Spatial variation in periphyton fatty acid composition in subtropical streams

Fen Guo*, †, Martin J. Kainz‡, Fran Sheldon*, Stuart E. Bunn*
*Australian Rivers Institute, Griffith University, Nathan, Queensland, Australia
†State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing, People’s Republic of China
‡WasserCluster Lunz – Inter-university Centre for Aquatic Ecosystem Research, Lunz am See, Austria

Author for correspondence: Fen Guo, fen.guo@griffithuni.edu.au

Postal address:
Australian Rivers Institute,
Griffith University, Nathan campus,
170 Kessels Road, Nathan, Queensland 4111, Australia

Running head: Periphyton fatty acid composition in subtropical streams

Keywords: food quality, food webs, riparian canopy, nutrients, grazers

Abbreviations: FA, fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; DHA, docosahexaenoic acid (22:6ω3); EPA, eicosapentaenoic acid (20:5ω3); ARA, arachidonic acid (20:4ω6); ALA, α-linolenic acid (18:3ω3); LIN, linoleic acid (18:2ω6); palmitic acid (16:0); palmitoleic acid (16:1ω7).
SUMMARY

1. Benthic algae play an important role in many stream food webs, not only serving as a major carbon source but also influencing energy transfer as the primary source of polyunsaturated fatty acids (PUFA). Dietary deficiency in PUFA can limit animal growth and reproduction. Algal PUFA content can vary considerably in response to abiotic factors.

2. We conducted a field investigation in streams in South-East Queensland, Australia, to identify environmental factors affecting the fatty acid composition of periphyton.

3. Riparian canopy cover and NO$_x$-N concentrations explained most of the observed variation in periphyton fatty acid profiles. NO$_x$-N concentrations showed negative relationships with most saturated fatty acids (SAFA), while mono-unsaturated fatty acids (MUFA) and the fatty acids 16:0 and 16:1$\omega$7 were negatively correlated with canopy cover. In contrast, the percentage of highly unsaturated fatty acids (HUFA) in periphyton was greater with increasing canopy cover regardless of NO$_x$-N concentrations.

4. Variation in riparian canopy cover and nutrients gave rise to opposite outcomes in terms of periphyton food quality and quantity. The highest periphyton food quality, measured by HUFA content, occurred in streams with high canopy cover and low nutrients, while the highest periphyton food quantity occurred in streams with low canopy cover.

5. Our study suggests that changes in riparian vegetation and/or nutrient inputs can significantly alter the fatty acid content of periphyton, with important implications for aquatic consumers. We recommend that future studies on impacts of riparian clearing or nutrient enrichment in stream ecosystems place more attention on the effects of food quality rather than quantity.
Introduction

Benthic algae play an important role in many streams as the trophic base of food webs, and influence energy transfer efficiency to consumers. In addition to their available biomass, algae directly affect the growth and reproduction of herbivores through their nutritional quality (Piepho, Arts & Wacker, 2012). Compared with allochthonous sources, algae are usually recognized as higher quality food for herbivores because of their high levels of essential elements (nitrogen and phosphorus) and polyunsaturated fatty acids (PUFA) (Hill, Rinchard & Czesny, 2011). The elemental limitation reflects the degree of nutrient constraints on consumers, and thus indicates food quality (Sterner, 2002), but it is insufficient to completely explain the energy transfer efficiency from primary sources to consumers (Müller-Navarra, 1995; Brett & Müller-Navarra, 1997). PUFA, in particular eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA, 22:6ω3) and arachidonic acid (ARA, 20:4ω6), are only abundant in certain groups of algae, but are required for animal growth and reproduction. They help regulate cell membrane fluidity and serve as precursors for animal hormones (Brett & Müller-Navarra, 1997). In spite of their nutritional importance, most aquatic consumers are unable or have limited ability to synthesize PUFA de novo from saturated fatty acids (Brett & Müller-Navarra, 1997; Kelly & Scheibling, 2011). Animals must thus obtain PUFA from their diets to meet physiological requirements. Therefore, PUFA in aquatic organisms have been widely studied to assess food quality, trophic relationships and even ecosystem functions of aquatic systems.

