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Comparison of fatty acids and elemental nutrients in periphyton, invertebrates, and cutthroat trout (*Oncorhynchus clarki*) in conifer and alder streams of western Washington state

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Abstract

Organism growth and reproduction are often limited by nutrient availability in freshwater ecosystems where, in some cases, food webs are primarily supported by allochthonous organic matter. Therefore, we hypothesized that the composition of riparian vegetation would influence the variability of N, P and fatty acid content of in-stream consumers. Specifically, we predicted that organisms living in alder streams would have higher levels of N, P, and polyunsaturated fatty acids than organisms in coniferous streams. To determine this, we sampled fresh and aged leaf litter, periphyton, invertebrates, and cutthroat trout (*Oncorhynchus clarki*) from 6 streams in western Washington state: 3 streams had high densities of nitrogen-fixing red alder (*Alnus rubra*) in the riparian zone, whereas 3 had high densities of conifers. We found fresh alder litter had twice the total polyunsaturated fatty acid concentrations of hemlock vegetation while there were few statistical differences among aged alder and aged hemlock vegetation. Multidimensional plots showed fatty acid profiles were unique to vegetation and fish while periphyton and invertebrates shared the same multidimensional space. We used a mixed model to determine the relative importance of vegetation type (fixed factor: conifer or alder), trophic levels (fixed factor: periphyton, primary consumer, or fish) and streams (random factor) on individual fatty acid concentrations. Total polyunsaturated fatty acids, 16:0, 20:1, 20:3n6 and total n3 were the only fatty acids influenced by stream vegetation (vegetation + stream model or full model. 67% of the fatty acids were best supported by the trophic +stream model. Nitrogen, P, Ca, Fe, C:N, N:P and C:N:P were all best supported by the trophic level + stream model and Zn was the only nutrient supported best by the full model. Correlations of n3 and n6 fatty acid concentrations between periphyton and primary consumers, and primary consumers with trout indicated several fatty acid metrics, such as n3:n6, showed food resources may affect relative fatty acid

abundances of consumers. Although vegetation type did not influence relative fatty acids of stream organisms, the importance of trophic level likely indicates organisms have different physical requirements for fatty acids. The significance of a random factor, ‘stream,’ suggests that the relative abundances of fatty acids in periphyton, invertebrates and trout are more related than similar organisms from another stream.

Introduction

There is a strong connection between riparian vegetation and forest stream food webs (Cummins et al. 1989; Richardson 1990; Kiffney et al. 2003). In general, food webs in these streams depend on biomolecules from terrestrial sources, such as leaf litter or soil runoff (Vannote et al. 1980; Barlocher 1992; Webster and Meyer 1997). The availability and quality of riparian leaf litter varies widely, and this variability potentially influences consumer populations (Volk 2004). Therefore, quantitatively assessing the chemical quality of a stream food web might be useful for predicting in-stream production and fish growth. Food quality is commonly assessed using C:N and N:P ratios, but essential fatty acids are an alternative measure of food quality that has recently been applied to lake and stream ecosystems (Arts 1998; Arts et al. 2009). Fatty acid indicators are unique in that many animals, including humans, lack the desaturation enzymes that act at the n3 and n6 positions of polyunsaturated fatty acids (PUFAs). These fatty acids are critical for hormone production and membrane fluidity, and since they cannot be produced they are essential dietary nutrients (Sargent et al. 1999). Furthermore, high dietary concentrations of PUFAs, specifically n3 and n6 fatty acids, promote growth and reproductive rates for aquatic invertebrates (Ravet et al. 2003; Brett et al. 2006). Few fatty acid studies have assessed fatty acid compositions across trophic levels in natural systems (Torres-Ruiz et al. 2007) or environmental factors that might affect fatty acid composition (e.g. Peeters et al. 2004) even though general fatty acid profiles of algae, invertebrates and fish are well summarized by Arts et al. (2009).

Streams in the Pacific Northwest are generally oligotrophic and, depending on underlying geology, can be limited by N, P or NP co-limited (Volk et al. 2008; Kiffney 2008; Sanderson et al. 2009). Red alder, *Alnus rubra*, is a common nitrogen-fixing species found along riparian corridors and disturbed landscapes of the Pacific Northwest. Alder leaf litter and underlying soils are rich in N and P, and our earlier research showed annual detrital inputs were about 3.5 × higher in streams dominated by riparian red alder relative to streams bordered primarily by conifers (Volk 2004). Concentrations of a number of important biomolecules (e.g., N, P) in water and fluvial particulate organic matter were also higher in alder-dominated streams. Alder additions occur throughout the year with a large pulse during leaf fall in autumn, resulting in annual total inputs of N and P to select streams of the Olympic Peninsula, WA, USA of 8.0 and 0.25 g/m^2/year, respectively (Volk 2004), which are 5 to 8 × higher than inputs to nearby conifer dominated streams. Others have also shown that riparian alder forests are associated with variability in the trophic productivity of freshwater ecosystems in the western US (Goldman 1961; Compton et al. 2003; Volk et al. 2003).

Therefore, we hypothesized the chemistry of detrital subsidies from riparian vegetation may affect the chemical composition of local aquatic biota since N, P and fatty acids are essential for survival, growth, and reproduction (Müller-Navarra 1995; Bendiksen et al. 2003). To test this hypothesis, we quantified concentrations of PUFAs, C, N, P, Ca, Fe, and Zn in fresh vegetation, stream-aged leaf litter, periphyton, invertebrates, and coastal cutthroat trout (*Oncorhynchus clarki*) from 6 independent watersheds in western Washington: three stream riparian corridors were dominated by alder and three by coniferous vegetation. Our objectives were to compare n3 and n6 fatty acid concentrations and elemental nutrient concentrations of: 1) food webs in alder and conifer-dominated streams; and 2) among trophic levels.

Methods

Study sites

Six headwater tributaries to the Hoh (47° 48' 36", -124° 5' 12") and Clearwater rivers (47° 42' 7”, -124° 10' 23") on the western Olympic Peninsula in Washington state were used as study sites (Table 1). We selected these sites because of the predominance of alder or conifer within 30 m of the stream bank, low accessibility to anadromous salmon and vehicle accessibility. Bridge Creek, Bull Creek and Hook Creek were classified as coniferous streams because their riparian corridors were dominated by ~75 year old second-growth Sitka spruce (*Picea sitchensis*), western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophyla*), and Douglas-fir (*Pseudotsuga mensizeii*). Christmas, Shale, and Maple Creeks were classified as alder streams and were predominantly vegetated with red alder within the riparian corridor. Classifications were assessed by measuring litter flux to streams using 5 baskets placed within the bankfull channel of a 200 m reach; alder sites were required to have over 90% of the litterfall composed of alder vegetation. We calculated the catchment area upstream of sample sites (NHDPlus hydrogrpahy) and then the percent of land within this catchment covered by hardwood species (data from Landsat Vegetation Mapping (1998) and GAP vegetation coverages (1991)) (Table 1). Alder is the dominant hardwood within these coastal streams and we considered the hardwood and broadleaf GIS layers a reasonable proxy for alder composition within watersheds.

