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Elevated light and nutrients alter the nutritional quality of stream periphyton

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SUMMARY

1. The biochemical composition of primary food resources may affect secondary production, growth, reproduction and other physiological responses in consumers and may be an important driver of food-web dynamics. Changes in land use, riparian clearing and non-point nutrient inputs to streams have the potential to alter the biochemical composition of periphyton, and characterising this relationship may be critical to understand the processes by which environmental change can affect food webs and ecosystem function.

2. We conducted a manipulative, *in situ* experiment to examine the effect of light and nutrient availability on stream periphyton biomass, nutrient content, stoichiometry and fatty acid composition. Greater light increased periphyton biomass [chl-*a*, ash-free dry mass (AFDM)], periphyton carbon concentrations and monounsaturated fatty acids (MUFAs), but decreased saturated fatty acids (SAFAs). Greater light availability also increased levels of <20C polyunsaturated fatty acids (PUFAs), but decreased quantities of several long-chain (20–22 C), highly unsaturated fatty acids (HUFAs).

3. Nutrient (+N, +P) addition had no significant effect on periphyton biomass in the study streams, but did increase periphyton carbon content. For fatty acids, despite non-significant effects on periphyton biomass, nutrient additions resulted in an increased ratio of SAFA to PUFA, greater concentrations of stearidonic acid (18:4ω3) and near-significant increases in α-linolenic acid (18:3ω3). Nutrient additions also resulted in decreased quantities of the physiologically important docosahexaenoic acid (DHA; 22:6ω3) and increased the ratio of omega-3 to omega-6 fatty acids. An interaction between nutrient addition and greater light also resulted in decreased percentages of another physiologically important fatty acid, eicosapentaenoic acid (EPA; 20:5ω3).

4. Results from this study suggest that increased light and nutrients, a condition common in urban and agricultural catchments, would increase 18C PUFA intermediates that would be available in periphyton food sources, but decrease the availability of essential 20-22C HUFAs. If stream consumers are unable to elongate PUFAs into essential HUFAs, there may be important effects on food webs, including shifts by invertebrate consumers from herbivory to omnivory or carnivory, or changes in the species composition of aquatic consumers.

5. Our results demonstrate that fatty acid measures were more sensitive to changes in light and nutrient conditions than more traditional measures of biomass, nutrient content and stoichiometry. We recommend routine measurement of fatty acid content in monitoring the effect of changing environmental conditions in rivers and streams, particularly studies on food quality and ecosystem health.

Keywords: fatty acids, food quality, light, nutrients, periphyton

Introduction

Primary production and the availability of food resources have traditionally been understood to be key factors limiting consumer productivity in aquatic food webs, but recent studies have demonstrated that biochemical composition, also known as food quality, is one of the most important factors in trophic efficiency

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and energy transfer across the plant–animal interface (Sterner & Hessen, 1994; Brett & Müller-Navarra, 1997; Müller-Navarra *et al.*, 2000; Rosemond, Mulholland & Brawley, 2000; Urabe *et al.*, 2002; Hill, Smith & Stewart, 2010). Food webs that lack key biochemical components within primary producers may demonstrate trophic decoupling, leading to a significant increase in primary productivity with little corresponding increase in consumer biomass (Brett & Müller-Navarra, 1997).

Algae are a fundamental food source for aquatic consumers due to their higher nutrient content, lower C : N : P ratios and greater concentrations of biologically essential fatty acids (EFAs) than in allochthonous material (Delong & Thorp, 2006; Torres-Ruiz, Wehr & Perrone, 2007; Lau, Leung & Dudgeon, 2008; Brett *et al.*, 2009). Studies of lake ecosystems have shown that fatty acid content is a better predictor of secondary productivity than nutrient stoichiometry and that previous results using nitrogen and phosphorus contents as a measure of food quality may be actually driven by co-occurrence of specific EFAs (Müller-Navarra, 1995). Fatty acids serve a vital function in a wide range of important physiological roles, including maintenance of membrane fluidity at low temperatures (Michaud & Denlinger, 2006). Saturated fatty acids (SAFAs), which contain no C-C double bonds, and monounsaturated fatty acids (MUFAs), which contain one C-C double bond, are readily synthesised by most organisms and serve as energy storage molecules (Napolitano, 1994). Highly unsaturated fatty acids (HUFAs), a subclass of long-chain ($\geq$20 C) polyunsaturated fatty acids (PUFAs) that contain more than 2 double bonds, are necessary for macroinvertebrate growth and reproduction (Ederington, McManus & Harvey, 1995; Demott & Müller-Navarra, 1997; Brett *et al.*, 2009), successful metamorphosis (Stanley-Samuelson & Dadd, 1984) and food conversion efficiency (Brett & Müller-Navarra, 1997; Müller-Navarra *et al.*, 2000). However, very few animals are known that can directly synthesise HUFAs *de novo* or elongate specific PUFA precursors (e.g. 18:3ω3, 18:3ω6, 20:3ω6) into biologically important HUFAs (Stanley-Samuelson, 1994; Brett & Müller-Navarra, 1997; Torres-Ruiz, Wehr & Perrone, 2010). Thus, most consumers must acquire HUFAs from their food. Since algae generally contain greater concentrations of fatty acids for aquatic consumers than do allochthonous sources (Torres-Ruiz *et al.*, 2007, 2010) and certain algal taxa produce characteristic fatty acids, such as 20:5ω3 in many diatoms (Parrish *et al.* 1995, Napolitano *et al.* 1990) and 18:3ω3 in green algae (Ahlgren, Gustafsson & Boberg, 1992; Steinman *et al.*, 2011; Torres-Ruiz *et al.*, 2010), it is important to understand how environmental change may affect the concentration and composition of these limiting resources.

