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Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers

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Abstract. Our study is a first attempt to characterize seasonal fatty acid (FA) profiles of foodweb components in a small forested stream. We measured FA content of autochthonous food sources (aquatic primary producers = periphyton, green algae, red algae, bryophytes), allochthonous food resources (terrestrial matter = benthic and transported organic matter [BOM and TOM, respectively]), and macroinvertebrate consumers (*Hydropsyche* spp., *Ephemerella* spp., isopods, oligochaetes). We examined whether FAs could be used as trophic markers and tried to identify which food sources best provided macroinvertebrates with essential FAs (EFA, ω3 and ω6 groups). Primary producers consistently had greater content of several EFAs (18:2ω6 and 18:3ω3 in green algae, 20:5ω3 in diatoms, 20:4ω6 in bryophytes) than did terrestrial matter. The ratio of Σω3/Σω6 FAs, a putative marker of the relative amount of autochthonous vs allochthonous matter, was greatest in macroinvertebrates, followed by algae, and was significantly correlated with chlorophyll *a* content of food sources (periphyton, ultrafine BOM, and TOM). The seasonal dynamics of EFA content of BOM and TOM varied with particle size. Other FAs were identified as specific markers for diatoms (20:5ω3 [eicosapentaenoic acid], 16:1ω7, 16:ω4s, 16C-polyunsaturated FAa [PUFAa]), green algae (18:3ω3 [α-linolenic acid], 18:2ω6 [linoleic acid], 16C-PUFAb), and bryophytes (20:4ω6, 20:3ω3). Terrestrial matter had higher levels of bacterial and fungal FAs than did primary producers. Nonmetric multidimensional scaling analyses based on FA spectra of foodweb components in early spring (open canopy) and mid-summer (shaded canopy) confirmed that *Ephemerella* and *Hydropsyche* consumed mainly autochthonous food sources, even during the shaded summer period. Isopods and oligochaetes consumed a mixed diet of terrestrial matter and algae. Autochthonous food sources may be a more important part of the diets of benthic macroinvertebrates in forested streams than previously recognized.

Key words: fatty acids, stream, food webs, food quality, seasonal changes, lipids, macroinvertebrates, algae, detritus, autochthonous matter, bryophytes, periphyton.

Most studies and conceptual models pertaining to stream food webs have focused on the quantity of each foodweb component and the movement of energy and materials from allochthonous and autochthonous food sources to consumers (Vannote et al. 1980, Allan 1995). Less attention has been given to the quality of these food sources and seasonal variations in their supply and composition (Feminella and Hawkins 1995). Seasonal variation in factors such as discharge, temperature, and riparian shading can affect food sources profoundly through changes in energy content, community composition, system productivity, and foodweb patterns (Vaughn 1986, Tavares-Cromar and Williams 1996, Thompson and Townsend 1999). However, these temporal and nutritional features have not been adequately incorporated into stream foodweb theory (Hicks 1997).

The source, identity, and quality of foods for macroinvertebrate consumers in streams are likely to change over the course of a year because of changes in physical and chemical conditions within the watershed. Such temporal dynamics can cause dietary shifts in consumers. In temperate forested streams, benthic algae are limited primarily by light during the summer (Hill et al. 1995), whereas winter and spring are times of increased algal production (Thompson and Townsend 1999). Macroinvertebrate shredders may face seasonal resource shortages of well-conditioned detritus during the late spring period (Wallace et al. 1995). Consumers track these changes and show dietary shifts as a function of food availability (Chapman and Demory 1963, Haapala et al. 2001). Few primary consumers in

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streams are still considered true specialists because many studies suggest that they consume both allochthonous and autochthonous sources of C (Mihuc and Mihuc 1995, Mihuc 1997, Reiso and Brittain 2000).

Food sources for stream invertebrates include autumn-shed leaves, bacteria and fungi colonizing these leaves, benthic algae, and bryophytes. Stream food webs traditionally have been regarded as mainly heterotrophic and fueled by allochthonous primary production (terrestrial matter; Vannote et al. 1980, Allan 1995), but recent studies suggest that autochthonous primary production (algae and bryophytes) can be important for many macroinvertebrates, even those that usually are considered shredders of terrestrial leaf litter (Mulholland et al. 2000, Finlay 2001, Dangles 2002, Thorp and Delong 2002, Lancaster et al. 2005, Delong and Thorp 2006).

Terrestrial matter differs greatly in chemical composition from aquatic matter, having greater amounts of refractory compounds and higher C:N ratios, making it a lower-quality food source for invertebrates (Allan 1995). However, bacteria and fungi colonizing decomposing leaves can increase their nutritional quality (Suberkropp 2001). At some times of the year, benthic algal communities, which are composed of a variety of taxa over an annual cycle (Wehr 1981), can be a greater energy source and, in particular, a higher-quality food source than terrestrial matter for the proper development and reproduction of macroinvertebrates (Anderson and Cummins 1979, Lamberti 1996). Algal food sources can remain qualitatively important throughout the year, even when their quantities are small, in part because of their lower C:N and C:P ratios (Frost et al. 2002) and higher protein and lipid content than terrestrial matter (Lamberti 1996). For these reasons, lipids might be particularly critical for understanding foodweb linkages in streams.

Little is known about the fatty acid (FA) requirements of macroinvertebrates in streams. Like most animals, invertebrates have certain lipid requirements that must be fulfilled through their diet. Such FAs are designated as essential (EFA) because they cannot be synthesized de novo to meet physiological demands (Burr and Burr 1930, Arts et al. 2001). Polyunsaturated fatty acids (PUFA) in the ω3 and ω6 families (e.g., 20:5ω3, 20:4ω6 [arachidonic acid]) are thought to maintain membrane fluidity in insects during periods of low ambient temperature (Hodkova et al. 1999), and some PUFAs are precursors of eicosanoids, which are critical for growth and reproduction (Stanley-Samuelson 1994). The essential nature of ω3 and ω6 PUFAs has been documented for several freshwater and marine invertebrates (D'Abramo and Sheen 1993, Demott and Mueller-Navarra 1997, Muller-Navarra et al. 2000, Von Elert 2002), but few studies have examined the FA profiles of stream macroinvertebrates or their food sources. Long-chain PUFAs, in particular 20:5ω3, seem to be ubiquitous among aquatic insects in temperate streams (Hanson et al. 1985, Bell et al. 1994, Ghioni et al. 1996, Sushchik et al. 2003). Evidence from marine and lacustrine systems indicates that invertebrates in these systems obtain ω3 PUFAs primarily from algae (Ahlgren et al. 1992), but data from lotic systems are sparse (Ghioni et al. 1996).

Another important application of FAs for stream studies may be their use as trophic markers. FA markers have provided insights into trophic links in open-ocean, estuarine, and lake food webs (Ahlgren et al. 1997, Napolitano et al. 1997, Napolitano 1999, Muller-Navarra et al. 2000). For example, 16:1ω7 (palmitoleic acid) and 20:5ω3 have been considered diagnostic for diatoms (Parrish et al. 1995, Napolitano et al. 1997), odd-chain FAs have been used as bacterial markers (Kharlamenko et al. 1995), and perhaps most relevant for stream studies, the ratio (Σω3 FAs)/(Σω6 FAs) has been proposed as a marker for terrestrial (<1.0) vs aquatic (>1.0) matter (Pollero et al. 1981, Desvilettes et al. 1994).

