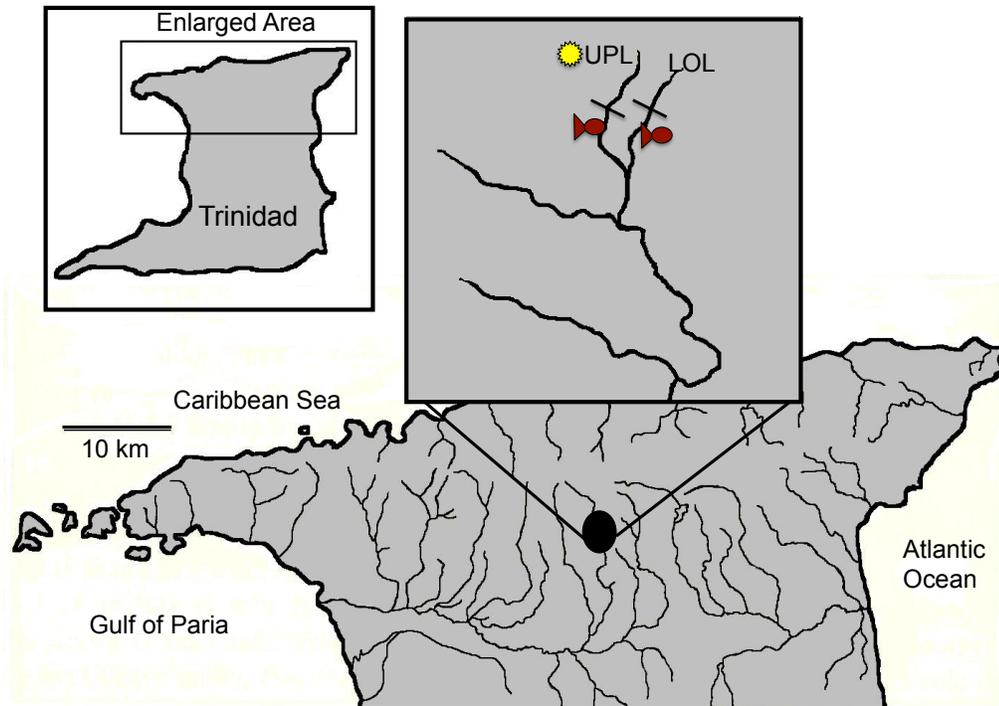


Figure 4.2: Locations of study streams. The thinned canopy stream (UPL, yellow sun symbol) and natural canopy stream (LOL) are in the Guanapo River drainage in the Northern Range of Trinidad. Both have a downstream guppy introduction reach (red fish symbols) that is separated from an upstream control reach by barrier waterfalls (black lines).

Table 1: Characteristics of four study reaches, including stream water chemistry, dry mass of primary uptake components and stoichiometry of primary uptake components. Means are for a pooled sample of three replicates per reach on each of two sampling dates (March, May) with standard deviations in parentheses. Significant contrasts identified from linear models described in the results section are noted in the right column.

	Thinned Canopy (Upper LaLaja)		Natural Canopy (Lower LaLaja)		Significant effects (guppy, stream, stream×guppy)
	Guppy Introduction	No Guppy Reference	Guppy Introduction	No Guppy Reference	
Discharge (L/sec)	17.6	17.6	13.8	13.8	----
Light (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$)	183	162	94	110	----
Whole-stream metabolism					
GPP ($\text{mg L}^{-1} \text{day}^{-1}$)	2.44 (0.16)	1.50 (0.27)	1.64 (0.74)	0.83 (0.18)	Stream ($p<0.001$), Guppy ($p<0.001$)
R ($\text{mg L}^{-1} \text{day}^{-1}$)	19.5 (0.31)	12.1 (1.2)	22.8 (3.0)	20.4 (0.72)	Stream ($p<0.001$), Guppy ($p<0.001$), Stream×Guppy ($p<0.001$)
Stream Chemistry					
NH ₄ ($\mu\text{g/L}$)	2.58	2.16	3.45	2.68	----
NO ₃ ($\mu\text{g/L}$)	213	218	200	204	----
SRP ($\mu\text{g/L}$)	23.8	23.6	37.3	29.1	----
DOC (mg/L)	0.582 (.027)	0.582 (.027)	0.626 (.073)	0.626 (.073)	----
Primary uptake - Dry mass					
Epilithon AFDM (g/m^2)	31.1 (40)	48.9 (46)	30.7 (22)	46.5 (38)	NS
Epi chl a (mg/m^2)	14.6 (19)	14.1 (12)	11.6 (7.2)	13.3 (13)	NS
CBOM (g/m^2)	70.9 (67)	86.3 (121)	131 (114)	71.5 (57)	NS
FBOM (g/m^2)	701 (492)	2026 (1418)	---	---	
Seston (mg/L)	0.893 (0.34)	0.505 (0.22)	0.388 (0.19)	1.49 (0.54)	Stream ($p<0.01$), Guppy ($p<0.01$), Stream×Guppy ($p<0.01$)
Primary uptake - C:N					
Epilithon	9.88 (1.7)	9.15 (1.5)	10.6 (1.7)	11.2 (1.6)	Stream ($p<0.001$), Stream×Guppy ($p=0.02$)
CBOM	32.9 (6.7)	35.4 (8.4)	37.8 (9.3)	36.9 (5.6)	NS
FBOM	13.2 (1.4)	13.2 (0.66)	14.4 (0.39)	13.9 (0.46)	NS
Seston	12.6 (1.3)	12.7 (1.2)	13.1 (1.5)	13.5 (1.4)	Stream ($p=0.02$)

canopy-thinned stream) in both the guppy introduction reach and control reach. We maintained the open canopy through continued removal of vegetation from 2007-2010. No canopy manipulations were conducted on the second stream, Lower LaLaja (LOL, or natural canopy stream).

We continuously monitored light using Hobo light loggers and monitored discharge using Hobo stage loggers (Onset Computer Corporation, Bourne, MA). We converted data from Hobo light loggers (lumen units) to photosynthetically active radiation (PAR) using methods described by Thimijan & Hines (1983). Canopy thinning in UPL resulted in a 30% increase in fluxes of photosynthetically reactive radiation (PAR) that were consistent over 2007-2010 (Kohler *et al.* 2012) and approximately 1.6 times higher light in the canopy-thinned stream (UPL) than the natural canopy stream (LOL) during the 2010 dry season (Table 4.1).

We used guppies (*Poecilia reticulata*) for our fish manipulations. Guppies are omnivorous, eating a combination of detritus, algae and invertebrates with diet varying depending on environmental conditions (Zandona *et al.* 2011). Guppies were introduced to the “introduction reach” of both study streams in March 2008 as part of a large study focusing on the consequences of guppy population evolution on stream ecosystem processes (*e.g.*, Palkovacs *et al.* 2009, Bassar *et al.* 2010, Zandona *et al.* 2011, Kohler *et al.* 2012, Marshall *et al.* 2012). Guppy populations have been quantified monthly since introduction through mark-recapture studies. An upstream no-guppy control reach of each stream was separated from the introduction reach by a waterfall or dam that prevented upstream guppy movement. No guppies were observed in control reaches during monthly visual surveys from 2008-2010.

Metabolism measurements

During the isotope tracer releases in March 2010, we quantified primary production in each study reach through whole-stream metabolism measurements. We continuously measured dissolved oxygen concentrations and water temperature at 10-minute intervals during blocks of 5 to 12 days. Gross primary production (GPP) and ecosystem respiration (ER) rates were determined using an open-system, single station diel approach (Roberts *et al.* 2007). Diel dissolved O₂ (DO) and temperature were collected using YSI Model 600 OMS V2 sondes equipped with YSI model 6150 Optical DO probes (YSI Inc., Yellow Springs, OH, USA). In all study reaches, reaeration coefficients (k_{O_2}) were determined for a 50-m section by simultaneously injecting propane gas (volatile tracer) and concentrated NaCl solution as a conservative tracer (for detailed methodology, Roberts *et al.* 2007). Using a YSI Model 600 OMS sonde we measured specific conductance while reaeration coefficients were determined from steady-state propane concentrations corrected for dilution using conductivity values. Gas samples were analyzed on a Hewlett Packard Model 5890 Series II gas chromatograph equipped with an Agilent HP-PLOT AI203 column and a flame ionization detector.

Daily GPP and ER rates were calculated from the diel DO and temperature data as described by Roberts *et al.* (2007). Ecosystem metabolism rates were determined from the rate of change in DO concentration over 10-min intervals using the following equation:

$$\Delta DO = GPP - ER + E$$

where ΔDO is the change in DO concentration ($\text{mg O}_2 \text{ m}^{-3}$), GPP is volumetric gross primary production ($\text{mg O}_2 \text{ m}^{-3}$), ER is volumetric ecosystem respiration ($\text{mg O}_2 \text{ m}^{-3}$), and E is net exchange of O_2 with the atmosphere ($\text{mg O}_2 \text{ m}^{-3}$) between consecutive measurements. The net exchange of O_2 with the atmosphere is the product of the O_2 reaeration coefficient k_{O_2} and the average DO deficit (DO concentration at 100% saturation minus the DO concentration in stream water) over the measurement interval.

Food web biomass sampling

Biomass of food web compartments and water chemistry in both streams was monitored in March 2010 before isotope tracer releases began and in May 2010 after sampling concluded. Each stream had six biomass sampling sites, three in the guppy introduction reach and three in the upstream control reach. We sampled one pool and one riffle at each of the six sampling sites for a total of 12 samples per stream. Food web compartments in the biomass samples include water chemistry, basal resources and invertebrate consumers. Biomass estimates for fish consumers (guppies and killifish) were obtained from concurrent mark-recapture studies in both streams (detailed in Fraser & Lamphere 2013, Arendt *et al.* 2014).

We collected filtered water samples for soluble reactive phosphorus (SRP) and nitrate (NO_3) that were frozen and returned to the US for analysis. Nitrate samples were analyzed using a Dionex ICS-90 ion chromatography system with Chromeleon software (Dionex Corporation) and SRP samples were analyzed on a Pharmacia LKB Ultraspec III spectrophotometer (model 80-2097-62; Pharmacia Biotech) using a

method developed by Murphy & Riley (1962). We analyzed ammonium (NH_4) water chemistry in the field using fluorometric methods with an Aquaflor handheld fluorometer (Turner Designs, Sunnyvale, CA). Ammonium samples collected in brown opaque bottles and kept cold for up to six hours until they were reacted with orthophthaldialdehyde (OPA). Fluorescence was measured 2-3 hours after the OPA reagent was added. We created a standard curve with stream water samples to correct for matrix effects and converted fluorescence to NH_4 concentration (Taylor *et al.* 2007).