Algae are the most efficient primary producers of PUFA (Sushchik et al., 2006). Elevated content of highly unsaturated fatty acids (HUFA), a subset of PUFA with 20 or more C in their acyl chains, especially EPA and DHA, indicates higher algal food quality and consequently enhances animal growth and reproduction (Müller-Navarra, 1995; Brett & Müller-Navarra, 1997). Diatoms, containing EPA and DHA, are considered high quality food sources (Brett & Müller-Navarra, 1997; Torres-Ruiz, Wehr & Perrone, 2007), while green algae, often considered medium dietary quality, contain primarily shorter chain PUFA, such as α-linolenic acid (ALA, 18:3ω3) and linoleic acid (LIN,18:2ω6) (Napolitano, 1999). Cyanobacteria are poor in PUFA and generally lack sterols, another group of organic molecules important in vitamin production (Martin-Creuzburg, von Elert & Hoffmann, 2008). Among algal-derived PUFA, EPA is a good predictor of secondary production and can stimulate energy transfer to higher trophic levels in lakes, reservoirs and ponds (Müller-
A number of environmental factors can affect algal PUFA composition, and an understanding of these could potentially reveal the underlying mechanisms of ecosystem processes, such as energy flow from algae to herbivores and then ultimately to fish and humans. Light intensity, nutrient availability and temperature have been suggested to be important environmental factors affecting algal PUFA content (Guschina & Harwood, 2009), with each environmental factor differing in the mechanistic way in which it affects PUFA content. Algae adjust to low temperatures by increasing the degree of unsaturation within their fatty acids, leading to an increase in PUFA content, which can be incorporated into the lipids of thylakoid membranes and assist in maintaining membrane fluidity (Morgan-Kiss et al., 2006; Piepho, Arts & Wacker, 2012). Light stimulates algal fatty acid (FA) synthesis, growth, and the formation of membranes (particularly chloroplasts) (Guschina & Harwood, 2009). Low light generally increases the relative content of unsaturated fatty acids, whereas high light intensity is required for the synthesis of saturated fatty acids (SAFA), which are often used for energy storage (Brett & Müller-Navarra, 1997; Guschina & Harwood, 2006). Nutrient levels can also result in changes in algal PUFA composition. Nutrient limitation leads to a steady decline in cell division rate, which in turn slows down algal growth, and typically results in increasing cellular production of triacylglycerols (TAG) and decreasing proportions of PUFA in most algae (Guschina & Harwood, 2009), whereas greater aqueous nutrient levels cause an increase in the content of galactolipids, which are rich in PUFA (Guschina & Harwood, 2006). Despite these apparent broad patterns, studies often only focus on individual algal species with limited relevance to entire assemblages or community level processes.

Information on environmental factors affecting algal PUFA content in situ in stream ecology is limited. One study conducted in artificial streams with controlled light intensity and phosphorus concentrations (Hill, Rinchard & Czesny, 2011) demonstrated that stream periphyton FA profiles were highly sensitive to light intensity and phosphorous concentration: the proportion of PUFA decreased with light augmentation and increased with phosphorous enrichment, while SAFA and monounsaturated fatty acids (MUFA) increased with light intensity and decreased with phosphorous conditions. By contrast, a recent field study, with manipulated light levels and enriched fertilizer (Cashman, Wehr & Truhn, 2013), found that
open canopy resulted in an increase of MUFA percentage but decreases of SAFA and HUFA, while nutrient addition showed no significant effect on the proportions of SAFA, MUFA and most PUFA. Such differing results suggest that the PUFA content of periphyton in natural streams is often influenced simultaneously by different environmental factors. The above studies focused on light and nutrients as factors, but did not consider fully the interaction between these and other environmental conditions, such as stream habitat change, which could constrain their effectiveness.

The present study was carried out in natural streams across a gradient of riparian canopy cover. This is the first field investigation to test effects of environmental factors on algal FA content in subtropical streams. The key objective was to identify the environmental factors affecting FA composition of benthic algae in Australian subtropical streams. We predicted that (1) riparian canopy cover was more important than other factors in determining PUFA content; (2) the proportions of PUFA would increase with shaded canopy and decrease with open canopy, and (3) the interaction of different environmental factors may contribute to the inconsistent trends of some FA responses to individual environmental factors.

Methods

Study streams

The study streams are located in the Logan-Albert catchment in South-East Queensland, Australia (Figure 1). This area has a sub-tropical climate with distinct wet and dry seasons and about 70% of the annual precipitation occurs in summer (December to March). The upper catchment is heavily forested, the middle catchment is mainly used for cattle grazing, intensive agriculture and rural residential, while the lower catchment contains a mix of urban and rural residential development. Samples were collected from nine streams spanning the upper and middle catchment from November 14 – 27, 2012. In each stream, two riffles dominated by cobble substrata (upstream and downstream) with similar canopy cover were sampled. The distance between those two riffles was less than 100 meters.

Sample collection

Periphyton was scraped with brushes from the upper surface of cobbles. At each riffle, 4 independent replicate samples were collected for FA analysis, and 3 replicates for algal community structure. Terrestrial organic matter, macroinvertebrates and particles were
removed by hand to ensure relatively clean samples. All samples were immediately placed in zip-lock plastic bags, stored on ice and kept in the dark in a portable freezer. Samples were brought to the laboratory within 6 hours. All FA samples were placed at -80°C freezer until processing, and samples for taxonomic identification were kept in lugol’s iodine preservative and in the dark without refrigeration.