The biochemical composition of algae has been shown to be partly dependent on in-stream habitat conditions. In particular, the stream environment may be affected by changing land use, especially through the loss of riparian shading and increased nutrient loading (Allan, 2004). Light and nutrient availability have previously been shown to be important factors driving food quality. For example, experiments under lake and laboratory conditions have demonstrated interacting effects of light and nutrients (P and N) on nutrient concentrations in periphyton (Hillebrand & Kahlert, 2001), phytoplankton (Hessen, Færøvig & Andersen, 2002) and zooplankton (Urabe *et al.*, 2002). Substantially less research has focused on the effects of light and nutrients on algal fatty acid composition. Data suggest that greater light levels may result in decreased PUFA concentrations in phytoplankton through oxidative damage (Guschina & Harwood, 2009), while laboratory experiments have indicated that elevated light may decrease ω3-FAs in the unicellular alga *Nannochloropsis* sp. (Fábregas *et al.*, 2004). In addition, nutrient limitation may cause an increase in the concentrations of shorter-chain FAs and a decrease in long-chain FAs in marine and freshwater phytoplankton (Reitan, Rainuzzo & Olsen, 1994; Elser, Hayakawa & Urabe, 2001). These studies have primarily examined the effects of light and nutrients under controlled laboratory experiments or in lake plankton studies, but few data exist on the effect of environmental conditions on the biochemical composition of algae in streams. One study examined the effect of light and nutrients on biochemical composition in an experimental flume system (Hill, Rinchard & Czesny, 2011), demonstrating that the proportion of SAFAs and MUFAs in periphyton increased in response to elevated light levels, but declined in response to phosphorus addition.

Here, we extend prior studies with validation in a field setting. We predicted that an open canopy (resulting in greater light availability) would increase periphyton biomass in streams and energy storage (based on SAFA and MUFA concentrations), but decrease nitrogen, phosphorus, PUFA and HUFA concentrations in periphyton. We hypothesised that nutrient addition would increase periphyton biomass, nitrogen and phosphorus concentrations, and PUFA and HUFA concentrations, but decrease periphyton C : N and C : P ratios, and SAFA and MUFA concentrations. Overall, we predicted greater light and higher nutrient concentrations should decrease food quality, mainly due to decreased PUFAs and HUFAs, even when increasing food quantity.

Methods

Study sites

This study was conducted in three streams, the Bronx, Muscoot and Saw Mill Rivers, located in Westchester County, New York, USA. The streams are located in predominantly suburban, forested catchments, dominated by cobble and boulder substrata, and have semi-eutrophic conditions (Table 1). The Saw Mill River site is a fourth-order forested stream that starts in Chappaqua, New York, runs south–southwest and empties into the Hudson River in Yonkers, New York. The sample reach was located in Hawthorne, New York. The Bronx River site is a third-order forested stream, which begins in Valhalla, New York, flows south and empties into the East River in New York City. The sample reach was located in Bronxville, New York. The Muscoot River site is a third-order stream in a forested catchment that starts at the Amawalk Reservoir and empties into the Croton Reservoir, and has been described in a previous study (Torres-Ruiz *et al.*, 2007). The sample reach was located approximately 4 km downstream of the Amawalk Reservoir.

Pre-treatment tile colonisation

The experiment was designed to evaluate the effects of nutrient additions and light availability on existing (rather than colonising) periphyton assemblages, so tiles were pre-incubated *in situ* prior to initiating treatments. Light availability was measured in each river with a LI-COR quantum sensor (LI-COR Environmental, Lincoln, NE, U.S.A) when conditions were uniformly overcast at midday to quantify differences between open-canopy and closed-canopy reaches (<15% irradiance of open canopy). Beginning on 2 July 2010, unglazed terracotta tiles (62.5 cm^2) were placed in each open-canopy reach, allowing for algal colonisation prior to the start of experimental treatments. For the Bronx and Saw Mill, periphyton was allowed to colonise over 22 days. This colonisation time was based on previous colonisation experiments and the time required for biomass to plateau in these systems (M. Cashman, unpubl. data). Due to greater stream velocity and lower water temperatures, Muscoot tiles had a lower rate of colonisation and were allowed 39 days for colonisation.