One recent lotic study inferred the diet of invertebrates from FA profiles (Sushchik et al. 2003), but thus far, no studies have tested the usefulness of FAs as trophic markers over an annual cycle in any stream ecosystem. Therefore, our goals were to: 1) confirm and compare long-chain PUFAs in foodweb components (allochthonous and autochthonous food sources and macroinvertebrates) in a small, forested stream, 2) quantify seasonal changes in several EFAs in these foodweb components, 3) investigate whether specific food sources could be linked with consumers using ω3 and ω6 EFAs, and 4) determine which FAs could be used as trophic markers specifically for stream food webs. Temporal variation was assessed by sampling foodweb components in the stream at different times of the year. We hypothesized that changes in the production of benthic algae during open-canopy periods (late winter–spring) would induce a shift in diet toward herbivory among detritivorous or omnivorous invertebrates, as reflected by increases in specific EFAs and FA markers in their tissues. We also predicted that the FA content of stream consumers would indicate a greater degree of herbivory on autochthonous food sources throughout the year than is predicted by current stream ecosystem theory.

Study Site

The study was conducted in a 20-m reach of the Muscoot River, a 3rd-order stream that feeds the

Croton River watershed in southeastern New York. The study site is located 5 km downstream of Amawalk Reservoir (lat 41°16.453′N, long 73°44.773′W) and is part of the New York City drinking-water system (Burns et al. 2005, Arscott et al. 2006, Bott et al. 2006, Kaplan et al. 2006). The streambed consists primarily of boulders (>256 mm, 60%) and cobbles (>64–256 mm, 25%), with smaller amounts of gravel (>2–64 mm, 2%) and sand (0.06–2 mm, 10%). Woody debris typically makes up <5% of submersed substrata. The reach consists mostly of riffle (50%) and run (30%) habitat with a few pools (20%). During typical baseflow conditions (average of 3 dates), stream width = 7.8 m, mean depth = 31 cm, specific conductance = 364 μS/cm, pH = 8.0, NH_4^+ = 100 μg N/L, NO_2^- + NO_3^- = 1000 μg N/L, Si = 1.5 mg/L, total dissolved P = 80 μg/L, and Ca = 20 mg/L. Land use is primarily undisturbed mixed-deciduous forest (*Quercus rubra, Acer saccharrum, Acer rubrum, Platanus occidentalis, Tsuga canadensis*). The stream is shaded from May to October (mean canopy cover = 75%), leaf fall is from late October to November, and maximum light penetration (80–90% open sky) occurs from late November to early April.

Methods

Field procedures

Food sources in the stream were sampled 5 times (September and November 2002, March, May, and July 2003). Five replicate samples from each foodweb component were collected on each date.

Allochthonous food sources (terrestrial matter) were defined as benthic and transported particulate organic matter (BOM and TOM, respectively) in 3 size categories: coarse (C; >1 mm), fine (F; >250 μm–1 mm), and ultrafine (UF; <250 μm–0.7 μm) (Cummins and Klug 1979). CTOM and FTOM samples were collected in the field by filtering 100 L (per replicate) of stream water through 2 nested cylinders with 1-mm and 250-μm Nitex® mesh (Wildco, Buffalo, New York) to separate the CTOM and FTOM fractions, respectively. Samples were rinsed from the sieves into plastic jars using distilled water, stored on ice, and returned to the laboratory for further processing. The final 5 L of sieved water was returned to the laboratory for collection of UFTOM (see below). BOM was collected from the upper 10 cm of sediments in pools using a plastic coring tube (5-cm inner diameter) attached to a vacuum hand pump. Core contents were stored on ice in plastic jars and returned to the laboratory for sieving into size fractions.

Autochthonous food sources (aquatic primary producers) were defined as mixed periphyton (primarily diatoms, but no dominant species was apparent), filamentous algae (mainly *Cladophora glomerata* or *Audouinella hermannii*), and bryophytes (mainly *Hygrohypnum luridum*). Algae and bryophytes were identified to species in the laboratory prior to chemical analyses. Five replicate cobbles (15–30 cm) were collected for mixed periphyton; filamentous algae and bryophytes were collected as nearly pure (single species) stands by hand. All samples were placed in zip-lock plastic bags, stored on ice, and returned to the laboratory for processing.

Macroinvertebrate consumers were collected from 5 random locations within the reach using a Surber sampler fitted with a 243-μm-mesh net. Macroinvertebrates clinging to the net were washed into a tray and later into plastic containers with stream water. All samples were placed on ice and brought to the laboratory within 2 h of collection.

Laboratory processing

UFTOM was collected from the reserved filtered (see above) stream water by filtering a known volume through Whatman GF/F filters (0.7-μm pore size). CTOM samples were blended briefly (≤5 s) to homogenize them before final processing. When the total mass of TOM was <10 mg/sample (generally the case), replicates were combined to create a pooled sample for each size fraction. BOM was filtered through 2 nested sieves (1-mm and 250-μm mesh size) to collect CBOM and FBOM, respectively. CBOM and FBOM samples were rinsed from the sieves into plastic jars using distilled water. UFBOM was collected from water that passed through both sieves by filtering a known volume through Whatman GF/F filters (0.7-μm pore size). Mixed periphyton was scraped with brushes from rocks into distilled water. Bryophytes and filamentous algae were sorted and rinsed following procedures described in Wehr et al. (1983); fragments that were heavily covered by diatoms were discarded. Material was split into 2 parts and processed as for macroinvertebrate consumers (see below).

Each sample of CTOM, FTOM, UFTOM, CBOM, FBOM, UFBOM, and mixed periphyton was collected on preashed Whatman GF/F filters for determination of ash-free dry mass (AFDM; 1 filter), chlorophyll *a* (chl *a*; 1 filter), and FA (2 filters) analyses. Samples for AFDM determination were dried at 80°C to constant mass, weighed, and ashed in a muffle furnace at 450°C (≥2 h). Dry mass and AFDM were determined to the nearest 0.0001 g. Samples for chl *a* determination were extracted in 90% neutral acetone and stored at −20°C until analysis. Chl *a* concentrations of periphyton samples were determined by spectroscopy and cor-

rected for pheophytin *a* (Lorenzen 1967). Samples for FA analyses (described below) were stored in a centrifuge tube under N_2 and frozen (−20°C) for FA extraction.

Macroinvertebrates were first sorted to order or family by eye and allowed 20- to 30-h gut-clearance time while stored in clean stream water in 50-mL centrifuge tubes, loosely capped, in the refrigerator (4°C). Sorting of 2 major insect groups, mayflies and caddisflies, was completed using a dissecting microscope. Two net-spinning caddisflies (*Hydropsyche* sp., *Chimarra* sp.), 1 rhyacophilid caddisfly (*Rhyacophila* sp.), and 3 genera of mayflies (*Ephemerella* sp., *Baetis* sp., and an unidentified Heptageneiidae) were identified, but only *Hydropsyche* spp. and *Ephemerella* spp. were abundant and present in the stream on all sampling dates. Other common macroinvertebrate taxa (isopods and oligochaetes) were identified to order and class, respectively. A few individuals from each group were preserved as reference samples. The rest of the individuals belonging to the most common taxa (*Hydropsyche* spp., *Ephemerella* spp., isopods, oligochaetes) were used for FA analyses. Half of the individuals in each sample were placed in 15-mL plastic tubes under N_2 and frozen (−20°C) for FA analyses; the rest were dried (80°C) for determination of dry mass and AFDM.