Riparian canopy cover was measured using a densiometer in situ (Table 1). At each riffle, duplicate ambient water samples were collected to determine the concentrations of total nitrogen (TN) and total phosphorus (TP). Ambient water samples were filtered in situ through 0.45 μm Sartorius Minisart (Sartorius Australia, East Oakley, Australia) single use filter to determine the ambient concentrations of dissolved inorganic nutrients, specifically nitrate + nitrite (NO$_3$-N), ammonium nitrogen (NH$_4$-N), and soluble reactive phosphorus (SRP). Samples were stored on ice and kept in the dark in a portable freezer in the field, and put at -20°C in the laboratory. Current velocity was measured by a current velocity meter (Open stream current velocity meter model 2100, Swoffer Instruments, Inc) (Table 1).

**Laboratory processing**

Periphyton samples for FA analysis were freeze-dried (Virtis Genesis Freeze Dryer). A sub-sample of 5-10 mg periphyton (dry mass) from each sample was used for lipid extraction. Samples <5 mg from the same riffle were pooled, and eventually 2-3 samples were taken from each riffle, and in total 5-6 samples from each stream. Lipids were extracted according to a modified version of the methods presented in Kainz, Johannsson and Arts (2010). Briefly, periphyton samples were extracted in chloroform: methanol (2:1 v/v), followed by sonication, vortex and centrifugation to remove non-lipid materials. This step was repeated three times. Extracted lipids were evaporated to a final volume (1.5 mL) under nitrogen. Duplicate aliquots (100 μL each) were dispensed into pre-weighed tin cups for gravimetric assessment of total lipids. The remaining extract was stored at -20°C until FA were derivatized to methyl esters (FAME). For FAME formation, sulphuric acid in methanol (1:100 mixture used as methylation reagent) and toluene were added to the lipid extract and the solution was incubated for 16 hours at 50°C in a water bath. Thereafter, potassium hydrogen carbonate and hexane were added, and the sample was then shaken, vortexed and centrifuged. The upper organic layer of the solution was transferred and hexane was added again. This step was repeated twice. All FAME containing layers were pooled and concentrated under nitrogen.
Fatty acids were analysed as FAME using a gas chromatograph (THERMO Trace GC) as in Heissenberger, Watzke and Kainz (2010). All samples were extracted and analysed at WasserCluster – Biologische Station Lunz, Austria.

Algal samples were identified to genus level. Soft algae (i.e., green algae and cyanobacteria) were identified and enumerated using a Sedgewick Rafter counting chamber under a microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan) at 400x magnification. Enumeration stopped only on the condition that either the most common taxa had a minimum of 23 units (trichomes/filaments or colonies) or 40 squares were counted. The following step was to scan the slide to 200 squares at 200x magnification in order to identify any possible algae which were not previously observed (Entwisle, Sonneman & Lewis, 1997). Diatoms were mounted on permanent slides using Naphrax™ after oxidizing the organic material with acid following the methods of Chessman et al. (1999). Samples were identified at 1000x magnification with a minimum of 400 valves per slide. Taxa were combined into categories (diatoms, cyanobacteria and green algae) for analysis (Table 1).

Dissolved nutrients (NO₃-N, NH₄-N, and SRP) were analysed using a SmartChem200 discrete chemical analyser (Westco Scientific Instruments, Brookfield, CT, USA) (Apha, 1995) at Griffith University, Queensland, Australia (Table 1). Total nutrients (TN and TP) were digested using a simultaneous persulfate digestion method (Hosomi & Sudo, 1986) before being analysed on the same machine (Table 1).

**Data analysis**

Constrained ordination analysis was applied to identify the most important environmental factors explaining the variability in periphyton FA composition. The percentage data of FA were arcsine-square-transformed for normal distribution approximation. Environmental factors were log₁₀ transformed except the percentage data of canopy cover, which were arcsine-square-transformed. Preliminary detrended correspondence analysis of the FA data set produced the gradient lengths of 0.765 and 0.792 for the first two axes, respectively, indicating that the response was monotonic and thus a linear model (rather than an unimodal model) was more suitable to explain the FA data structure (Ter Braak & Prentice, 1988; Jongman, Ter Braak & Van Tongeren, 1995). Therefore, a multivariate direct gradient
analysis, redundancy analysis (RDA) (Legendre & Legendre, 1998), was used to analyse the
data set.