Because both streams contained many large rocks and bedrock that could not be removed from the stream and scrubbed, we sampled epilithon with modified Loeb samplers (Loeb 1981). Seven Loeb samples per sampling site were combined into a single sample for analysis. Epilithon samples were subsampled and filtered through glass fiber filters (Whatman GF/F; 0.7 μm pore size) to analyze chlorophyll-*a* and ash free dry mass (AFDM). Suspended organic matter (seston) was collected on Whatman GF/F filters in the field by filtering a known quantity of stream water, between 1 and 2 L depending on seston concentration. Fine benthic organic matter (FBOM) was sampled by sinking a plastic cylinder (bottom area = 530 cm^2) into an area of soft sediment, measuring the water depth in the cylinder, mixing the surface layer of organic matter into the water, and removing a known quantity of slurry from the cylinder. Coarse benthic organic matter (CBOM) was sampled by haphazardly selecting a location on the stream and removing all leaf litter and woody material in a 0.5 m wide transect that stretched across the width of the stream at the selected location. We dried epilithon AFDM, seston, FBOM and CBOM samples at 50°C until

they reached a constant mass and recorded all biomasses. After recording the mass of epilithon AFDM filters, we ashed filters at 450°C for six hours and recorded the mass of the filter plus ash.

Invertebrates were sampled at the same transects as basal resource compartments using a Hess sampler with a 0.032 m² area and a 250 µm net. We preserved invertebrate samples in 90% ethanol and returned them to the US for processing and enumeration. Biomass was calculated using standard length-mass relationships (Benke *et al.* 1999, Baumgartner & Rothhaupt 2003, Sabo *et al.* 2002, Miyasaka *et al.* 2008, T. Heatherly, unpublished data).

¹³C-acetate and ¹⁵N-ammonium addition to quantify food web fluxes

We added ¹⁵N labeled ammonium (as dissolved ¹⁵NH₄Cl) to all four study reaches: canopy thinned guppy introduction reach (thinned guppy), canopy thinned reference reach (thinned no guppy), natural canopy guppy introduction reach (canopy guppy), and natural canopy reference reach (canopy no guppy) and ¹³C labeled acetate (as dissolved sodium acetate-2-¹³C) to the thinned guppy and canopy guppy reaches. It was only possible to add ¹³C to two of the four reaches because of logistical and funding constraints, and we chose to hold guppies constant and contrast light availability because the difference between total fluxes and fluxes from heterotrophic bacteria have a clear connection to light availability and primary production. We added isotope tracers using a continuous drip with an injection rate of 10 mL min⁻¹ over a 10-day period from March 7- 16, 2010. The amount of C isotope we added increased the concentration of ¹³C in stream water by 1.25 µg L⁻¹, which elevated the

$\delta^{13}\text{C}$ of the DOC pool to approximately 100‰. The N injections increased the $\delta^{15}\text{N}$ of dissolved ammonium to approximately 20,000‰. Despite the lower enrichment in ^{13}C relative to the DOC pool, acetate is a highly labile compound and is taken up rapidly relative to bulk DOC, so uptake compartments became comparably enriched in both ^{13}C and ^{15}N (Figure 3), except for leaf CBOM, which did not become as enriched in ^{13}C as it did in ^{15}N , likely due to high C:N ratio of detrital leaf material. The target enrichments were not intended to fertilize the system, and the concentration of ^{15}N added was <5% of ambient NH_4 and the concentration of ^{13}C added was several orders of magnitude less than ambient DOC (Table 1). We also added rhodamine fluorescent dye as a conservative tracer throughout the course of the isotope release, which we used to correct for dilution along the study reach. We assumed that algal uptake of the ^{13}C tracer was minimal relative to bacterial uptake (Wright & Hobbie 1966) and fungal uptake should have been negligible because the half saturation constants for fungal uptake are several orders of magnitude higher than the concentration at which we added acetate to the streams (Newell 1984, Hall & Meyer 1998, Simon *et al.* 2003).

We sampled food web compartments to track the fate of the isotope tracers at three stations downstream of each point of isotope release (approximately 15, 30 and 60m downstream depending on the reach). Samples were collected on three days during the 10-day isotope release (Days 3, 7 and 10), and on five days during the month following the isotope release (Days 13, 17, 20, 30 and 40). Sampled food web compartments included: water chemistry ($^{15}\text{NO}_3$ and $^{15}\text{NH}_4$), epilithon, FBOM (sampled from the sediment surface via suction), CBOM, seston, eight common

invertebrate taxa representing all functional feeding groups (predators, grazers, collector-gatherers, collector-filterers and shredders), guppies, and killifish. Invertebrate taxa selected were sufficiently large-bodied and abundant that they could be collected by hand with minimal disturbance to the streambed. Many of the invertebrate taxa we collected (*Eudaniela*, *Euthyplocia*, *Psephenus*, *Leptonema*, *Tricorythodes* and *Argia*) are among the dominant taxa in both streams (Table 4.2), but two of our sampled invertebrate groups, *Petrophila* sp. and *Phylloicus* sp. were not among the most abundant invertebrate taxa but represented functional feeding groups (scraper and shredder, respectively) distinct from those dominant in the streams. We were unable to collect some small-bodied but abundant taxa (e.g., chironomids) because we could not collect enough individuals for isotope sample analysis without causing major disturbance to the streambed. Guppies and killifish were sampled on a more limited number of days (Days 10, 20, 30, 40) because they have longer turnover times and we wanted to avoid significant changes to fish population sizes. We also collected background samples from each compartment to correct for background isotopic values. Background samples were collected either prior to the start of the experiment or from upstream of the control reach tracer addition point.

We dried all samples at 50°C and conducted isotopic analyses at the University of Georgia isotope analysis facility. We used elemental analysis data from isotope analyses to quantify the ratio of C:N in basal resources, which is often used as a proxy of food quality. Basal resource sampling protocols were the same as the biomass sampling techniques, but for invertebrates, we hand-picked individuals by turning over rocks and sorting through organic matter in plastic trays to minimize disturbance and

Table 4.2: Biomass dominant invertebrate taxa in the two study streams. Means are for the three replicates per reach and three sampling dates (March, April, May) with standard deviations in parentheses. “Sampled” column indicates whether the taxon was sampled in our experiment.

Order	Family	Genus/Tribe	Thinned Canopy (Upper LaLaja)		Natural Canopy (Lower LaLaja)		Sampled?
			Guppy Intro Dry Mass mg/m ²	No Guppies Dry Mass mg/m ²	Guppy Intro Dry Mass mg/m ²	No Guppies Dry Mass mg/m ²	
Decapoda	Pseudothelphusidae	Eudaniela	9475 (2622)	6785 (5440)	5278 (2096)	6945 (5162)	Yes
Ephemeroptera	Euthyplociidae	Euthyplocia	0 (0)	3611 (3967)	1404 (2431)	1764 (2563)	Yes
Oligochaeta	Tubificidae	Tubifex	1133 (229)	655 (619)	598 (479)	632 (667)	No
Coleoptera	Psephenidae	Psephenus	824 (170)	868 (559)	547 (159)	303 (159)	Yes
Diptera	Chironomidae	Non-tanypodinae	574 (212)	533 (384)	319 (135)	898 (269)	No
Trichoptera	Hydropsychidae	Leptonema	881 (75)	159 (116)	464 (768)	7 (9)	Yes
Ephemeroptera	Leptohyphidae	Tricorythodes	235 (205)	591 (609)	54 (24)	143 (128)	Yes
Diptera	Chironomidae	Tanypodinae	126 (80)	107 (12)	73 (36)	147 (33)	No
Coleoptera	Elmidae	Heterelmis	116 (122)	74 (23)	99 (45)	10 (20)	No
Odonata	Coenagrionidae	Argia	62 (47)	105 (59)	59 (27)	46 (49)	Yes

ensure that sufficient numbers of each taxon were collected for isotopic analysis. We also measured water column ^{15}N , which we measured with a filter pack diffusion technique (Sigman *et al.* 1997, Holmes *et al.* 1998). Specifically, we collected 900 mL of water in 1 L plastic cubitainers for $^{15}\text{NH}_4$ samples and added a 60 μg spike of N as NH_4 to increase N mass to a level that is detectable by a mass spectrometer. We collected 500 mL of water for $^{15}\text{NO}_3$ samples and transferred samples in 250 mL Nalgene bottles after boiling to reduce volume to approximately 100 mL. Ammonium from all samples was allowed to diffuse onto a 1.0 cm Whatman GF/D (2.7 μm pore size) filter sealed in Teflon tape for at least three weeks before filters were harvested and dried at 50°C.

Turnover and flux calculations

Turnover rates for primary uptake compartments were calculated using a simple exponential decline model fit to data from the days following the isotope tracer release (*i.e.*, drip days 11-40). Turnover, k , is represented by the negative slope of a linear regression of log-transformed isotope data over time. Although exponential decline models are the preferred method of calculating turnover, they are not effective unless the data series is long enough to represent three turnovers of the food source of a given compartment (J. Webster, unpublished). This assumption was met for the primary uptake compartments, because we collected 30 days of decline data after the isotope drip, which would easily represent more than 3 turnover times of water column C or N. The average turnover times for primary uptake compartments in this study (16.2 days for N and 15.0 days for C) would have required approximately 45

days of post-drip decline data to achieve three turnover cycles, so we could not model consumers with exponential decline methods.

We used a dynamic compartment model to calculate turnover and flux rates of consumers as described in detail by Dodds *et al.* (in press) and in Appendix D. Briefly, we used observed patterns of ^{15}N or ^{13}C accumulation and loss in a given taxon and its presumed food source over the course of our tracer release and post-release sampling period. We estimated uptake and loss rates by calculating a flux rate of ^{15}N and ^{14}N (or ^{13}C and ^{12}C) from the food pool into the consumer pool between each sampling date. We accounted for the change in ^{15}N (or ^{13}C) in the consumer after each time step to calculate the new beginning size of the consumer pool for the following time step. The number of time steps for each model usually included 5-8 samples at a frequency of 3-10 days between samples, depending on sample availability. We used the Solver function in Microsoft Excel to minimize the sum of error terms for each time step and visually checked that observed and modeled patterns of $\delta^{15}\text{N}$ (or $\delta^{13}\text{C}$) were similar. Despite criticisms of using the Solver function to fit functions (McCullough & Heiser 2008), it is effective for other isotope tracer data sets with similar sampling frequency as ours, including data from 19 streams in temperate and tropical regions across the world and tests on simulated data sets to assure that it is effective for stream isotope tracer data (Dodds *et al.* in press). We calculated turnover rate for each consumer pool at each downstream sampling station in a reach and calculated the average turnover rate for the reach. To calculate flux rate, we multiplied the turnover rate for the reach by the average N (or C) mass for the reach.

Prior diet assumptions were based on feeding mode of invertebrates, qualitative inspection of gut contents, and literature values of diet proportions (Zandona *et al.* 2011). For omnivores that ate more than one food source, we weighed each individual food source based on estimated diet proportions in the diet to create a composite food pool for the consumer. Information for insect functional feeding groups, killifish and guppies was available, but no published diet data existed for the highly abundant freshwater crab, *Eudaniela*, especially for the small, young crabs collected in our study streams (*i.e.*, carapace width of a few cm or less). However, *Eudaniela* are thought to be detritivores and it is known that other freshwater crab species in the Caribbean consume terrestrial detritus (*e.g.*, Fraiola 2004). We assumed that *Eudaniela* eat a mix of fine and coarse detrital organic matter in our models. All diet proportions and food web model inputs are summarized in Appendix E.

