

Limited seasonal variation in food quality and foodweb structure in an Adirondack stream: insights from fatty acids

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Abstract: Together terrestrial and aquatic resources fuel stream food webs. Past work suggests that both terrestrial and aquatic resources can vary in multiple metrics of food quality, such as elemental, macronutrient, and fatty acid (FA) composition, and that resource quality may vary seasonally in temperate systems. However, studies on FA composition in stream food webs, especially those quantifying seasonal patterns, remain scarce. We documented foodweb structure and examined FA composition as a metric of food quality in an Adirondack stream throughout the temperate growing season to understand from where stream consumers derived energy, how food quality varied among resources, and whether these patterns shifted seasonally. In spite of major seasonal shifts in environmental factors, such as light availability and temperature, we found limited seasonal variation in the FA composition of basal resources and macroinvertebrates. Instead, we found consistent differences in FA composition between aquatic and terrestrial basal resources and between macroinvertebrate functional feeding groups. Foodweb structure also was consistent throughout the growing season, and macroinvertebrates and fish relied on a mix of terrestrial and aquatic resources. Our results suggest that stream consumers in heavily forested reaches rely upon low-quality terrestrial resources supplemented with higher-quality aquatic resources throughout the year.

Key words: streams, food webs, stable isotopes, fatty acids, survey

Many stream food webs are subsidized by inputs of energy and nutrients from terrestrial sources, and these inputs vary seasonally (Cummins 1974, Hynes 1975, Nakano and Murakami 2001). Terrestrial resources entering stream food webs vary by season and trophic level, ranging from inorganic nutrients that fuel in-stream primary production and are washed into streams during rainstorms to organic nutrients in the form of autumn leaf-litter pulses that feed shredding invertebrates (Cummins 1974, Hynes 1975) or summer fluxes of terrestrial arthropods that feed fishes (Nakano and Murakami 2001). Various terrestrial resources can fuel stream food webs throughout the year, but they differ from freshwater resources in composition, especially food quality. At the level of basal resources, leaves and other forms of terrestrial plant detritus are much lower-quality food than biofilms because they have high C : N : P ratios (Elser

et al. 2000) and contain indigestible structural materials like cellulose (Webster and Benfield 1986, Thorp and Delong 2002). In addition, unless colonized by algal biofilms (Guo et al. 2016b), terrestrial leaves are devoid of highly unsaturated omega-3 fatty acids (HUFAs), a group of physiologically vital fats for freshwater animals (Brett and Müller-Navarra 1997, Stanley and Kim 2014, Brett et al. 2017). Terrestrial arthropods have similar elemental ratios to those of aquatic invertebrates (Elser et al. 2000), but they are much lower in HUFAs (Stanley-Samuelson et al. 1988, Jardine et al. 2015). The major differences in food quality between aquatic and terrestrial resources may be problematic for stream consumers. For example, terrestrial resources like leaves often require trophic upgrading by aquatic microbes to become more palatable and nutritious for stream consumers (Thorp and Delong 2002, Tanentzap et al. 2014, Collins et al. 2016).

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For stream consumers, differences in the quality of organic compounds between terrestrial and aquatic resources may be especially important because autotrophs, including both terrestrial plants and algae, can synthesize all of the organic compounds that they need, but animals require organic nutrients, such as vitamins and complex fats, for growth, development, and health. In most cases, animals must obtain these organic compounds directly from their diets (Koussoroplis et al. 2008).

HUFAs are a physiologically important group of organic compounds with high potential for a food-quality mismatch between terrestrial availability and stream animal needs (Twining et al. 2016). HUFAs are necessary for animal growth and development, especially the development of nerve tissue (Ahlgren et al. 2009). Many animals are incapable of synthesizing all of the HUFAs they require from molecular precursors and must obtain HUFAs directly from their diet (Kainz et al. 2004). Stream animals can obtain HUFAs directly from biofilms or other animals or indirectly by converting precursor fatty acids (FAs) into HUFAs, a process that requires energy and functional enzymes for FA elongation and desaturation (Twining et al. 2016).

Investigators have documented mismatches between the HUFA content of resources and freshwater animal HUFA requirements and the detrimental effects of HUFA limitation on freshwater animal growth and performance (Brett and Müller-Navarra 1997, Guo et al. 2016b). However, these studies have overwhelmingly focused on lakes, and many basic questions are unresolved in lotic systems (Guo et al. 2016b). For example, many freshwater zooplankton researchers have documented the effects of the HUFA eicosapentaenoic acid (EPA; 20:5n-3) limitation in cladocerans and the HUFA docosahexaenoic acid (DHA; 22:6n-3) limitation in copepods (Brett and Müller-Navarra 1997). The few studies done on aquatic macroinvertebrates in streams suggest that many stream invertebrates also have a high potential for HUFA limitation (Guo et al. 2016a), but this potential appears to vary greatly across taxa based on their ability to convert the HUFA precursor alpha linolenic acid (ALA; 18:3n-3) into EPA (Goedkoop et al. 2007, Brett et al. 2009, Chen et al. 2012). Work in fish aquaculture and captive rearing also suggests that many stream-dwelling freshwater fish require HUFAs directly from their diet for optimal growth and development (Henderson and Tocher 1987, Sargent et al. 1999). Seasonal differences in the relative availability of high- and low-HUFA resources also may have major effects on stream animals. However, additional research on the HUFA content of stream resources vs needs of stream consumers is necessary to assess the potential for HUFA mismatches.

In particular, few investigators have examined the effects of seasonality on FA composition in stream food webs (but see Torres-Ruiz et al. 2007). Studies done in lakes have shown that aquatic basal resources tend to be much richer in HUFAs than terrestrial basal resources, but aquatic re-

sources can also undergo seasonal shifts in quality. For example, FA composition in phytoplankton is highly variable across taxa and environmental conditions (Ahlgren et al. 1997, Galloway and Winder 2015). Lake phytoplankton and stream periphyton both undergo well-documented seasonal taxonomic shifts. Thus, stream periphyton probably undergoes FA compositional shifts similar to those in lake phytoplankton. Low-light, high-nutrient conditions appear to increase algal HUFA content in lakes and streams (e.g., Ahlgren et al. 1992, Hill et al. 2011, Guo et al. 2015, 2016c), but studies in lakes suggest that effects of abiotic drivers vary considerably across species groups (Piepho et al. 2012, Galloway and Winder 2015).

Temperature can have important effects on the FA composition of lake phytoplankton. Warm temperatures generally decrease phytoplankton HUFA content while increasing saturated FA content (Piepho et al. 2012, Galloway and Winder 2015). Changes in stream algal FA composition may also result in shifts in the FA composition of stream consumers. For example, Guo et al. (2016a) found that the HUFA content of periphyton in a tropical stream increased in experimental treatments with shading and shading plus nutrients, and the FA composition of 2 invertebrate grazers in both treatments became more similar. Torres-Ruiz et al. (2007) also found similar but less consistent patterns in FA composition associated with seasonal changes in canopy cover and stream temperature during the growing season in a temperate stream.

Stream macroinvertebrates (e.g., Bell et al. 1994, Ghioni et al. 1996, Guo et al. 2016a) and fish (Wang et al. 2016) also show considerable variation in HUFA content across taxa and functional feeding groups (FFGs). For example, grazers generally have higher amounts of EPA (Guo et al. 2016b), which usually is the most abundant FA in diatoms (Galloway and Winder 2015, Twining et al. 2016). Grazer FA profiles are similar to those of algae, whereas shredders and collector-gatherers have lower HUFA levels and FA profiles similar to those of bacteria and fungi (Guo et al. 2016b). Among stream macroinvertebrates, predators often have the highest HUFA levels (Bell et al. 1994, Ghioni et al. 1996). Studies in lakes suggest that zooplankton can selectively retain HUFAs, such that HUFAs bioaccumulate at successive trophic levels (e.g., Kainz et al. 2004, Hessen and Leu 2006, Gladyshev et al. 2011, Strandberg et al. 2015), but whether this pattern occurs in stream invertebrate taxa is unclear. Temperate stream fishes have higher HUFA levels than lake fishes, and invertivores have higher HUFA levels than other feeding guilds like piscivores and algivores (Wang et al. 2016). Few investigators have examined the consequences of seasonal changes in diet on the FA composition of macroinvertebrates or fish or have compared seasonal changes with FFG-based differences in FA composition (but see Torres-Ruiz et al. 2007).

