Temporal variability of benthic algal $\delta^{13}C$ signatures influences assessments of carbon flows in stream food webs.

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This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to Hydrobiologia.

Abstract

Stream food web function is often assessed using carbon stable isotope assessments of the relative contribution of autochthonous and allochthonous sources of organic matter to consumer diets. As a result, variability in source signatures can strongly influence the assessment of carbon flows. To examine the implications of temporal source variability on food web interpretations, benthic algal $\delta^{13}C$ signatures were measured over an eight week period in five streams in subtropical Queensland, Australia. All of the sampled food webs were determined to be largely driven by benthic algal carbon, however substantial week-to-week variation in benthic algal $\delta^{13}C$ signatures modified the calculated contributions of algae to consumer diets, with differences in autochthonous contributions of up to 11% between weeks. In addition, variable algal signatures led to many occasions in which the $\delta^{13}C$ signatures of some consumers was beyond the range of available sources, meaning the mixing model analyses did not have a valid solution. Together, these findings suggest that temporal variability in algal $\delta^{13}C$ signatures can strongly influence the interpretation of carbon flows in stream food webs. Future food web studies should assess the temporal variability of sources prior to sampling consumers, in order to characterise end member signatures and their relevance to consumers at the time of collection.

Introduction

Food webs are often represented as the biotic components of energy and nutrient flows through ecosystems (Gu et al., 1996; Jepsen & Winemiller, 2002). As a result, conceptual models depicting carbon flows in lotic environments (e.g. those of Vannote et al., 1980; Junk et al., 1989; Thorp & Delong, 1994) emphasise the importance of accurately determining the relative importance of allochthonous and autochthonous sources of organic matter to higher trophic levels. Recent tests of these conceptual models have relied heavily on stable isotope analyses of carbon and nitrogen (Thorp et al., 1998; Bunn et al., 2003; Hoeinghaus et al., 2007), which have been shown to be relatively robust elements for discriminating between allochthonous and autochthonous sources in aquatic food webs (Boon & Bunn, 1994; France, 1995).

Whilst stable isotope analyses have been used to resolve patterns of energy flow in many freshwater food webs, spatial and temporal variability in the carbon isotope signatures of primary producers can introduce considerable uncertainty in the analysis and interpretation of food webs (Finlay et al., 1999). For example, Hill & Middleton (2006) found that for benthic algal stable isotope signatures in second and third order streams in Tennessee, USA, algal $\delta^{13}C$ values often overlapped with those of terrestrial sources. Such a result violates one of the critical requirements of stable isotope assessments of food webs (that source signatures are distinct) and makes assessment of the relative contributions of autochthonous and allochthonous carbon sources to consumers impossible to determine (France, 1995; Fry, 2006).

In addition to occasionally overlapping terrestrial source signatures, algal $\delta^{13}C$ signatures have been shown to be highly variable in space and time (France & Cattaneo, 1998; Hill & Middleton, 2006; Syvaranta et al., 2006). This variability has been shown to be driven by a wide range of context-dependent factors, ranging from changes in water velocity and the associated boundary layer effects, to changes in light intensity and penetration, to changes in thermal regime (France, 1995; France & Cattaneo, 1998; MacLeod & Barton, 1998). The rate of change in isotope signatures in response to these environmental factors is also variable, but given that benthic algae can have fast tissue turnover rates under favourable conditions, algal isotope signatures can often track changes in local environmental conditions over relatively short timeframes (i.e. less than 2 hours) (Lajtha & Michener, 1994; Vymazal, 1995; Hadwen & Bunn, 2005). These fluctuations in stable isotope signatures can have significant implications for the interpretation of carbon flows in food web studies, as the timing of changes (and of sampling) mediates the degree to which the collected algal samples are directly relevant (as a basal resource) to the sampled consumers (Boon & Bunn, 1994; Vander Zanden & Rasmussen, 2001).