It is common for primary consumers to become more enriched than presumed food sources in stream isotope tracer studies, likely due to selective assimilation of the active parts of bulk organic matter pools (Dodds *et al.* in press). When consumer enrichment exceeded bulk food pool enrichment, we solved for a multiplier that increased the isotopic label of the food source by a constant factor. The multiplier was constrained by the maximum $\delta^{15}\text{N}$ of water column NH_4 , which should represent the isotope label of active algae and bacteria at equilibrium. For $\delta^{13}\text{C}$, we constrained the multiplier by creating an upper bound that was based on the ratio of water column $^{15}\text{NH}_4$ to bulk basal resource $\delta^{15}\text{N}$. Secondary consumers generally did not require a multiplier; presumably predators assimilate food sources that are stoichiometrically

similar to their own tissue content so selective assimilation of high quality parts of food sources was not necessary.

We compared turnover and flux rates among guppy and light treatments using fixed effects linear models, with the three sampling transects per study reach as our unit of replication. Although transects in the same stream reach may be viewed as pseudoreplicates, it is not possible to conduct whole-system isotope tracer experiments in more than a couple of streams simultaneously, so within-reach sampling transects were the best possible estimates of replication. We corrected p-values for multiple comparisons using Benjamini & Hochberg's false discovery rate method (Benjamini & Hochberg 1995). We calculated effect size (Cohen's d) to compare the strength of guppy and light effects on N fluxes to consumers. All statistical tests were conducted in R (R Core Team 2013).

Results

Population sizes of the introduced guppy populations in our two study streams reached a peak during the 2010 dry season compared with other censuses between 2008-2011 with guppy biomass estimates of 2.86 g/m² in the thinned canopy stream (UPL) and 1.43 g/m² in the natural canopy stream (LOL). Increased PAR in the canopy-thinned stream (Table 4.1) corresponded with an increase in gross primary production during 2010 March-May dry season period (fixed effects linear model, F=25.1, df=1, p<0.001, Table 4.1) and lower rates of ecosystem respiration (fixed effects linear model, F=97.1, df=1, p<0.001) but did not result in significantly different amounts of epilithon chlorophyll-*a* or AFDM (fixed effects linear model,

F=0.36, df=1, p=0.55, Table 4.1). Gross primary production was also higher in guppy introduction reaches (fixed effects linear model, F=36.5, df=1, p<0.001, Table 4.1) and rates of ecosystem respiration were also higher (fixed effects linear model, F=70.1, df=1, p<0.001), but did not differ in the standing stock of chlorophyll-*a* (fixed effects linear model, F=0.04, df=1, p=0.85, Table 4.1) or epilithon AFDM (fixed effects linear model, F=0.81, df=3, p=0.49). Biomass of CBOM did not differ among any of the reaches (fixed effects linear model F=0.83, df=3, p=0.49) and biomass of FBOM could not be compared due to missing samples in the natural canopy stream (LOL). Seston biomass differed among all reaches, with dry mass declining in the following order: natural canopy no guppy, thinned guppy, thinned no guppy, natural canopy guppy (fixed effects linear model, F=5.92, df=3, p=0.02, Table 4.1).

Turnover times of basal resource compartments varied by compartment, with fastest turnover of CBOM, moderate turnover of epilithon and seston and slow turnover of FBOM (fixed effects linear model, F=7.4, df=3, p<0.001, Table 4.3). Areal uptake rates of N and C into basal resource compartments also varied by compartment, with high uptake rates to FBOM and CBOM due to their relatively high biomass, and very low uptake rates for seston (fixed effects linear model, F=13.8, df=3, p<0.001, Table 4.3). Although basal resource quantity did not often differ due to guppies or light availability, elemental composition of basal resources was significantly different among reach types for epilithon and seston, with higher quality resource pools (*i.e.*, lower C:N) in thinned canopy reaches relative to natural canopy (epilithon fixed effects linear model, F=8.84, df=3, p<0.001, seston fixed effects linear model, F=2.72, df=3, p=0.04).

Table 4.3: Uptake rates and turnover times of primary uptake compartments in each study reach. Means are shown with standard deviations in parenthesis.

	Thinned Canopy (Upper LaLaja)		Natural Canopy (Lower LaLaja)	
	Guppy Introduction	No Guppy Reference	Guppy Introduction	No Guppy Reference
N uptake rate (mg N m⁻² day⁻¹)				
Epilithon (day ⁻¹)	17.6 (12)	24.9 (9.7)	11.6 (3.9)	6.52 (1.3)
CBOM (day ⁻¹)	426 (--)	270 (175)	165 (82)	50.5 (2.4)
FBOM (day ⁻¹)	45.9 (28)	244 (6.2)	78.9 (51)	128 (52)
Seston (day ⁻¹)	0.446 (0.064)	0.222 (0.036)	0.176 (0.031)	0.553 (0.11)
N turnover time (days)				
Epilithon (days)	12.35 (6.9)	15.38 (5.9)	17.24 (7.6)	35.71 (7.0)
CBOM (days)	2.39 (--)	4.29 (3.9)	10.00 (8.8)	17.86 (7.9)
FBOM (days)	71.43 (74.9)	35.71 (10.3)	43.48 (31.6)	29.41 (8.1)
Seston (days)	18.52 (3.2)	16.13 (2.7)	23.26 (8.3)	21.28 (3.7)
C uptake rate (mg C m⁻² day⁻¹)				
Epilithon (day ⁻¹)	100 (63)		182 (41)	
CBOM (day ⁻¹)	3117 (--)		5637 (--)	
FBOM (day ⁻¹)	1701 (--)		2283 (--)	
Seston (day ⁻¹)	6.97 (0.80)		4.00 (1.3)	
C turnover time (days)				
Epilithon (days)	19.61 (14)		10.99 (2.6)	
CBOM (days)	10.42 (1.0)		10.53 (--)	
FBOM (days)	25.71 (--)		22.08 (--)	
Seston (days)	14.71 (1.6)		13.16 (3.8)	

The isotope tracer release resulted in high enrichment of all sampled food web compartments in the four study reaches in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Figure 4.3). In many cases, invertebrate primary consumers were more enriched in ^{15}N than presumed bulk food sources, but still less enriched than water column $\delta^{15}\text{N-NH}_4$ (Figure 4.3). Many primary consumers became more enriched than all basal resources (*i.e.*, epilithon, FBOM, CBOM, seston) in $\delta^{13}\text{C}$ (Figure 4.3). Guppies and killifish also became enriched at levels of several hundred per mil above background levels, which are represented by data on Day 0, collected before the tracer release began (Figure 4.3).

Our invertebrate sampling included most of the biomass-dominant taxa found in both streams (Table 4.2). Fluxes of N from primary uptake compartments to primary consumers differed by invertebrate taxon, stream, and guppy vs. control reach (Figure 4.4, statistical contrasts detailed for each species in Table 4.4). Grazers, collector-gatherers and collector-filterers had significantly higher N fluxes in the thinned canopy stream than the natural canopy stream, and significantly higher N fluxes in guppy reaches than control reaches (Table 4.4). Sample sizes for fluxes of bacterial C from basal resources to primary consumers were too small for statistical comparison because only the two sampling stations closest to the isotope injection had sufficient C label in the biota to calculate flux, but means suggest differences by invertebrate taxon and stream (Figure 4.5), with higher fluxes to grazers and collector-gatherers in thinned canopy reaches compared to natural canopy reaches. There were no guppy or light effects on C or N flux rates for shredder invertebrates (Figures 4.4, 4.5, Table 4.4). Fluxes of N and C to predatory invertebrates (*Argia* and *Euthyplocia*) were small relative to primary consumer taxa, guppies and killifish (Figure 4.6) and

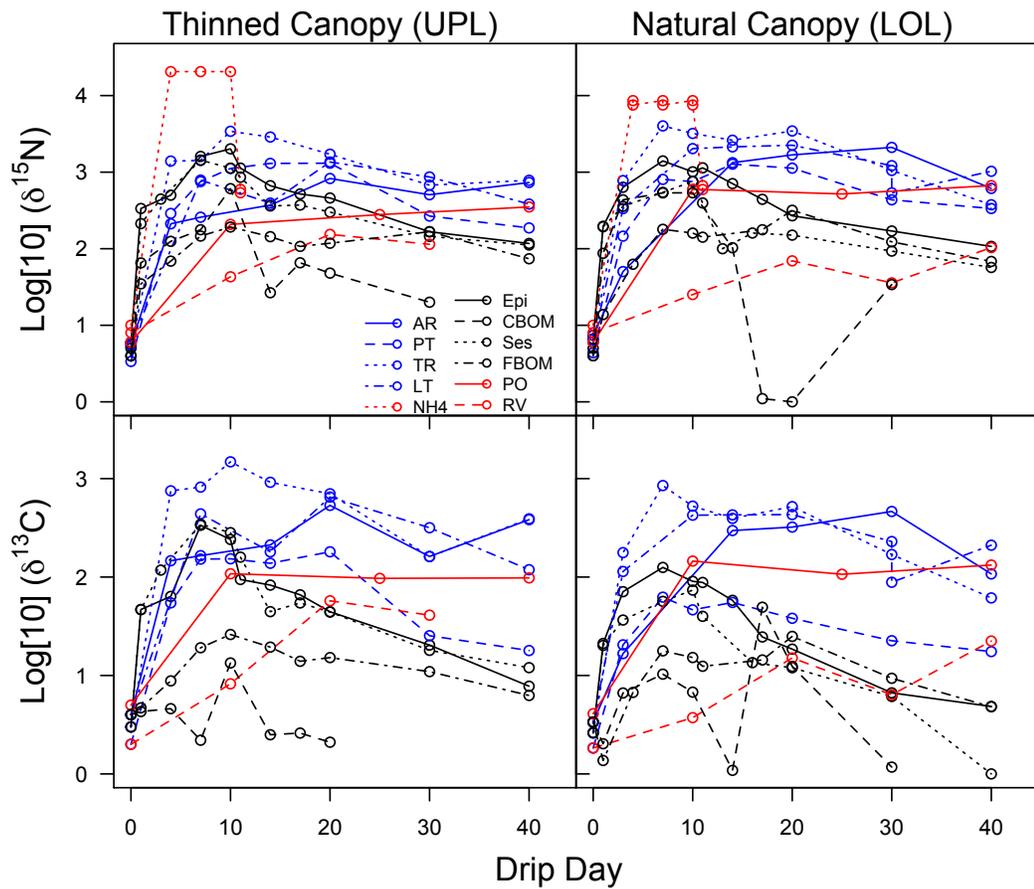


Figure 4.3: We detected isotope tracer in all food web compartments. Invertebrate taxa are shown in blue, primary uptake compartments in black and fishes in red. Invertebrate and fish taxa are coded as follows: AR= *Argia*, PT= *Petrophila*, TR= *Tricorythodes*, LT= *Leptonema*, RV= *Rivulus*, PO= *Poecilia reticulata*, Epi= epilithon, Ses = seston. Invertebrate taxa represent several key functional feeding groups (predator, scraper, collector-gatherer, and collector-filterer, respectively).