FA analyses

Samples for FA analysis were weighed (fresh mass), homogenized, and extracted using a modified method of Parrish (1999). Lipids were extracted in chloroform:methanol (2:1 v/v), methylated using BF_3 (10–15% w/v in methanol), resuspended in hexane, and concentrated under N_2. FA methyl esters (FAMEs) were separated, and their relative percentages were measured using a Hewlett–Packard 5890 gas chromatograph fitted with a Supelco™ (Bellfonte, Pennsylvania) Omegawax 320 capillary column (30 m × 0.32 mm) running in splitless mode. Individual FAs were identified by comparing retention times with certified standard mixtures (e.g., Supelco 37 Component FAME mix, Supelco menhaden oil: PUFA-3, and Supelco bacterial acid methyl esters mix [BAME]). A few single FAME standards (Supelco) were used to resolve the identities of specific FAs. Peaks with retention times matching those in the BAME standard but not positively identified were recorded as bacterial FAs. A few peaks with retention times not matching any of the standard components were termed unknown, but assigned approximate chain length (C number) on the basis of retention times. A peak with a % area <0.5% of total area was defined as undetectable. Reagent blanks were processed and analyzed in an identical manner to samples. A sample peak with a % area similar to its corresponding blank peak was regarded as a contaminant; a peak was not excluded if the % area in the sample was 10× greater than % area in its corresponding blank.

Data analysis

Total, Σω3, and Σω6 FAs were represented as concentration per unit dry mass. A standardized procedure was used to calculate individual FA concentrations per unit mass using regression curves on the basis of known standard peak areas. Calibration curves were linear and highly significant, but slopes differed slightly depending on chain length, number of double bonds, and concentration range, as observed previously by Ahlgren et al. (1992). It was judged that percentages compared FAs across samples more reliably than absolute concentrations (Ahlgren et al. 1992, Napolitano et al. 1997). The ratio Σω3 FAs/Σω6 FAs (Σω3/Σω6) was tested as a marker for the relative amount of aquatic (values >1.0) vs terrestrial (values <1.0) matter in a sample, as proposed elsewhere (Pollero et al. 1981, Desvilettes et al. 1994).

Nonmetric multidimensional scaling (NMS) was used to examine which food sources were most similar to macroinvertebrate consumers in their combined FA profiles. NMS ordinations were done on Bray–Curtis distances using PC-ORD (multivariate analysis of ecological data, version 4.0; MjM Software, Gleneden Beach, Oregon). NMS was less sensitive than principal components analysis to non-normal and 0 values in the FA data set. FAs used in these analyses were: 1) EFAs used in seasonal comparisons (18:2ω6, 20:4ω6, 18:3ω3, 20:5ω3); 2) sums of saturated FAs (SAFA), monounsaturated FAs, and PUFAs; 3) FAs defined as common (>1% in >5 categories); and 4) potential biomarker FAs, including diatom markers 16:1ω7 and 16:2ω4, bacterial markers 18:1ω7 and odd-chain FAs, and Σω3/Σω6 (Desvilettes et al. 1994, Kharlamenko et al. 1995, Napolitano 1999).

NMS plots were produced for food webs in March (open canopy) and July (closed canopy) to test whether open-canopy conditions resulted in different FA patterns in food sources and consumers from those in summer shaded conditions. NMS selected the number of dimensions beyond which additional axes provided only small (<5%) reductions in stress (measure of departure from monotonicity in the relation between original distances and distances in the reduced ordination space). The slow-and-thorough option (PC-ORD) was used to summarize similarities among total food sources and consumers, on the basis

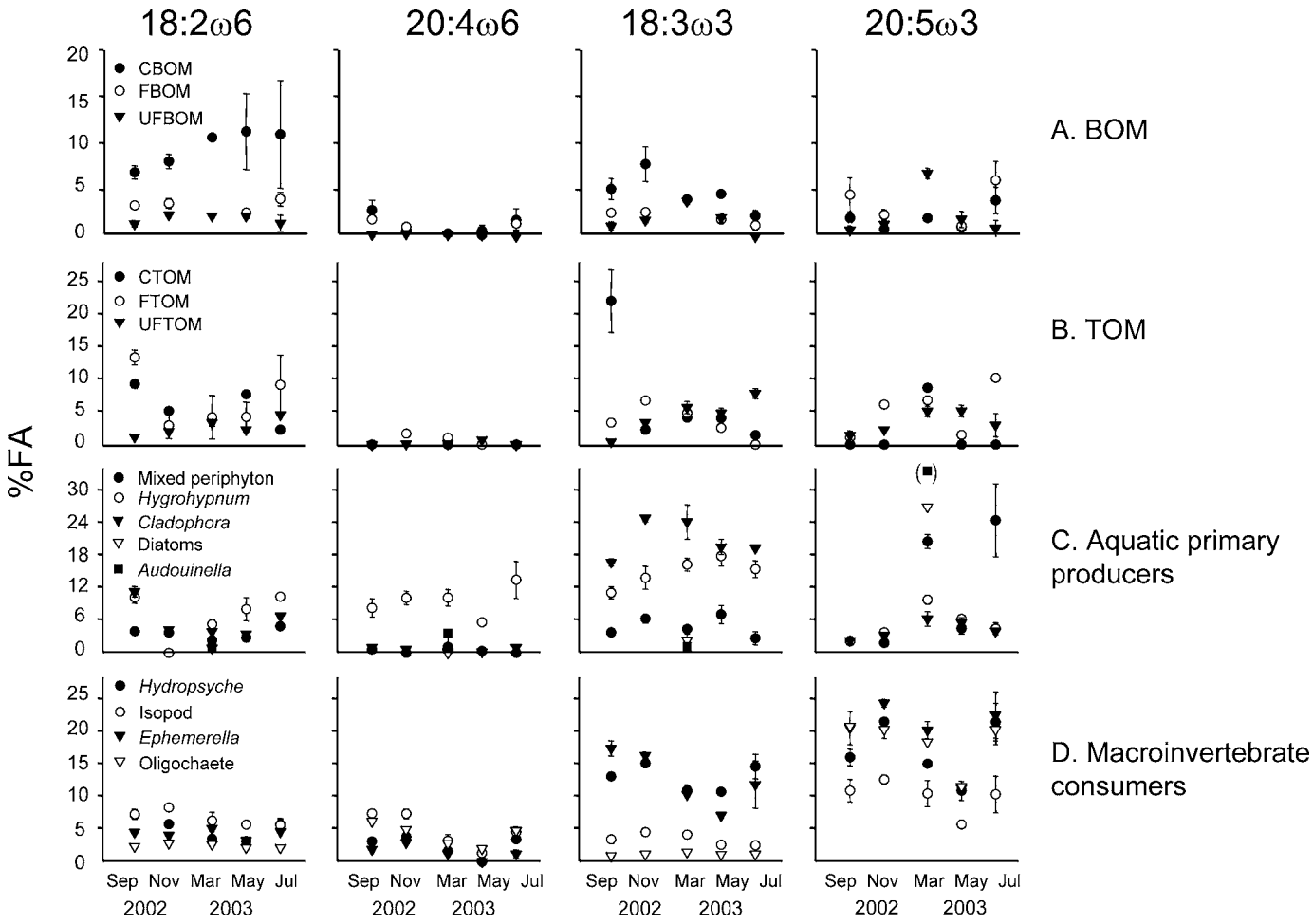

FIG. 1. Mean (±1 SE) % of total fatty acid (FA) content consisting of 18:2ω6 (linoleic acid), 20:4ω6 (arachidonic acid), 18:3ω3 (α-linolenic acid), and 20:5ω3 (eicosapentaenoic acid) in benthic organic matter (BOM) (A), transported organic matter (TOM) (B), aquatic primary producers (C), and macroinvertebrate consumers (D) in the Muscoot River from September 2002 to July 2003. CBOM = coarse benthic organic matter, FBOM = fine benthic organic matter, UFBOM = ultrafine benthic organic matter, CTOM = coarse transported organic matter, FTOM = fine transported organic matter, UFTOM = ultrafine transported organic matter, *Cladophora* = *Cladophora glomerata*, *Audouinella* = *Audouinella hermannii*, *Hygrohypnum* = *Hygrohypnum luridum*, *Hydropsyche* = *Hydropsyche* spp., *Ephemerella* = *Ephemerella* spp. Value of 20:5ω3 in *Audouinella* in March = 40%.

of their % FA profiles. Correlations between distances in the reduced NMS space and distances in the original space were used to quantify the proportion of total variation explained by NMS, an analysis similar to that used with FA profiles of soil microbial communities (Shutter and Dick 2000, Butler et al. 2003).