As $\delta^{13}C$ signatures of consumers reflect those of their food sources and because stable isotope analyses of food webs rely on the proximity of source and consumer $\delta^{13}C$ signatures to infer feeding relationships, highly variable algal $\delta^{13}C$ signatures can adversely affect the capacity to accurately interpret energy pathways (Boon & Bunn, 1994; France, 1995; Trudeau & Rasmussen, 2003; Hill & Middleton, 2006). In addition, limited knowledge of turnover rates of consumer tissues (O'Reilly et al., 2002; Gratton & Forbes, 2006) , and the lag time between consumption of particular sources and their transfer into consumer tissues further reduces the ability to make

robust assessments of carbon flows (Pinnegar & Polunin, 1999). Furthermore, given that many food web studies are conducted with low spatial and temporal replication, the relevance of algal stable isotope signatures to those of the consumers sampled at the same time, and from the same location, may be somewhat limited (France, 1995; O'Reilly et al., 2002). Consequently, depicting food webs using end member data collected from a single time point introduces an often unquantified, but potentially substantial degree of uncertainty in assessments of trophic relationships (O'Reilly et al., 2002).

Whilst it is rarely undertaken in food web studies, assessing the extent of temporal variation of source $\delta^{13}C$ signatures may enable more accurate assessments of carbon flow pathways in stream food webs (Boon & Bunn, 1994; France, 1995). To examine this issue, we examined spatial and temporal variation in bulk algal $\delta^{13}C$ signatures in five streams of southeast Queensland over an eight-week period that encompassed a range of hydrological conditions. Our emphasis was very much on collecting bulk algal or biofilm samples in the manner frequently done in isotope ecology studies. To this end, we did not examine species composition of the biofilm or interrogate differences in isotopic signatures among species. We predicted that there would be substantial temporal variability in algal $\delta^{13}C$ signatures. We quantified the potential effects of this variability on the interpretation of food web structure and function at these sites by calculating the relative contribution of autochthonous and allochthonous sources to all consumers using the observed algal $\delta^{13}C$ signatures from each sampling period. We predicted that the solvability of mixing models and the interpretation of carbon flows would be significantly affected by variability in benthic algal end member $\delta^{13}C$ signatures.

Materials and methods

Study area

We sampled five upland streams in the Logan-Albert catchment; a moderate sized coastal catchment (4195km^2) in southeast Queensland, Australia. The primary land use in the catchment is cattle production and much of the middle and lower catchment is cleared for this purpose. However, riparian vegetation, composed mostly of native species, was largely intact at all five study sites. The five study streams were selected on the basis of having similar relative positions in the catchment, nearby land use, elevation and orientation. Each study site (one per stream) constituted a 50 m reach encompassing a similar range of hydraulic habitat conditions (in terms of width, depth and velocity), including shallow fast-flowing riffles and deeper slow flowing runs and pools.

Food web sampling

Initial sampling of all food web components was conducted between 28 January 2007 and 8 February 2007, with the sampling at each site conducted within a single day. At all sites, representatives of the most abundant components of the food webs were sampled. Primary sources of carbon included riparian vegetation, algae and benthic particulate organic matter (POM). Riparian vegetation and algae were collected by hand. Invertebrates were sampled using a dip net and mayfly, caddisfly, damselfly and dragonfly nymphs were also sampled by hand from beneath cobbles. Fish and decapod crustaceans were sampled by dragging a small fine mesh (< 1 mm) seine net constructed from shade cloth and weighed down at the bottom with a chain. At least five individuals of each fish species and 20 individuals of each crustacean species

were collected from each site. All samples were placed in labelled zip lock bags and were immediately placed on ice in the field, prior to being transferred to a freezer upon return to the laboratory.

Temporal algae sampling

The collection of algal samples was conducted in two stages. Initially, algae from all five sites were sampled between 28 January 2007 and 8 February 2007 as part of the food web sampling described above (hereafter referred to as sampling trip T1). Thereafter, replicate ($n \geq 3$) algal samples were collected weekly between 15 February 2007 and 5 April 2007 (sampling trips T2 to T9). Importantly, this sampling period encompassed several small but locally intense rainfall events in the Logan-Albert catchment (Figure 1) that resulted in periods of visibly elevated discharge, hydraulic turbulence and turbidity at all study sites, particularly between T1 and T6.