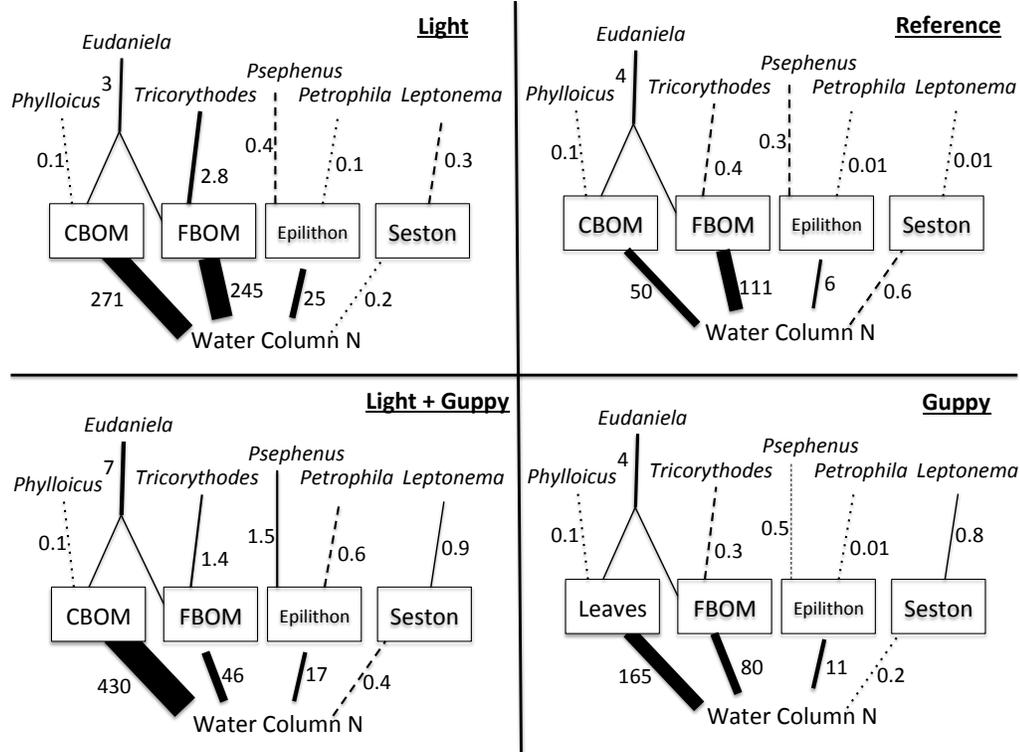


Figure 4.4: Flux rates of N to primary uptake compartments and invertebrate primary consumers in the four study reaches. Numbers next to the lines represent N flux in units of $\text{mg N m}^{-2} \text{ day}^{-1}$ and correspond to weight of the line (small dashed lines represent minor fluxes and large black lines represent major fluxes).

Table 4.4: Results of fixed effects linear models comparing N fluxes to different invertebrate groups in study reaches. Significant contrasts (at the $p < 0.05$ level after correction for multiple comparisons using Benjamini & Hochberg’s false discovery rate method) are described. Non-significant contrasts are denoted by “NS”.

Taxon	Functional group	Guppy effect	Light effect	Light-Guppy Interaction
<i>Argia</i>	Predator	NS	NS	NS
<i>Eudaniela</i>	Detritivore	NS	NS	NS
<i>Euthyplocia</i>	Predator	NS	NS	NS
<i>Leptonema</i>	Collector-filterer	F=169, df=3, p=0.005	F=25.3, df=3, p=0.03	NS
<i>Petrophila</i>	Grazer	F=26.3, df=3, p=0.03	F=46.6, df=3, p=0.02	F=19.4, df=3, p=0.03
<i>Phylloicus</i>	Shredder	NS	NS	NS
<i>Psephenus</i>	Grazer	F=11.6, df=3, p=0.04	F=13.8, df=3, p=0.04	NS
<i>Rivulus</i>	Predator	NS	NS	NS
<i>Tricorythodes</i>	Collector-gatherer	NS	F=19.2, df=3, p=0.05	NS

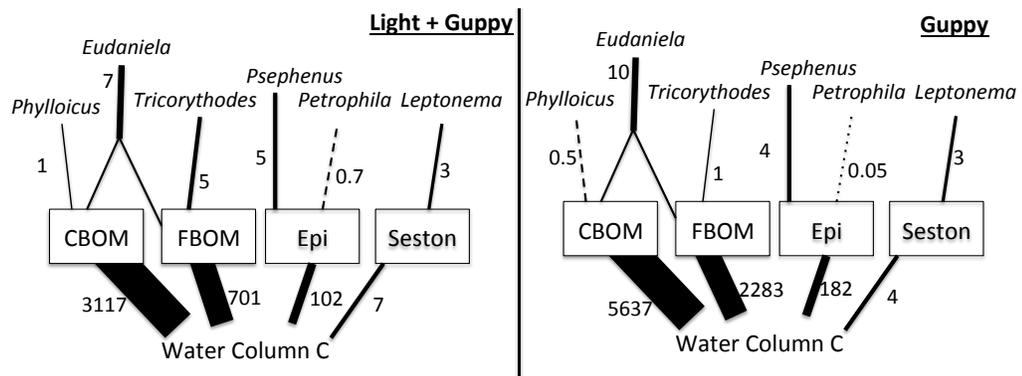


Figure 4.5: Flux rates of bacterial C to primary uptake compartments and invertebrate primary consumers in the guppy introduction reach of each study stream. Numbers next to the lines represent bacterial C flux in units of $\text{mg C m}^{-2} \text{ day}^{-1}$ and correspond to weight of the line (small dashed lines represent minor fluxes and large black lines represent major fluxes).

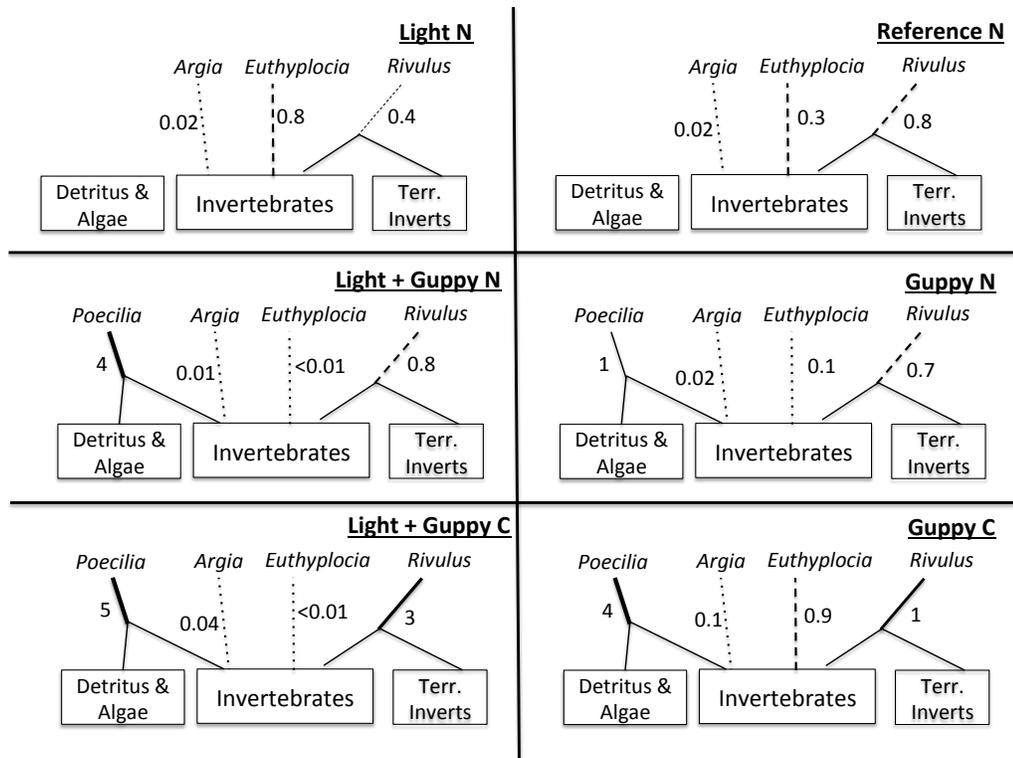


Figure 4.6: Flux rates of N or C to predatory invertebrates and fishes in the four study reaches. Numbers next to the lines represent N or C flux in units of $\text{mg N m}^{-2} \text{ day}^{-1}$ or $\text{mg C m}^{-2} \text{ day}^{-1}$ and correspond to weight of the line (small dashed lines represent minor fluxes and large black lines represent major fluxes).

were not affected by light or guppy treatments (Table 4.4). N fluxes to killifish were also not significantly different among reaches (Figure 4.6, Table 4.4).

Combined, reach N fluxes were higher in the thinned canopy stream (UPL) than the natural canopy stream (LOL), and higher in guppy reaches than control reaches, especially for the canopy-thinned, guppy introduction reach (Figure 4.7). Across reaches, we observed high areal flux rates of N for guppies, *Eudaniela* and *Tricorythodes* relative to other consumer taxa (Figure 4.7). Total N flux was much greater in the guppy reach of the thinned canopy stream compared to the reference reach despite reduced fluxes to *Tricorythodes* in the guppy introduction reach (Figure 4.7).

The effect size (Cohen's *d*) of guppy versus canopy treatments on N flux to consumers differed among consumer taxa (Figure 4.8). The largest effect was guppy presence on filter feeding caddisflies (*Leptonema*), followed by the effect on light on collector-gatherer mayflies (*Tricorythodes*). Effects of canopy and guppies were similar for both grazer invertebrate taxa (*Psephenus* and *Petrophila*), and effects on predators and shredders were relatively small compared to other functional groups (Figure 4.8).