Results

Seasonal patterns of EFAs

Aquatic primary producers and macroinvertebrate consumers had consistently higher levels of the 4 major EFAs (18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3) than BOM and TOM (Figs 1A–D). EFA content of BOM was relatively low (e.g., <0.1% 20:4ω6; Fig. 1A); the most abundant EFA in BOM was 18:2ω6. Collectively, EFAs constituted ~13% of total FA in CBOM (Fig. 1A), and 18:2ω6 and 18:3ω3 typically were 2 to 7× higher in CBOM than in FBOM or UFBOM. 20:5ω3 content of UFBOM was highest in March (7%). Absolute concentrations of EFAs in BOM were low throughout the year (0.005–0.15 mg/g; Fig. 2A, B).

Total mass of TOM and concentrations of EFAs in TOM were highly seasonal. In general, EFA content of TOM was low, ranging from <0.1 to 10% of total FA (Fig. 1B). UFTOM was the poorest source of EFAs (Figs 1B, 2A, B). Temporal dynamics of 18:2ω6 and 18:3ω3 in TOM appeared to depend on particle size. 18:2ω6 content of CTOM and FTOM was highest (9–13%) in September, as was 18:3ω3 content of CTOM (23%),

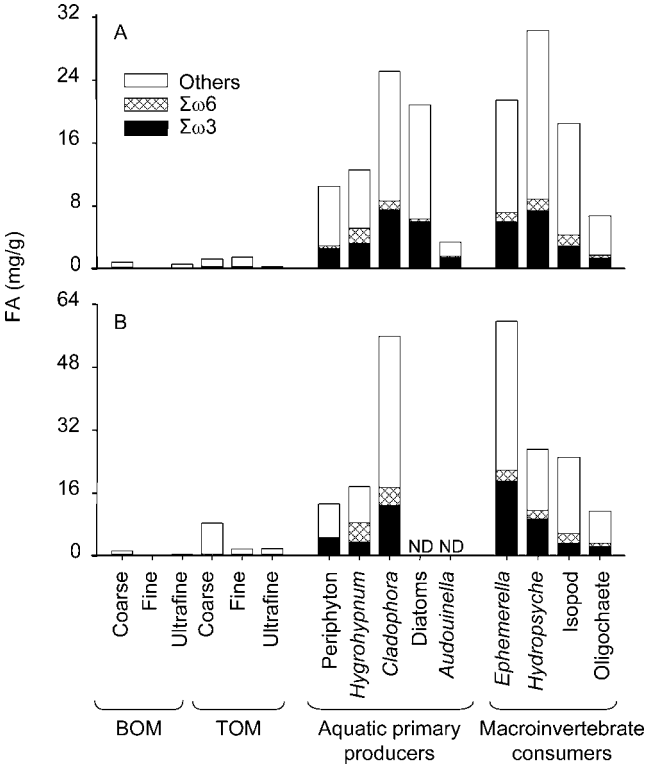

FIG. 2. Mean (±1 SE) concentrations of ω3 and ω6 fatty acids (FA) in foodweb components in the Muscoot River in March (open-canopy conditions) (A) and July 2003 (closed-canopy conditions) (B). ND = no data (algal group not observed on this date).

whereas 18:2ω6 content of UFTOM was highest in March (Fig. 1B). Minor peaks in 20:5ω3 content occurred in March for CTOM (10%), in July for FTOM (11%), and in May for UFTOM (5%). Absolute amounts of total FA in all fractions of TOM were relatively low throughout the year (0.05–8.2 mg/g; Fig. 2A, B).

Concentrations of ω3 FAs were 3 to 6× higher in autochthonous than in allochthonous food sources. Temporal patterns varied by FA and type of autochthonous food source (Fig. 1C). EFA content ranged from <0.1% (20:4ω6 in most food sources) to 40% (20:5ω3 in the filamentous red alga *A. hermannii* in March) (Fig. 1C). EFA content of mixed periphyton typically was low, but 20:5ω3 content peaked in March (~24%) when diatoms were most abundant (MT-R, personal observation). The moss *H. luridum* was the only autochthonous food source in the stream with 20:4ω6 content >5% on all dates. Total FA concentrations of *H. luridum* ranged from 4.6 mg/g (November) to 28.3 mg/g (March) (Fig. 2A, B).

The green alga *C. glomerata* consistently had the highest percentages of 18:3ω3 among all food sources in the stream, ranging from ~18% in July and September to 24% in November and March (Fig. 1C). Total FA concentrations also were higher in *C. glomerata* than in any other food source sampled, with a maximum of 55 mg/g in July (Fig. 2B). In March, the biomass of *C. glomerata* decreased, whereas diatoms and *A. hermannii* were more abundant. Total FA concentrations (3.3 mg/g) were lower in *A. hermannii* than in any other autochthonous food source (Fig. 2A, B). The % of 20:5ω3 was higher in *A. hermannii* than in any other autochthonous food source, but concentrations of most other EFAs were low (at or near detection limits) in *A. hermannii* (Fig. 1C). The FA composition of the winter–spring diatom assemblage was similar to that of mixed periphyton, with high levels of 20:5ω3 and low levels of 18:3ω3, 18:2ω6, and 20:4ω6. Percentages of individual EFAs in diatoms varied from <0.1% (20:4ω6) to >26% (20:5ω3) (Fig. 1C). Diatoms had the 2nd-highest total FA concentration among all food sources in March (20.8 mg/g) (Fig. 2A).

FA profiles and concentrations, especially ω3 content, differed among macroinvertebrate consumers (Figs 1D, 2A, B). *Hydropsyche* spp. and *Ephemerella* spp. had high percentages of ω3 FAs (e.g., 10–23% 20:5ω3) and low percentages of ω6 FAs (0–6%) (Fig. 1D). Temporal patterns of EFAs were similar between *Hydropsyche* spp. and *Ephemerella* spp., with higher percentages of 18:3ω3 in months when the stream was shaded (September, November, and July), and lower percentages in spring (March and May). However, concentrations of total FAs differed between *Hydropsyche* spp. and *Ephemerella* spp. In March, FA concentrations were 30% higher in *Hydropsyche* spp. (30.2 mg/g) than in co-occurring *Ephemerella* spp. (22.2 mg/g; Fig. 2A). In July, this difference was reversed; FA concentrations were 60.8 mg/g in *Ephemerella* spp. and 26.9 mg/g in *Hydropsyche* spp. larvae (Fig. 2B).