Benthic algae were collected using a standardised sampling protocol that was consistent for all sites and sampling occasions. Each site was searched for all available algal sources for 30 minutes. Filamentous algae (*Spyrogyra* spp.) were collected by hand and rock biofilm was scraped from cobbles using a scalpel blade. Where possible, replicate ($n \geq 3$) samples of each algal type were collected from all hydraulic habitat types (i.e. riffles, runs and pools) to account for within-site spatial variability. All algal samples were immediately placed in labelled zip-lock bags and stored on ice before being frozen upon return to the laboratory.

Stable isotope sample processing

Stable isotope sample processing followed the protocols outlined in Hadwen et al. (2007). Briefly, all animal and plant samples were dried in an oven at 70^{o}C for at least 24 hours before being ground into a fine powder with a mortar and pestle or a puck and mill ring grinder. They were then combusted in a continuous flow-isotope ratio mass spectrometer (Micromass Isoprime EuroVector EA300, Manchester, UK) at Griffith University. Both carbon and nitrogen were analysed for each sample and signatures were determined in relation to laboratory standards of ANU sucrose for $\delta^{13}C$ and atmospheric N for $\delta^{15}N$.

Data analysis

Variation in benthic algae $\delta^{13}C$

For sites with sufficient quantities of benthic algae on all sampling occasions, we examined if there was significant spatial and temporal variation in $\delta^{13}C$ signatures of rock biofilm and filamentous algae using two-way analysis of variance with repeated measures (Zar, 1996). In this analysis, we used sites as subjects, and tested the within-subject effect of time (with errors modelled allowing for correlation). For significant effects of time and site-by-time interactions, we used pre-planned orthogonal contrasts to distinguish significant differences between each successive time period. All analyses were conducted using SPSS (SPSS Inc., 2003). Possible causes of significant temporal changes in algal carbon signatures were interpreted with reference to antecedent rainfall conditions and consequent changes to the aquatic environment.

Stable isotope analysis of the food web

In addition to the traditional $\delta^{13}C$ *versus* $\delta^{15}N$ bi-plots (Fry, 2006), we used Isosource mixing model software (Phillips & Gregg, 2003) to examine feeding relations at each site. Specifically, we sought to determine the relative importance of each primary carbon source to consumer nutrition. Isosource is used to calculate (in increments set by the user – we used 1%) the possible combinations of end member isotope signatures that explain the signatures of the sampled consumers (Phillips & Gregg, 2003). In our IsoSource analyses we only used the carbon stable isotope signatures of sources and consumers, as the level of $\delta^{15}N$ trophic fractionation in the sampled organisms and the study streams are unknown (*sensu* Hadwen et al., 2007; Hadwen & Arthington, 2007). Furthermore, as the emphasis of our study was on the assessment of proportional contributions of allochthonous and autochthonous carbon sources to consumer diets, irrespective of trophic level, it was not necessary to include the nitrogen stable isotope data to reveal trophic relationships.

Our IsoSource mixing model analyses sought to fit consumer $\delta^{13}C$ signatures against all algal and riparian sources from each site. Benthic POM signatures were not included in the analysis as POM is not a true end member; rather it represents a mixture of primary sources and its inclusion in the mixing model analyses would have made calculations of the relative importance of autochthonous and allochthonous sources impossible (*sensu* Hadwen & Bunn, 2004; 2005).