Discussion

Our results demonstrate that both light availability and guppy presence can influence energy and material fluxes through food webs in Trinidadian streams. Differences in food web fluxes between the stream with reduced canopy cover and the stream with natural canopy cover indicate that increased light availability led to

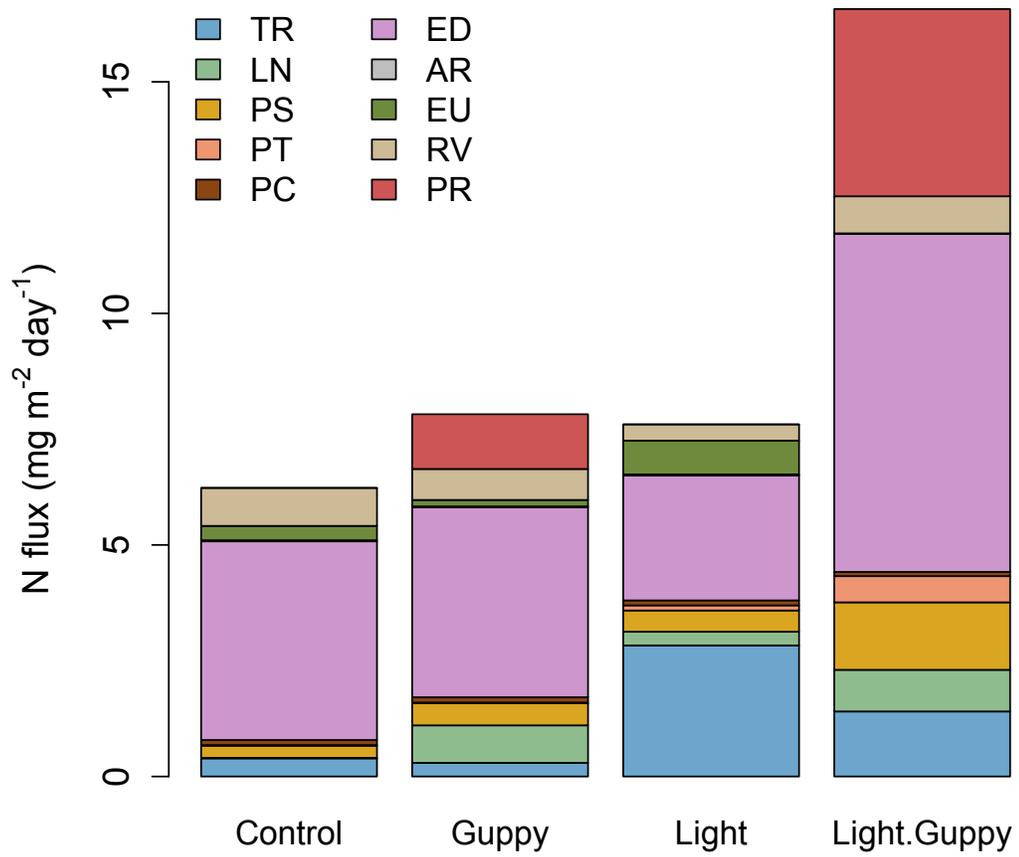


Figure 4.7: Combined average N fluxes in different treatment reaches. Taxa abbreviations are: TR= *Tricorythodes*, LN = *Leptonema*, PS = *Psephenus*, PT = *Petrophila*, PC = *Phylloicus*, ED = *Eudaniela*, AR = *Argia*, EU = *Euthyplocia*, RV = *Rivulus* (killifish), PR = *Poecilia reticulata* (guppies).

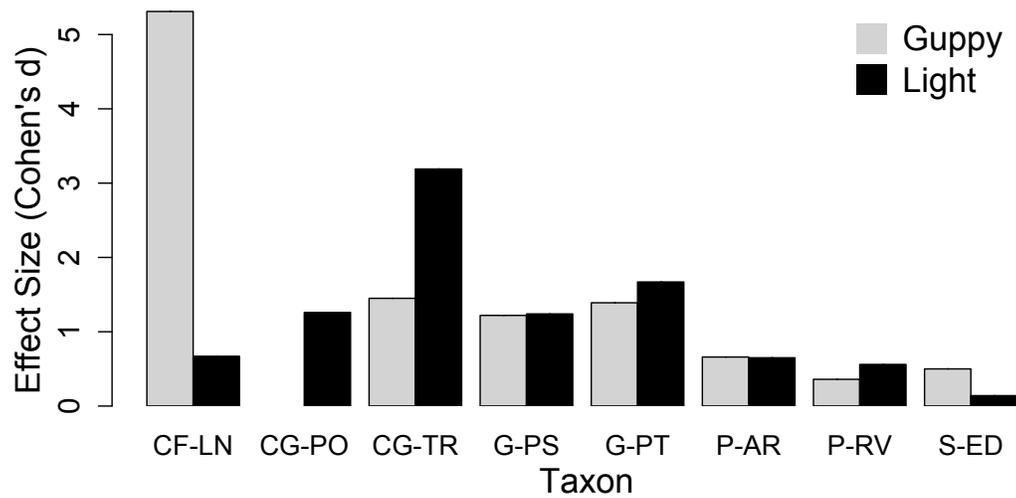


Figure 4.8: Effect size (Cohen's d) from guppies and light on N flux to consumer taxa. Taxa are arranged by functional group as follows: filter feeders (*Leptonema*, CF-LN), collector gatherers (guppies, CG-PO, *Tricorythodes*, CG-TR), grazers (*Psephenus*, G-PS, *Petrophila*, G-PT), predators (*Argia*, P-AR, *Rivulus*, P-RV), and shredders/detritivores (*Eudaniela*, S-ED) are shown.

increased fluxes of both N and bacterial C to some invertebrate taxa in the thinned canopy stream compared to the natural canopy stream. Specifically, grazers (*Psephenus* and *Petrophila*) and collector-gatherers (*Tricorythodes*) had higher N and apparently higher bacterial C fluxes in the control reach of the thinned canopy stream relative to the control reach of the natural canopy stream and the introduction reach of the thinned canopy stream relative to the introduction reach of the natural canopy stream (Figures 4.4, 4.5). Consistent increases in N and bacterial C fluxes to grazers and collector-gatherers in the canopy-thinned stream suggest that canopy thinning stimulated the production of both algae and heterotrophic bacteria in two primary uptake compartments (epilithon and FBOM). We also noted higher fluxes of N to filter feeders (*Leptonema*) in both introduction and control reaches of the canopy-thinned stream, but apparently no differences in fluxes of bacterial C, indicating that increased algal production due to canopy thinning had an effect on filter-feeding invertebrates but bacterial production did not contribute to filter feeders. Despite increased light availability and elevated primary production, we did not see an increase in epilithon chlorophyll *a* in the thinned canopy stream, which is likely due to increased grazing rates and higher fluxes of N to consumers (Hill *et al.* 2001, Hill *et al.* 2010).

In other tropical stream ecosystems, removal or addition of vertebrates can have obvious impacts on standing stocks of organic matter (*e.g.* grazing tadpoles Whiles *et al.* 2013, or detritivorous fish Flecker 1996, Flecker & Taylor 2004, Taylor *et al.* 2006), but we noted no effects of guppy presence on biofilm biomass or chlorophyll *a* standing stock despite increased primary production in guppy reaches.

Since guppies are omnivorous (Zandona *et al.* 2011), they are also unlikely to exert top-down effects of the same magnitude as a voracious predator. However, our results suggest that effects of guppies on the food web nevertheless exist and that effect sizes are similar to effects of canopy removal (Figure 4.8), including differences in N flux rates to primary consumers between guppy and control reaches. Since we only added ¹³C tracer to the introduction reaches, we were not able to evaluate the effect of guppy presence on fluxes of bacterial C.

Specifically, fluxes of N to *Leptonema* sp., a filter-feeding caddisfly, were higher in the guppy introduction reaches of both streams compared with control reaches (Figure 4.4) and the effects size of guppy treatments on *Leptonema* was higher than any effect on any other consumer (Figure 4.8). Guppies are epibenthic feeders that consume high proportions of fine detritus and guppy feeding activity on the benthos likely led to suspension of fine organic matter in the water column. However, fluxes of N to *Tricorythodes*, a collector-gatherer, were lower in guppy reaches than control reaches (Figure 4.4). Guppies could have both direct and indirect negative effects on *Tricorythodes* including predation, competition for high quality organic matter, and alteration of *Tricorythodes* feeding behavior because guppies are present in high densities on patches of fine organic matter, but guppy effects were not as strong as light effects (Figure 4.8). Fluxes of N to grazers (*Psephenus* and *Petrophila*) were usually higher in guppy introduction reaches than in control reaches, with the exception of no difference between *Petrophila* in control and introduction reaches in the natural canopy stream (Figure 4.4). Increased primary production in guppy reaches would have increased food availability for grazers, and both species have body

types that are unlikely to be susceptible to predation by guppies: *Petrophila* are lepidopterans that live in cases that are flat and attached to rocks and *Psephenus* (water pennies) are coleopterans that have a flat body shape and are tightly attached to rocks.

N fluxes to shredder caddisflies (*Phylloicus*) and detritivorous crabs (*Eudaniela*) and killifish (*Rivulus*) did not differ among light or guppy treatments. While fluxes of N to *Phylloicus* were low relative to other primary consumers, fluxes of N to *Eudaniela* were higher than any other taxon (Figures 4.4, 4.5). There were also no apparent light treatment effects on C fluxes to *Phylloicus*, *Eudaniela* and *Rivulus*, though low sample size prevented statistical comparison. High biomass of crabs relative to other invertebrates (Table 4.2) drove the magnitude of flux rates even though crabs have relatively slow turnover rates compared with insects. The high biomass of crabs and high flux rates of both N and bacterial C indicate that detritivory by macroconsumers is an important pathway in our study system that does not appear to be influenced by light or fish introductions. Similarly, killifish diets are composed of high proportions of detritus (including ~35% terrestrial invertebrates, B. Lamphere, unpublished data), also suggesting that detrital food web linkages are not as influenced by light or guppy manipulations.

Although we sampled many of the highly abundant invertebrate groups in our two study streams (Table 4.2), guppy effects might have been large for smaller bodied taxa that were not possible to collect during our isotope tracer release. For example, invertebrates in the family Chironomidae comprise a high proportion of the invertebrate biomass in guppy diets (Zandona *et al.* 2011) and studies in mesocosms

show that guppy density and phenotype can have a strong influence on chironomid biomass (Bassar *et al.* 2010). Collecting sufficient biomass of small-bodied insect taxa for isotopic analysis would have required that large areas of streambed be disturbed, dislodging organic matter and other invertebrates. The sampling design for isotope tracer studies relies on sampling several downstream stations from the point of tracer release, and it would be problematic to contaminate stations with more enriched material from upstream. Some sort of characterization of the role of chironomids and other smaller invertebrates would improve the scope of our results and perhaps identify stronger effects of guppy introduction because of the known trophic linkage between guppies and chironomids. Isotope tracer results are not often accompanied by natural abundance data (with some exceptions, *e.g.* Mulholland *et al.* 2000) but would be strengthened by comparing tracer results with natural abundance isotope data for invertebrate taxa that are not possible to sample during the tracer release.

The C and N dual isotope tracer approach was effective for quantifying food web fluxes and for specifically evaluating total fluxes versus fluxes of carbon from heterotrophic bacteria. Our comparison suggests that bottom up effects (*i.e.*, increased light availability and primary production) can have an influence on heterotrophic pathways in the food web as well as autotrophic pathways. Although our C tracer release was limited to the downstream guppy introduction reaches of each stream, we would expect that increased primary production in guppy reaches might also lead to increased bacterial C fluxes. Results from temperate systems indicate that bacteria are likely to be an important trophic link in streams (Hall & Meyer 1998, Simon *et al.* 2003, Chapter 3), but fluxes of bacterial energy to higher trophic levels have not been

previously studied in tropical stream ecosystems. Our results are in accordance with results from temperate streams that bacteria are important in shaded, headwater systems that are fueled by detritus, but also demonstrate that the light environment can alter the role of bacteria as a food source for stream invertebrates.

Our results also support the results of experimental studies in mesocosms (Palkovacs *et al.* 2009, Bassar *et al.* 2010), which indicate that guppy presence, phenotype and density can affect stream ecosystems. Further analysis of the results from the mesocosm data presented by Bassar *et al.* (2010) indicates that some effects of guppies on ecosystem processes are predominantly due to effects of guppy phenotype while others are driven mostly by ecological differences in guppy density (Ellner *et al.* 2011). Although our experiment was intended to compare guppy presence versus absence, introduced guppy populations were allowed to grow for several years, which resulted in almost twice the guppy density in the canopy-thinned stream relative to the natural canopy thinned stream. While we suspect that differences in food webs between the two streams are primarily due to experimental changes in the light environment, differences in guppy density might also be a contributing factor. Characterizing food webs in our study streams over several years as introduced guppy populations adapt to a predator-free environment could provide a venue to examine feedbacks between guppy evolution and ecosystem processes, but effects of guppy presence, density and phenotype would be difficult to evaluate given the differences in density between the two streams.