Experimental design

At the end of the pre-treatment colonisation period, four tiles from each river were randomly sampled to establish initial (0 days) conditions. The remaining tiles were subjected to a 2 × 2 factorial experiment (treatments: light, nutrients) across the three streams, which functioned as blocks (4 treatments × 4 replicates × 3 blocks = 48 tiles). The light treatment consisted of a 20-m reach of pre-existing open canopy or shaded, closed canopy (<15% irradiance of open) at each site. Nutrient enrichment was added via mesh plastic bags containing 80 g of Osmocote® Smart-Release Plant Food fertiliser (14% N, 14% P, 14% K, Scott's®, www.scotts.com) attached to the upstream edge of the tile (e.g. Steinman *et al.*, 2011). Mesh plastic bags containing 80 g of similar-sized non-diffusing plastic beads functioned as a control on the effect of experimental design on flow. These 'ambient' tiles, which were exposed only to ambient stream nutrient conditions, were placed approximately 5 m upstream from nutrient tiles to minimise cross-contamination. Nutrient enrichment plastic bags were replaced with new bags 15 days into the experimental period to ensure constant diffusion throughout the experiment. Nutrient enrichment was determined to increase ambient concentrations of NO_3^- by 2%, NH_4^+ by 4% and TDP by 12%.

At the end of the full experimental period (+31 days), tiles were sampled for periphyton using a razor blade and rinsed with stream water according to Stevenson & Bahls (1999). The collected suspension was mixed and immediately placed on ice. Upon return to the laboratory, samples were stored in a refrigerator at 4 °C and processed within 48 h.

Table 1 Nutrient concentrations (n = 18) and other characteristics (n = 2) of the three study streams

	Sample latitude (N)	Sample longitude (W)	Stream order	Width (m)	Current velocity (m s^{-1})	Temperature (°C)	pH	Specific conductance (μS cm^{-1})	NH_4^+ (μg N L^{-1})	NO_3^- (μg N L^{-1})	TDP (μg P L^{-1})
Bronx	40°56′32″	73° 50′16″	3	8.7	0.29	25.3	8.0	972	93.4 ± 36.9	398 ± 65	32.3 ± 2.4
Muscoot	41°16′18″	73°44′18″	3	8.4	0.80	15.8	7.7	502	99.4 ± 10.1	1041 ± 228	26.0 ± 4.4
Saw Mill	41°5′42″	73°48′47″	4	6.4	0.35	20.6	8.2	851	60.1 ± 5.9	719 ± 23	23.6 ± 2.9

Periphyton processing and laboratory analyses

Periphyton samples were homogenised using a hand blender for 10 s, followed by processing for chlorophyll-*a* (chl-*a*), ash-free dry mass (AFDM) and nutrient stoichiometry (C : N : P). Dry mass (DM) and AFDM were quantified by filtering the suspension through a pre-ashed, pre-weighed, Whatman GF/F 47-mm glass fibre filter (Whatman International, Florham Park, NJ, U.S.A). Filters were dried overnight at 80 °C, weighed for DM, ashed at 450 °C for 2 h and reweighed for final AFDM values. Phaeophytin-corrected chlorophyll concentrations were measured following extraction with 90% buffered acetone (Lorenzen, 1967; Arar, 1997). Algal carbon (algal-C) and nitrogen (algal-N) contents were analysed from aliquots dried in 9 × 10 mm tin cups using a Thermo Scientific Flash 2000 CHN analyzer (Thermo Scientific, West Palm Beach, FL, U.S.A). Algal phosphorus (algal-P) was measured after digestion following Solorzano & Sharp (1980) and analysed as SRP (Kopp & McGee, 1983). All stoichiometric ratios were determined on a molar basis.

Fatty acid analysis

Fresh periphyton suspensions were filtered onto pre-ashed, Whatman GF/F glass fibre filters (Whatman International, Florham Park, NJ, U.S.A) and stored under N_2 at −30 °C until extraction. Extraction followed a modified method of Parrish (1999) as adapted by Torres-Ruiz *et al.* (2007). Filters were ground using a tissue homogeniser (BioSpec Products, Bartlesville, OK, U.S.A), and lipids were extracted in chloroform/methanol (2 : 1 v/v). The extract was methylated with BF_3 (10–15% w/v in methanol), suspended in hexane and concentrated under N_2. Resulting fatty acid methyl esters (FAMEs) were measured on a Shimadzu GC-2014 gas chromatograph fitted with a Supelco™ Omegawax 320 capillary column (30 m × 0.32 mm; Sigma-Aldrich, St. Louis, MO, U.S.A) running in splitless mode. Methyl nonadecanoate (19:0) was used as an internal standard, and individual peaks were identified by comparing retention times using a Supelco 37 Component FAME mix (Supelco-Sigma-Aldrich, St. Louis, MO, U.S.A). Quantitative analysis was conducted using GCSolution software (Shimadzu Scientific Instruments, Columbia, MD, U.S.A), and automatic peak detection was determined with the settings of width = 3 s, slope = 2000 μV min^{-1}, drift = 0 μV min^{-1}, T.DBL = 0 min and minimum area/height = 1000 counts. Any peak less than <0.5% of total peak area was defined as undetectable and ignored unless detected at sufficient concentrations ($\geq$0.5%) in other samples. Individual FA concentrations were calculated from a standard concentration curve of known FA concentrations calibrated against the internal standard. Calibration curves were linear and highly significant, but slopes varied with degree of saturation and chain length, as observed in previous studies (Ahlgren *et al.*, 1992, Torres-Ruiz *et al.*, 2007). Fatty acids were examined by two methods: FA concentration (standardised per unit dry mass) and percentage FA (% of total fatty acids in sample).