To examine the importance of each primary carbon source to consumer diets and the influence of algal variability on these assessments, we first ran the IsoSource mixing model on the initial sample (T1) of the food web at each of the five study sites. These

analyses sought to fit individual consumer species $\delta^{13}C$ signatures to those of the primary carbon sources, to evaluate the relative contribution of autochthonous and allochthonous carbon pools to each species. To examine how source variability influenced the assessment of carbon flows through the entire food web at each site, we then pooled (averaged) the mean IsoSource output for each consumer for each site to generate a measure of the degree to which the entire food web was reliant on autochthonous sources of carbon (*sensu* Hadwen & Bunn, 2004, Hadwen & Arthington, 2007). This metric, referred to as the mean percent (± SE) contribution of autochthonous carbon to consumers, is the component most likely to vary along with variable algal $\delta^{13}C$ signatures, so much of our analyses revolved our investigations of changes in response to sampling period-specific algal $\delta^{13}C$ signatures.

Following the initial assessment of food web structure and function (samples from T1), we subsequently re-ran the mixing model analyses a further four times, replacing the initial algal signatures with those measured at each site at every two weeks of the temporal sampling program. The $\delta^{13}C$ signatures of riparian vegetation measured at T1 were used throughout these re-run mixing models, as the $\delta^{13}C$ signatures of riparian trees have generally been found to be constant over time (e.g. France, 1995; Finlay et al., 1999). We also continued to use the consumer signatures from T1 for the following reasons. First, consumer stable isotope signatures are less variable than primary producers over short time periods, principally due to physiological constraints and longer tissue turnover times (O'Reilly et al., 2002; Gratton & Forbes, 2006). Second, weekly or bi-weekly sampling of the entire food web, or even just the primary consumers, would have significantly reduced the abundance of some organisms, making food web relations difficult to establish or, perhaps, altering the

structure and function of the food web through top-down processes (Pinnegar & Polunin, 1999). As a result of these limitations, our approach was to simply examine the degree to which the measured variation in $\delta^{13}C$ of algae could influence our assessment of carbon flows in each of the five study streams.

In addition to assessing the influence of algal $\delta^{13}C$ variability on calculations of the degree to which autochthonous sources fuel food webs, we also sought to examine the relative 'solvability' of feeding relations in light of the observed bi-weekly algal signatures. Specifically, some consumer $\delta^{13}C$ signatures occasionally fall outside the range of source signatures at any given time point, making calculation of trophic relations impossible (Benstead et al., 2006; Fry, 2006). This is a relatively common occurrence in stable isotope food web studies and is generally attributed (without substantiating evidence) to source variability or incomplete sampling of potential source materials (e.g. Bunn et al., 2003; Hadwen & Arthington, 2007). Given that these 'unsolvable' consumers add considerable uncertainty to the assessment of the relative roles of allochthonous and autochthonous sources in supporting entire stream food webs, we quantified this metric of 'solvability' in this study to identify how source signature variability can potentially reduce the applicability of stable isotope approaches to stream food web studies.

Results

Temporal variation in algal $\delta^{13}C$ signatures

Algal $\delta^{13}C$ signatures were highly temporally variable over the course of the study, although the range of observed signatures differed between sites and across the sampled algal types (Table 1). For example, there were considerable site-to-site

differences in the degree to which all algal signatures varied over the course of the study, with relatively low variation among algal samples from sites LW, LHB and CC and much greater temporal change in samples from sites SC and WC (Table 1). Across all sampled algal types, the variation in $\delta^{13}C$ signatures of SC and WC samples ranged from 6.63‰ (stringy algae) to 17.00‰ (filamentous algae) and 2.48‰ (stringy algae) to 19.71‰ (filamentous algae), respectively. In contrast, variation in algal samples from LHB, LW and CC ranged from 5.37‰ (rock biofilm) to 11.79‰ (filamentous algae), 2.92‰ (rock biofilm) to 8.79‰ (filamentous algae), and 3.96‰ (rock algae) to 11.08‰ (filamentous algae), respectively. Furthermore, rock biofilm variation was less than that observed for filamentous algae at all sites where both algal types were sampled (Table 1).