FA composition is not the only facet of food quality likely to vary over the growing season in stream food webs. C : N

is an established metric of food quality in streams, and numerous studies suggest that C : N, like FAs, varies considerably between freshwater and terrestrial resources (Cross et al. 2003, Lau et al. 2009). Basal resource quality, measured in terms of stoichiometric change in biofilms (Drake et al. 2012) or as the relative availability of basal resources, shifts over the growing season (Lauridsen et al. 2012), as do food quality and quantity, measured in terms of relative availability of different basal resources. Algal resource availability peaks when riparian canopy cover is low and light levels and temperature are conducive to growth in late autumn and early spring, whereas terrestrial leaf litter peaks in autumn (Tank et al. 2010). Thus, in addition to our focus on seasonal shifts in stream foodweb FA composition, we also considered shifts in basal-resource quality and quantity over time.

We evaluated the effects of seasonality in several key abiotic factors (e.g., light, water chemistry, and stream temperature) on foodweb structure (feeding relationships based on stable isotopes) and food quality in a north-temperate forested stream from May (before leafout) to October (during leaf fall). We examined how the relative importance of terrestrial vs aquatic resources for stream consumers and the quality of these resources in terms of HUFAs varied over the season. We used FAs and C : N ratios to assess the quality of stream resources (leaf litter, algal biofilms, and invertebrates) and bulk stable isotopes ($\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$) to trace the movement of these resources in the stream food web.

Based on previous research in this river system (Collins et al. 2016), we expected that autochthonous resources would be most abundant and most important in spring when light levels were high enough to increase biofilm abundance, but stream temperatures were low enough to keep HUFA content high. In summer, we expected the quality and importance of aquatic resources to decline as the stream warmed and canopy cover shaded algal biofilms. We expected the HUFA content of biofilms to increase again in autumn with the onset of cooler stream temperature, but expected that the relative importance of biofilms as an invertebrate food source would decrease during leaf fall. Numerous investigators have characterized seasonal changes in stream resource abundance, but our study provides a unique window into how seasonal variation may affect food quality and foodweb interactions by documenting the quality, in terms of FA composition, of food resources and the movement of those resources through a temperate stream food web.

METHODS

Field surveys

We surveyed Little Moose Outlet (lat 43°39'45.27" N, long 74°56'53.82" W), an ~4.5-m-wide forested tributary of the Moose River that drains Little Moose Lake (~2.5 km

north of the sampling reach), approximately once per month from mid-May to mid-October 2013. During each survey, we measured % canopy cover, water temperature, pH, and total dissolved solutes (TDS), and we collected water samples for additional chemical analyses. We measured % canopy cover at 3 locations in the ~50-m reach with a gridded densitometer and water temperature, pH, and TDS with an Oakton Multi-Parameter Testr 35 Series (Oakton Instruments, Vernon Hills, Illinois). We also collected fresh American beech (*Fagus grandifolia*) and sugar maple (*Acer saccharum*) leaves, periphyton, invertebrate, and fish samples. All samples were flash frozen in the field with dry ice and transported to the laboratory where they were stored at a maximum of -20°C until processing. Samples for FA analyses were processed <1 mo after arrival in the laboratory.

In the field, we scrubbed a standardized surface area (38.5 cm²) of each of 3 replicate rocks with toothbrushes and rinsed the brushes with flowing water until no biofilm pieces were stuck to them. We retained the slurry and measured areal chlorophyll *a* (Chl *a*) and ash-free dry mass (AFDM). We also collected 3 replicate samples of periphyton from entire rocks and in-stream terrestrial detritus samples for stable-isotope, %C, %N, and FA analyses. We collected coarse detritus from the stream reach by placing a 25-cm² quadrat on the stream bottom at 3 haphazardly chosen spots and collecting all terrestrial plant material (i.e., leaves, stems, sticks) in the quadrat. We used kick nets to collect ≥ 3 individuals of the most abundant (in terms of biomass) macroinvertebrates for stable-isotope, stoichiometric, and FA analyses. Invertebrates included: philopotamid and hydropsychid caddisflies, crayfish (*Cambarus* spp.), heptageniid mayflies, aeshnid odonates, and perlid stoneflies. We collected fish for stable-isotope analyses with the aid of an AbP-3 Pulsed DC backpack electrofishing unit (ETS Electrofishing, Madison, Wisconsin). Fish included: Blacknose Dace (*Rhinichthys atratulus*), Brook Trout (*Salvelinus fontinalis*), Common Shiner (*Luxilus cornutus*), Creek Chub (*Semotilus atromaculatus*), Longnose Dace (*Rhinichthys cataractae*), and White Sucker (*Catostomus commersonii*). We collected ≥ 3 fish per species and sampling date.

Laboratory analyses

We filtered 3 replicates of 1 to 5 mL of biofilm slurry from each rock section onto preweighed, precombusted 47-mm glass-fiber filters (Pall Gelman, Port Washington, New York) to measure AFDM and Chl *a*. We dried filters and weighed them to estimate dry mass, combusted the filters at 500°C for ≥ 1 h, reweighed the filters, and subtracted the difference to obtain AFDM. We measured Chl *a* content by fluorometry on a TD-700 fluorometer (Turner Designs, Sunnyvale, California). We extracted chlorophyll from samples with 25 mL of 90% ethanol for 24 h prior to analysis. We calculated the autotrophy index (AI) as the ratio of Chl *a* per unit area to AFDM per unit area.

We measured total P (TP), total dissolved N (TDN), and dissolved organic C (DOC) concentrations in water samples from each sampling date. We measured total P via spectrophotometry with acid molybdate–antimony and ascorbic acid reagent on a Shimadzu UVmini 1240 spectrophotometer (Shimadzu Scientific Instruments, Columbia, Maryland) following digestion with potassium persulfate (Parsons et al. 1984). We measured TDN and DOC on a Shimadzu TNM-1 and TOC- V_{CPN} after water was filtered through a precombusted Whatman GF/F filter (GE Healthcare Life Sciences, Pittsburgh, Pennsylvania).

We extracted FAs from basal resources (biofilm, microbially colonized in-stream detritus, and fresh leaves) and a subset of macroinvertebrate taxa representing 3 different functional feeding groups (FFGs; mayfly grazers, crayfish shredders, and stonefly and odonate predators). We analyzed the whole body of all invertebrates, pooling multiple mayfly and stonefly individuals to reach ~200 mg tissue/sample. All FA samples were analyzed from wet samples (previously frozen until extraction process). We extracted FA methyl esters (FAMES) with a modified 1-step method, which is preferable for polyunsaturated FA (PUFA) extraction from solid samples (Garcés and Mancha 1993, Zhou et al. 2008). First, we added an aqueous reagent of methanol, 2,2-dimethoxypropane, and sulfuric acid and then an organic reagent of heptane and toluene to samples in test tubes. We vortexed and then shook samples in a water bath at 80°C for 2 h. After samples returned to room temperature, we added water saturated with NaCl to each sample, and vortexed and then centrifuged samples for 10 min at 3500 rpm. We transferred the top lipid layer to a clean test tube and added heptane to the initial tube followed by vortexing and another 10 min of centrifugation. We transferred the top lipid layer to new test tube, which was then dried down under N_2 gas. We transferred N_2 -dried samples to stock vials in heptane and stored them at –80°C until quantification of FAMES.