Statistical analysis

A two-way factorial design examined the effect of light and nutrient addition (treatment variables) on periphyton biomass, nutrient content and fatty acid composition using a general linear model (GLM) with 'river' as a block. This also permitted a test of light x nutrient interaction effects. Data were treated as the percentage change ('net change') of periphyton biomass and chemistry from pre-treatment conditions ('initial', 0 days) to the endpoint of the study ('final', +31 days). Net change was used to standardise for different starting conditions in each river and to account for relative effect sizes of each treatment, as has been used in similar experimental designs (e.g. Steinman, Mulholland & Beauchamp, 1995). Data were log- or square-root-transformed as appropriate to best fit the assumptions of normality and equal variance. Statistical analyses were performed using Systat v. 13, and α was set at 0.05 for all tests (Systat, Chicago, IL, U.S.A).

Results

Periphyton biomass

Across all streams, tiles under open canopy had significantly greater accumulations of biomass as measured by AFDM (F = 35.2, P < 0.0001; Fig. 1a) and chl-*a* (F = 16.2, P = 0.0003) compared to tiles under a closed canopy. There was no significant effect of nutrient addition on net accumulation of AFDM (F = 0.355, P = 0.5550) or chl-*a* (F = 1.68, P = 0.2028), but there was a significant interaction of canopy x nutrient enrichment, resulting in greater chl-a concentrations (F = 6.72, P = 0.0135).

Nutrient content and stoichiometry

Periphyton nutrient concentrations (C, N, P) had few significant responses to either treatment type, although

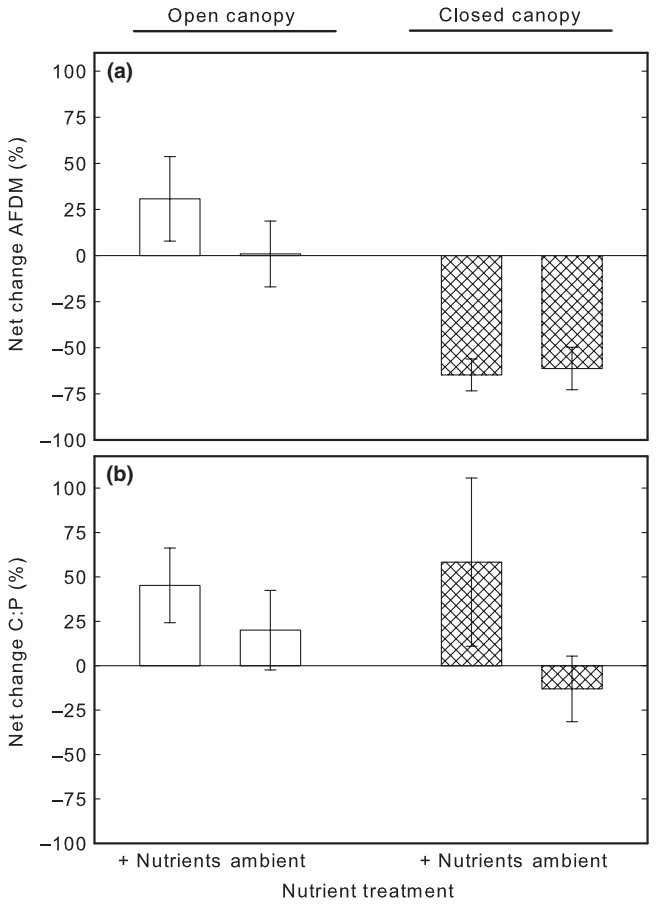

Fig. 1 Net change in (a) ash-free dry mass (AFDM) values and (b) C : P ratios from initial conditions (0 days) to the end point of the study (+31 days) for all treatment types. Bars represent means ± 1 SE.

open-canopy conditions resulted in significantly greater periphyton-C concentrations than closed habitats ($F = 8.27$, $P = 0.0068$; data not shown). Periphyton-N and periphyton-P were not affected by either treatment (data not shown). Nutrient addition resulted in higher C : P ratios ($F = 4.39$, $P = 0.0434$; Fig. 1b), but no other stoichiometric ratios responded significantly to either treatment.

Fatty acid composition

SAFAs were the most abundant fatty acid category in the initial sampling of periphyton from all three streams (36–44% of total FA), and open-canopy conditions resulted in a significant decrease in SAFAs as a percentage of total FAs ($F = 18.3$, $P = 0.0001$; Table 2) and a near-significant decrease in absolute concentration per unit dry mass ($F = 4.07$, $P = 0.0512$; Table 3). More specifically, open canopy resulted in significantly decreased percentages of nearly all SAFAs, namely 17:0, 18:0, 20:0, 21:0 and 22:0 (Table 2). Open canopy also resulted in decreased concentrations of 15:0, 17:0, 18:0, 20:0, 21:0 and 22:0 (Table 3). Nutrient addition resulted in increased concentration of 17:0, but no other SAFA effects were detected (Table 3).