However, our results show that it is possible to detect effects of guppy presence on food webs in natural streams as well as in mesocosm experiments. We

expect that the effects we saw on food web fluxes may be more pronounced than most dry season experiments in Trinidad because the extent and severity of dry weather in 2010 was greater than any other year from 2008-2011, which led to stable flows and high guppy densities in both streams. A complex suite of drivers affects the biomasses of epilithon (Kohler *et al.* 2012) and invertebrate communities (Heatherly 2012) in Trinidadian streams, and it is impossible to hold other variables constant in natural streams. Hydrologic disturbance and seasonal differences in rainfall are particularly difficult to control yet are critical drivers of food web pathways in Trinidad and in other Neotropical stream ecosystems (Frauendorf *et al.* 2013). While comparisons across years will be difficult to evaluate due to differences in dry season severity and rainfall, attempting to detect both density and phenotype effects of guppies in natural stream environments would shed additional light on how ecological and evolutionary processes interact to influence ecosystem function.

In summary, our results demonstrate that both light availability and fish introductions can influence the architecture of food webs in tropical stream ecosystems. This provides additional evidence from tropical streams that both top-down and bottom-up changes in the environment can influence ecosystem function (Flecker *et al.* 2002, Moulton *et al.* 2010), and that changes in primary production can have strong effects in systems that are fueled mostly by detritus (Boyero *et al.* 2008). Comparison of fluxes of N and bacterial C demonstrate that increased light availability not only stimulated primary production and N fluxes, but also increased fluxes of heterotrophic bacteria to higher trophic levels. Although contrasting the effects of fish presence and fish phenotype may be more complex in natural streams compared with

controlled mesocosms, our results demonstrate that comparing food web fluxes in whole-ecosystem manipulations is possible using isotope tracer techniques.

REFERENCES

- Arendt, J.D., D.N. Reznick and A. Lopez-Sepulcre. 2014. Replicated origin of female-biased adult sex ratio in introduced populations of the Trinidadian guppy (*Poecilia reticulata*). *Evolution* 68(8): 2343-2356.
- Ashkenas, L.R., S.L. Johnson, S.V. Gregory, J.L. Tank and W.M. Wollheim. 2004. A stable isotope tracer study of nitrogen uptake and transformation in an old-growth forest stream. *Ecology* 85: 1725-1739.
- Ask, J., J. Karlsson, L. Persson, P. Ask, P. Bystrom, and M. Jansson. 2009. Terrestrial organic matter and light penetration: Effects on bacterial and primary production in lakes. *Limnology & Oceanography* 54(6): 2034-2040.
- Bassar, R.D., M.C. Marshall, A. Lopez-Sepulcre, E. Zandonata, S.K. Auer, J. Travis, C.M. Pringle, A.S. Flecker, S.A. Thomas, D.F. Fraser and D.N. Reznick. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. *Proceedings of the National Academy of Sciences* 107(8): 3616-3621.
- Baumgartner, D. and K.O. Rothhaupt. 2003. Predictive length-dry mass relationships for freshwater invertebrates in a pre-alpine lake littoral. *International Review of Hydrobiology* 88: 453-463.
- Benjamini, Y. and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B* 57: 289-300.
- Benke, A.C., A.D. Huryn, L.A. Smock and J.B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North American with particular reference to the southeastern United State. *Journal of the North American Benthological Society* 18: 308-343.
- Benstead, J.P. and C.M. Pringle. 2004. Deforestation alters the resource base and biomass of endemic stream insects in eastern Madagascar. *Freshwater Biology* 49: 490-501.
- Boyero, L., P.A. Rincon, and R.G. Pearson. 2008. Effects of a predatory fish on a tropical detritus-based food web. *Ecological Research* 23: 649-655.
- Boyero, L., A. Ramirez, D. Dudgeon and R.G. Pearson. 2009. Are tropical streams really different? *Journal of the North American Benthological Society* 28(2): 397-403.
- Carpenter, S.R. and J.F. Kitchell. 1988. Consumer control of lake productivity. *Bioscience* 38(11): 764-769.

- Cheshire, K., L. Boyero and R.G. Pearson. 2005. Food webs in tropical Australian streams: shredders are not scarce. *Freshwater Biology* 50: 748-769.
- Coat, S., D. Monti, C. Bouchon and G. Lepoint. 2009. Trophic relationships in a tropical stream food web assessed by stable isotope analysis. *Freshwater Biology* 54: 1028-1041.
- Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*, 2nd ed. Hillsdale, NJ: Lawrence Earlbaum Associates.
- Cole, J.J., S. Findlay and M.L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Marine Ecology Progress Series* 43: 1-10.
- Davis, A.M., M.L. Blanchette, B.J. Pusey, T.D. Jardine and R.G. Pearson. 2012. Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. *Freshwater Biology* 57: 2156-2172.
- Davis, J.M., A.D. Rosemond, S.L. Eggert, W.F. Cross and J.B. Wallace. 2010. Nutrient enrichment differentially affects body sizes of primary consumers and predators in a detritus-based stream. *Limnology and Oceanography* 55: 2305-2316.
- Davis, J.M., A.D. Rosemond, S.L. Eggert, W.F. Cross and J.B. Wallace. 2010. Long-term nutrient enrichment decouples predator and prey production. *Proceedings of the National Academy of Sciences* 107: 121-126.
- De Nadai-Monoury, E., F. Gilbert and A. Lecerf. 2014. Forest canopy cover determines invertebrate diversity and ecosystem process rates in depositional zones of headwater streams. *Freshwater Biology* doi:10.1111/fwb.12364
- Dodds, W.K., M.A. Evans-White, N.M. Gerlanc, L. Gray, D.A. Gudder, M.J. Kemp, A.L. Lopez, D. Stagliano, E.A. Strauss, J.L. Tank, M.R. Whiles and W.M. Wollheim. 2000. Quantification of the nitrogen cycle in a prairie stream. *Ecosystems* 3: 574-589.
- Dodds, W.K., S.M. Collins, S.K. Hamilton, J.L. Tank, S. Johnson, J.R. Webster, K.S. Simon, M.R. Whiles, H.M. Rantala, W.H. McDowell, S.D. Peterson, T. Riis, C.L. Crenshaw, S.A. Thomas, P.B. Kristensen, B.M. Cheever, A.S. Flecker, N.A. Griffiths, T. Crowl, E.J. Rosi-Marshall, R. El-Sabaawi and E. Marti. In press. You are not always what we think you eat: selective assimilation across multiple whole-stream isotopic tracer studies. *Ecology*.

- Dudgeon, D., F.K.W. Cheung and S.K. Mantel. 2010. Foodweb structure in small streams: do we need different models for the tropics? *Journal of the North American Benthological Society* 29(2): 395-412.
- Ellner, S.P., M.A. Geber and N.G. Hairston, Jr. 2011. Does rapid evolution matter? Measuring the rate of contemporary evolution and its impacts on ecological dynamics. *Ecology Letters* 13: 989-977.
- Findlay, S., K. Howe and D. Fontvieille. 1993. Bacterial-algal relationships in streams of the Hubbard Brook Experimental Forest. *Ecology* 74(8): 2326-2336.
- Flecker, A.S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical ecosystem. *Ecology* 77: 1845-1854.
- Flecker, A.S. and B.W. Taylor. 2004. Tropical fishes as biological bulldozers: density effects on resource heterogeneity and species diversity. *Ecology* 85(8): 2267-2278.
- Flecker, A.S. and C.R. Townsend. 1994. Community-wise consequences of trout introductions in New Zealand streams. *Ecological Applications* 4: 798-807.
- Flecker, A.S., B.W. Taylor, E.S. Bernhardt, J.M. Hood, W.K. Cornwell, S.R. Cassatt, M.J. Vanni and N.S. Altman. 2002. Interactions between herbivorous fishes and limiting nutrients in a tropical stream ecosystem. *Ecology* 83: 1831-1844.
- Fraiola, K.M.S. 2004. Juvenile *Epilobocera sinuatifrons* growth rates and ontogenetic shifts in feeding in wild populations. MS Thesis, University of Hawaii at Manoa.
- Fraser, D.F. and B.A. Lamphere. 2013. Experimental evaluation of predation as a facilitator of invasion success in a stream fish. *Ecology* 94: 640-649.
- Frauendorf, T.C., C. Colon-Gaud, M.R. Whiles, T.R. Barnum, K.R. Lips, C.M. Pringle and S.S. Kilham. 2013. Energy flow and the trophic basis of macroinvertebrate and amphibian production in a neotropical stream food web. *Freshwater Biology* 58: 1340-1352.
- Grether, G.F., D.F. Millie, M.J. Bryant, D.N. Reznick and W. Mayea. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology* 82(6): 1546-1559.
- Gruner D.S., J.E. Smith, E.W. Seabloom, S.A. Sandin, J.T. Ngai, H. Hillebrand, J.J. Elser, E.E. Cleland, M.E.S. Bracken, E.T. Borer and B.M. Bolker. 2008. A

- cross-system synthesis of consumer and nutrient resource control on producer biomass. *Ecology Letters* 11(7): 740-755.
- Hairston Jr., N.G. and N.G. Hairston, Sr. 1993. Cause-effect relationships in energy flow, trophic structure and interspecific interactions. *American Naturalist* 142: 379-411.
- Hairston Jr., N.G., S.P. Ellner, M.A. Geber, T. Yoshida and J.A. Fox. Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters* 8: 1114-1127.
- Hall, R.O. and J.L. Meyer. 1998. The trophic significance of bacteria in a detritus-based stream food web. *Ecology* 79: 1995-2012
- Hambright, K.D. 1994. Can planktivorous fish really affect lake thermal dynamics? *Archiv fur Hydrobiologie* 130: 429-438.
- Hambright, K.D., N.G. Hairston Jr., W.R. Schaffner, and R.W. Howarth. 2007. Grazer control of nitrogen fixation: phytoplankton taxonomic composition and ecosystem functioning. *Fundamental and Applied Limnology* 170: 103-124.
- Hamilton, S.K., J.L. Tank, D.F. Raikow, E.R. Siler, N.J. Dorn and N.E. Leonard. 2004. The role of instream vs. allochthonous N in stream food webs: modeling the results of an isotope addition experiment. *Journal of the North American Benthological Society* 23(3): 429-448.
- Hansson, L. 1992. Factors regulating periphytic algal biomass. *Limnology and Oceanography* 37(2): 322-328.
- Heatherly II, T. 2012. Flow regime, guppy introduction and light manipulate influence invertebrate assemblages in Trinidadian streams. PhD Dissertation, University of Nebraska Lincoln.
- Hill, W.R., J.G. Smith and A.J. Stewart. 2010. Light, nutrients and herbivore growth in oligotrophic streams. *Ecology* 91(2): 518-527.
- Hill, W.R., M.G. Ryon and E.M. Schilling. 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. *Ecology* 76: 1297-1309.
- Hill, W.R., P.J. Mulholland and E.R. Marzolf. 2001. Stream ecosystem response to forest leaf emergence in spring. *Ecology* 82(8): 2306-2319.
- Holmes, R.M., J.W. McClelland, D.M. Sigman, B. Fry and B.J. Peterson. 1998. Measuring N-15-NH₄⁺ in marine, estuarine and fresh waters: An adaptation of

the ammonia diffusion method for samples with low ammonium concentrations. *Marine Chemistry* 60: 235-243.