We quantified FAMES with the aid of a BPX-70 (SGE Inc., Ringwood, Australia) column and a HP5890 series II GC-FID (Agilent Technologies, Santa Clara, California). We processed chromatogram data with PeakSimple 2.83 software (SRI Instruments, Torrance, California). We calculated response factors based on the reference standard 462a (Nu-Check Prep, Waterville, Minnesota). We identified FAMES with the aid of a Varian (Agilent Technologies) Saturn 2000 ion trap with a Varian Star 3400 gas chromatography mass spectrometer run in chemical ionization mass spectrometry mode using acetonitrile as reagent gas and H_2 as a carrier gas. We expressed FA composition data as % total FA.

All samples for stable-isotope analysis were oven-dried at ~50°C for >48 h. Samples for δ^2H analyses were equilibrated in Ithaca, New York, for ≥ 8 wk prior to analysis. Samples for stable-isotope and elemental analysis were ground and homogenized. We weighed ~0.5 mg of sample into Sn capsules for %C, %N, $\delta^{13}C$, and $\delta^{15}N$ analyses and ~0.3 mg

of sample into Ag capsules for H and δ^2H analyses. Percent C, %N, $\delta^{13}C$, and $\delta^{15}N$ were analyzed at the Yale Earth System Center for Stable Isotopic Studies in New Haven, Connecticut, on a Thermo Fisher Scientific (Waltham, Massachusetts) Delta Plus Advantage mass spectrometer with a Costech (Valencia, California) ECS 4010 elemental analyzer with ConFlo III interface. $\delta^{13}C$ was standardized to Vienna Pee Dee Belemnite, and $\delta^{15}N$ was standardized to N_2 of atmospheric air based on cocoa, trout, and beech laboratory internal standards and US Geological Survey standards 40 (L-glutamic acid) and 41 (L-glutamic acid enriched in $\delta^{13}C$ and $\delta^{15}N$; <https://isotopes.usgs.gov/lab/referencematerials.html>). We calculated C : N ratios as %C divided by %N. δ^2H of water and solid samples and %H of solid samples were analyzed at the Cornell Stable Isotope Laboratory in Ithaca, New York, on a Thermo Delta V Advantage isotope ratio mass spectrometer with a temperature conversion elemental analyzer and ConFlo III interface. δ^2H was standardized to Vienna Standard Mean Ocean Water based on internal laboratory standards including keratin and benzoic acid.

Statistical analyses

We first applied nonmetric multidimensional scaling (NMDS) to understand how FA composition varied among basal resources and among macroinvertebrates over the growing season. We removed FAs present in only 1 taxon prior to analyses. We ran NMDS using metaMDS within the *vegan* package in R (version 3.3.3; R Project for Statistical Computing, Vienna, Austria) with default options (Bray–Curtis distance, Wisconsin double standardization, 3 dimensions, and \sqrt{x} transformations) and checked for a run stress < 0.2. We also fit environmental factors (stream temperature, cover, TP, TDN, and DOC) to our NMDS as environmental predictors using standard options and 1000 permutations in *vegan*. We used general linear models (GLMs) to analyze differences in environmental variables with month as a factor and found no significant differences (Table S1). We did not analyze cover or temperature with GLMs because these variables were single-point measurements.

We used GLMs to assess differences in the quality of basal resources (in-stream detritus, fresh leaves, and biofilm) in terms of %ALA, %EPA (the only HUFA present in basal resources), the ratio of total ω -3 : total ω -6 FAs, and C : N ratios, using identity (fresh leaves, conditioned detritus, or biofilm) as a factor. We also used GLMs to analyze differences in the quantity and quality of biofilm over the season using month as a factor. We assessed biofilm quantity over the season in terms of Chl *a* content (g/m^2 stream bed) and AFDM (g/m^2 stream bed) and biofilm quality in terms of %ALA, %EPA (the only HUFA present in basal resources), the ratio of total ω -3 : total ω -6 FAs, and C : N ratios, again using month as a factor. Last, we used GLMs to compare seasonal vs taxonomic differences between the per-

centages of 3 major ω -3 FAs (ALA, EPA, DHA) and C : N in macroinvertebrate consumers. We analyzed season and taxonomic group as factors in the following ways: 1) group plus month, and 2) group plus month and their interaction and then compared between the 2 models based on Akaike's Information Criterion (AIC). Only significant differences between groups and our lowest AIC GLM are presented. We calculated least squares means and standard error for all variables in GLMs using the *lsmeans* package in R. We also used 2-sample *t*-tests to examine differences between biofilm and detritus $\delta^{13}\text{C}$ and $\delta^2\text{H}$. All analyses were performed in R.

RESULTS

Like most small temperate deciduous forest streams, Little Moose Outlet experienced major shifts in canopy cover and temperature over the course of the growing season. Both peaked in midsummer with >90% cover and a stream temperature of 25.2°C (Fig. 1A, B). Inorganic nutrients in stream water were low throughout the season and

peaked in autumn with a TP concentration of 19.6 $\mu\text{g/L}$ in October (Fig. 1C) and TDN concentration of 0.43 mg/L in October (Fig. 1D). DOC was consistently low (<5 mg C/L) throughout the growing season. Water chemistry variables did not vary significantly across the growing season (Table S1).

Based on FA composition, basal resources clustered strongly by group (i.e., fresh leaves, conditioned in-stream terrestrial detritus, or algal biofilm), but not by season (Fig. 2A, B, Tables 1, 2, S2). None of the environmental factors that we fit to NMDS were significant predictors of overall FA composition (all $p > 0.05$; Table S2). Therefore, we represent FA composition for groups as a whole aggregated throughout the season.

In terms of FA composition, in-stream detritus was the lowest-quality resource throughout the season and never contained more than trace amounts of ω -3 FAs (Figs 2A, 3A, S1). In-stream detritus and biofilm were both characterized by high percentages of branched-chain FAs (BCFAs; e.g., iso and anteiso FAs), which are typically thought of as mi-