In the initial sampling, MUFA abundance was comparable to that of SAFAs in all three streams (36–42%), but in open canopy conditions MUFAs increased as a percentage of total FA ($F = 7.38$, $P = 0.0102$). This increase was specifically seen in increased percentages of 14:1 and 17:1 (Table 2), but a net decrease of 18:1ω9. The sum of MUFA concentrations did not change with either treatment type, but an open canopy resulted in consistent decreases in concentrations of 14:1, 15:1, 18:1ω9, 20:1ω9 and 22:1ω9, and greater concentrations of 18:1ω7 (Table 3). Nutrient enrichment resulted in near-significant decreased percentages of 17:1 (Table 2).

Polyunsaturated fatty acids accounted for 24–30% of total FA in the initial sampling of all three streams. The open-canopy treatment resulted in significantly greater PUFAs as a percentage of all FAs ($F = 16.5$, $P = 0.0003$), but did not significantly affect the total sum of PUFA concentrations. Open canopy resulted in an increased percentage of 18:2ω6 and greater percentages and concentrations of 16:4ω1, 18:3ω3 (α-linolenic acid) and 18:4ω3 (stearidonic acid), as compared to closed-canopy conditions (Tables 2 and 3). Nutrient addition resulted in increased concentrations of 18:4ω3 (Fig. 2a), and an interaction between nutrient addition and open canopy increased concentrations of 18:3ω3 (Table 3).

The percentage and concentration of total HUFAs did not change with either treatment type; however, treatments did result in significant changes to specific HUFAs. Open canopy resulted in reductions in the concentration and percentages of several HUFAs, particularly 20:3ω3 and 20:3ω6 (Fig. 2), and also in 20:2 and 22:5ω3 (docosapentaenoic acid; Tables 2 and 3). Nutrient enrichment resulted in significantly decreased percentages of 20:2 and 22:6ω3 (Table 2). Together, an interaction between nutrient addition and open canopy resulted in decreased percentages of the essential fatty acid eicosapentaenoic acid (EPA, 20:5ω3).

The percentage of PUFA and the sum of total PUFA (∑ PUFA = (PUFA + HUFA)/total FA) increased with greater light availability ($F = 16.5$, $P = 0.0003$; $F = 4.88$, $P = 0.0335$), but did not increase per unit dry mass. In this fraction, the ratio of PUFAs to HUFAs significantly increased under increasing light conditions ($F = 7.48$, $P = 0.0096$), but there was no significant change in the percentage or absolute concentration of 20C HUFAs

Table 2 Results of 2-way GLM testing the effects of nutrient addition and light availability on relative concentrations of periphyton fatty acids expressed as a percentage of total FA.

		Light			Nutrients			Interaction		
Fatty acid	Transform used	*F*	*P*	+/−	*F*	*P*	+/−	*F*	*P*	+/−
SAFA										
17:0	Log	**8.593**	**0.0058**	−	2.706	0.1086		0.377	0.5432	
18:0	Sqrt	**27.988**	**<0.0001**	−	0.271	0.6061		3.107	0.0864	
20:0	Sqrt	**16.565**	**0.0002**	−	0.506	0.4814		0.375	0.5441	
21:0	Log	**4.614**	**0.0385**	−	0.009	0.0926		0.309	0.5819	
22:0	Log	**11.681**	**0.0016**	−	2.433	0.1275		0.000	0.9988	
MUFA										
14:1	Log	**9.446**	**0.0040**	**+**	3.151	0.0843		0.517	0.4769	
17:1	Sqrt	**13.114**	**0.0009**	**+**	3.688	0.0628		0.089	0.7671	
18:1ω9		**8.664**	**0.0056**	−	1.037	0.3154		0.842	0.3651	
PUFA										
16:4ω1	Log	**17.597**	**0.0002**	**+**	0.571	0.4547		0.078	0.7813	
18:2ω6	Log	**6.508**	**0.0151**	+	2.527	0.1207		1.303	0.2612	
18:3ω3	Sqrt	**29.721**	**<0.0001**	**+**	1.771	0.1917		**5.200**	**0.0286**	**+**
18:4ω3	Log	**34.669**	**<0.0001**	**+**	0.603	0.4424		2.484	0.1238	
HUFA										
20:2	Log	**4.450**	**0.0419**	−	**9.098**	**0.0047**	−	0.143	0.7070	
20:3ω3	Log	**10.397**	**0.0027**	−	0.225	0.6382		0.138	0.7121	
20:3ω6	Log	**7.144**	**0.0112**	−	0.107	0.7451		0.096	0.7590	
20:5ω3	Log	0.018	0.8942		0.248	0.6218		**6.476**	**0.0154**	−
22:5ω3	Log	**4.130**	**0.0496**	−	0.008	0.9275		0.022	0.8826	
22:6ω3	Log	0.004	0.9458		**7.091**	**0.0115**	−	0.121	0.7300	

HUFA, highly unsaturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SAFA, saturated fatty acids. FAs are grouped into SAFA, MUFA, PUFA and HUFA classifications ['+/−' indicates whether treatment increased (+) or decreased (−) FA percentages; significant effects in boldface].

with light availability. Nutrient enrichment resulted in a decreased percentage of HUFAs ($F = 5.34$, $P = 0.0446$) and significantly increased the ω3/ω6 ratio of fatty acids in the periphyton ($F = 7.06$, $P = 0.0117$; Fig. 3).