Hotchkiss, E.R. and R.O. Hall Jr. In press. Whole-stream ^{13}C tracer addition reveals distinct fates of newly fixed carbon. *Ecology*.

Johnson, B.R. and J.B. Wallace. 2005. Bottom-up limitation of a stream salamander in a detritus-based food web. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 301-311.

Kohler, T.J., T.N. Heatherly III, R.W. El-Sabaawi, E. Zandona, M.C. Marshall, A.S. Flecker, C.M. Pringle, D.N. Reznick and S.A. Thomas. 2012. Flow, nutrients and light availability influence Neotropical epilithon biomass and stoichiometry. *Freshwater Science* 31(4): 1019-1034.

Lau, D.C.P., K.M.Y. Leung and D. Dudgeon. 2009. What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong. *Freshwater Biology* 54: 127-141.

Li, A.O.Y. and D. Dudgeon. 2008. Food resources of shredders and other benthic macroinvertebrates in relation to shading conditions in tropical Hong Kong streams. *Freshwater Biology* 53: 2011-2025.

Loeb, S.L. 1981. An *in situ* method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. *Limnology and Oceanography* 26: 394-399.

Magurran, A.E. 2005. *Evolutionary Ecology: The Trinidadian Guppy*. Oxford Series in Ecology and Evolution. Oxford University Press, UK.

March, J. G. and C.M. Pringle. 2003. Food web structure and basal resource utilization along a tropical island stream continuum, Puerto Rico. *Biotropica* 35(1): 84-93.

Marshall, M.C., A.J. Binderup, E. Zandona, S. Gouette, R.D. Bassar, R.W. El-Sabaawi, S.A. Thomas, A.S. Flecker, S.S. Kilham, D.N. Reznick and C.M. Pringle. 2012. Effects of consumer interactions on benthic resources and ecosystem processes in a neotropical stream. *PLOS One* 7(9): e45230.

McCullough, B. and D.A. Heiser. 2008. On the accuracy of statistical procedures in Microsoft Excel 2007. *Computational Statistics and Data Analysis* 52: 4570-4578.

- Miyasaka, H., M. Gentai-Kato, Y. Miyake, D. Kishi, I. Katano, H. Doi, S. Ohba and H. Khuara. 2008. Relationships between length and weight of freshwater invertebrates in Japan. *Limnology* 9: 75-80.
- Moulton, T.P., M.L. Souza, R.M.L. Silveira, F.A.M. Krsulovic, M.P. Silveira, J.C.F. de Assis and C.N. Francischetti. 2010. Patterns of periphyton are determined by cascading trophic relationships in two neotropical streams. *Marine and Freshwater Research* 61: 57-64.
- Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J. Peterson, J. R. Webster, and J. L. Meyer. 2000. Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a ¹⁵N tracer addition. *Journal of the North American Benthological Society* 19:145-157.
- Murphy, J., and J. P. Riley. 1962. A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* 26:31–36.
- Newell, S.Y. 1984. Bacterial and fungal productivity in the marine environment: a contrastive overview. *Colloques Internationaux Centre National Recherches Scientifique (Marseilles)*: 331: 133-139. Pace, M.L., J.J. Cole, S.R. Carpenter and J.F. Kitchell. 1999. Trophic cascades revealed in diverse ecosystems. *Trends in Ecology & Evolution* 14(12): 483-488.
- Palkovacs, E.P., M.C. Marshall, B.A. Lamphere, B.R. Lynch, D.J. Weese, D.F. Fraser, D.N. Reznick, C.M. Pringle and M.T. Kinnison. 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philosophical Transactions of the Royal Society B-Biological Sciences* 364: 1617-1628.
- Persson, L. 1999. Trophic cascades: abiding heterogeneity and the trophic level concept at the end of the road. *Oikos* 85(3): 385-397.
- Polis, G.E. and D.R. Strong. 1996. Food web complexity and community dynamics. *American Naturalist* 147: 813-846.
- Power, M.E. 1990. Effects of fish in river food webs. *Science* 250: 811-814.
- Power, M.E. 1992. Top-down and bottom-up forces in food webs – do plants have primacy? *Ecology* 73(3): 733-746.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

- Reznick, D.N., H. Bryga and J.A. Endler. 1990. Experimentally induced life-history evolution in a natural population. *Nature* 346: 357-359.
- Roberts, B.J., P.J. Mulholland and W.R. Hill. 2007. Multiple scales of temporal variability in ecosystem metabolism rates: Results from 2 years of continuous monitoring in a forested headwater stream. *Ecosystems* 10: 588-606.
- Sabo, J.L., J.L. Bastow and M.E. Power. 2002. Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. *Journal of the North American Benthological Society* 21: 336-343.
- Schindler, D.W. 1997. Evolution of phosphorus limitation in lakes. *Science* 195: 260-262.
- Schoener, T.W. 2011. The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science* 331: 426-429.
- Sigman, D.M., M.A. Altabet, R. Michener, D.C. McCorkle, B. Fry and R.M. Holmes. 1997. Natural abundance-level measurement of nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine Chemistry* 57: 227-242.
- Simon, K.S., E.F. Benfield and S.A. Macko. 2003. Food web structure of epilithic biofilms in cave streams. *Ecology* 84(9): 2395-2406.
- Taylor, B.W., A.S. Flecker and R.O. Hall, Jr. 2006. Loss of a harvest fish species disrupts carbon flow in a diverse tropical river. *Science* 313: 833-836.
- Taylor, B.W., C.F. Keep, R.O. Hall Jr., B.J. Koch and L.M. Tronstad. 2007. Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. *Journal of the North American Benthological Society* 26(2): 167-177.
- Thimijan, R.W. and R.D. Heins. 1983. Photometric, radiometric and quantum light units of measure: a review of procedures for interconversion. *Horticultural Science* 18: 818-822.
- Walsh, M.R., D.F. Fraser, R.D. Bassar and D.N. Reznick. The direct and indirect effects of guppies: implications for life-history evolution in *Rivulus hartii*. *Functional Ecology* 25: 227-237.
- Whiles, M.R., R.O. Hall Jr., W.K. Dodds, P. Verburg, A.D. Huryn, C.M. Pringle, K.R. Lips, S.S. Kilham, C. Colon-Gaud, A.T. Rugenski, S. Peterson and S. Connelly. 2013. Disease-driven amphibian declines alter ecosystem processes in a tropical stream. *Ecosystems* 16: 146-157.

- Wright, R.T. and J.E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* 47: 447-464.
- Yule, C.M. 1996. Trophic relationships and food webs of the benthic invertebrate fauna of two aseasonal tropical streams on Bougainville Island, Papua New Guinea. *Journal of Tropical Ecology* 12(4): 517-534.
- Zandona, E., S.K. Auer, S.S. Kilham, J.L. Howard, A. Lopez-Sepulcre, M.P. O'Connor, R.D. Bassar, A. Osorio, C.M. Pringle, and D.N. Reznick. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Functional Ecology* 25: 964-973.

APPENDIX A

End members used in percent allochthony simulations in Chapter 2. Most allochthonous end members were point estimates in Trinidad based on empirical measurements of leaf litter. Distributions for autochthonous end members were estimated based on discrimination from water, samples that were known to be pure algae, and correction factors for epilithon. Uniform distributions include the two endpoints of the distributions and normal distributions include the mean and standard deviation. Point estimates are shown as single numbers.

	Autochthonous end member	Estimated or empirical	Allochthonous end member
Trinidad			
Quare River HP 2009	Uniform -162, -134	Empirical	-70.4
Quare River HP 2013	Uniform -172, -162	Empirical	-72
Quare River LP 2009	Uniform -167, -161	Estimated	-70
Quare River LP 2013	Uniform -148, -134	Estimated	-72
Quare Rjver RO 2009	Uniform -172, -112	Estimated	-60
Quare Rjver RO 2013	Uniform -183, -173	Estimated	-68
Aripo River HP 2009	Uniform -164, -145	Empirical	-82
Aripo River HP 2013	Normal -211, 21.3	Empirical	-70
Aripo River LP 2009	Uniform -175, -172	Estimated	-99
Aripo River LP 2013	Uniform -200, -172	Estimated	-70
Aripo Rjver RO 2009	Uniform -204, -174	Estimated	-82
Aripo Rjver RO 2013	Uniform -200, -173	Estimated	-71
Marianne River HP 2009	Uniform -155, -150	Estimated	-57
Marianne River HP 2013	Uniform -175, -150	Estimated	-65
Marianne River LP 2009	Uniform -148, -134	Estimated	-45
Marianne River LP 2013	Uniform -172, -149	Estimated	-55
Marianne Rjver RO 2009	Uniform -153, -126	Estimated	-45
Marianne Rjver RO 2013	Uniform -172, -153	Estimated	-60
Adirondacks			
Moose River	Uniform -249, -229	Empirical	-143
Combs Headwater	Uniform -239, -219	Estimated	Normal -132, 6
Combs Trail	Uniform -247, -227	Estimated	-128
Combs Hatchery	Uniform -243, -223	Estimated	Normal -129, 7
Otter Headwater	Uniform -247, -227	Estimated	Normal -132, 3
Otter Camp	Uniform -247, -227	Estimated	Normal -134, 1
Blues Brook	Uniform -246, -226	Estimated	-130

APPENDIX B

$\delta^2\text{H}_{\text{water}}$ from all sites sampled in Chapter 2. Values for $\delta^2\text{H}_{\text{water}}$ were similar among sites within a drainage or region, but differed greatly between temperate and tropical regions.

	Average $\delta^2\text{H}$	Standard Deviation $\delta^2\text{H}$
Trinidad		
Quare downstream	-2.26	2.7
Quare midstream	-13.1	1.3
Quare upstream	-11.8	2.4
Aripo downstream	-4.31	3.8
Aripo midstream	-11.7	2.4
Aripo upstream	-13.6	4.5
Marianne downstream	9.95	3.4
Marianne midstream	11.1	6.5
Marianne upstream	7.18	4.9
Adirondacks		
Moose River	-69.3	0.34
Blues Brook	-78.9	1.2
Combs Headwater	-77.8	2.3
Combs Trail	-73.9	5.5
Combs Hatchery	-77.6	2.5
Otter Headwater	-77.1	1.1
Otter Camp	-76.3	0.76

APPENDIX C

In Chapter 3, we used field experiments to estimate information needed for the $^{13}\text{CO}_2$ mixing model that we used to calculate $^{13}\text{CO}_2$ that was respired by bacteria. We ran 16 concurrent incubations in sealed mason jars in the each study stream using the same methods as we did for field incubations during the isotope tracer releases. In each stream, 8 jars contained coarse benthic organic material (leaves) and 8 contained fine benthic organic matter (sediment). Of the 8 jars for each type of organic matter, 4 were untreated and 4 were treated with sodium azide to kill all microorganisms and stop respiration. From this experiment, we were able to calculate $\delta^{13}\text{C}$ of CO_2 from inorganic sources (*i.e.*, not from respiration) by measuring $\delta^{13}\text{C}$ of headspace CO_2 in sodium azide treatments. We were also able to infer the proportion of headspace CO_2 that was derived from inorganic sources based on the ratio of $[\text{CO}_2]$ in treatments with sodium azide over $[\text{CO}_2]$ in treatments with both inorganic diffusion and respiration. We calculated the proportion of headspace CO_2 from respiration by difference. Those data provided the information needed for our mixing model that is described in the methods section of the paper. Values we calculated from the experiment and used in the mixing model are detailed below in a table. We also corrected to account for the fact that both bacterial and fungal respiration contribute to the organic/respiration fraction of CO_2 in the incubation headspace (f_{organic}). We estimated a ratio of respiration due to bacteria versus fungi in each organic matter type based on literature values from other systems (described in the methods section) and used that ratio to correct f_{organic} to find f_{bacteria} .