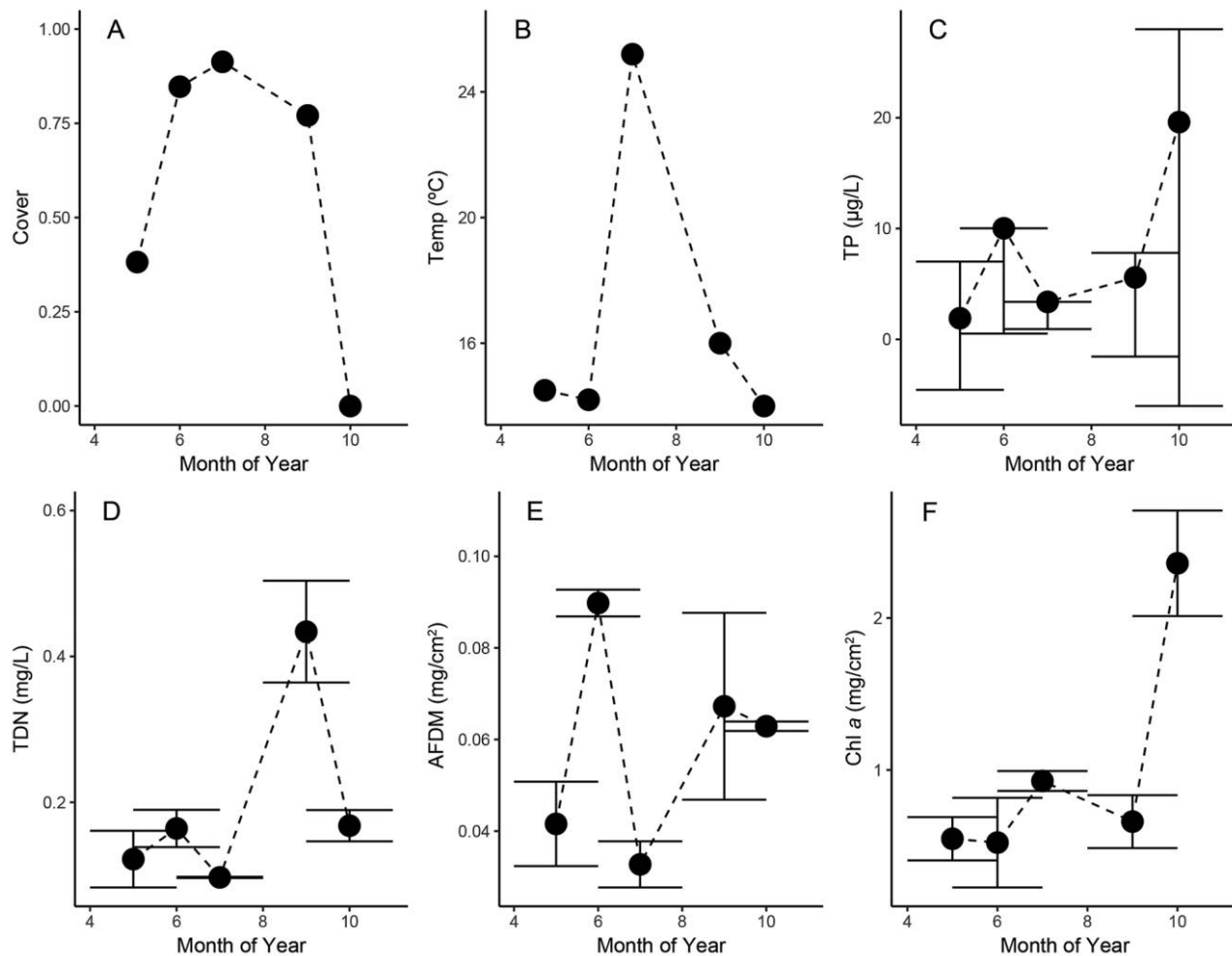


Figure 1. Mean (\pm SD) % canopy cover (A), stream temperature (Temp) (B), total P (TP) (C), total dissolved N (TDN), biofilm ash-free dry mass (AFDM) (E), and chlorophyll *a* (Chl *a*) (F) over the growing season in a temperate forested stream.

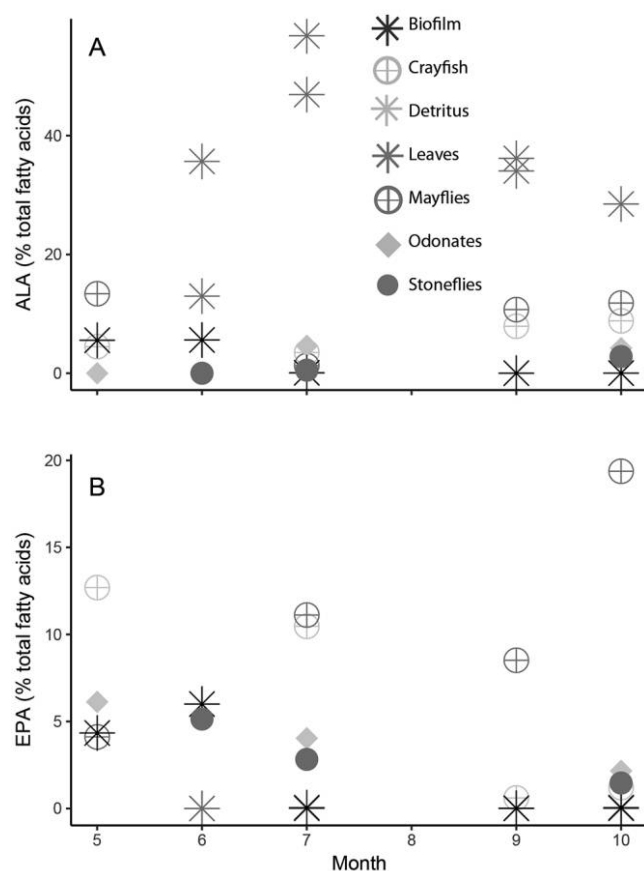


Figure 2. Mean (each sample was run in triplicate) % alpha linolenic acid (ALA; a highly unsaturated ω -3 fatty acid [HUFA] precursor) (A) and % eicosapentaenoic acid (EPA, a HUFA) (B) in basal resources and macroinvertebrates across the growing season. Detritus did not contain ALA or EPA.

crobiologically derived. This result suggests that biofilms on detritus and rocks had overlapping microbial communities (Fig. S1). However, biofilm had much higher percentages of highly unsaturated (≥ 20 -C) FAs, including EPA (Figs 2B, 3C, S1), commonly found in diatoms (Galloway and Winder 2015, Twining et al. 2016), and microbe-associated ω -7 FAs than either fresh leaves or in-stream detritus (Torres-Ruiz et al. 2007). Fresh leaves had the highest percentages of 18-C FAs including common terrestrial PUFAs like the HUFA precursor ALA (Figs 2A, 3A) and the 18-C ω -6 FAs linoleic and γ -linolenic acids (Hixson et al. 2015; Figs 3B, S1). Fresh leaves had the highest overall %ALA and as a result, the highest ω -3 : ω -6 ratios (Table 1, Fig. 3A). Biofilm had the highest %EPA (Table 1, Fig. 3C), and the lowest C : N ratios (Table 1). Biofilm and terrestrial leaves also contained the 18-C ω -6 FA linoleic acid (LA; Fig. 3B), but had little to none of the 20-C ω -6 FA arachidonic acid (AA; Fig. 3D), which is physiologically vital for fish (Sargent et al. 1999).

We expected biofilm quantity and quality to vary substantially over the season, but found little to no seasonal

change in autochthonous stream basal resources. We found no significant differences in biofilm quantity over the growing season in terms of AFDM (Fig. 1E), but a small increase in terms of Chl *a* content in October (Fig. 1F, Table 2). Biofilm quality, in terms of FA and stoichiometric composition, also remained consistent over the growing season. We found small differences in biofilm ω -3 : ω -6 ratios over the season (Table 2), and ω -3 : ω -6 ratios were highest in June. Percent EPA did not vary over the growing season (Fig. 2B, Table 2).

$\delta^{13}\text{C}$ and $\delta^2\text{H}$ did not differ between aquatic and terrestrial basal resources ($t = 0.58$, $df = 5.672$, $p = 0.59$) and ($t = -1.66$, $df = 4.74$, $p = 0.16$; Fig. 4A–E), probably because of the large quantity of allochthonous input that fuels heterotrophic bacterial biofilms on rocks and leaves in our system (see Collins et al. 2016). This lack of variability limited our ability to apply multi-isotope mixing models to estimate contributions of allochthonous and autochthonous resources to consumers. However, aquatic and terrestrial basal resources exhibited large differences in $\delta^{15}\text{N}$ and maintained them throughout the season (Fig. 4A–J). Aquatic basal resources were consistently enriched in $\delta^{15}\text{N}$ relative to conditioned in-stream detritus and fresh terrestrial leaves.

$\delta^{13}\text{C}$ (Fig. 4A–E) and $\delta^2\text{H}$ (Fig. 4F–J) values of basal resources were not very distinctive, but consumers appeared to feed on distinctly different resources that generally followed FFG predictions (Fig. 4A–J). These differences also were reflected in FA composition for primary consumers (Fig. 5A–E). Both NMDS and GLMs of FA composition suggested that macroinvertebrates clustered more strongly by taxonomic group than by season (Fig. S2, Tables 3, S2). Percent EPA was high in all macroinvertebrate taxa (Figs 2B, 5B, Table 3), making EPA a poor foodweb tracer for within-stream comparisons, but highlighting its importance across macroinvertebrate taxa (Figs 2B, 5B, S2). Macroinvertebrate C : N did not differ by taxonomic identity or month (Table S3).