Discussion

The key objective of this study was to examine the effects of light and nutrient availability on both quantity (biomass) and nutritional quality (nutrient and FA content) of established periphyton assemblages in temperate streams. Enhanced light conditions and nutrient enrichment each resulted in significant but different changes in the biochemical composition of algal assemblages in the study streams. Greater light availability provided by an open canopy resulted in changes in periphyton biomass, carbon concentration and fatty acid composition. The effect of nutrient addition was less pronounced than the effects of light availability, most likely due to the existing elevated ambient nutrient conditions. Data indicate that periphyton growth in these streams was primarily light limited, as nutrient additions resulted in increased algal biomass only in open-canopy conditions. Importantly, even in conditions where a biomass effect was not detected, nutrient addition resulted in several significant changes in nutrient content and fatty acid composition, most notably increased C : P ratio, ω3:ω6 ratio, and changes to specific FA abundances and concentrations, especially of the essential fatty acid 22:6ω3 (docosahexaenoic acid, DHA). Such results demonstrate that changes in riparian cover and increased nutrient loads collectively can have profound effects on the biochemical, and hence nutritional, composition of periphyton food resources even when changes in biomass may not be evident.

Greater light availability not only significantly increased the concentration of fatty acids in periphyton (per unit dry mass), but also significantly altered the FA fractions that were available. As the relative percentage of SAFAs decreased with greater light, there was a corresponding increase in MUFAs and 16–18C PUFAs. While the relative abundance of 20–22C long-chain HUFAs did not change with greater light availability, nutrient addition resulted in a decrease in the proportion of HUFAs.

We predicted increases in MUFAs and SAFAs under greater light, as they are associated with energy storage in nutrient-limiting conditions (Napolitano, 1994) and as

Table 3 Results of 2-way GLM testing the effects of nutrient addition and light availability on periphyton fatty acid concentrations (with stream as a block).

		Light			Nutrients			Interaction		
Fatty acid	Transformused	*F*	*P*	+/−	*F*	*P*	+/−	*F*	*P*	+/−
SAFA										
15:0	Log	**10.023**	**0.0031**	−	3.213	0.0815		0.017	0.8980	
17:0	Log	**13.013**	**0.0009**	−	**6.253**	**0.0171**	+	0.422	0.5196	
18:0	Log	**21.867**	**<0.0001**	−	0.449	0.5069		0.649	0.4259	
20:0	Log	**22.949**	**<0.0001**	−	1.174	0.2858		0.076	0.7845	
21:0	Log	**12.700**	**0.0011**	−	2.358	0.1334		0.635	0.4308	
22:0	Log	**23.779**	**<0.0001**	−	0.159	0.6922		0.072	0.7895	
MUFA										
14:1	Log	**12.269**	**0.0012**	−	0.014	0.9064		0.528	0.4720	
15:1	Log	**5.625**	**0.0232**	−	0.004	0.9528		0.026	0.8723	
18:1ω7	Log	3.820	0.0584	−	0.428	0.5169		0.023	0.8800	
18:1ω9	Log	**6.270**	**0.0169**	−	0.260	0.6131		0.440	0.5112	
20:1ω9	Log	**6.788**	**0.0133**	−	0.012	0.9135		0.408	0.5272	
22:1ω9	Log	**9.285**	**0.0044**	−	0.059	0.8100		0.461	0.5016	
PUFA										
16:4ω1	Log	**6.421**	**0.0158**	+	0.032	0.8585		0.007	0.9710	
18:3ω3	Sqrt	**5.930**	**0.0200**	+	2.968	0.0935		2.568	0.1177	
18:3ω6	Log	1.256	0.2699		3.387	0.0739		0.385	0.5387	
18:4ω3	Sqrt	**10.828**	**0.0022**	+	**5.289**	**0.0274**	+	4.054	0.0516	
HUFA										
20:2	Log	**9.989**	**0.0032**	−	3.551	0.0676		0.310	0.5814	
20:3ω3	Log	**17.155**	**0.0002**	−	2.511	0.1218		0.266	0.6090	
20:3ω6	Log	**12.416**	**0.0012**	−	1.000	0.3240		0.008	0.9295	
22:5ω3	Sqrt	**13.340**	**0.0008**	−	3.781	0.0597		0.073	0.7887	

HUFA, highly unsaturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SAFA, saturated fatty acids. FAs are grouped into SAFA, MUFA and PUFA classifications ['+/−' indicates whether treatment increased (+) or decreased (−) FA concentration as a percentage of dry mass; all interaction effects detected were between high-light and nutrient enrichment; significant effects in boldface].

has been demonstrated in one flume-based study (Hill *et al.*, 2011). However, our results with *in situ* experiments showed that the proportions of SAFAs decreased in greater light availability, perhaps suggesting different functions of these two classes of fatty acids. Irradiances recorded in our study in open-canopy conditions were 2- to 10-fold greater than the maxima experimentally supplied by Hill *et al.* (2011) and may account for the different responses in SAFAs. The SAFA responses in our study may also have been influenced by temperature differences, as overall mean temperatures were ~12 °C in the experimental stream study by Hill *et al.* (2011), substantially lower than the streams in this study (15–25 °C). Certain classes of fatty acids are integral to the maintenance of cell membrane fluidity (Arts & Kohler, 2009), and the percentage of SAFAs has been shown to be greater in high temperatures and decreased in cold temperatures (Ravet, Brett & Arhonditsis, 2010). Thus, SAFA synthesis may be affected by light availability differently across temperature ranges.