Sample collections

In September 2003, alder and hemlock vegetation, periphyton, invertebrates, and cutthroat trout were collected from a 200m reach of each stream for C, N, P, Fe, Ca, Zn and fatty acid analyses. Freshly senesced alder leaves and hemlock needles were shaken from three trees of each species and collected. Periphyton was scraped from rocks (5 samples/stream) with a toothbrush, rinsed with deionized water, filtered onto Whatman GF/F) filters, and frozen. Baetidae (n_{alder} = 3 individuals from a total of 2 streams, $n_{conifer}$ = 7 individuals from a total of 2 streams), Heptageniidae (n_{alder} = 11 from 2 a total of 2 streams, $n_{conifer}$ = 4 from 1 stream), and Glossosomatidae (n_{alder} = 6 individuals from a total of 2 streams, $n_{conifer}$ = 6 individuals from a total of 2 streams) invertebrates were hand collected and frozen. Macroinvertebrate collections were limited to these predominantly herbivorous and dominant families due to a limited abundance of detritivores and shredders at the time of sampling. Five to ten cutthroat trout (fork length of 6-15 cm) were collected from each stream using a single pass electrofishing survey, weighed (nearest 0.1 g), measured (nearest 1 mm), and frozen. Single-pass electrofishing was deemed a sufficient capture method as no fish population abundance estimates were planned for the study (e.g. Bateman et al. 2005). All samples were frozen at -80 ºC until fatty acid extraction and C, N and P and micronutrient analyses.

In addition to freshly senesced vegetation, we aged leaf litter in streams to simulate in-stream detritus. We placed 10 g packets of senesced hemlock or alder in mesh bags (15 cm diameter bags with 0.4 mm mesh) and zip-tied packets to large rocks in Christmas (alder vegetation type) and Hook (conifer vegetation type) Creeks (note only one stream for each vegetation type was used for packet placement and we considered individual packets as replicates). Packets were submerged on 9/11/2003 and after 18 days packs were recovered and placed in plastic bags for biomass measurements. We submerged leaf packs long enough to allow diffusion of most nutrients and partial mass loss, as most leaf litter nutrients are lost within 24 hours of submersion in water (Gessner and Schwoerbel 1989) and 40% mass loss can be found after four weeks (Braatne et al. 2007). In the lab, invertebrates were removed from packets and remaining biomass was dried (30^{o}C) to a constant weight and weighed. All weights used within analyses are total biomass and were not corrected for inorganic matter accumulation.

Fatty acid extraction

A simultaneous extraction of wax esters and total fatty acids (Kattner and Fricke 1986; Doerthe C. Müller-Navarra, University of Hamburg, Hamburg, Germany, personal communication) was used for extraction of n3 and n6 fatty acids. Subsamples of leaf litter (fresh and aged), periphyton, fish dorsal muscle tissue and whole invertebrates were freeze dried for 4 hours and weighed (sample weights ranged from 0.25-1.5mg, pending tissue type). Ten µl of the internal standard, 21:0 (10mg/10ml methanol; Sigma #H-5149, heneicosanoic acid) was added prior to three dichloromethane:methanol (2:1v/v) extractions. The first extraction was overnight (15 hours) with 5ml of dichloromethane:methanol mixture, followed by a second and third extraction of the sample material with 3 and 2ml of dichloromethane:methanol solution for 2-3 hours and 0.5 hours, respectively. Suspension liquid was removed, set aside, and chilled (32ºC) between extractions of source material. After all extractions were complete, set aside suspensions were recombined and then evaporated with N_2 gas (30ºC heat block), and resuspended with 2ml of 3% sulfuric acid in methanol and 5ml 16% n-hexane addition. This mixture was heated for 4 hours at 80 ºC, converting all Fatty Acids to Methyl Esters (FAMEs), which are soluble in hexane. FAMEs were separated from the sulfuric acid matrix with four additional extractions with n-hexane (water added to solution before extraction to dilute sulfuric acid matrix and facilitate hexane:sulfulric acid solution separation), then N_2 gas evaporated to dryness and dissolved in 1.5ml n-hexanes. All FAMEs were frozen (–80 ºC) until injection (5µl) into the gas chromatograph (GC).

Fatty acids were analyzed on an HP6890 series GC with an Agilent DB-WAX (30 m) + guard column (10m; 0.32 mm, 0.25 µm film) and PTV inlet. Gas chromatography program specifics were 5 minutes at 40 °C (ramp rate = 10 °C/min), 5 minutes at 150 °C (ramp rate = 2 °C/min), 24 minutes at 220 °C (ramp rate = 2°C/min). The program was optimized for 18C fatty acids and had a detection limit of 0.112mg dry weight. All program specifications were per the University of California-Davis (Goldman Laboratory) specifications from Kattner and Fricke (1986) and Doerthe C. Müller-Navarra (University of Hamburg, Hamburg, Germany, personal communication). All fatty acids with retention times between 20 and 65 minutes were identified by retention time on the chromatograph with comparison to reference standard (37 FAME, Supelco Mix C4-C24). Reference peaks of interest included 10:0, 11:0, 12:0, 13:0, 14:0, 14:1, 15:0, 15:1, 16:0, 16:1, 17:0, 17:1, 18:0, 18:1n9, 18:2n6 cis and trans, 18:3n6, 18:3n3, 18:4n3, 20:0, 20:1, 20:2n*, 20:3n6, 20:4n6, 20:3n3 20:5n3 22:0, 22:1n9, 22:2n6, 23:0 24:0, 22:6n3, and 24:1. Once peaks were identified through comparison with the reference standard, areas for each sample peak were corrected for the recovery volume of the internal standard, 21:0, and multiplied by the total amount of sample (mg). No inferences on non-reference peaks were made. Fatty acid analyses were replicated only within the study design (e.g. 6 detritus samples per stream) and not for fatty acid analyses (e.g. 1 sample from stream extracted and run through the GC multiple times). At least two blanks were included in each extraction and an additional 2 standards were included in each GC sample run.

Elemental Nutrients

Fresh alder and hemlock, aged alder and hemlock, periphyton, and trout muscle tissue were analyzed for C, N, P, Ca, Fe, K, and Zn. Material for elemental nutrient analyses was extracted from the same samples as fatty acids when there was ample material. Tissues were freeze dried for 4 hours and ground for C and N analysis in a CE440 Elemental Analyzer (Leeman Labs, Inc., University of Washington Oceanography Technical Services Laboratory, Seattle, WA). To determine P, Ca, Fe, and Zn concentrations, freeze dried material was digested with nitric acid for 12 hours, heated to 120 ºC for 1 hour, oxidized with H_2O_2 until colorless and resuspended in 5% HCl (modified P digest from Jones et al. (1991)). Samples were run on an ICP (Inductively Coupled Plasma Analyzer, NOAA, Northwest Fisheries Science Center, Seattle, WA) and nutrient concentrations were calculated using standard curves for laboratory standards.

Physical measurements

One surface water grab sample in (September 2003) was collected for total N, total P, ammonium, nitrite, nitrate, and phosphate analyses (Valderrama 1981). All water samples were frozen (-80 º C) and analyzed within 1 month of field collection. Additional physical habitat details from a 200m survey of each stream during August-September 2003 are summarized in Table 1 and described in detail in Volk (2004).

Statistical analyses

Since we assumed individual plants and leaf packs were independent samples, a one-way ANOVA was used to compare nutrient composition (fatty acids or elemental nutrients) between: a) fresh alder and fresh conifer vegetation and b) aged alder and aged conifer detritus. All data were tested for normality (Shapiro test and Q-Q plots) and non-normal data were *ln (x)* transformed to meet assumptions of normality. Low C:N ratios and high Ca, N, P, Fe, and Zn content were used as indicators of food quality for comparisons within the study.