Grazing mayflies had depleted $\delta^{13}\text{C}$ and $\delta^2\text{H}$ (more aquatic; Fig. 4A–J) values close to those of biofilm but did not have notably enriched $\delta^{15}\text{N}$ signatures compared to biofilm, suggesting that they consumed both biofilm and terrestrial resources. Over the growing season, grazing mayfly $\delta^2\text{H}$ and $\delta^{15}\text{N}$ values became less depleted, a result hinting at a subtle shift to greater use of terrestrial resources. Throughout the season, grazing mayflies had high levels of several BCFAs, a result indicating possible microbe consumption, an abundance of <20-C PUFAs, and significantly higher ALA levels than other macroinvertebrate taxa (Figs 2A, 5A, S2, Table 3). Crayfish had the highest $\delta^{13}\text{C}$ and $\delta^2\text{H}$ (more terrestrial; Fig. 4A–J) and the highest levels of 22-C FAs, including the HUFA DHA (Figs 5C, S2) and several 20+ -C ω -6 FAs (Fig. S2). Other macroinvertebrates contained only trace amounts of DHA (Fig. 5C). Net-spinning caddisflies generally had intermediate $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values, suggesting

Table 1. General linear model (GLM) comparing elements of food quality (% alpha linolenic acid [ALA; 18:3n-3], % eicosapentaenoic acid [EPA; 20:5n-3], ratio of total ω -3 : total ω -6 fatty acids [n3 : n6], and ratio of %C:%N) between terrestrial and aquatic basal resources. Direction is the direction of any significant differences between resources, ID = specific basal resource, LSM = least squares mean, and LSMSE = least squares mean standard error.

Model/variable	<i>t</i>	<i>p</i>	Direction	ID	LSM	LSMSE
ALA						
Intercept	0.00	1.00	Detritus = biofilm < leaves (<i>p</i> < 0.01)	Detritus	0	7.67
Leaves	3.49	<0.01		Leaves	31.39	4.70
Biofilm	0.47	0.65		Biofilm	4.56	5.94
Null deviance: 5594.3 on 15 df						
Residual deviance: 2296.7 on 13 df						
EPA						
Intercept	0.00	1.00	Detritus = leaves < biofilm (<i>p</i> < 0.01)	Detritus	0	0.54
Leaves	0.00	1.00		Leaves	0	0.33
Biofilm	6.321	<0.01		Biofilm	4.29	0.42
Null deviance: 74.63 on 15 df						
Residual deviance: 11.25 on 13 df						
n3 : n6						
Intercept	0.00	1.00	Detritus = biofilm	Detritus	0	1.46
Leaves	2.50	<0.05	Detritus < leaves (<i>p</i> < 0.01)	Leaves	4.28	0.89
Biofilm	0.57	0.57	Biofilm < leaves (<i>p</i> < 0.10)	Biofilm	1.06	1.13
Null deviance: 137.63 on 15 df						
Residual deviance: 83.07 on 13 df						
C : N						
Intercept	5.412	<0.01	Biofilm < leaves = detritus (<i>p</i> < 0.01)	Detritus	31.82	5.88
Leaves	-0.195	0.85		Leaves	30.48	3.60
Biofilm	-3.022	<0.01		Biofilm	9.35	4.55
Null deviance: 2941.2 on 15 df						
Residual deviance: 1348.6 on 13 df						

a diet intermediate between those of grazing mayflies and crayfish (Fig. 4A–J).

Fishes and predatory macroinvertebrates (perlid stoneflies and odonates) appeared to consume a mix of macroinvertebrates from across FFGs as evidenced by $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values that were intermediate between grazers and shredders throughout the season (Fig. 4A–J). Fishes tended to have more depleted $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values, whereas predatory macroinvertebrates had more enriched $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values (Fig. 4A–J), suggesting that fish may have relied more on aquatic energy pathways, whereas predatory invertebrates may have relied more on terrestrial subsidies. However, over the season, fish may have shifted to more terrestrial resources (terrestrial invertebrates or aquatic macroinvertebrates consuming terrestrial resources) because their $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values shifted in the direction of terrestrial basal resources (although freshwater and terrestrial basal resources were not statistically different). Throughout the season, fishes had slightly elevated $\delta^{15}\text{N}$ values compared to predatory macroinvertebrates (Fig. 4A–J), suggesting that they may have in-

cluded small amounts of predatory macroinvertebrates in their diet. Stoneflies and odonates had high EPA throughout the season (Figs 2B, 5B, S2), making them a high-quality large-bodied food source for fish.

DISCUSSION

We examined foodweb relationships and food quality in terms of FA composition throughout the growing season in an Adirondack stream. In particular, we were interested in understanding whether and how seasonally variable abiotic factors, such as light, nutrients, and temperature, affect food quality and foodweb pathways in a single stream. Foodweb structure, described based on stable isotopes stayed fairly consistent throughout the season, and resource use varied among FFGs and trophic positions, rather than seasons. We found major differences in FA composition based on the origin of basal resources (terrestrial or aquatic) and macroinvertebrate FFGs, but we found only limited seasonal variation in FA composition in spite of major shifts in

Table 2. General linear model (GLM) comparing elements of food quality (% alpha linolenic acid [ALA; 18:3n-3], % eicosapentaenoic acid [EPA; 20:5n-3], ratio of total ω -3 : total ω -6 fatty acids [n3 : n6], and ratio of %C:%N, chlorophyll *a* [Chl *a*], and ash-free dry mass [AFDM]) of biofilm among months. Direction is the direction of any significant differences between resources, ID = specific month, LSM = least squares mean, and LSMSE = least squares mean standard error.

Model/variable	<i>t</i>	<i>p</i>	Direction	ID	LSM	LSMSE
ALA						
Intercept	3.22	<0.05	No differences among months	May	5.55	1.72
June	0.02	0.9853		June	5.60	1.72
July	0.91	0.3843		July	7.78	1.72
September	-1.64	0.1358		September	1.08	2.11
October	-1.14	0.2825		October	2.77	1.72
Null deviance: 150.29 on 13 df						
Residual deviance: 80.12 on 9 df						
EPA						
Intercept	1.61	0.14	No differences among months	May	4.34	2.68
June	0.44	0.67		June	6.00	2.68
July	0.09	0.93		July	4.70	2.68
September	-0.67	0.52		September	1.51	3.29
October	-0.71	0.50		October	1.64	2.68
Null deviance: 236.85 on 13 df						
Residual deviance: 194.55 on 9 df						
n3 : n6						
Intercept	2.17	<0.10	Oct. < June (<i>p</i> < 0.05)	May	0.93	0.43
June	1.89	<0.10		June	2.07	0.43
July	-0.21	0.84		July	0.80	0.43
September	0.20	0.85		September	1.06	0.52
October	-0.85	0.42		October	0.41	0.43
Null deviance: 26.545 on 13 df						
Residual deviance: 19.841 on 9 df						
C : N						
Intercept	9.55	< 0.01	No differences among months	May	8.19	0.86
June	1.39	0.20		June	9.88	0.86
July	1.10	0.30		July	9.52	0.86
September	0.35	0.74		September	8.65	1.05
October	1.40	0.19		October	9.89	0.86
Null deviance: 12.9865 on 13 df						
Residual deviance: 5.1189 on 9 df						
Chlorophyll <i>a</i>						
Intercept	3.08	<0.05	June < Oct. (<i>p</i> < 0.05)	May	1.34	0.43
June	-1.33	0.21	July < Oct. (<i>p</i> < 0.05)	June	0.52	0.43
July	-1.29	0.23	Sept. < Oct. (<i>p</i> < 0.05)	July	0.55	0.43
September	-1.39	0.20		September	0.39	0.53
October	1.65	0.13		October	2.36	0.43
Null deviance: 12.99 on 13 df						
Residual deviance: 5.12 on 9 df						
AFDM						
Intercept	2.32	< 0.05	No differences among months	May	0.05	0.02
June	1.63	0.14		June	0.09	0.02
July	-0.46	0.66		July	0.03	0.02
September	0.49	0.63		September	0.06	0.02
October	0.65	0.53		October	0.06	0.02
Null deviance: 0.02 on 13 df						
Residual deviance: 0.01 on 9 df						