In the RDA analysis the transformed FA data were used as the response variables, with
environmental factors the independent variables. Fatty acids percentages and sample sites
were each assigned scores on the ordination axes, of which the first two axes were displayed
on the ordination biplot. The significance of the RDA model and canonical axes were tested
using permutation procedures (Oksanen et al., 2007), with the marginal effects of all
measured environmental factors tested in the same manner. In addition, correlation analysis
was used to explore relationships between PUFA, or FA categories, and gradients of canopy
cover and nutrient conditions. All statistical analyses were conducted in the statistical
software R version 3.0.3 (R Core Team, 2014), with the package Vegan (Oksanen et al., 2007)
used for the constrained ordination analysis and the package Hmisc (Harrell & Dupont, 2012)
for correlation analysis.

Results

Fatty acid composition
SAFA were the most abundant FA category in periphyton (41-61% of total FA), followed by
MUFA (24-36%) and PUFA (15-25%). Palmitic acid (16:0), palmitoleic acid (16:1ω7) and
EPA were the principal SAFA, MUFA and PUFA, respectively. These three FA represented
48 - 63% of the total FA across nine streams. Both 16:1ω7 and EPA were used as diatom
biomarkers. The ratios of DHA/EPA (ranging from 0.03-0.13) were < 1, confirming the
dominance of diatoms in the algal biofilm (Dalsgaard et al., 2003; Parrish, 2009). The
shorter chain C18 PUFA, i.e., ALA and LIN, biomarkers of most green algae and a number
of cyanobacteria, accounted for only 5-13% of the total FA. HUFA represented between 5.4
and 7.4% of the total FA while EPA, DHA, and ARA accounted for 82-91% of the total
amount of HUFA.

RDA analysis
An RDA of the FA composition of all samples indicated that NOx-N and riparian canopy
cover were the most important environmental predictors of periphyton FA profiles (Figure 2).
The RDA model was significant ($F$-value = 3.107, $p = 0.005$). The first four canonical axes
explained 94% of the variation in the FA data set, but only the first two axes were significant
(axis 1: F-value = 7.559, p = 0.005; axis 2: F-value = 4.843, p = 0.005) with the explanations of FA variation 40.4% and 25.6%, respectively. The first axis was strongly correlated with NO$_x$-N ($\alpha$ = -0.802) with the second axis mainly correlated with riparian canopy cover ($\alpha$ = -0.817). The marginal effects for all environmental variables showed that only NO$_x$-N (F-value = 3.483, p = 0.005) and riparian canopy cover (F-value = 3.818, p = 0.005) were significant.

The correlations between NO$_x$-N, riparian canopy cover and FA compositions of periphyton were revealed by the first two axes. NO$_x$-N concentrations showed negative correlations with most SAFA and MUFA regardless of riparian canopy cover, while canopy cover indicated positive relationships with most SAFA and MUFA, but negative relationships with 16:0 and 16:1ω7. As for PUFA, along the first axis, contents of ALA, LIN, EPA and DHA were greater with increasing NO$_x$-N concentrations, but ARA was lower at higher NO$_x$-N concentrations; high NO$_x$-N concentration (0.21 mg N L$^{-1}$) versus low NO$_x$-N concentration (0.02 mg N L$^{-1}$) in EPA = 6.8% versus 5.1%, DHA = 0.6% versus 0.2%, ARA = 1.2% versus 1.6%, ALA = 7.3% versus 2.9% and LIN = 5.1% versus 3.6%. Along the second axis, EPA, DHA and ARA contents were higher at higher canopy cover, but ALA and LIN contents were lower; high canopy cover (86%) versus low canopy cover (20%) in EPA = 6.2% versus 2.5%, DHA = 0.4% versus 0.3%, ARA = 1.7% versus 0.5%, ALA = 2.2% versus 4.5% and LIN = 3.5% versus 4.6%.

The first two axes reflected the separation of periphyton nutritional quality among different stream conditions. Different combinations of riparian canopy cover and NO$_x$-N corresponded with different periphyton HUFA compositions. Stream S14, with high riparian canopy cover and low NO$_x$-N concentration, contained higher contents of HUFA EPA and ARA, but lower contents of ALA in comparison with stream S16 that had low canopy cover and high NO$_x$-N concentration; S14 versus S16 in HUFA = 9.4% versus 5.0%, EPA = 6.2% versus 3.6%, ARA = 1.7% versus 0.5%, ALA = 2.2% versus 9.6%. The higher HUFA content in S14 compared with other streams indicated higher nutritional quality of periphyton.

The first two axes also strongly differentiated major taxonomic groups. Regardless of riparian canopy cover, the biomarkers of diatoms (EPA and 16:1ω7) dominated streams with low NO$_x$-N concentrations, while under high NO$_x$-N concentration and low canopy cover

9
conditions, it was the biomarkers of green algae and cyanobacteria (ALA and LIN) that dominated.