Fatty acid and elemental nutrient data for fresh alder and hemlock, aged alder and hemlock, periphyton, Baetidae, Glossosomatidae, Heptageniidae, and trout were compared among all six streams using three mixed models in an information-theoretical approach (Burnham and Anderson 1998). With fatty acids and elemental nutrient data as response metrics, the models were designed such that vegetation type (classified by alder or conifer-dominated vegetation) and trophic level (fresh vegetation-alder or hemlock, aged vegetation-alder or hemlock, periphyton, invertebrates and fish) were used as predictive, fixed factors. ‘Stream’ was used as a random factor to capture inherent natural differences among streams (aka sites).

Models

Model A (full model):

Response = vegetation + trophic level + stream

$y_i = \alpha_{veg\ i} + \beta_{trophic\ i} + b_i + \varepsilon_i$

Model B: Response = vegetation + stream

$y_i = \alpha_{veg\ i} + b_i + \varepsilon_i$

Model C: Response = trophic level + stream

$y_i = \beta_{trophic\ i} + b_i + \varepsilon_i$

y_i is the *i*th response data point across all streams

$\alpha_{veg\ i}$ has two values: alder and conifer

$\beta_{trophic\ i}$ has five values: vegetation, detritus, periphyton, invertebrates, and trout

$b_i \sim N(0, \sigma_{stream})$

$\varepsilon_i \sim N(0, \sigma_{residual})$

Response metrics were averages of each fatty acid for each trophic level in a stream and were transformed to meet assumptions of normality prior to analyses. Most fatty acids were transformed with log, square root, or cubed root transformations. We used arcsine-root transformations for C, N and P percentages. Akaike’s Information Criteria corrected for small sample sizes (AICc) were compared among models to assess the relative importance of vegetation type and trophic level on nutrient composition of sampled organisms. The relative

Akaike weight (w_i) is the relative likelihood of each model divided by the sum of all weights for all models and was calculated for each model. We considered models with greater than 0.70 relative weights as strongly supported (AICc differences >2 from best model), relative weights between 0.40 and 0.70 as moderately supported (AIC differences between 1 and 2 from best model), and models with less than 0.40 relative weights as minimally supported by the dataset (Burnham and Anderson 1998). Because vegetation type was a binary fixed factor (alder or conifer), support for this model indicated alder and conifer response metrics are different. Coefficients of the model were used to determine directionality associated with alder and conifer differences.

We utilized a multi-dimension scaling (MDS) plot of ln (x+1) to describe the relative fatty acid composition of in-stream organisms in multivariate space (Primer 6 Software, Clarke and Gorley 2006). To investigate whether fatty acids are correlated between trophic levels, we created a correlation matrix of stream averages of periphyton (n = 6) and primary consumers Heptageniidae (n = 3), Baetidae (n = 3), Glossosomatidae (n = 4) and trout (n = 6) for each fatty acid. Since not all invertebrate families were found in all streams, n values varied among invertebrate families. We also correlated all primary consumers with trout similarly to periphyton and invertebrates. Pearson's correlation coefficients were used to determine significance of correlations.

Results

Comparison of fatty acids among trophic levels and vegetation types

Of the three models, the trophic + stream model (Model C) best explained the variability for 67% of the fatty acids (Table 2). All monosaturated fatty acids and 7 of 9 saturated fatty acids were best supported by this model. The n3 and n6 fatty acids were of particular interest as they are essential fatty acids; half of the n3 and 5 of 6 of the n6 fatty acids were strongly supported by this model, all with relative weights greater than 0.70 (Table 2). We found similar results with elemental nutrients, where 7 of 8 elemental nutrients or nutrient ratios (e.g. N:P) were best supported by the trophic + stream model (Table 2).

Mean relative abundances of individual fatty acids were similar between alder and conifer streams for periphyton, invertebrates and trout (Appendix 1). Although we expected relative abundances of fatty acids would be influenced by riparian vegetation type, there was almost no evidence that vegetation type influenced variation in fatty acid profiles, as the vegetation + stream model (Model B) had low w_i values compared to the full and trophic + stream models (Table 2). Two fatty acids were best supported by Model B (20:1 and 20:3n6) and 3 fatty acid metrics (16:0, n3 and total PUFA) by the full model (Model A), but only five of these showed relative abundances higher in alder than conifer; total PUFA was marginally higher in conifer streams compared to alder streams. Despite limited support for relative abundances of fatty acids, the influx of fatty acids from alder vegetation into streams may still be important to stream ecosystems. We calculated the annual biomass inputs of fatty acids from senesced vegetation by multiplying relative abundances of fatty acids by the estimated biomass of litterfall from alder and conifer streams (Volk 2004) (Appendix 2). Annual alder biomass inputs were ~3.5 times greater than conifer biomass inputs, this difference in detrital flux offers some perspective on the total contribution of fatty acids to stream ecosystems from alder forests.

The general patterns of relative abundances of fatty acids among trophic levels can be seen in Table 3. Invertebrates and trout had higher relative abundances of n3 fatty acids than periphyton. Trout had highest levels of 22:6n3 relative to all other trophic levels. Vegetation and periphyton had higher relative abundances of n6 fatty acids than invertebrates and trout. Relative abundances of saturated fatty acids (SAFA) were also highest in vegetation and declined as trophic level increased (Table 3).

There was some evidence that fatty acids were conserved in these stream food webs. The n3:n6 ratio was positively correlated between periphyton and consumers (r= 0.67, p = 0.03, n =10, Figure 1) and consumers and trout (r = 0.57, p = 0.08, n = 10) (Figure 2). Furthermore, the relative concentration of 18:3n3, was positively correlated between periphyton and consumers (r = 0.76, p < 0.01, n = 10). Variability in 18:3n3 were positively correlated between consumers and trout but this was not statistically significant (r = 0.53, p = 0.11, n = 10). Percent PUFA was also positively correlated between primary consumers and trout (r = 0.63, p = 0.03, n = 10) but not between periphyton and consumers (r = 0.13, p = 0.70, n = 11). The other n3 and n6 polyunsaturated fatty acids exhibited no correlations between different trophic levels (results not shown).

Results from our multidimensional plots showed fatty acid profiles of trout were tightly grouped and distinct from vegetation, periphyton and invertebrates. All vegetation (fresh alder, aged alder, fresh hemlock and aged hemlock) showed considerable overlap, but were distinct from periphyton, invertebrates and trout (Figure 3). The invertebrate families (Baetidae, Heptageniidae, and Glossosomatidae) did not separate in multidimensional space. Similarly, periphyton fatty acid profiles were highly variable and overlapped with all three invertebrate families.

Nutrient concentrations of decomposing alder and conifer litter in streams

We compared fatty acid concentrations from the two streams (1 alder and 1 conifer) after 18 days of decomposition (samples were used as replicates). Eighteen carbon fatty acids were 10-30% lower in both aged alder and hemlock compared to fresh litter concentrations, and the relative abundances of 50% of measured PUFAs dropped (Table 3). N3 and n6 fatty acid concentrations decreased by 30% in alder but increased by 5-25% in hemlock (Figure 4 and Table 3). In alder, 18:4n3 concentrations declined by 77%, but increased by 40% in hemlock, while 18:2n6 concentrations were reduced in both hemlock and alder (44 and 50%, respectively) (Figure 1). 18:3n3 was the only polyunsaturated fatty acid where aged alder concentrations were statistically higher than aged hemlock concentrations (n = 12, $p < 0.01$).