Previous studies have shown greater light availability to decrease the proportion of 18–22C PUFAs in marine diatoms, perhaps due to photoacclimation (Thompson, Harrison & Whyte, 1990; Thompson, Guo & Harrison, 1993; Leu *et al.*, 2010) and oxidative damage (Guschina & Harwood, 2009). In contrast, our results showed that greater light resulted in increased proportions of shorter-chain 16–18C PUFAs in stream periphyton. In particular, greater light resulted in increases in several notable PUFA precursors: 18:2ω6, 18:3ω3, 18:3ω6 and 18:4ω3. Although there was no change in the overall proportion of long-chain HUFAs with light, there were consistent declines among those HUFAs that responded to light, including 20:3ω3, 20:3ω6 and 22:5ω3, which are generally regarded as essential for most animals (Brett *et al.*, 2009).

Nutrient additions in our study resulted in significantly greater concentrations (per dry mass) of 17:0 and several PUFAs (18:3ω6, 18:4ω3 and 20:3ω6), despite negligible effects on algal biomass. Such a biochemical shift

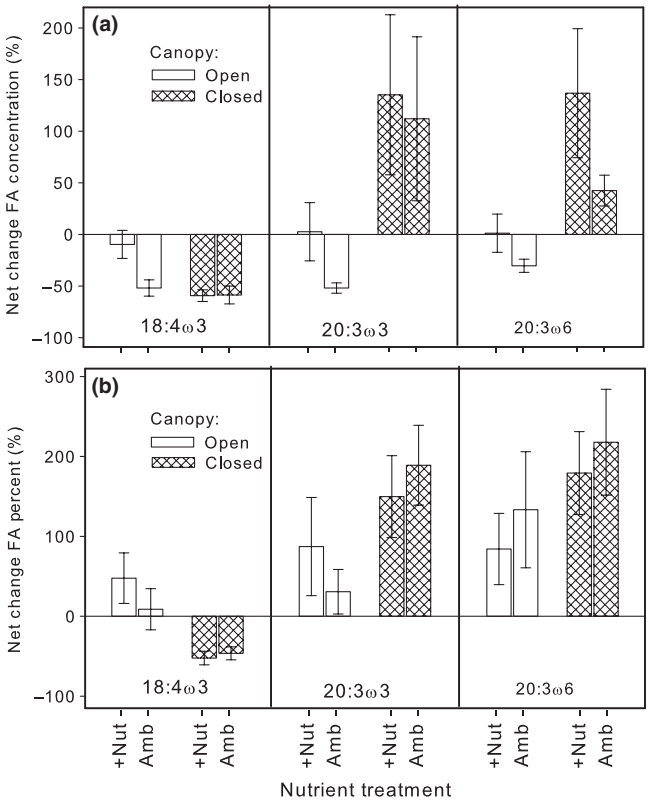

Fig. 2 Net change in (a) selected polyunsaturated fatty acids (PUFA) concentrations and (b) selected%-PUFA (% of total FAs) from initial conditions (0 days) to the end point of the study (+31 days) for light (canopy: open or closed) and nutrient treatments. Bars represent means ± 1 SE.

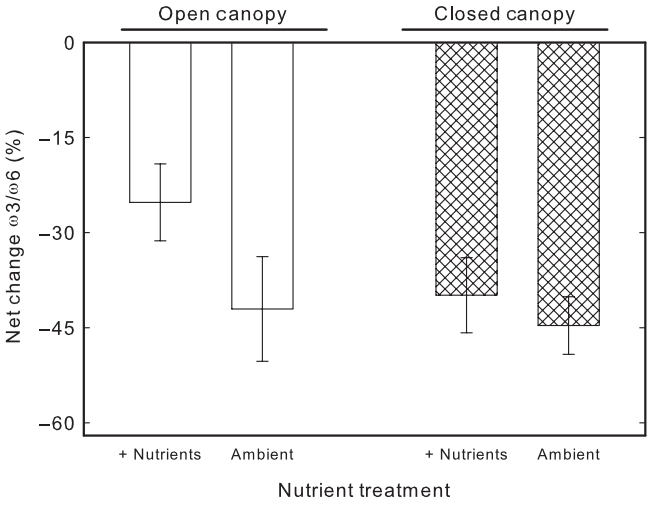

Fig. 3 Net change in ω3/ω6 ratio from initial conditions (0 days) to the end point of the study (+31 days) for all treatment types. Bars represent means ± 1SE.