**Correlation analysis**
The correlations between PUFA (EPA, DHA, ARA, ALA and LIN) and environmental factors (NO$_x$-N concentrations and riparian canopy cover) were consistent with the RDA results, but only the trends in ARA with canopy cover and in ALA and DHA with NO$_x$-N concentrations were significant (p<0.05, Figure 3). In addition, correlation results also indicated that the relationships between environmental factors (NOx-N concentrations and riparian canopy cover) and FA categories varied. Both SAFA% and MUFA% were lower with increasing canopy cover and nutrient concentrations, but PUFA% and HUFA% were higher. Only the trends in HUFA with canopy cover and in PUFA with NO$_x$-N were significant (p<0.05, Figure 4). The correlation coefficients in all significant relationships ranged from 0.28 to 0.44.

**Discussion**
This study has illustrated the complex relationships between riparian canopy cover, dissolved nutrients and periphyton nutritional quality in natural streams. Periphyton FA profiles most strongly correlated with differences in stream water NO$_x$-N concentrations and riparian canopy cover. NO$_x$-N, rather than phosphorus, was identified as the most important nutrient predictor of FA profiles, which is perhaps not unexpected given most streams in the region are nitrogen limited (Mosisch, Bunn & Davies, 2001). Although we predicted that riparian canopy cover would be a more important factor, local land use (catchment forest cover, cattle grazing and intensive agriculture) also creates a gradient of NO$_x$-N concentrations, which may strengthen the influence of NO$_x$-N in this study. The observed relationships between environmental factors and periphyton FA composition in these natural streams differ from laboratory studies that focused primarily on individual algal species and single environmental factors. Our study thus provides field evidence that algal FA profiles are sensitive to a combination of environmental changes. The significant relationships obtained in the RDA analysis were not apparent when canopy cover and NO$_x$-N concentrations were examined in isolation. This indicated the complexity of the natural environment, and the possible interactions between additional environmental factors.
The observed relationships between NOx-N concentrations and most SAFA, MUFA and PUFA in our study were consistent with previous experimental findings (Guschina & Harwood, 2006; Guschina & Harwood, 2009). In particular, PUFA% was significantly higher in streams with higher NOx-N concentrations. Riparian canopy cover was negatively correlated with only the most abundant SAFA and MUFA (16:0 and 16:1ω7), which likely explains the negative relationships between SAFA%, MUFA% and canopy cover.

The higher SAFA% and lower PUFA% observed in this study compared with others (Hill, Rinchard & Czesny, 2011; Cashman, Wehr & Truhn, 2013) may be attributed to different temperature regimes. Saturated FA have been reported to accumulate more in high temperatures and decrease in cold temperatures, whereas PUFA tend to accumulate more in cold temperatures to increase cell membrane fluidity (Guschina & Harwood, 2006). Our study was conducted in early summer in subtropical areas with the stream temperature range 17-25°C, thus SAFA% appeared to be higher and PUFA% lower. A field investigation in six headwater streams in western Washington State (Volk & Kiffney, 2012) with a 7-day average temperature of 11.5 - 12°C, showed a similar proportion of periphyton SAFA (48%) to our results but PUFA (28%) were lower than the artificial stream study (Hill, Rinchard & Czesny, 2011). These differences confirm that periphyton FA compositions are influenced by the interplay of several different environmental factors.

Variations in the proportions of HUFA and C18 PUFA corresponded with differences in riparian canopy cover and NOx-N concentrations. Both EPA and DHA were positively correlated with canopy cover and NOx-N concentrations, whereas ALA and LIN were positively correlated with NOx-N, but negatively with canopy cover, suggesting taxonomic shifts among the proportions of diatoms, cyanobacteria and green algae in the algal biofilm. ARA showed an interesting trend different from other HUFA: the content was lower with increasing NOx-N concentrations, which is inconsistent with the previous artificial stream study (Hill, Rinchard & Czesny, 2011). Nonetheless, laboratory studies have shown that nitrogen starvation can induce a strong increase in ARA proportion of total FA in freshwater green algae (Khozin-Goldberg et al., 2002; Solovchenko et al., 2008). This might be due to the fact that compared with field observation and laboratory experiments, manipulative studies only focused on certain factors and did not consider fully the interaction between these and other environmental conditions, which may influence periphyton FA patterns and
contribute to the unexplained variance. ARA is the most efficiently retained PUFA in organisms of planktonic food webs in oligotrophic lakes (Kainz, Arts & Mazumder, 2004), and is also essential during early development of marine finfish (Izquierdo et al., 2000), but its function in streams has not been clarified. Moreover, EPA and DHA play an important role in normal neural development and vision functions, and are broadly required for cellular membrane functioning (Arts & Kohler, 2009; Parrish, 2009). Therefore, the variation in algal HUFA content could potentially influence the production of herbivores and even higher trophic levels, such as fish and humans.