Despite these differences in variability across sites and algal types, there were no consistent trends in the rise and fall of $\delta^{13}C$ signatures over time. For all algal types and all sites (within algal types) the timing and magnitude of change in carbon stable isotope signatures was not consistent, as evidenced by the variability in the sampling period in which the 'most depleted' and 'least depleted' $\delta^{13}C$ signatures were recorded (Table 1). Statistical analyses of changes in algal signatures over time were only able to be conducted on rock biofilm and filamentous algae data, at two and four sites, respectively (Figure 2), as the other algal types were not collected sufficiently frequently to facilitate temporal analyses. Two-way repeated measure analysis of variance of rock biofilm data indicated a significant interaction between site and time ($P<0.001$, Table 2). This indicates that the $\delta^{13}C$ signature of rock biofilm varied significantly between sites and times, but that the pattern of temporal variation differed across the sites. For example, $\delta^{13}C$ signatures of rock biofilm at SC and WC

generally decreased between times T3 and T5, before remaining relatively stable between T5 and T8 (Figure 2A). Conversely, rock biofilm $\delta^{13}C$ signatures at LHB tended to decrease throughout the study period, whereas those from samples collected from the LW site were relatively stable over the course of the temporal sampling (Figure 2A). Contrasts between each successive sampling point showed a minor but significant difference in $\delta^{13}C$ signatures between T4 and T5 ($p=0.013$), a major change between T5 and T6 ($p<0.001$), and a minor change between T6 and T7 ($p=0.029$) for all sites (Table 1). A significant interaction was evident for comparisons between T5 and T6 ($p<0.001$), indicating that there was a major change in $\delta^{13}C$ signatures between these time periods, but that their effect was not consistent across sites (Figure 2A).

As observed with the rock biofilm samples, the $\delta^{13}C$ signature of filamentous algae also varied significantly between sites and times ($p=0.003$, Table 2), but the pattern of temporal variation differed between sites (Figure 2B). Contrasts between each successive time period showed a significant increase in $\delta^{13}C$ signatures of filamentous algae between T5 and T6 ($p<0.001$). For this result, the lack of a significant interaction effect indicates that the increase in filamentous algae $\delta^{13}C$ signatures was consistent for both LHB and WC sites ($p=0.219$).

Food web analyses at T1

At T1, the $\delta^{13}C$ signatures for riparian vegetation and filamentous algae from site SC were similar in $\delta^{13}C$ (–30.5‰ and –30.9‰ respectively) (Figure 3A). Filamentous algae spanned between –30.6‰ and –29.4‰ in $\delta^{13}C$. Rock biofilm was more enriched with a $\delta^{13}C$ mean of -16.7±0.5‰. The $\delta^{13}C$ signatures of the majority of consumers at

SC fell between those of rock biofilm and filamentous algae, but appeared to gravitate more towards filamentous algae (Figure 3A). IsoSource analyses revealed that across all consumers 68 % (±8%) of tissue carbon was derived from benthic algal sources (Table 3).

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At LHB, the three primary carbon sources were distinct from one another (Figure 3B). Riparian vegetation had a mean $\delta^{13}C$ of –30.7‰ (±0.5), filamentous algae had a mean $\delta^{13}C$ signature of –24.3‰ (±0.2) and rock biofilm was the most ^{13}C-enriched source with a mean $\delta^{13}C$ signature of –21.4 (±0.7). The $\delta^{13}C$ values of all consumers in LHB
325 generally fell between those of the two autochthonous sources, with no consumers matching the ^{13}C depleted signature of riparian vegetation (Figure 3B). At this site, Isosource analyses across all consumers revealed that 81% (±10) of consumer carbon was derived from algal sources (Table 3).

330 At the LW site, the primary carbon sources again showed distinct separation on the $\delta^{13}C$ axis (Figure 3C). Riparian vegetation was most depleted in $\delta^{13}C$ with a mean (± SE) of –29.4 ‰ (±0.6). Filamentous algae were the most ^{13}C-enriched source, with a mean (± SE) of –23.7 ‰ (±0.4). Rock biofilm had a mean (± SE) $\delta^{13}C$ signature of –25.4 ‰ (±0.9). Averaged across all consumers, Isosource analyses revealed that 86 %
335 (±6) of consumer carbon was derived from algal sources (Table 3).