Elemental concentrations of Ca, N and P were significantly higher in fresh alder than hemlock vegetation, and C:N ratios were significantly lower (percent by weight) (Table 4). Nitrogen, C, and Zn were significantly higher, and C:N ratios were almost 3 times lower in aged alder detritus relative to aged hemlock. Percent P was not statistically different between aged vegetation types (n = 18, p = 0.75, Table 4). In alder, 36-67% of Ca was lost over 18 days whereas C loss rates were twice as fast in hemlock than alder (1.00 and 0.53% per day, respectively).

Discussion

This is one of the first studies to investigate fatty acids in natural stream food webs, and factors that may affect the variability of these essential biomolecules. Overall, most of the variability in fatty acids was attributed to trophic level and stream, with vegetation type as an important covariate for only a few fatty acids. We speculate the importance of trophic level and stream suggests organisms were not limited in essential fatty acids and that metabolic differences likely accounted for the significance of trophic level for almost all fatty acid and nutrient response metrics.

One of the more intruiging results was the positive correlation among the relative abundance of fatty acids in different trophic levels (periphyton, consumers, and fish). The positive correlation between stream trophic levels for some fatty acids suggests consumers reflect the chemical composition of their food resources. Specifically, levels of n3 and n6 fatty acids in periphyton were positively correlated with fatty acids in primary consumers and trout, suggesting these materials were conserved as they moved up the food chain. Feeding trials of phytoplankton (cryptophytes, chlorophytes and cyanophytes) to *Daphnia pulex* showed high correlations of 20:5n3 + 22:6n3 and 20:4n6 between food sources and *Daphnia* (Brett et al. 2006). Similarly, feeding trials using 18:2n6, 18:3n3, 20:4n6 or an n3 PUFA mix for laboratory reared Arctic charr found a general dominance of n3 PUFA in fish muscle tissue when diets were composed of 18:3n3 (Olsen et al. 1991), supporting our observations that a high PUFA content in invertebrates correlated to high PUFA content in fish tissue. However, our study was observational and exploratory, which limits our inference, but suggest some potential future studies evaluating how fatty acids may influence food webs in stream ecosystems. For example, does natural variation in essential fatty acids influence growth rate of stream fish through higher growth efficiencies?

For elemental nutrients, lower C:N ratios in periphyton than terrestrial vegetation indicates that aquatic primary producers were a higher quality food for consumers and predators than terrestrial producers, and similar conclusions have been drawn by Reiners (1986) and Elser et al. (2000). This is likely due to the high proportion of structural carbon material in terrestrial vegetation (McGroddy et al. 2004). Furthermore, all else being equal, the lower C:N of algae and alder may result in differences in growth efficiencies of higher trophic levels relative to systems dependent on more recalcitrant sources of energy such as conifer needles, as conifer needles break down more slowly than deciduous litterfall (Alberińo and Balseiro 2002). In both terrestrial and lake environments, a reduction in the conversion efficiency of carbon into new biomass ('gross growth efficiency') has been correlated with higher food C:N and C:P ratios (Elser et al. 2000).

Trout, vegetation and invertebrates separated well in multidimensional space while periphyton tended to overlap with vegetation and invertebrates. The high percentages of PUFA (56%), and 22:6n3 (5 times higher relative abundance than other trophic levels) likely contributed to the separation of trout from other trophic levels. Metabolic differences among trout and invertebrates likely account for the separation of the two consumers, as the essential fatty acid requirements for growth and cell structure are different (Ackman 1998). The considerable overlap between periphyton and primary consumers was expected, as these invertebrates were predominantly grazers, scrapers, and collectors that feed on periphyton (Cummins 1973). In general, Baetidae feed by scraping algae and fine detritus from submerged rocks or other submerged materials, such as woody debris. Heptageniidae are surface feeding collectors or scrapers (mineral or organic scrapers) and Glossosomatidae are typically mineral scrapers (Cummins 1973). The overlap between the fatty acid profiles of these invertebrate families and periphyton is therefore not surprising and we assume periphyton is the dominant food source for the sampled invertebrates in the study streams.

Results from this study suggest the relative abundance of a few fatty acids or n3:n6 ratios of fatty acids can be used to assess the potential biological (e.g., growth, survival, productivity) importance of different chemical components in stream food web (riparian plants, periphyton, primary consumers, and trout. This suggests that streams with high relative abundance of these fatty acids in periphyton will have similarly high abundances of these fatty acids in grazers and trout. However, the majority of correlations among trophic levels for n3 and n6 fatty acids were not significant, suggesting that some fatty acids may be better tracers than others. Further studies considering feeding habits and trials in mesocosm environments with natural food sources are needed especially those that quantify whether these fatty acids actually contribute to variation in performance in higher trophic levels.

One of our main objectives of this study was to determine if leaf litter inputs from alder vegetation influence the relative abundance of fatty acids of in-stream organisms because primary producers are the only source of these fatty acids for higher trophic levels. We did find higher abundances of PUFA, 18:3n3, 20:5n3, 20:4n6 and 22:6n3 fatty acids, and higher N and P in leaf material from alder relative to hemlock vegetation (N and P results similar to Volk 2004). However, the results presented here provided little evidence that fatty acids and elemental nutrients in periphyton, invertebrates and trout were strongly influenced by vegetation type. We suggest three potential reasons for this result: 1) the relative abundance of only 5 fatty acids was higher in alder than conifer vegetation, suggesting there are very few fatty acids where we would have expected to see influences from alder vegetation; 2) low sample sizes, especially for invertebrates, did not provide enough power of detection for this study; and 3) polyunsaturated fatty acids are not limiting food webs in these small streams and therefore relative abundances do not change with the presence of additional fatty acid resources.

Instead, our model results indicated trophic level and streams were important covariates predicting variation in biomolecules. The importance of trophic level likely indicates organisms at different trophic levels have different metabolic requirements for fatty acids, particularly essential fatty acids. The significance of a random factor, 'stream,' suggests that the relative abundances of fatty acids in periphyton, invertebrates and trout are stream-specific and are responding to local environmental or communal variables. Physical aspects or food resources unique to each stream could influence the fatty acid profiles of these food webs. For example, this 'stream effect' may be linked to differences in the composition of in-stream primary producers, litterfall, other inputs of biomolecules (e.g. plant reproductive structures) or feeding relationships. However we investigated a limited number of food resources and physical aspects of a site that may contribute to this random variation in fatty acid profiles.

Aging leaf litter in streams reduced the relative abundances of n3 and n6 fatty acids, and this may be due to an accumulation of inorganic material on aged leaf litter. This hypothesis may be supported by the large increase in Fe between fresh and aged material, as Fe is common element in inorganic minerals. Other changes in n3 and n6 fatty acid content between fresh and aged material may have been due to algal or microbial colonization, but these communities were not directly studied.