The observed changes in periphyton FA composition can be explained by physiological acclimation. When light is abundant (below photoinhibitory levels), the synthesis of light-capturing pigments and photosynthetic membranes (rich in PUFA) decline, leading to less N required by algal cells (Hessen, Færøvig & Andersen, 2002; Hill, Rinchard & Czesny, 2011). Conversely, under low light intensity, higher concentrations of N in the algal cell are required to produce more photosynthetic pigments to maximize their ability to harvest light (Mock & Kroon, 2002). Marine diatoms have been observed to decline in PUFA% with increasing light intensity due to photoacclimation (Thompson, Harrison & Whyte, 1990; Thompson, Guo & Harrison, 1993; Leu et al., 2010), while the PUFA% of stream diatoms was reported not to decline as light intensity increased, being attributed more to increasing production of MUFA and SAFA rather than to photoacclimation (Hill, Rinchard & Czesny, 2011). In our study, PUFA% were significantly higher with higher NOx-N and slightly higher as canopy cover increased, indicating photo-acclimation may contribute to the changes in periphyton FA. Alternatively, another possible pathway is the alteration in periphyton community composition. Periphyton FA profiles strongly reflected the algal biofilm community composition in terms of the proportions of diatoms, cyanobacteria and green algae in our study. Changes in the composition of FA biomarkers 16:1ω7, EPA, LIN and ALA from low to high NOx-N concentrations (RDA axis 1) and from high to low canopy cover (RDA axis 2) suggested taxonomic shifts from diatoms to cyanobacteria and green algae. Nevertheless, periphyton taxonomic composition did not show any significant relationships with canopy cover, NOx-N concentrations, and FA biomarkers. Therefore, we suggest that the observed relationships between environmental factors and periphyton FA profiles were mainly due to physiological acclimation.
Optimal light intensity and nutrient conditions should lead to the highest algal food quality. In our study, the highest periphyton food quality (highest HUFA %) occurred in S14 (9.4%) with high canopy cover and low nutrients, whereas the lowest was in S8 (3.9%) with low canopy cover and low nutrients. This is in contrast to observed changes in food quantity, with increases in GPP associated with reduced canopy cover and increased nutrients in these streams (Bunn, Davies & Mosisch, 1999; Fellows et al., 2006). This suggests a clear trade-off between periphyton food quality and food quantity for herbivores. They cannot acquire enough carbon (GPP) under low light levels, and they obtain less HUFA as light intensity increases. Nevertheless, dietary HUFA threshold for herbivore somatic growth in rivers are still poorly studied, and how this threshold compares to the availability of PUFA is as yet unresolved.

Previous studies have observed that removal of riparian vegetation led to an increase in light intensity, causing a shift in aquatic plant composition from diatoms to filamentous green algae and ultimately macrophytes (Davies et al., 2008), and also resulting in increases in the biomass and density of macroinvertebrate functional feeding groups except grazers (Moldenke & Ver Linden, 2007). However, few studies have been concerned with the mechanisms behind these two processes. Our study shows that algal food quality differs with riparian vegetation removal and nutrient inputs, with likely consequences to energy flow from algae to consumers.

Acknowledgments
The authors are grateful to Carolyn Polson, Dominic Valdez, Wing Ying Tsoi and Xiang Tan for their assistance with sample collection, water nutrient analysis and/or algae identification. The authors also thank Katharina Winter and Katharina Hader for help with lipid analysis, and two anonymous reviewers for their valuable comments on the manuscript. The research was supported by a PhD grant from Griffith University to FG.
References


### Tables

Table 1 Field measured environmental characteristics, ambient nutrient concentrations and periphyton composition obtained from the nine study streams in South-East Queensland, Australia (Figure 1)