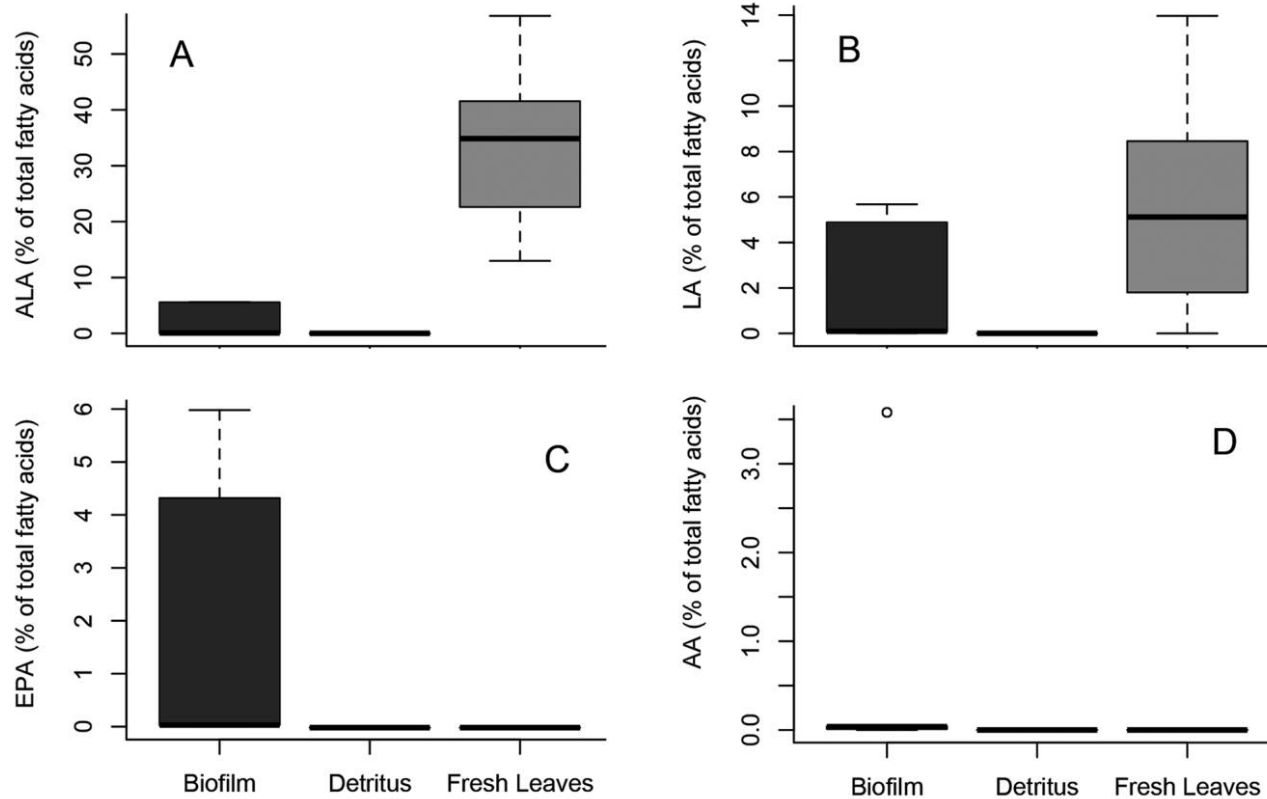


Figure 3. Box-and-whisker plots of % alpha linolenic acid (ALA; 18:3n-3) (A), % linoleic acid (LA; 18:2n-6) (B), % eicosapentaenoic acid (EPA; 20:5n-3) (C), and % arachidonic acid (AA; 20:4n6) (D) in basal resources. Heavy lines in boxes show medians, box ends show quartiles, whiskers show first quartile minus 1.5 times the interquartile range and the third quartile plus 1.5 times the interquartile range, and circles show outliers.

temperature and canopy cover. Overall, these results suggest that within the study stream, the quantity of food varied much more than its quality over the growing season, and that many consumers remained remarkably consistent in their feeding preferences despite changes in resource availability.

The major differences between the FA quality of aquatic and terrestrial basal resources (Table 1) throughout the season were consistent with those found in other studies (e.g., Torres-Ruiz et al. 2007, Cashman et al. 2016, Brett et al. 2017). Biofilm, conditioned in-stream detritus, and fresh terrestrial leaves had distinctive FA profiles, which our analyses suggested were not significantly affected by seasonally variable abiotic environmental factors or time of year (Figs 2A, B, S1, Table 1). In-stream detritus, which contained no ω -3 FAs and had a high C : N ratio, was an extremely poor-quality food source in terms of FA composition compared to either fresh terrestrial leaves or biofilm. The most notable seasonal change was an increase in ALA in fresh terrestrial leaves from spring to summer followed by a decrease from summer to autumn (Fig. 2A). Throughout the season, fresh leaves had the highest percentage of the HUFA precursor ALA and the highest ratio of ω -3 : ω -6 FAs (Table 1, Fig. 3A),

and biofilm was the only basal resource containing any HUFA (EPA) and had the lowest C : N ratio (Table 1, Fig. 3C).

The quantity and quality of biofilm varied little throughout the growing season (Table 2). This result suggests that biofilm probably provides stream macroinvertebrates with a consistent high-quality resource throughout the season, whereas leaves provide a medium-quality, but highly pulsed resource in this stream. However, most stream macroinvertebrates do not consume ALA-rich fresh leaves throughout the year. Instead, they usually consume microbially conditioned leaves (Thorp and Delong 2002, Collins et al. 2016), which lack any ω -3 FAs in our system (Fig. 3A). Moreover, the relative value of biofilm vs fresh terrestrial leaves to stream invertebrates also depends upon the ability of stream macroinvertebrates to convert the HUFA precursor ALA into EPA, a process likely to be highly variable across taxa (Guo et al. 2016a).

Throughout the season, stream macroinvertebrates that differed in diet (Fig. 4A–J) based on FFGs also differed consistently in their FA composition (Fig. 5A–E, Table 3) and, thus, their food quality for fishes. In contrast, macroinvertebrates exhibited no significant seasonal and FFG variation in C : N ratios (Table S3). Percent ALA and %EPA,

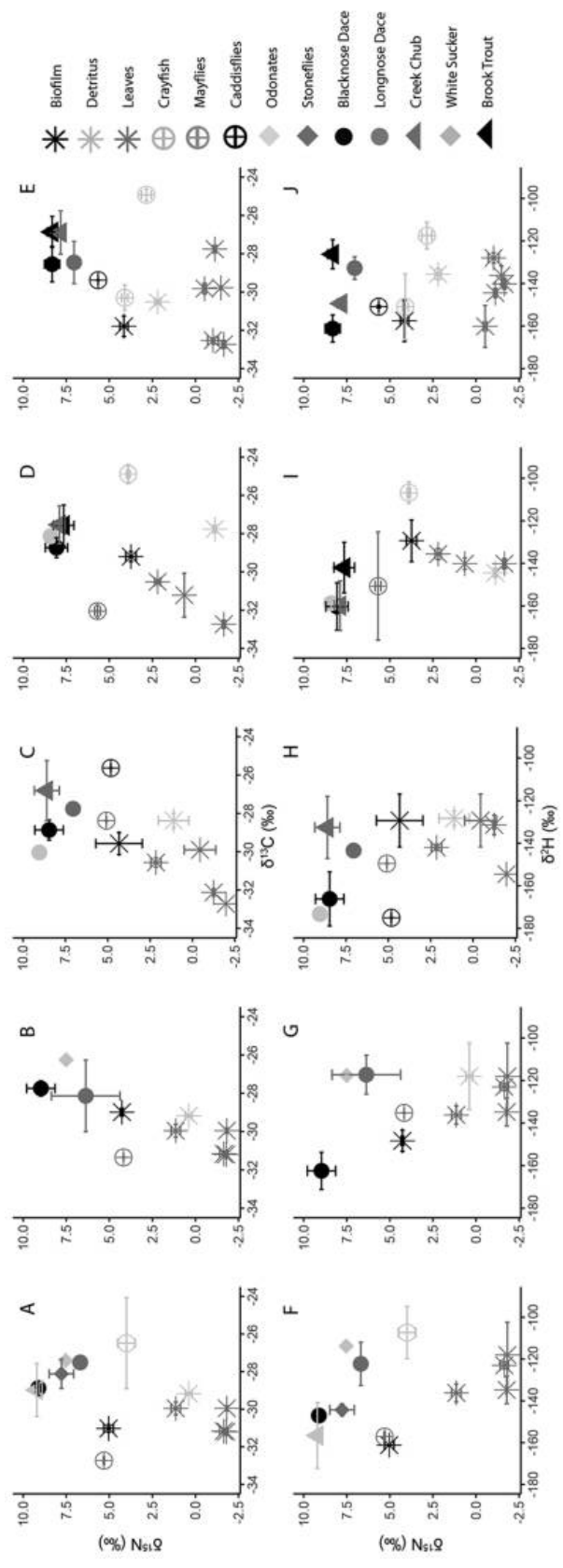


Figure 4. Stable isotope biplots showing mean (\pm SD) values for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (A–E) and $\delta^{15}\text{N}$ and $\delta^2\text{H}$ (F–J) of basal resources and consumers in May (A, F), June (B, G), July (C, H), September (D, I), and October (E, J). Note terrestrial primary producer and detritus values shown for May were inferred from values measured in June.