may be ecologically significant. The FA 18:4ω3 is a precursor of essential fatty FAs 20:5ω3 (EPA) and 22:6ω3 (DHA), while 18:3ω6 and 20:3ω6 are precursors of arachidonic acid (20:4ω6), an essential ω6-HUFA. However, while nutrient addition increased concentrations of these precursors, it also decreased percentages of DHA in the periphyton and of EPA when in combination with elevated light levels. DHA is a component of neural cellular membranes in fish and is found in high concentrations in fish eggs (Sargent *et al.*, 1995), while EPA is necessary for the synthesis of hormone-like eicosanoids (Stanley-Samuelson, 1994; Heckmann *et al.*, 2008). Both of these FAs are required for proper cellular membrane functioning (Arts & Kohler, 2009). Therefore, reductions in DHA and EPA in algal assemblages could limit the production of macroinvertebrates (Müller-Navarra *et al.*, 2000; Arendt *et al.*, 2005) and ultimately of fish (Parrish, 2009). Lastly, nutrient addition in our study also resulted in a greater proportion of ω6 to ω3 fatty acids in the algae, which suggests decreased overall food quality, since consumers must balance their dietary proportions of ω3 to ω6 PUFAs for correct physiological function (Sargent, McEvoy & Bell, 1997).

The fatty acid compositional change seen in our study may have occurred through two main pathways: a change in biochemical and cellular pathways and also a change in algal species composition. While we did not specifically examine species composition, changes in concentrations of some characteristic fatty acids, such as 20:5ω3 (common in diatoms; Parrish *et al.*, 1995, Napolitano *et al.*, 1990) and 18:3ω3 (common in green algae; Ahlgren *et al.*, 1992), suggest a taxonomic shift from diatoms to green algae as a result of these treatments. Further experiments are planned that will test these alternative explanations.

The results of our study reveal a complex set of relationships between light, nutrients and nutritional composition of periphyton, and also how biochemical changes may influence food quality for consumers. Physiological threshold limits, or the minimum amount of specific FAs necessary in consumer diets, have not yet been determined for most EFAs in aquatic ecosystems. In addition, these dynamics may depend on the ability of stream consumers to elongate and convert FA precursors into specific long-chain essential FAs. In our study, greater light availability resulted in greater concentrations and percentages of PUFA precursors in periphyton, but also in lower proportions and concentrations of HUFAs. Few macroinvertebrates species are known to have the ability to elongate these 16- and 18-C PUFA precursors into physiologically important HUFAs, but this ability may vary among species (Ahlgren, Vrede & Goedkoop, 2009). This ability has been shown to be lacking in the net-spinning *Hydropsyche* sp., a common

stream consumer (Torres-Ruiz *et al*., 2010). Therefore, in forested streams where Hydropsychid caddisfly larvae are common, factors that limit essential HUFAs may result in a nutrient-limited consumer community. Such biochemical constraints may lead to increased carnivory among consumers, shift the consumer community composition towards taxa that have the ability to elongate PUFA precursors into essential HUFAs, or may result in reduced secondary production.

More broadly, our data suggest that changes to the local catchment, through nutrient inputs and/or canopy removal, may lead to trophic instability by increasing dependence of the food web on a limited number of species able to elongate these precursors or synthesise essential HUFAs. Previous studies have reported changes in macroinvertebrate and fish populations following riparian clearing in some systems (Bilby & Bisson, 1992). Other studies have observed that open canopy can result in significant decreases of shredders in favour of other feeding guilds (Hawkins, Murphy & Anderson, 1982), and increased numbers of Chironomidae, *Baetis* sp., *Drunella* sp. and *Cinygmula* sp., but decreased numbers of Simuliidae (Behmer & Hawkins, 1986). However, since there is still very little known about the biochemical abilities of these species and feeding groups, it is unknown whether such community shifts may have been affected by an (in)ability of consumers to utilise FA precursors. Changes in food quality may underlie many other observed shifts in community composition in relation to physical habitat conditions, but these connections have not yet been studied. Identifying species that have or lack the ability to elongate PUFA precursors may be important to understand the previously unknown importance of individual species in regulating ecosystem stability.

Light and nutrient treatments significantly altered the biochemical composition of periphyton in our study streams, with fatty acid concentration (and their percentages) most sensitive to these treatments. Recent studies have shown that moderate, chronic nutrient enrichment may have little detectable effect on periphyton biomass in streams (Greenwood & Rosemond, 2005), but our study suggests that biomass measures alone may miss a vital aspect of periphyton responses. Small biomass effects may be judged to be of minor concern, but our data suggest that changes to habitat conditions can result in significantly altered biochemical composition, which has the potential to profoundly affect stream food webs. In our study, light and nutrient treatments led to significantly increased concentrations of PUFA precursors and decreased concentrations of specific HUFAs. This may result in an increased pressure on stream consumers to obtain essential fatty acids. However, few of these processes have been incorporated into current models of stream food webs or the roles of consumers. Our study suggests that greater importance should be placed on a deeper understanding of the biochemical composition of food sources, especially in the face of environmental change. Further research is especially needed to determine the abilities of stream consumers to synthesise or elongate PUFA precursors into physiologically important HUFAs and their role in stabilising ecosystems with limiting HUFA availability.

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