Stream	Organic Matter Type	f inorganic	f organic	$\delta^{13}\text{C}$ inorganic	bacteria:fungi	f bacteria
Blues Brook	Coarse (leaves)	0.83	0.17	-23.5	1 to 10	0.02
Blues Brook	Fine (sediment)	0.81	0.19	-23.5	1 to 1	0.10
Combs Brook	Coarse (leaves)	0.86	0.14	-22.8	1 to 10	0.01
Combs Brook	Fine (sediment)	0.79	0.21	-22.8	1 to 1	0.11

We also used data for $\delta^{13}\text{C}$ of CO_2 from our field incubations during the isotope tracer release as an estimate of the total CO_2 pool (both respiration and inorganic). We collected data on the three sampling days during the course of the 10-day isotope drip (Days 3, 7, 10) and during the sampling day following the isotope drip (Day 14). Data for $\delta^{13}\text{C}$ of CO_2 at the two sampling stations (10 m downstream of the point of isotope addition and 30 m downstream of the point of isotope addition) are summarized in the following table.

Stream	Organic Matter Type	Downstream Distaince	Year	$\delta^{13}\text{C}$ Day 3	$\delta^{13}\text{C}$ Day 7	$\delta^{13}\text{C}$ Day 10	$\delta^{13}\text{C}$ Day 14
Combs Brook	Coarse (leaves)	10	2009	29.3	96.7	74.7	-4.1
Combs Brook	Coarse (leaves)	30	2009	-4.1	49.2	2.5	4.2
Blues Brook	Coarse (leaves)	10	2009	104.3	72.4	70.8	-0.5
Blues Brook	Coarse (leaves)	30	2009	-0.6	46.4	20.1	-4.3
Combs Brook	Fine (sediment)	10	2009	1.7	0.2	0.5	-8.1
Combs Brook	Fine (sediment)	30	2009	-9.4	-6.6	-0.5	-11.3
Blues Brook	Fine (sediment)	10	2009	29.1	37.8	25.4	13.3
Blues Brook	Fine (sediment)	30	2009	-0.5	13.2	15.2	-5.2
Combs Brook	Coarse (leaves)	10	2010	44.2	104.3	20.8	-6.7
Combs Brook	Coarse (leaves)	30	2010	-7.7	2.2	-11.6	-17.8
Blues Brook	Coarse (leaves)	10	2010	57.3	543.6	314.7	9.9
Blues Brook	Coarse (leaves)	30	2010	18.1	34.3	104.1	-7.6
Combs Brook	Fine (sediment)	10	2010	-1.0	-0.3	-5.1	-14.7
Combs Brook	Fine (sediment)	30	2010	-4.3	-4.2	-12.1	-13.0
Blues Brook	Fine (sediment)	10	2010	-0.4	6.1	41.2	-9.6
Blues Brook	Fine (sediment)	30	2010	-8.5	10.6	17.6	-9.6

APPENDIX D

In Chapters 3 and 4, we used a linked dynamic compartment model approach based on observed isotopic enrichment of food (food pool, or FP) and consumers (consumer pool, or CP). We collected data during the 10 days that the system was enriched with tracer ^{15}N and ^{13}C and for 20 days after the addition stopped and ^{15}N and ^{13}C declined. From this point forward, we describe terms of the model for N for the sake of simplicity, but ^{13}C and ^{12}C could be used throughout the model instead of ^{15}N and ^{14}N .

We tracked the mass of each isotope (either ^{15}N and ^{14}N) over time in each pool and gains and losses of isotopes were based on uptake ($U_{15\text{N}}$ and $U_{14\text{N}}$) and loss ($L_{15\text{N}}$ and $L_{14\text{N}}$) of both isotopes expressed as mass per unit area per unit time (t). These uptake and loss fluxes were calculated by tracking the ^{15}N and ^{14}N in the consumer pool ($CP_{15\text{N}}$ and $CP_{14\text{N}}$, respectively) as a function of the $\delta^{15}\text{N}$ of up to three potential food pools labeled a, b, and c ($FP_{\delta^{15}\text{N},a}$; $FP_{\delta^{15}\text{N},b}$; $FP_{\delta^{15}\text{N},c}$).

The composite food pool $\delta^{15}\text{N}$ ($FP_{\delta^{15}\text{N}}$) was estimated by weighting the $\delta^{15}\text{N}$ of individual diet sources ($FP_{\delta^{15}\text{N},i}$) by the proportion of that food source in the consumer diet (P_i ; Eq. 1). The proportion of each food source was estimated using prior knowledge of consumer diets or by functional feeding group classification. For organisms that were presumed to eat fewer than three food sources, the number of sources was reduced accordingly.

$$FP_{\delta^{15}\text{N}} = FP_{\delta^{15}\text{N},a} \times P_a + FP_{\delta^{15}\text{N},b} \times P_b + FP_{\delta^{15}\text{N},c} \times P_c \quad (1)$$

The ^{15}N atomic ratios of the *FP* (*ARFP*) and *CP* (*ARCP*) were calculated from $\delta^{15}\text{N}$ values to allow separate mass-balance tracking of ^{15}N and ^{14}N based on the ratio of ^{15}N and ^{14}N in the atmosphere of 0.003663:

$$ARFP = \frac{FP_{15N}}{FP_{14N} - FP_{15N}} = (FP_{\delta^{15}N}/1000) \times 0.003663 + 0.003663 \quad (2)$$

At each sampling point, the ^{15}N and ^{14}N fluxes into the consumer pool were assumed to be proportional to the atomic ratio of the food pool(s), and N uptake and loss were the sum of the ^{15}N and ^{14}N uptake rates (U_{15N} and U_{14N} , respectively):

$$U = U_{15N} + U_{14N} \quad (3)$$

We used the change in the consumer pool of ^{15}N between time steps 1 and 2, representing the time between sampling, to calculate the new size of the consumer pool at time step 2 ($CP_{15N, t=2}$) from net uptake, which depends on *ARFP* and the uptake from the *FP* over time (t) as well as the loss from the *CP* (L), the atomic ratio of the consumer pool (*ARCP*), and time (Eq. 4):

$$CP_{15N, t=2} = CP_{15N, t=1} + (U \times ARFP \times t) - (L \times ARCP \times t) \quad (4)$$

Similarly, net uptake of ^{14}N was calculated as:

$$CP_{14N, t=2} = CP_{14N, t=1} + [U \times (1 - ARFP) \times t] - [L \times (1 - ARCP) \times t] \quad (5)$$

The calculated *ARCP* can lead to model instability if the time steps are too large (i.e., many days between samples). Thus, *ARCP* was reset to the observed value at each sampled time step in the model. In our study systems, biomass of *CP* was constant over the time period, then $U = L$.

We created a multiplier (M) to evaluate the degree to which we were not accounting for $\delta^{15}\text{N}$ label mismatch in the food pool by adjusting the peak food source label to fit the observed peak animal isotopic signal. The bounds on the range of M

values were set such that the lowest possible value for M adjusted the food source to match the $\delta^{15}\text{N}$ of its consumer ($CP_{15\text{N}}/FP_{15\text{N}}$). In contrast, the highest M value was dependent on the measured or calculated $\delta^{15}\text{N-NH}_4^+$ in the water column during the experiment ($\text{water}_{15\text{N}}/FP_{15\text{N}}$).

The model was created in Microsoft Excel and the “Solver” function was used to fit observed to modeled values of $\delta^{15}\text{N}$ by minimizing the sum of square of errors and changing U , U/L , and M . Because there was no change in biomass over the experiment, U/L was set to 1. We observed the model output graphically to assess the quality of fit.

APPENDIX E

The dynamic compartment model requires specification of diet proportions for consumers. For invertebrate groups with known functional feeding groups, we specified diets based on FFG classification. For *Eudaniela* crabs, we were not sure of classification beyond that they eat detritus and that similar species eat detritus in streams as juveniles (Fraiola 2004), so we specified a proportion of 50% FBOM and 50% CBOM. Since FBOM and CBOM have relatively similar isotope signatures throughout the course of the tracer study (Chapter 4, Figure 3), changes in proportion between the two types would not have a substantial effect on the output of food web models. For guppies, we used diet proportions published by Zandona *et al.* 2011 and for killifish, we used unpublished diet data from B. Lamphere. Diet priors are detailed in the table below.

Since the duration of the experiment was during the dry season, we assumed that biomass of consumer pools did not change during the experiment. Although it is possible that invertebrate biomasses would have changed over the course of a 2-month period, biomass samples are highly variable and the resolution of our sampling would have been insufficient to detect differences on that fine of a time-scale. The multiplier (M, detailed in Methods and Appendix D) was required for several consumer groups (specified in the table below). For grazers (*Petrophila*, *Psephenus*) the multiplier was only needed in some model runs.

Taxon	Functional Feeding Group	Diet Proportions	Multiplier required?	Source
Invertebrates				
<i>Argia</i>	Predator	100% inverts (<i>Tricorythodes</i>)	No	FFG classification
<i>Eudaniela</i>	Detritivore	50% CBOM, 50% FBOM	Yes	Fraiola 2004
<i>Euthyplocia</i>	Predator	100% inverts (<i>Tricorythodes</i>)	No	FFG classification
<i>Leptonema</i>	Collector-filterer	100% seston	Yes	FFG classification
<i>Petrophila</i>	Grazer	100% Epilithon	Sometimes	FFG classification
<i>Phylloicus</i>	Shredder	100% CBOM	Yes	FFG classification
<i>Psephenus</i>	Grazer	100% Epilithon	Sometimes	FFG classification
<i>Tricorythodes</i>	Collector-gatherer	100% FBOM	Yes	FFG classification
Fishes				
<i>Rivulus</i> (killifish)	Insectivore	50% aquatic inverts (<i>Tricorythodes</i> , <i>Argia</i>), 50% terrestrial inverts (unenriched)	No	B. Lamphere, unpublished diet data
<i>Poecilia</i> (guppies)	Omnivore	30% FBOM, 30% Epilithon, 40% inverts (<i>Tricorythodes</i>)	Yes	Zandona <i>et al.</i> 2011