Isopods had higher percentages of 18:2ω6 (6–8%) and 20:4ω6 (3–7%) (Fig. 1D) than any other macroinvertebrate consumers, but their percentages of 18:3ω3 (~2%) and 20:5ω3 (~10%) were low. Deposit-feeding oligochaetes consistently had lower percentages of 18:2ω6 and 18:3ω3 (≤2%) than any other macroinvertebrate consumers (Fig. 1D). Oligochaetes had relatively high 20:5ω3 content (~18%) annually, but overall, they had the lowest total FA concentrations of all macroinvertebrate consumers, with a minimum of 6.7 mg/g in March (Fig. 2A).

Trophic-marker FAs

Temporal patterns of 3 nonessential FAs differed distinctly between autochthonous and allochthonous foodweb components (Fig. 3A–D). 16:1ω7 and the sum

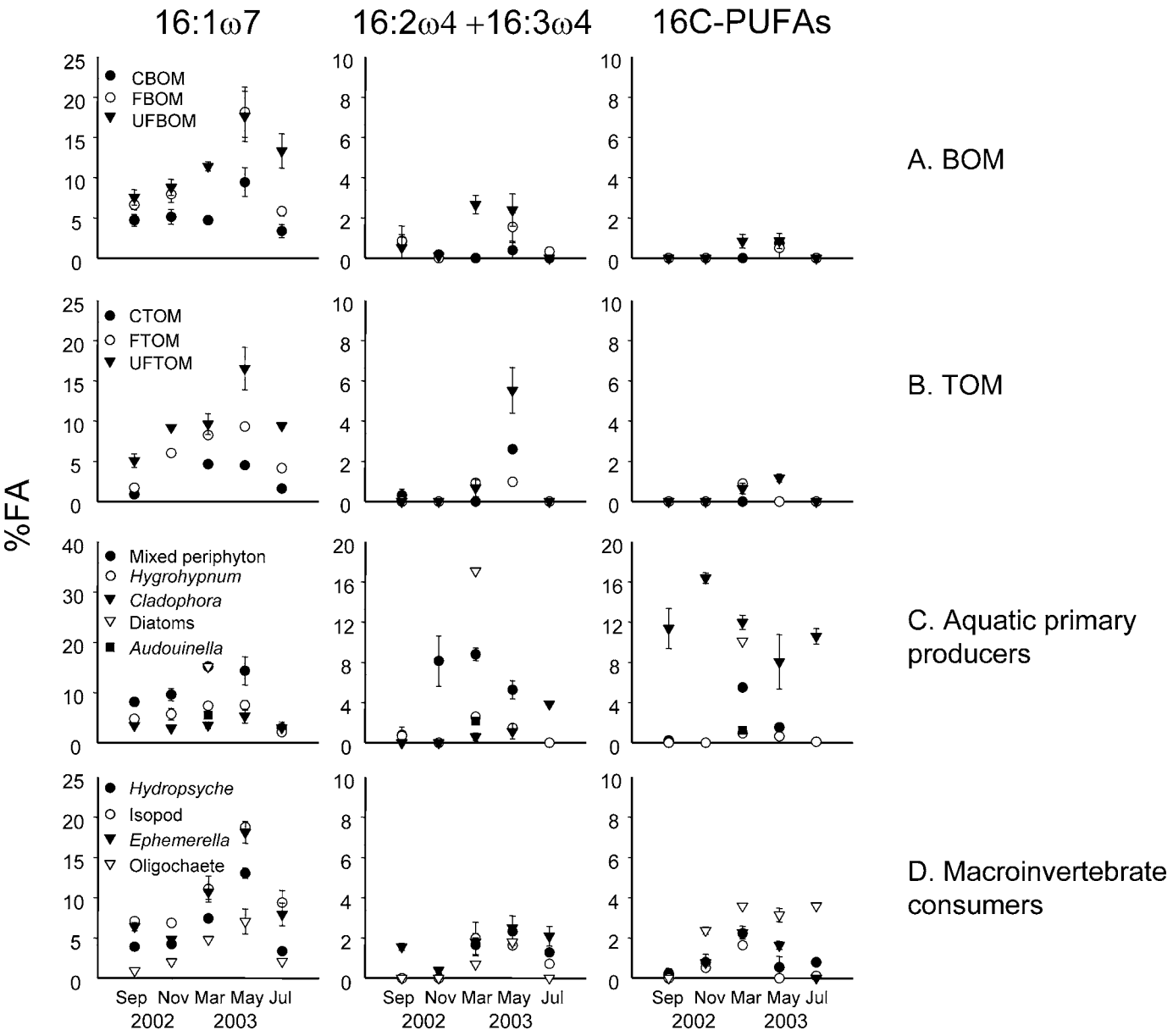

FIG. 3. Mean (±1 SE) % of total fatty acid (FA) content consisting of 16:1ω7 (palmitoleic acid) and 16:2ω4 + 16:3ω4 (diatom markers) and 16C-polyunsaturated fatty acids (PUFA) (algae marker) in benthic organic matter (BOM) (A), transported organic matter (TOM) (B), aquatic primary producers (C), and macroinvertebrate consumers (D) in the Muscoot River from September 2002 to July 2003. Note differences in *y*-axis scales. Abbreviations are as in Fig. 1.

of 16:2ω4 + 16:3ω4 (16:ω4s) were especially abundant in diatoms and diatom-rich matter (Fig. 3C). The sum of 2 unidentified 16C-PUFAs was consistently a major FA component in *C. glomerata* (Fig. 3C). Other potential FA markers were considered in the multivariate analysis. Across all food categories, 16:1ω7 was present in higher levels than 16:ω4s and 16C-PUFAs (Fig. 3A–C). BOM and TOM typically had low (<5%) percentages of autochthonous marker FAs, although some size fractions had somewhat higher levels of these algal markers in spring than at other times of the year (Fig. 3A, B). For example, in May, FBOM and UFTOM had peaks (~17%) for the diatom marker 16:1ω7 and UFBOM and UFTOM had peaks (2.5% and 5.5%, respectively) for 16:ω4s.

Temporal patterns of these 3 FA markers also differed among aquatic primary producers, with diatoms having higher percentages (10–15%) than other aquatic primary producers of all 3 markers (Fig. 3C). 16:1ω7 content of mixed periphyton in-