Conclusions

Relationships between 1) periphyton and invertebrate primary consumers and 2) invertebrate primary consumers and fish suggest that the relative abundance of these resources were conserved. Further observational and experimental studies are needed to improve our understanding of the nutritional ecology of freshwater ecosystems, because this understanding may help us conserve and restore ecologically and economically important fish species and their ecosystems. Although the widespread abundance of riparian red alder in the Pacific Northwest provides particulate and dissolved nutrient resources, it is difficult to discern the role of these resources for primary and secondary consumers unless the limiting resources of the local ecosystems are known. Moreover, we need a better understanding of the linkages between watershed and riparian conditions that may affect chemical constituents potentially important in the trophic productivity of freshwater food webs because this understanding may improve our restoration and management of forested watersheds with economically and ecologically important fish (Wipfli and Baxter 2010).

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Table 1. Physical stream habitat characteristics of study sites

	Stream					
	Maple	Shale	Christmas	Bridge	Hook	Bull
Vegetation	Alder	Alder	Alder	Conifer	Conifer	Conifer
Alder in watershed, %	16.0	25.0	62.0	46.0	0	0
Alder leaf litter in riparian area, %	100.0	97.0	91.0	10.0	34.0	18.0
Alder leaf litterfall in riparian area, g/m^2	15.2	59.8	81.5	1.8	12.5	8.2
Conifer leaf litterfall in riparian area, g/m^2	0	2.5	6.9	7.9	16.4	12.0
Stream gradient, %	2.7	2.4	1.2	5.1	3.6	1.8
Discharge, L/s	16.9	21.6	17.4	1.3	153.0	1.9
7d average T, °C	12.0	11.5	12.0	11.8	11.7	11.5
TP, µg/L	26.0	35.0	25.0	25.0	29.0	28.0
TN, µg/L	102.0	169.0	85.0	219.0	161.0	165.0
PO_4, µg/L	4.4	4.8	2.9	7.5	4.0	2.6
NO_3, µg/L	64.0	30.0	13.0	195.0	84.0	81.0
NH_4, µg/L	6.7	5.7	8.0	4.4	1.4	5.2

Table 2. AIC scores and relative model weights (w_i) for the full, vegetation and trophic models. NA = Data not analyzed using mixed models due to high zero counts in data that led to violations in normality assumptions. Response metrics are relative fatty acid abundance or elemental nutrient concentrations per sample. * values calculated using subset of data for model due to abundance of zeros within trophic levels. [+] data did not conform well to assumptions of normality. [++] 3 models not completed due to limited amount of non-zero data in all trophic levels.

	AICc scores			w_i		
Response metric	Full model (Model A)	Vegetation + stream (Model B)	Trophic + stream (Model C)	Full model	Vegetation + stream	Trophic + stream
14:0	-26.29	28.49	-31.49	0.07	0.00	0.93
15:0	-181.63	-178.34	-187.56	0.05	0.01	0.94
16:0	756.96	799.82	757.76	0.60	0.00	0.40
17:0	-194.43	-172.64	-201.57	0.03	0.00	0.97
18:0	-47.20	-70.42	-52.86	0.00	1.00	0.00
20:0	133.15	165.79	129.53	0.14	0.00	0.86
22:0	191.45	207.96	187.86	0.14	0.00	0.86
23:0	NA	NA	NA	NA	NA	NA
24:0*	112.65	127.95	109.29	0.16	0.00	0.84
SAFA	-218.74	-173.69	-225.29	0.04	0.00	0.96
14:1[++]	NA	NA	NA	NA	NA	NA
15:1[++]	NA	NA	NA	NA	NA	NA
16:1	707.06	788.95	708.77			
17:1[+]	164.85	194.02	161.01	0.13	0.00	0.87
18:1n9	256.17	263.12	252.70	0.15	0.00	0.85
20:1	0.048	-6.60	-4.44	0.03	0.73	0.25
22:1n9[++]	NA	NA	NA	NA	NA	NA
24:1[++]	NA	NA	NA	NA	NA	NA
MUFA	-122.45	-88.43	-129.29	0.03	0.00	0.97
18:3 n 3	305.95	350.38	304.45	0.32	0.00	0.68
18:4 n 3	-40.38	-44.40	-45.83	0.04	0.32	0.64
20:5 n 3	43.93	170.12	38.62	0.07	0.00	0.93
20:3 n 3*	60.82	62.77	57.21	0.13	0.05	0.82
22:6 n 3[+]	203.65	469.65	201.25	0.23	0.00	0.77
n3	848.63	1009.01	850.91	0.76	0.00	0.24
18:2 n 6	-106.36	-47.67	-111.17	0.08	0.00	0.92
18:3 n 6	117.40	125.15	113.06	0.10	0.00	0.90
20:3 n 6[+]	68.43	63.51	65.085	0.06	0.65	0.30
20:4 n 6	-65.06	23.04	-69.87	0.08	0.00	0.92
22:2 n 6*	NA	51.90	NA	NA	NA	NA
n6	-97.02	-92.27	-102.92	0.05	0.00	0.95
n3:n6	-85.82	14.96	-92.06	0.04	0.00	0.96
PUFA	871.74	989.69	874.67	0.81	0.00	0.19
C[+]	-153.33	-74.43	-160.53	0.03	0.00	0.97
N	-244.45	-96.48	-252.08	0.02	0.00	0.98
P	64.00	119.59	60.82	0.17	0.00	0.83
Ca	70.23	91.02	67.35	0.19	0.00	0.81
Fe	86.26	218.36	82.42	0.13	0.00	0.87
Zn	110.21	114.95	110.25	0.48	0.04	0.47
C:N	-188.55	-76.31	-193.77	0.07	0.00	0.93
N:P	36.50	46.72	34.48	0.27	0.00	0.73
C:N:P	38.25	123.95	32.74	0.06	0.00	0.94

Table 3. Summary of fatty acids in aquatic food web. Values are relative percentages of fatty acid (or fatty acid ratio) averaged across all streams. N indicates the number of replicates (total for all streams). Baetidae not collected from Hook Creek and Glossomatidae not collected from Christmas Creek.