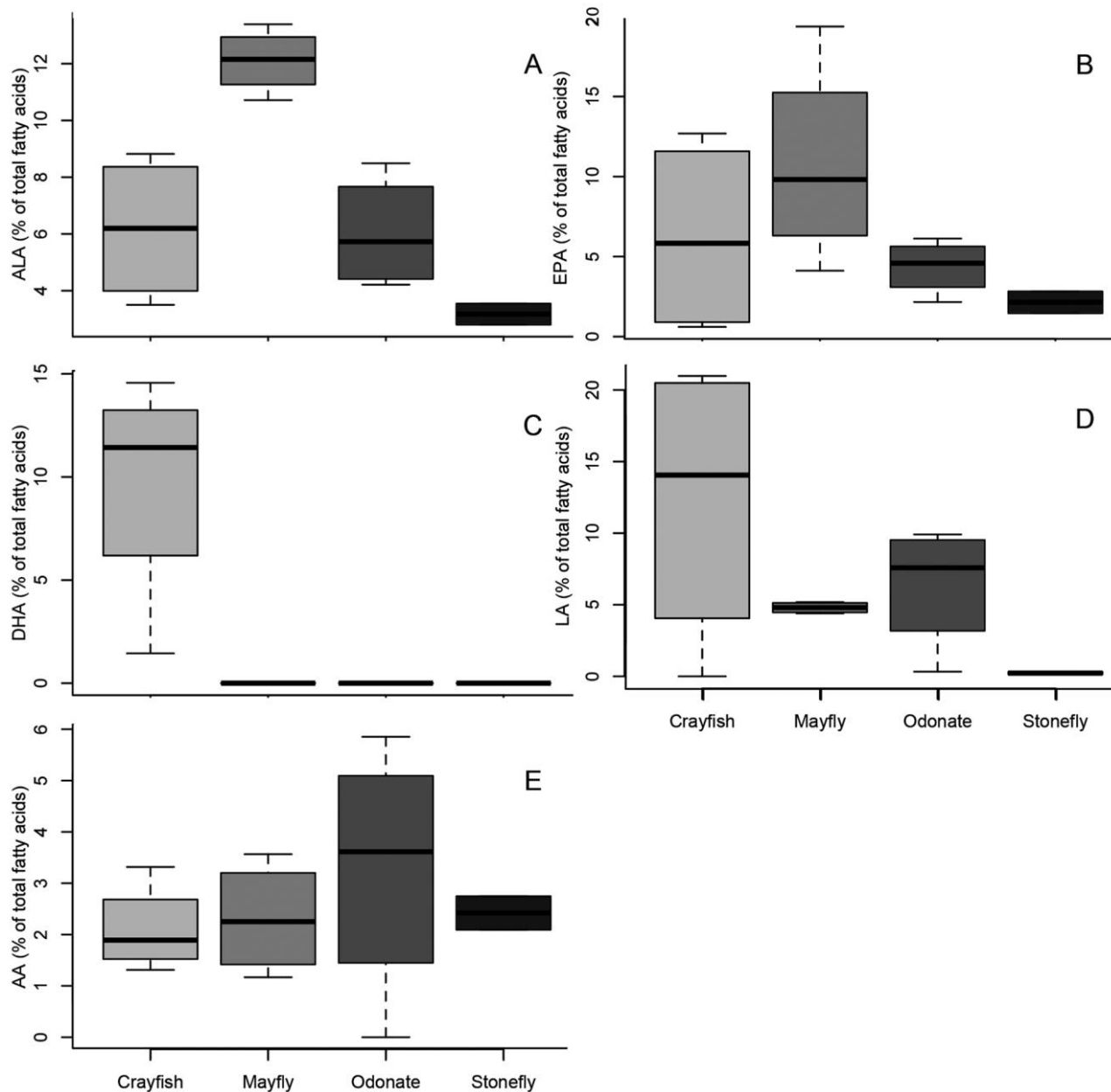


Figure 5. Box-and-whisker plots of % alpha linolenic acid (ALA; 18:3n-3) (A), % eicosapentaenoic acid (EPA; 20:5n-3) (B), % docosahexaenoic acid (DHA; 22:6n-3) (C), % linoleic acid (LA; 18:2n-6) (D), and % arachidonic acid (AA; 20:4n6) (E) in macroinvertebrates. Heavy lines in boxes show medians, box ends show quartiles, whiskers show first quartile minus 1.5 times the interquartile range and the third quartile plus 1.5 times the interquartile range, and circles show outliers.

which were both present in biofilm, were high in mayflies (Fig. 5A, B, Table 3), a result suggesting that they probably obtained HUFAs directly from aquatic resources. However, stable-isotope analyses suggested that mayflies consumed a mixed diet of both aquatic and terrestrial basal resources because their $\delta^{15}\text{N}$ was not elevated relative to biofilm and their $\delta^{13}\text{C}$ overlapped with both biofilm and terrestrial resources throughout the season (Fig. 4A–E). This result raises the possibility that they could have obtained HUFAs from

terrestrial sources rich in the HUFAs precursor ALA. Shredding crayfish were rich in DHA (Fig. 5C, Table 3) even though their relatively high $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values suggested that they primarily consumed terrestrial basal resources (Fig. 4A–J). We did not find DHA in aquatic or terrestrial basal resources. Thus, shredding crayfish may derive DHA by converting ALA in fresh leaves or algae in biofilms into HUFAs either directly or with the help of gut microbes, instead of obtaining DHA directly from diet.

Table 3. Generalized linear model (GLM) comparing fatty acid composition (% alpha linolenic acid [ALA; 18:3n-3], % eicosapentaenoic acid [EPA; 20:5n-3]) among consumers and months. Direction is the direction of any significant differences between resources, ID = specific month or invertebrate, LSM = least squares mean, and LSMSE = least squares mean standard error.

Model/variable	<i>t</i>	<i>p</i>	Direction	ID	LSM	LSMSE
ALA						
Intercept	4.05	<0.01	Stonefly < mayfly	May	7.59	1.41
June	-0.86	0.42	Crayfish < mayfly	June	5.13	2.58
July	-0.55	0.60	Odonate < mayfly	July	6.59	1.15
September	-0.15	0.88		September	7.26	1.78
October	-0.37	0.72		October	6.91	1.15
Mayfly	3.64	0.01		Crayfish	5.79	1.27
Odonate	0.22	0.84		Mayfly	11.71	1.27
Stonefly	-1.26	0.25		Odonate	6.18	1.22
				Stonefly	3.12	1.83
Null deviance: 173.71 on 13 df						
Residual deviance: 31.72 on 6 df						
EPA						
Intercept	1.71	0.14	No differences among months or taxonomic groups	May	6.12	3.57
June	-0.01	1.00		June	6.08	6.55
July	0.27	0.79		July	7.38	2.91
September	-0.89	0.41		September	1.25	4.50
October	-0.01	0.99		October	6.05	2.91
Mayfly	1.10	0.31		Crayfish	6.41	3.22
Odonate	-0.67	0.53		Mayfly	10.95	3.22
Stonefly	-1.05	0.34		Odonate	3.33	3.08
				Stonefly	0.80	4.65
Null deviance: 379.30 on 13 df						
Residual deviance: 203.69 on 6 df						