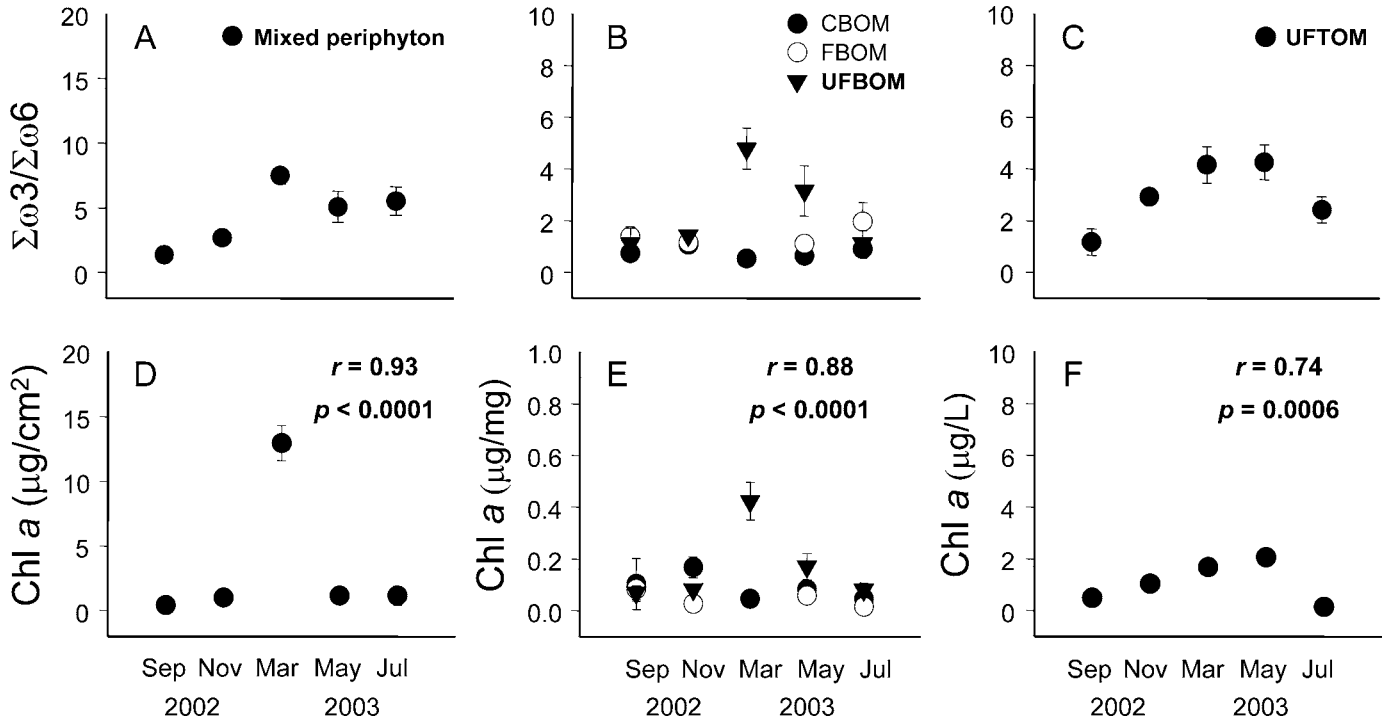

FIG. 4. Mean (±1 SE) Σω3/Σω6 fatty acid (FA) ratios (A, B, C) and chlorophyll *a* (chl *a*) (D, E, F) concentrations of mixed periphyton (A, D), benthic organic matter (BOM) (B, E), and ultrafine transported organic matter (UFTOM) (C, F) in the Muscoot River between September 2002 and July 2003. *r*- and *p*-values refer to correlations between chl *a* concentrations and Σω3/Σω6 in mixed periphyton, BOM, and UFTOM. Abbreviations are as in Fig. 1.

creased to 15% in March and remained high through May. 16:1ω4s (8%) and 16C-PUFAs (4%) content of mixed periphyton were greatest in March. *Hygrohypnum luridum* had relatively low percentages of these markers, although 16:1ω7 reached 8% in March (Fig. 3C). *Cladophora glomerata* had low levels of 16:1ω7 and 16:ω4s (<4%) throughout the year, but had higher levels of 16C-PUFAs than all other autochthonous food sources on all dates (Fig. 3C).

Levels of putative diatom FA markers in macroinvertebrates were higher in spring than in other seasons, but annual patterns differed among taxonomic groups (Fig. 3D). 16:1ω7 was the most abundant FA marker in macroinvertebrate consumers, as it was in most aquatic primary producers (Fig. 3D). Most macroinvertebrate consumers had higher 16:1ω7 content in late spring (May) than during the rest of the year. Isopods had higher 16:1ω7 content (6–18%) than other macroinvertebrate consumers throughout the year (Fig. 3D). *Ephemerella* spp. had higher 16:ω4s content than other macroinvertebrate consumers on most dates, but all macroinvertebrate consumers had peaks of these FAs in spring (Fig. 3D). Oligochaetes had higher 16C-PUFA content than other macroinvertebrate consumers during most of the year (~3%), with a peak in July to September, whereas 16C-PUFA content of other macroinvertebrate consumers peaked in March (Fig. 3D).

Seasonal variation in the proposed aquatic vs terrestrial marker, Σω3/Σω6, was compared among mixed periphyton (Fig. 4A), BOM (Fig. 4B), and UFTOM (Fig. 4C). Σω3/Σω6 increased in these food sources after leaf fall and peaked in March (open canopy). Values were higher (up to 7.5) in mixed periphyton (Fig. 4A) than in BOM (Fig. 4B) and UFTOM (Fig. 4C). Σω3/Σω6 was consistently ≤1.0 in CBOM (terrestrial matter dominant), but was >1.0 in FBOM and UFBOM in spring and summer (Fig. 4B). Σω3/Σω6 in UFTOM varied from 1.2 in September to 4.2 in March and May (Fig. 4C). Σω3/Σω6 was significantly positively correlated with chl *a* in mixed periphyton ($r = 0.93$, $p < 0.0001$; Fig. 4A, D), UFBOM ($r = 0.88$, $p < 0.0001$; Fig. 4B, E), and UFTOM ($r = 0.74$, $p = 0.0006$; Fig. 4C, F), and was positively correlated with 20:5ω3 when considered across all food categories ($r = 0.81$, $p < 0.00001$). Σω3/Σω6 was consistently >1.0 in *C. glomerata*, *A. hermannii*, *H. luridum*, and mixed periphyton.

Σω3/Σω6 was >1.0 in all macroinvertebrate consumers on all sampling dates (Fig. 5). Σω3/Σω6 increased in *Hydropsyche* spp. from 2.8 in September to 6.9 in March, whereas Σω3/Σω6 was relatively

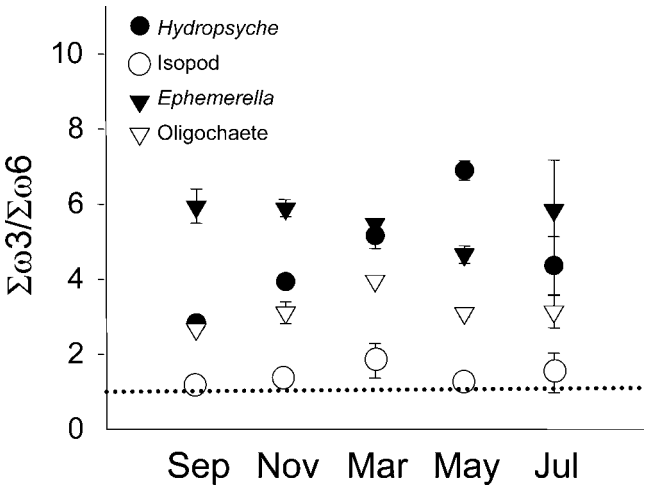

FIG. 5. Mean (±1 SE) Σω3/Σω6 fatty acid (FA) ratios of macroinvertebrate consumers in the Muscoot River between September 2002 and July 2003. The dashed line represents the threshold thought to separate ratios indicating terrestrial (<1) and aquatic (>1) diets.

constant (~5.5) in *Ephemerella* spp. Σω3/Σω6 was relatively low but constant (1.4–1.8) in isopods and intermediate (~3.5) in oligochaetes.

NMS ordination of stream food webs using FA content

Open-canopy period (March).—Axes 1 and 2 explained 54% and 30.5%, respectively, of the total variation in the data set. NMS axis 1 was positively correlated with bacterial (odd-chain and BAME) FA content ($r = 0.52$, $p < 0.0001$) and negatively correlated with 20:5ω3 content ($r = -0.62$, $p < 0.0001$). NMS axis 2 was positively correlated with 16C-PUFAb ($r = 0.67$, $p < 0.0001$) and 16:3ω4 ($r = 0.60$, $p < 0.0001$) content and negatively correlated with 18:2ω6 ($r = -0.8$, $p < 0.0001$), 22:1ω9 ($r = -0.76$, $p < 0.0001$), 22:0 ($r = -0.76$, $p < 0.0001$), and 20:1ω9 ($r = -0.75$, $p < 0.0001$) content. FA signatures generally were consistent among replicates for each foodweb component (Fig. 6A). *Ephemerella* spp., *Hydropsyche* spp., and isopods loaded near mixed periphyton, *H. luridum*, *C. glomerata*, and TOM, all of which were positively correlated with 20:5ω3 content. Oligochaetes loaded closest to UFBOM. CBOM loaded on the negative end of NMS axis 2 and was positively correlated with 18:2ω6 content. Diatoms loaded on the positive end of axis 2, consistent with greater 16C-PUFAb and 16:3ω4 content, whereas *A. hermanii* loaded on the negative end of axis 1, consistent with greater 20:5ω3 content.