	Vegetation								
Fatty acid	Fresh Alder	Fresh hemlock	Aged alder	Aged hemlock	Periphyton	Baetidae	Hept	Gloss	Trout
n =	6	6	6	6	27	10	15	12	36
14:0	3.00	3.75	3.92	4.59	4.52	1.25	0.92	3.29	0.80
15:0	0.38	0.66	0.49	0.41	0.78	0.26	0.69	033	0.18
16:0	25.88	20.10	24.45	22.37	30.25	27.66	20.25	20.96	21.29
17:0	0.56	0.85	0.69	0.55	0.49	1.19	1.53	0.84	0.63
18:0	6.88	11.98	5.61	8.74	8.06	9.32	13.14	4.32	7.03
20:0	3.69	4.08	3.80	3.72	1.08	0.49	1.88	0.45	0.20
22:0	6.53	2.75	2.53	4.02	1.02	0.39	2.28	0.43	0.15
23:0	0.43	3.02	0.42	0.15	0.02	0	0	0	0
24:0	3.73	3.97	3.99	7.49	1.44	0.01	0.06	0	0.01
SAFA	51.09	51.17	45.89	52.05	47.67	40.59	40.74	30.63	30.29
14:1	0.35	0.32	0.07	0.16	0.02	0	0.01	0.40	0
15:1	0	0.81	0	0	0	0	0.01	0.02	0
16:1	1.48	2.38	2.13	1.33	11.9	5.98	11.23	10.53	3.21
17:1	0.13	0.84	0.33	0	1.32	0.30	0.31	1.67	0.08
18:1n9	12.68	14.76	14.10	14.87	10.53	8.49	10.21	10.49	9.58
20:1	0.30	0	0	0	0.12	0.05	0.01	0.06	0.08
22:1n9	0	0.26	0	0.49	0.02	0.05	0	0	0.01
24:1	0	0	0	0	0.19	0	0	0.20	0.02
MUFA	14.63	19.37	16.63	16.85	24.11	14.88	21.78	23.01	12.98
18:3 n 3	18.98	8.79	21.17	10.37	8.01	19.18	8.26	17.37	7.41
18:4 n 3	1.78	0.74	0.67	0.64	1.38	2.21	0.98	3.64	1.21
20:5 n 3	0.78	0	1.14	3.43	7.16	14.08	19.39	16.62	11.87
20:3 n 3	0.22	0.29	0.21	0	0.06	0.18	0.35	0.23	0.70
22:6 n 3	0.27	5.38	0	3.38	1.07	0.29	0.22	0.23	28.41
n3	22.03	15.20	23.19	17.82	17.67	35.95	29.20	38.09	49.61
18:2 n 6	10.90	11.20	7.71	8.36	6.21	6.85	3.99	3.83	3.99
18:3 n 6	0.09	0.32	0	0	0.42	0.23	0.19	0.52	0.07
20:3 n 6	0	0	0	0.38	0.02	0.07	0.01	0.34	0.25
20:4 n 6	0.44	0	0.50	0	1.69	14.08	3.62	3.51	2.66
22:2 n 6	0	0	3.46	4.25	1.26	0	0	0	0.06
n6	11.43	11.52	11.67	12.99	9.70	8.34	7.81	8.20	7.03
n3:n6	2.09	1.52	2.32	1.73	2.26	4.57	3.96	5.71	7.51
PUFA	34.20	28.92	37.21	30.82	27.94	44.53	37.30	46.30	56.71
SAFA: (MUFA+PUFA)	1.05	1.06	0.85	0.92	0.95	0.78	0.61	0.44	0.43

Table 4. Summary of elemental nutrient concentrations for fresh and aged alder and hemlock litter. Significance values are for ANOVA analyses comparing fresh alder to fresh hemlock and aged alder to aged hemlock ($\alpha = 0.05$). Fe and Zn values not available for fresh hemlock due to sample limitations.

		Fresh			Aged		
	n	Alder	Hemlock	p	Alder	Hemlock	p
C (%)	12	47.00	59.08	0.20	36.35	41.60	0.30
N (%)	12	2.37	0.99	**0.02**	3.06	1.41	**0.03**
P (%)	18	0.27	0.13	**<0.01**	0.32	0.22	0.75
C:N (molar)	12	23.13	69.61	**<0.01**	14.38	34.28	**<0.01**
C:P (molar)	12	459.81	1344.46	**<0.01**	6.03	23.89	**0.04**
C:N:P (molar)	12	2.7×10^6	1.9×10^6	**<0.01**	2266.05	14261.83	**0.04**
Ca (µg/g)	18	22846.00	4704.00	**0.02**	15302.00	5817.00	**<0.01**
Fe (µg/g)	18	322.23	*na*	*na*	3957.00	2333.00	0.21
Zn (µg/g)	18	252.08	*na*	*na*	4976.00	148.00	**0.03**

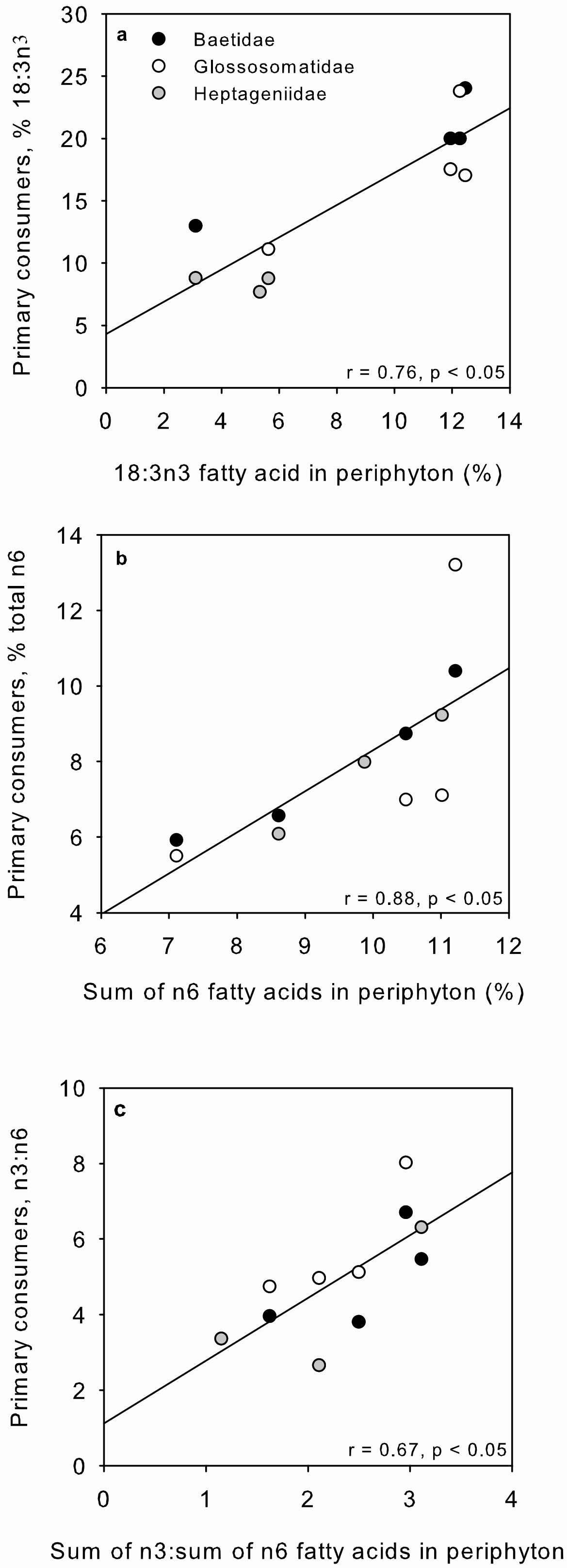
Fig 1 Correlations between periphyton and primary consumers for a) 18:3n3; b)n6 and c) n3:n6 fatty acids