ω -6 FAs, which are typically higher in terrestrial plants than in algae, can serve as markers for terrestrial resource use (Koussoroplis et al. 2008, Lam et al. 2013, Hixson et al. 2015). We observed the highest levels of the short-chain ω -6 FA LA (18:3n-6) in shredding crayfish (Fig. 5D), which fits with their terrestrial diet (Fig. 4A–J). Macroinvertebrates had similar levels of the ω -6 FA AA across groups (Fig. 5E) even though AA was all but absent from both aquatic and terrestrial basal resources (Fig. 3D). Biofilm and fresh leaves contained the AA precursor LA (Fig. 3B), suggesting that all of the stream macroinvertebrates we examined may be able to convert LA into AA (Fig. 5E). However, the efficiency of ω -6 FA elongation appears to vary across taxa. Crayfish, which had the highest %LA, and mayflies, which had much lower %LA, had similar %AA, a result suggesting that mayflies may be comparatively efficient at converting LA to AA, whereas crayfish, which consume resources rich in LA are comparatively inefficient at doing so.

We found BCFAs (e.g., iso-15:0 and anteiso-15:0) in both crayfish and mayflies (Fig. S2). BCFAs are biomarkers for bacteria found in microbe-rich areas (Kaneda 1991) in-

cluding freshwater fish skin (Wang et al. 2016). We found a wealth of BCFAs in microbially colonized detritus, but little in fresh leaves or biofilm (Fig. S1). This result and our stable isotope data (Fig. 4A–J) suggest that primary consumer macroinvertebrates from across multiple FFGs derive BCFA, other microbial products, and energy from in-stream detritus in spite of its high C : N ratio and complete lack of HUFAs and ω -3 FAs. We found much lower %BCFAs in predatory macroinvertebrates (Fig. S2), suggesting that these compounds are potentially good dietary markers for primary consumers but do not travel up the food chain in large quantities.

Throughout the season, fish in our stream appeared to concentrate their feeding upon soft, small-bodied macroinvertebrates because their depleted $\delta^2\text{H}$ and $\delta^{13}\text{C}$ signatures overlapped with those of mayflies and caddisflies (Fig. 4A–J). In contrast, DHA-rich crayfish had much higher $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values than fish (Fig. 4A–J), making it unlikely that they are major constituents of fish diets. This result is probably a consequence of gape-size limitations because the most common fish in our stream were small species like

Blacknose Dace and Creek Chub, which were only slightly larger than some of the larger crayfish we caught. Blacknose Dace and Creek Chub include small amounts of algae and plant material in their diets (Newsome and Gee 1978, Pappantoniou and Dale 1982), which also may have shifted their stable-isotope values slightly. However, even larger invertivores, such as White Suckers, and piscivores like Brook Trout, had more depleted $\delta^2\text{H}$ and $\delta^{13}\text{C}$ and were ≥ 1 trophic level ($\Delta\delta^{15}\text{N} > 3.4\text{‰}$) above crayfish (Fig. 4A–J), suggesting that crayfish were not a major contributor to fish diets in our stream. Thus, although crayfish were potentially the highest-quality food resource for predators because of their high %DHA, DHA in crayfish probably did not move up the food chain to higher-order consumers. Instead, stream fishes probably derive DHA by converting ALA into EPA and then DHA, a physiologically intensive process requiring $\Delta 5$ and $\Delta 6$ desaturase enzymes (Sargent et al. 1995), or by converting EPA in macroinvertebrates into DHA.

One of the strengths of stable-isotope analysis is that, unlike snapshot approaches like observation or gut-content analyses, it provides an integrated picture of diet (Post 2002). Thus, animals sampled at any given point may represent stable-isotope values of foods consumed long before the sampling period. For example, tissue turnover time for stream consumers may be on the order of ≥ 1 mo (McIntyre and Flecker 2006). Stream taxa, especially fish or invertebrates with longer multivoltine life cycles, might have stable-isotope values representative of past resources consumption. However, we found limited evidence of seasonal variation in basal resource quality and limited seasonal variation in the food-web structure and FA composition of consumers, so we were unable to assess the potential for lag effects.

The differences we observed in FA composition between aquatic and terrestrial basal resources and macroinvertebrate FFGs are generally consistent with those reported in a growing number of studies on macroinvertebrate FAs (e.g., Bell et al. 1994, Torres-Ruiz et al. 2007, Jardine et al. 2015, Cashman et al. 2016, Guo et al. 2016a). Biofilm was consistently the highest HUFA and lowest C : N resource for stream primary consumers and varied little in quantity over the season in our stream, but all of our stream primary consumers appeared to consume a mix of biofilm and terrestrial detritus (Fig. 4A–J). Our results suggest that although primary consumers, especially grazers, in forested Adirondack streams may rely on high-quality aquatic primary producers for HUFAs throughout the year, they also rely heavily upon terrestrial resources to satisfy their energetic and other nutritional demands. Thus, these stream food webs probably rely upon a consistently low quantity of high-HUFA aquatic basal resources supplemented by larger seasonal pulses of terrestrial basal resources, consistent with findings from a recent study on food quality in heavily forested freshwater systems (Tanentzap et al. 2014).

The authors of the limited number of previous studies on seasonal shifts in FA composition in temperate streams

have found greater seasonal variation in FA composition than we observed. We ascribe this contrast to different sampling schemes. Torres-Ruiz et al. (2007) sampled over a longer period and found greater seasonal variation within aquatic resources and individual macroinvertebrate taxa, but also found consistent patterns among taxa (Torres-Ruiz et al. 2007). Honeyfield and Maloney (2015) also observed seasonal variation in periphyton FA composition across several streams, but found the greatest difference in FA composition between summer/autumn and winter, when we were unable to access our site. We suspect that we would have captured more variability in FA composition as well as higher reliance on detritus throughout the food web had we sampled later in autumn and in early winter.

Considerable debate has arisen among aquatic ecologists working in lake and stream ecosystems about the relative importance of terrestrial basal resources vs aquatic basal resources (Brett et al. 2017). Researchers who used bulk stable-isotope techniques, including tracers, have frequently come to the conclusion that terrestrial subsidies are the dominant basal resource in stream food webs (Tank et al. 2010, but see contrasting studies by McCutchan and Lewis 2002, Thorp and Delong 2002) and contribute a surprising amount of energy to lake food webs (e.g., Pace et al. 2004, Carpenter et al. 2005, Cole et al. 2006). In contrast, researchers using a FA approach have often concluded that aquatic basal resources must dominate food webs in both streams and lakes because they contain important FAs not found in most terrestrial primary producers (Brett and Müller-Navarra 1997, Torres-Ruiz et al. 2007, Brett et al. 2009, 2017).

We combined bulk stable isotopes and FAs to examine a stream food web and have concluded that the answer probably lies somewhere in the middle. Our results indicate that terrestrial resources subsidize freshwater food webs, especially in forested streams, by providing energy and that aquatic resources, even in small quantities, are a source of important HUFAs that may help consumers survive on an otherwise lower-quality terrestrial diet (e.g., Lau et al. 2014, Jardine et al. 2015, Cashman et al. 2016). The relative importance of aquatic sources of HUFAs vs aquatic and terrestrial sources of ALA will depend on the degree to which individual consumers are able to convert terrestrial sources of ALA into HUFAs (Guo et al. 2016b). For example, some taxa, such as Chironomidae, appear to be relatively efficient at this process (Goedkoop et al. 2007), whereas other invertebrate taxa (Guo et al. 2016b) suffer major declines in performance when HUFAs are limited and, thus, appear more likely to rely on aquatic basal resources in streams. These processes are well understood for lake zooplankton like cladocerans and copepods (Brett and Müller-Navarra 1997), but further research into the HUFA needs of stream consumers like mayflies and crayfish is essential to understand the relative roles of terrestrial vs aquatic resources in stream food webs.

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