Shaded period (July).—Three NMS axes explained 82.2% of the total variation in the data (axes 1 [26.6% of total variation] and 2 [30.2%] shown). Axis 1 was positively correlated with 16C-PUFAb ($r = 0.75$, $p <$

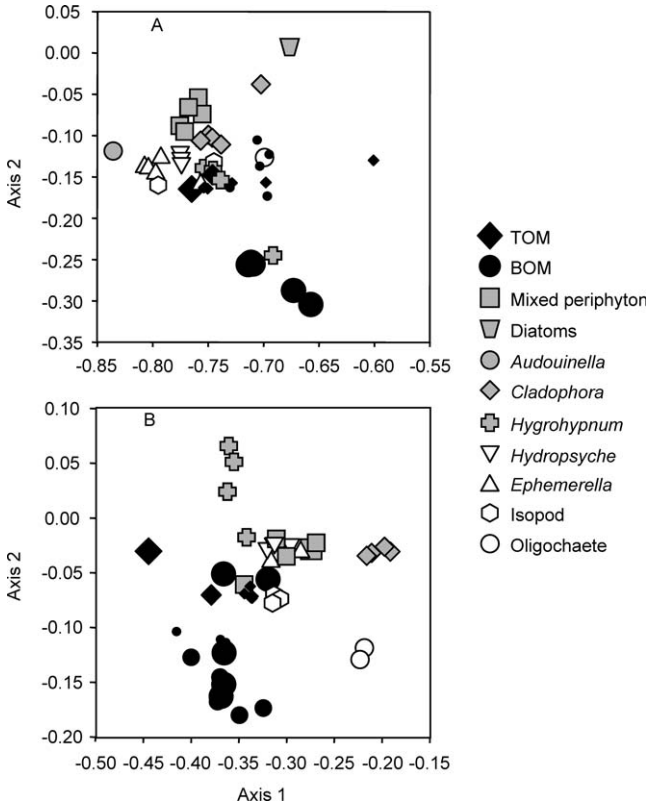

FIG. 6. Nonmetric multidimensional scaling plots based on the fatty acid profiles of foodweb components in the Muscoot River food web during March (open canopy) (A) and July (closed canopy) (B) 2003. Sizes of symbols for benthic organic matter (BOM) and transported organic matter (TOM) indicate particle sizes (large = coarse, medium = fine, small = ultrafine).

0.0001) and with 16:2ω4 ($r = 0.63$, $p < 0.0001$), 16:3ω4 ($r = 0.69$, $p < 0.0001$), and PUFA ($r = 0.60$, $p < 0.0001$) content; axis 1 was negatively correlated with SAFA ($r = -0.62$, $p < 0.0001$) content. Axis 2 was positively correlated with PUFA ($r = 0.71$, $p < 0.0001$) and 18:3ω3 ($r = 0.65$, $p < 0.0001$) content, and negatively correlated with 22:0 ($r = -0.64$, $p < 0.0001$) and 24:0 content ($r = -0.64$, $p < 0.0001$). Axis 3 was positively correlated with Σω3/Σω6 ($r = 0.67$, $p < 0.0001$) and 20:5ω3 content ($r = 0.60$, $p < 0.0001$) and negatively correlated with bacterial FA content ($r = -0.65$, $p < 0.0001$). *Ephemerella* spp. and *Hydropsyche* spp. loaded closest to mixed periphyton, consistent with higher Σω3/Σω6, 20:5ω3, and PUFA content in both foodweb components (Fig. 6B). Isopods loaded closest to UFTOM. Oligochaetes loaded closest to BOM along axis 2, but loaded close to *C. glomerata* along axis 1, reflecting similarities in 16C-PUFAb and 20:5ω3 content. *Hygrohypnum luridum* loaded near other aquatic primary producers, but closer to the positive end of axis 2,

consistent with its higher PUFA content (especially 18:3ω6 and 20:4ω6). CTOM was separated from all other foodweb components because of its negative loading on axis 1, consistent with its higher SAFA content (16:0, 18:0).

Discussion

Allochthonous vs autochthonous sources of FAs

FA analyses of the Muscoot River food web identified the food sources that best provided EFAs (18:3ω3, 20:5ω3, 18:2ω6, and 20:4ω6) for macroinvertebrate consumers and revealed specific marker FAs that can be used to determine trophic links within the stream. Taken as a whole, our data strongly suggest that autochthonous matter is a richer source than allochthonous matter of EFAs (especially the ω3 family) for most macroinvertebrates. Deposited terrestrial matter (especially FBOM and UFBOM) was the poorest source of total and essential FAs. $\Sigma\omega3/\Sigma\omega6$ was up to 6× higher in tissues of macroinvertebrate consumers than in allochthonous matter, suggesting a predominantly autochthonous diet (Desvilettes et al. 1994, Napolitano 1999).

TOM and BOM are regarded as having allochthonous origins in streams (Vannote et al. 1980, Allan 1995). However, recent observations indicate that most of these fractions consist of living (algal, fungal, or bacterial) and dead (terrestrial leaf) portions (Mulholland et al. 2000, Delong and Thorp 2006). Our study suggests that the nature of BOM and TOM changes seasonally in streams and that they are richer in FAs associated with autochthonous sources in the spring. Seasonal increases in 18:3ω3 and 20:5ω3 content of BOM and TOM could be explained by colonization of leaf litter by algae (e.g., dislodged periphyton), as evidenced by increases in chl *a* content and marker FAs specific for algae. Increases in 18:2ω6 content of CBOM could be explained by colonization of leaf litter by fungi and bacteria (MT-R, personal observation) during decomposition and by the prevalence of this EFA in fungi (Stahl and Klug 1996). Seasonal changes in the living portion of TOM and BOM have important implications when inferring macroinvertebrate diets from functional feeding group or gut-content analysis because the chemical nature of defined food sources and detritus can change with time (McArthur and Moorhead 1996).

Seasonal changes in FA profiles differed among aquatic primary producers and macroinvertebrate consumers. Similar changes have been demonstrated in a benthic lake food web (Goedkoop et al. 1998) and in a marine planktonic system (Jeffries 1979). Such changes in FA composition within foodweb components may reflect shifts in algal community composition (e.g., 20:5ω3 maximum during spring diatom peak) or varying abiotic factors (e.g., light) that, in turn, may affect algal or bryophyte chemistry (e.g., *C. glomerata* 18:3ω3 spring maximum and summer minimum). Changes in FA content of macroinvertebrates may reflect life-history changes linked to internal cues (e.g., pupation) or may be caused by variation in the quality of their food. Correlations between FA profiles of consumers and food sources in the Muscoot River suggest that FAs in macroinvertebrates probably depend on seasonal changes in the FA content of their diets. Experimental studies are needed to further clarify these links.