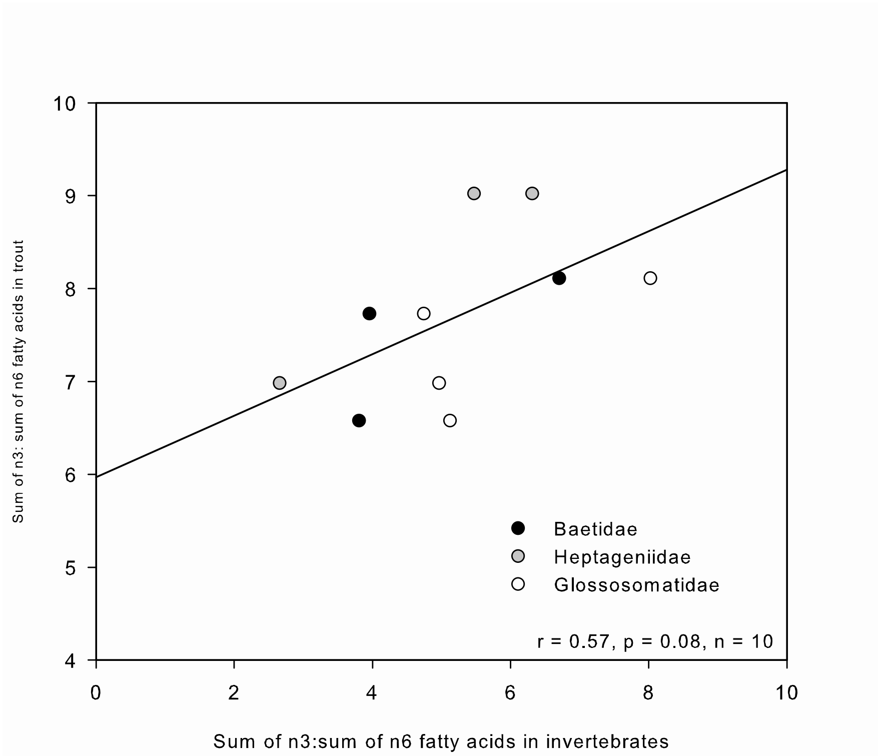
Fig 2 Correlation between primary consumers and trout for n3:n6 fatty acids

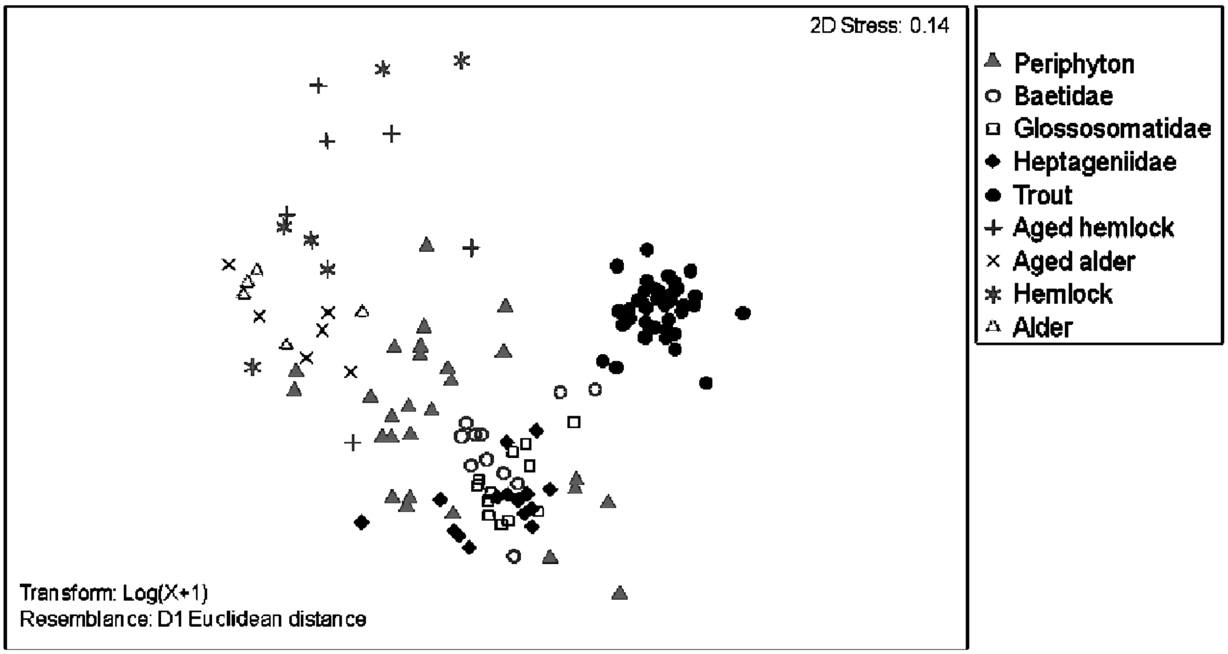
Fig 3 Fresh and aged leaf litter, periphyton, invertebrates and trout fatty acids in multidimensional space

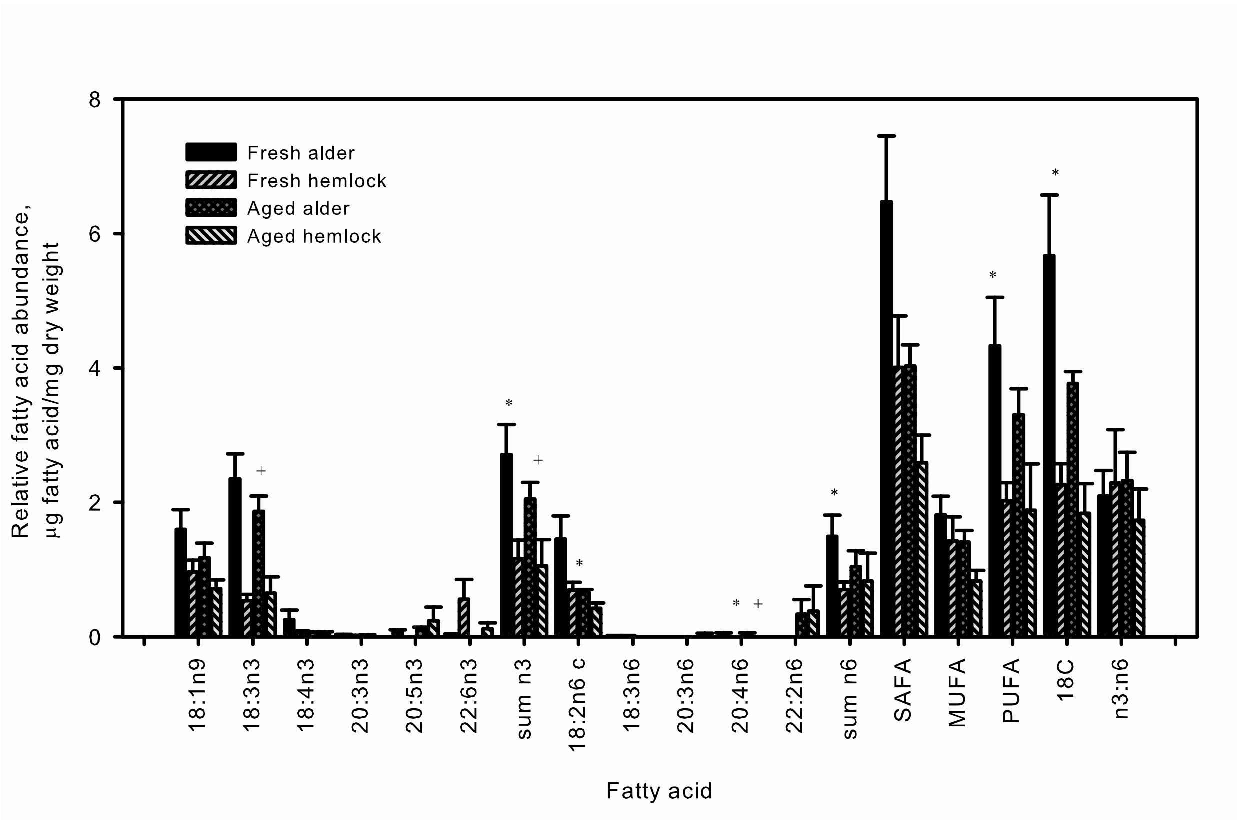
Fig 4 Major fatty relative abundances in fresh and aged vegetation. n= 6 for each series. sum n6 = sum of 18:2n6 (cis and trans) 18:3n6, 20:3n6, 20:4n6, and 22:2n6; SAFA = saturated fatty acids; MUFA= monosaturated fatty acids, PUFA = polyunsaturated fatty acids. Bars indicate standard error, * indicates significance between fresh alder and fresh hemlock at $p < 0.05$ and + indicates significance between aged alder and aged hemlock at $p < 0.05$