<table>
<thead>
<tr>
<th>Streams</th>
<th>Stream Number</th>
<th>Velocity (m s⁻¹)</th>
<th>Canopy (%)</th>
<th>TN (mg L⁻¹)</th>
<th>TP (mg L⁻¹)</th>
<th>NO₃-N (mg N L⁻¹)</th>
<th>NH₄-N (mg N L⁻¹)</th>
<th>SRP (mg P L⁻¹)</th>
<th>Diatoms (%)</th>
<th>Green algae (%)</th>
<th>Cyanobacteria (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stockyard Creek</td>
<td>S2</td>
<td>0.08</td>
<td>81</td>
<td>0.14</td>
<td>0.12</td>
<td>0.02</td>
<td>0.02</td>
<td>0.06</td>
<td>56.01</td>
<td>13.17</td>
<td>30.82</td>
</tr>
<tr>
<td>Left Hand Branch Creek</td>
<td>S3</td>
<td>0.13</td>
<td>12</td>
<td>0.17</td>
<td>0.07</td>
<td>0.02</td>
<td>0.04</td>
<td>0.05</td>
<td>73.21</td>
<td>1.98</td>
<td>24.81</td>
</tr>
<tr>
<td>Lost World Creek</td>
<td>S4</td>
<td>0.27</td>
<td>47</td>
<td>0.38</td>
<td>0.05</td>
<td>0.21</td>
<td>0.02</td>
<td>0.04</td>
<td>79.71</td>
<td>2.40</td>
<td>17.80</td>
</tr>
<tr>
<td>Christmas Creek at Stinson Park</td>
<td>S6</td>
<td>0.31</td>
<td>29</td>
<td>0.23</td>
<td>0.07</td>
<td>0.05</td>
<td>0.04</td>
<td>0.06</td>
<td>27.95</td>
<td>2.74</td>
<td>69.31</td>
</tr>
<tr>
<td>Running Creek</td>
<td>S8</td>
<td>0.39</td>
<td>20</td>
<td>0.15</td>
<td>0.05</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
<td>77.17</td>
<td>0.57</td>
<td>22.11</td>
</tr>
<tr>
<td>Christmas Creek at Burgess Park</td>
<td>S10</td>
<td>0.34</td>
<td>35</td>
<td>0.40</td>
<td>0.07</td>
<td>0.12</td>
<td>0.04</td>
<td>0.05</td>
<td>75.78</td>
<td>2.21</td>
<td>22.02</td>
</tr>
<tr>
<td>Mt Barney Creek</td>
<td>S13</td>
<td>0.41</td>
<td>78</td>
<td>0.40</td>
<td>0.02</td>
<td>0.18</td>
<td>0.00</td>
<td>0.01</td>
<td>55.69</td>
<td>2.70</td>
<td>41.60</td>
</tr>
<tr>
<td>Burnett Creek</td>
<td>S14</td>
<td>0.15</td>
<td>86</td>
<td>0.21</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>85.08</td>
<td>3.56</td>
<td>11.36</td>
</tr>
<tr>
<td>Upper Christmas Creek</td>
<td>S16</td>
<td>0.30</td>
<td>50</td>
<td>0.17</td>
<td>0.05</td>
<td>0.08</td>
<td>0.03</td>
<td>0.05</td>
<td>72.53</td>
<td>0.80</td>
<td>26.68</td>
</tr>
</tbody>
</table>

*Abbreviations: Canopy, riparian canopy cover; TN, total nitrogen; TP, total phosphorus; NO₃-N, nitrate + nitrite; NH₄-N, ammonium nitrogen; SRP, soluble reactive phosphorus.

*Algal percentage data were based on actual cell numbers rather than biovolume.
Table 2 Fatty acid compositions (%, percentages relative to total fatty acids, mean ± SE) of all periphyton samples from the nine study streams in South-East Queensland, Australia