EFAs

Most field studies of forested streams have shown that the largest source of energy or C supplied to stream macroinvertebrates is provided by terrestrial matter (Doucett et al. 1996, Hicks 1997). The mass of terrestrial matter inputs typically exceeds the mass of autochthonous C sources in forested streams, and terrestrial matter contains high percentages of some EFAs (especially 18:2ω6). However, autochthonous food sources are a richer source of nearly all other FAs, and autochthonous food sources also have greater total amounts of FA per unit mass than do allochthonous food sources.

Recent experiments using chemical markers (stable isotopes) suggest that algae can be very important food sources for lotic invertebrates (Mulholland et al. 2000, Finlay 2001, Dangles 2002, Thorp and Delong 2002, Lancaster et al. 2005, Delong and Thorp 2006). Our data also suggest that a single species or guild of algae may not be sufficient to meet the dietary FA requirements of most macroinvertebrates. Our study demonstrates the importance of algae in providing macroinvertebrates with EFAs, in particular 18:3ω3 (α-linolenic acid) and 20:5ω3 (eicosapentaenoic acid), each of which has been shown to be critical for growth and reproduction of other aquatic consumers (Muller-Navarra 1995, Von Elert 2002).

The importance of diatoms as an essential source of 20:5ω3 for planktonic invertebrates has been amply demonstrated with *Daphnia* (Muller-Navarra 1995, Muller-Navarra et al. 2000, 2004). Our study and others (Hanson et al. 1985, Ghioni et al. 1996) have shown that several stream macroinvertebrates also have substantial quantities of 20:5ω3 in their tissues. That this FA is essential for stream invertebrates seems likely, given the limited ability of other aquatic invertebrates to synthesize 20:5ω3 or 20:4ω6 from 18:3ω3 or 18:2ω6 (D'Abramo and Sheen 1993, Von Elert

2002). Experiments are underway to test this pathway with stream-dwelling insect larvae.

Bryophytes rarely have been considered an important food source for stream consumers (Bowden et al. 1999, Dangles 2002), but in the Muscoot River, the aquatic moss *H. luridum* was the only food source sampled that had amounts of 20:4ω6 (arachidonic acid) similar to those of macroinvertebrates. This EFA is required by at least some aquatic invertebrates (Dadd et al. 1986). High levels of 20:4ω6 measured in oligochaetes and isopods in the Muscoot River provide evidence that bryophytes could be an important food for at least some members of stream food webs.

FAs as trophic markers

We also investigated the usefulness of FAs as trophic markers in the stream. Diatoms were indicated by 20:5ω3, 16:1ω7, 16:ω4s, and 16C-PUFAs; green algae (*C. glomerata*) was indicated by 18:3ω3, 18:2ω6, and 16C-PUFAs; the moss *H. luridum* was indicated by 20:4ω6; and detritus colonized by fungi and bacteria was indicated by 18:1ω9, 18:1ω7, 16:1ω7, 16:0, 18:0, BAME, and odd-chain FAs. Our data clearly support earlier studies in lakes and marine systems that Σω3/Σω6 is positively related to the proportion of aquatic matter (algae + bryophytes in the Muscoot River) in the food source, as evidenced by the positive correlation between Σω3/Σω6 and chl *a* concentrations in food sources. Perhaps such relations are not surprising in planktonic food webs (Wainman et al. 1993), but our data raise important questions about the relative importance of aquatic vs terrestrial matter and the importance of EFAs in stream food webs.

FAs provide useful information about nutritional quality and trophic links, but typical FA profiles may result in 20 to 40 peaks, not all of which occur in all food sources or consumers. This complexity poses a challenge for understanding FAs in food webs. We were able to resolve and simplify some of this complexity using a multivariate analysis (NMS). However, caution is required when using ordination results as evidence of direct links between consumers and food sources because some FAs (e.g., 18:1ω9) may be synthesized by some macroinvertebrates. The FA metabolism of most aquatic macroinvertebrates is largely unknown, and certain FAs obtained from food sources may be elongated, shortened, accumulated, or simplified to meet an animal's needs. Nonetheless, NMS analyses of the Muscoot River food web corroborated seasonal patterns of individual EFAs and trophic-marker FAs.

The ordination plots of FA data and macroinvertebrate EFA and trophic-marker FA profiles reveal differences in food-source use. High levels of certain algal markers and ω3 EFAs in *Hydropsyche* spp. (particularly in spring) provide evidence that TOM sometimes contains algal FAs or that invertebrate consumers may shift from collecting TOM to grazing on algae or bryophytes to supplement their EFA requirements. This shift to a grazing feeding mode has been observed before (Fuller and Mackay 1980). High and relatively constant Σω3/Σω6 in *Ephemerella* spp. suggest that these mayflies fed selectively on algae year round, even when quantities were low. Further evidence of algal herbivory by both *Hydropsyche* spp. and *Ephemerella* spp. was provided by high 18:3ω3 and 20:5ω3 content year round and the presence of diatom markers (16:1ω7, 16:ω4s) in consumer tissues most times of the year.

Isopods and oligochaetes were identified only to order and class (respectively), but their low within-group variation in FA profiles suggests that members of these groups were consuming similar diets. Low Σω3/Σω6 ratios in isopods suggest a diet rich in ω6 FAs from sources that changed with time of year; allochthonous matter was important in summer and autumn, whereas bryophytes were important in spring. Oligochaetes were unusual in having low percentages of SAFAs, especially very low levels of 16:0, which was abundant in all other consumers. High 20:5ω3 and BAME content in oligochaetes suggests a diet of diatoms and bacteria, with greater consumption of algae in spring, probably from diatom-colonized UFBOM.

Our data strongly suggest that shredders and collectors (isopods, oligochaetes, net-spinning caddisflies [*Hydropsyche* spp.]), which typically feed on distinct size categories of BOM or TOM (Cummins and Klug 1979), probably receive a pulse of ω3 FAs from diatoms and other algae in winter and spring. This pulse may be critical for emergence (in the case of insects) and reproduction (Stanley-Samuelson 1994).

Implications for lotic food webs

Traditional models of lotic food webs predict that allochthonous (terrestrial) matter should be the main source of energy for macroinvertebrates in small and very large rivers. These models have been based mainly on invertebrate mouthparts, gut contents, or system-level production-to-respiration ratios (e.g., the River Continuum Concept; Vannote et al. 1980). Chemical markers have recently emerged as tools for understanding trophic relations and energy transfer in stream ecosystems. Most of these studies suggest that aquatic primary producers are critical for most lotic macroinvertebrate secondary production (Mulholland

et al. 2000, Finlay 2001, Lewis et al. 2001, Dangles 2002, Thorp and Delong 2002, Lancaster et al. 2005, Delong and Thorp 2006). Our study supports these conclusions. Differences in FA spectra among aquatic primary producers also suggest that streams with diverse primary producer communities may provide a wider variety of EFAs for consumers than streams with few types of primary producers. Studies are now needed on the specific EFA requirements of macroinvertebrate consumers and their abilities to synthesize and elongate FAs.

The study of stream and river food webs often has been limited to certain times of the year. This limitation could be a major barrier to understanding stream food webs because temperate streams are open, changing systems in which trophic interactions and energy flow could be greatly affected by seasonal changes in potential food sources. Some studies in large rivers suggest that algal matter remains important throughout the year in spite of seasonal changes in algal biomass (Kendall et al. 2001), but data are scarce for streams. Our study suggests that temporal changes can affect trophic relations in streams by altering the chemical composition of available food sources. Future studies of trophic relations using chemical markers, such as FAs, that include temporal variability will clarify our understanding of how energy flows in these systems.

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