597


a
Baetidae
Glossosomatidae
Heptageniidae
r = 0.76, p < 0.05
Primary consumers, % 18:3n3
18:3n3 fatty acid in periphyton (%)
b
r = 0.88, p < 0.05
Primary consumers, % total n6
Sum of n6 fatty acids in periphyton (%)
c
r = 0.67, p < 0.05
Primary consumers, n3:n6
Sum of n3:sum of n6 fatty acids in periphyton


Sum of n3: sum of n6 fatty acids in trout
Sum of n3:sum of n6 fatty acids in invertebrates
Baetidae
Heptageniidae
Glossosomatidae
r = 0.57, p = 0.08, n = 10
10
9
8
7
6
5
4
0
2
4
6
8
10


2D Stress: 0.14
Periphyton
Baetidae
Glossosomatidae
Heptageniidae
Trout
Aged hemlock
Aged alder
Hemlock
Alder
Transform: Log(X+1)
Resemblance: D1 Euclidean distance


Fresh alder
Fresh hemlock
Aged alder
Aged hemlock
Relative fatty acid abundance,
µg fatty acid/mg dry weight
0
2
4
6
8
18:1n9
18:3n3
18:4n3
20:3n3
20:5n3
22:6n3
sum n3
18:2n6 c
18:3n6
20:3n6
20:4n6
22:2n6
sum n6
SAFA
MUFA
PUFA
18C
n3:n6
Fatty acid

Appendix 1. Fatty acid summary for alder and conifer streams. Values are relative percentages of fatty acid (or fatty acid ratio) averaged across all streams. N indicates the number of replicates (total for all streams). Baetidae not collected from Hook Creek and Glossosomatidae not collected from Christmas Creek.

Fatty acid	Periphyton Alder	Periphyton Conifer	Baetidae Alder	Baetidae Conifer	Hept Alder	Hept Conifer	Gloss Alder	Gloss Conifer	Trout Alder	Trout Conifer
n =	14	13	3	7	11	4	6	6	13	23
14:0	5.82	3.13	1.59	1.21	0.92	0.92	3.68	2.90	0.77	0.83
15:0	0.97	0.57	0.23	0.27	0.79	0.40	0.29	0.37	0.17	0.19
16:0	31.47	28.94	26.84	27.74	19.97	21.02	22.30	19.63	21.64	21.01
17:0	0.56	0.41	0.90	1.22	1.61	1.31	0.70	0.99	0.57	0.67
18:0	8.93	7.13	6.22	9.64	13.68	11.65	3.78	4.87	6.63	7.35
20:0	1.38	0.75	0.28	0.51	1.96	1.65	0.37	0.53	0.15	0.25
22:0	1.23	0.79	0.15	0.42	2.40	1.96	0.39	0.47	0.10	0.19
23:0	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24:0	1.45	1.44	0.00	0.02	0.08	0.00	0.00	0.00	0.03	0.00
SAFA	51.86	43.16	36.21	41.02	41.41	38.91	31.50	29.75	30.05	30.50
14:1	0.04	0.00	0.00	0.00	0.01	0.00	0.06	0.02	0.00	0.00
15:1	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.01	0.00	0.00
16:1	11.61	12.22	10.08	5.57	12.50	7.76	10.41	10.66	2.84	3.51
17:1	1.37	1.27	0.47	0.28	0.31	0.30	1.68	1.65	0.07	0.08
18:1n9	9.22	11.94	7.62	8.59	10.27	10.04	10.85	10.14	10.65	8.70
20:1	0.20	0.04	0.00	0.05	0.00	0.04	0.10	0.02	0.10	0.07
22:1n9	0.03	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.01	0.01
24:1	0.16	0.22	0.00	0.00	0.00	0.00	0.00	0.40	0.04	0.00
MUFA	22.64	25.68	18.17	14.55	23.10	18.13	23.13	22.90	13.71	12.37
18:3 n 3	7.41	8.66	19.99	19.10	8.07	8.79	17.45	17.28	7.94	6.98
18:4 n 3	1.47	1.28	3.12	2.12	0.89	1.22	3.67	3.60	1.56	0.93
20:5 n 3	4.00	10.55	16.39	13.85	16.88	26.29	17.31	15.93	11.07	12.54
20:3 n 3	0.06	0.06	0.20	0.18	0.37	0.30	0.23	0.23	0.98	0.48
22:6 n 3	1.92	0.15	0.00	0.32	0.30	0.00	0.33	0.13	27.08	29.50
n3	14.86	20.70	39.70	35.57	26.51	36.60	39.00	37.18	48.63	50.41
18:2 n 6	5.03	6.66	4.73	7.06	4.34	3.03	3.20	4.46	4.44	3.62
18:3 n 6	0.15	0.72	0.38	0.22	0.19	0.17	0.46	0.57	0.07	0.07
20:3 n 6	0.03	0.00	0.00	0.07	0.02	0.00	0.11	0.58	0.31	0.20
20:4 n 6	1.48	1.92	0.81	1.23	3.89	2.88	2.53	4.49	2.54	2.75
22:2 n 6	2.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
n6	9.49	9.93	5.92	8.57	8.44	6.09	6.30	10.10	7.50	6.64
n3:n6	1.57	2.08	6.70	4.14	3.11	6.31	6.49	4.93	7.10	7.84
PUFA	25.02	31.09	45.62	44.42	35.25	42.94	45.30	47.30	56.22	57.11

Appendix 2. Annual biomass inputs of fatty acids from senesced vegetation.

Fatty acid	Annual Biomass Input (g/m^2/year) Fresh Alder	Fresh hemlock
14:0	10.5	3.75
15:0	1.33	0.66
16:0	90.58	20.1
17:0	1.96	0.85
18:0	24.08	11.98
20:0	12.92	4.08
22:0	22.86	2.75
23:0	1.51	3.02
24:0	13.06	3.97
SAFA	178.82	51.17
14:1	1.23	0.32
15:1	0	0.81
16:1	5.18	2.38
17:1	0.46	0.84
18:1n9	44.38	14.76
20:1	1.05	0
22:1n9	0	0.26
24:1	0	0
MUFA	51.21	19.37
18:3 n 3	66.43	8.79
18:4 n 3	6.23	0.74
20:5 n 3	2.73	0
20:3 n 3	0.77	0.29
22:6 n 3	0.95	5.38
n3	77.11	15.20
18:2 n 6	38.15	11.20
18:3 n 6	0.32	0.32
20:3 n 6	0	0
20:4 n 6	1.54	0
22:2 n 6	0	0
n6	40.01	11.52
n3:n6	7.32	1.52
PUFA	119.70	28.92
SAFA:(MUFA+PUFA)	3.68	1.06