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S6</th>
<th>S8</th>
<th>S10</th>
<th>S13</th>
<th>S14</th>
<th>S16</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>8.6 ± 1.9</td>
<td>4.6 ± 0.2</td>
<td>6.2 ± 0.9</td>
<td>4.4 ± 0.3</td>
<td>5.5 ± 0.5</td>
<td>13.4 ± 2.6</td>
<td>7.0 ± 0.3</td>
<td>4.6 ± 0.5</td>
<td>11.6 ± 0.8</td>
</tr>
<tr>
<td>15:0</td>
<td>0.5 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.0</td>
</tr>
<tr>
<td>16:0</td>
<td>34.1 ± 2.6</td>
<td>31.4 ± 0.8</td>
<td>27.8 ± 1.8</td>
<td>36.4 ± 2.0</td>
<td>36.8 ± 1.2</td>
<td>31.1 ± 3.1</td>
<td>30.9 ± 1.3</td>
<td>27.5 ± 2.4</td>
<td>30.3 ± 1.5</td>
</tr>
<tr>
<td>17:0</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>18:0</td>
<td>4.0 ± 0.4</td>
<td>3.1 ± 0.1</td>
<td>4.5 ± 0.5</td>
<td>4.7 ± 1.1</td>
<td>4.1 ± 0.5</td>
<td>5.2 ± 0.7</td>
<td>3.9 ± 0.3</td>
<td>6.9 ± 1.1</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>19:0</td>
<td>0.4 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.3 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>0.9 ± 0.3</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>20:0</td>
<td>0.2 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.5 ± 0.2</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>22:0</td>
<td>0.8 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>1.5 ± 0.2</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>24:0</td>
<td>0.8 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.7 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>1.4 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>MUFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:1o5</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.3 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>15:1o5</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>16:1o7</td>
<td>20.3 ± 2.7</td>
<td>24.6 ± 2.1</td>
<td>15.7 ± 0.8</td>
<td>18.1 ± 4.4</td>
<td>19.6 ± 1.4</td>
<td>14.5 ± 1.4</td>
<td>17.9 ± 1.1</td>
<td>18.3 ± 2</td>
<td>14.3 ± 1.3</td>
</tr>
<tr>
<td>16:1o9</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>18:1o6</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>2.0 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>18:1o7</td>
<td>3.2 ± 0.3</td>
<td>3.3 ± 0.6</td>
<td>3.7 ± 1.0</td>
<td>3.7 ± 0.5</td>
<td>3.8 ± 0.2</td>
<td>2.8 ± 0.5</td>
<td>4.2 ± 0.4</td>
<td>3.9 ± 0.4</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>18:1o9</td>
<td>4.3 ± 0.5</td>
<td>4.8 ± 1.1</td>
<td>4.4 ± 0.4</td>
<td>4.7 ± 0.6</td>
<td>4.4 ± 0.3</td>
<td>5.2 ± 0.5</td>
<td>3.7 ± 0.3</td>
<td>3.9 ± 0.4</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>18:1o12</td>
<td>0.6 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>2.9 ± 0.5</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>3.0 ± 0.6</td>
<td>2.1 ± 0.5</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>20:1o9</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>22:1o9</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>24:1o9</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>PUFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2o6</td>
<td>3.6 ± 0.3</td>
<td>5.3 ± 0.7</td>
<td>5.1 ± 0.3</td>
<td>5.1 ± 0.7</td>
<td>4.6 ± 0.3</td>
<td>2.7 ± 0.2</td>
<td>5.7 ± 0.3</td>
<td>3.5 ± 0.2</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>18:3o3</td>
<td>2.9 ± 0.4</td>
<td>4.2 ± 1.0</td>
<td>7.3 ± 0.7</td>
<td>7.5 ± 1.7</td>
<td>4.5 ± 0.6</td>
<td>3.9 ± 0.1</td>
<td>6.5 ± 0.9</td>
<td>2.2 ± 0.3</td>
<td>9.6 ± 1.9</td>
</tr>
<tr>
<td>18:3o6</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.0</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>18:4o3</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>20.2ω6</td>
<td>20.3ω3</td>
<td>20.3ω6</td>
<td>20.4ω3</td>
<td>20.4ω6</td>
<td>20.5ω3</td>
<td>22.2ω6</td>
<td>22.3ω3</td>
<td>22.4ω6</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Value</td>
<td>0.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>1.6 ± 0.2</td>
<td>5.1 ± 0.7</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>1.2 ± 0.2</td>
<td>6.8 ± 1.2</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>1.1 ± 0.3</td>
<td>3.7 ± 0.7</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>1.1 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>3.7 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>4.1 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>6.2 ± 1.4</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
</tbody>
</table>

*SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; DHA, docosahexaenoic acid (22:6ω-3); EPA, eicosapentaenoic acid (20:5ω-3).

*a SE of 0.0 means SE < 0.05.
Figure Legends

Figure 1 Map of the Logan-Albert catchment in South-East Queensland, Australia, showing the location of the nine study streams.

Figure 2 Position of each study stream (centroid ± SE) as well as individual replicates on the first two axes (RDA1 and RDA2) from the redundancy analysis (RDA) of periphyton fatty acid composition for all samples. DHA, docosahexaenoic acid (22:6ω-3); EPA, eicosapentaenoic acid (20:5ω-3); ARA, arachidonic acid (20:4ω6); ALA, α-linolenic acid (18:3ω3); LIN, linoleic acid (18:2ω6).
Figure 3 Relationships between NO$_2$-N concentration, canopy cover and periphyton essential fatty acids (EPA, DHA, ARA, LIN and ALA). Only significant relationships were showed (p<0.05). DHA, docosahexaenoic acid (22:6ω-3); ARA, arachidonic acid (20:4ω6); ALA, α-linolenic acid (18:3ω3).
$r = 0.44$

$p < 0.01$
\( r = 0.29 \)
\( p = 0.04 \)
$r = 0.30$

$p = 0.03$
Figure 4 Relationships between NO$_2$-N concentration, canopy cover and periphyton fatty acid groups (SAFA, MUFA, PUFA and HUFA). Only significant relationships were showed (p<0.05). PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids.
Canopy cover (%)

r = 0.28

p = 0.04
The scatter plot shows the relationship between PUFA (%) and NO\textsubscript{x} (mg/L). The correlation coefficient is $r = 0.29$ and the p-value is $p = 0.04$. This indicates a weak positive correlation, statistically significant at the 0.05 level.