At WC, riparian vegetation was the most carbon-depleted end member with a mean (± SE) $\delta^{13}C$ of –31.3 ‰ (±1.0) (Figure 3D). The $\delta^{13}C$ signature of filamentous algae was intermediate between those of rock biofilm and riparian vegetation, with a mean (±
340 SE) $\delta^{13}C$ signature of –28.7 ‰ (±1.0). As for all other sites, consumer $\delta^{13}C$ signatures

in WC more closely resembled those of the autochthonous sources than those of the allochthonous sources and Isosource analyses revealed that 78% (±6) of consumer carbon was derived from algal sources (Table 3).

At CC, the $\delta^{13}C$ signatures of riparian vegetation and filamentous algae overlapped (Figure 3E). Riparian vegetation had a mean (± SE) $\delta^{13}C$ signature of -30.6 ‰ (±2.0) and the mean (± SE) signature for filamentous algae was –30.6 ‰ (±0.5). Rock algae and rock biofilm samples had similar means (± SE) of –27.9 ‰ (±0.8) and -27.1 ‰ (±0.4), respectively. The most enriched end member was filamentous algae with mean (± SE) signatures of $\delta^{13}C$ –23.1±0.3‰ Most consumers had $\delta^{13}C$ signatures between those of filamentous algae and rock biofilm and Isosource analyses backed up this observation by suggesting that carbon derived from algal sources contributed, on average, 88 % (±7) of the carbon in consumer tissues (Table 3).

Although the food webs at all five sites were strongly driven by benthic algal sources of carbon, there was considerable variation in the degree to which algal carbon supported all consumer organisms. For example, the food web at CC was calculated to be the most reliant on autochthonous carbon, with 88% of consumer carbon derived from these sources (Table 3). In contrast, 32% of consumer carbon was derived from riparian vegetation at SC.

Food web analysis in the context of temporal variation in algae $\delta^{13}C$

The capacity of Isosource to solve the food webs at all five sites was strongly influenced by the temporal variability in algal biomass at each sampling occasion. For example, on three occasions, the biomass of algae was not sufficient for sampling (T8 for SC and T3 and T6 at CC – marked as N/A in Table 3), meaning that the food web

was ‘unsolvable’ using the Isosource mixing model (which requires at least three end members). For instances where IsoSource calculations were possible, the percent of consumer $\delta^{13}C$ signatures that fell between the $\delta^{13}C$ signatures of source materials varied from as low as 27% (T8 at LW) up to 100% (Table 3). The site with the least number of consumers explained by the source materials was LW, with a range of 27% to 71% across all analyses. In contrast, all of the consumers sampled at SC had $\delta^{13}C$ signatures that fell within the range of source $\delta^{13}C$ signatures, except for T8 when there were insufficient sources available for collection.

Whilst variability in the percent of consumer signatures falling within the range of source carbon stable isotope signatures was high, there was also some variability in IsoSource output with regard to the mean percent contribution of autochthonous sources to consumer signatures (Table 3). This variability was site specific, although some general trends were apparent. For example, the three sites with relatively consistent and moderate flow conditions displayed relatively minor variation in IsoSource output (82 – 88% for CC, 84 – 89% for LW and 81 – 88% for LHB, Table 3). In contrast, the other two sites, which were characterised by lower and more variable flow conditions, exhibited substantially higher variability in percent autochthonous contributions (68 – 77% variation for SC and 68 – 92% variation for WC, Table 3).

The influence of flow-driven changes in algal biomass and algal carbon signatures were reflected in both sample collections and the food web analyses. For example, elevated stream flows associated with the largest rainfall event (on 10 March 2007, see Figure 1) caused scouring of rocks in CC to the degree that insufficient biomass

was able to be collected to assess food web relations at the next sampling time (T6). For sites with sufficient algae, changes in $\delta^{13}C$ signatures (probably resulting from variation in stream flows) influenced the calculated percent autochthonous contribution to consumer tissues between sampling periods T4 and T6. This was particularly evident in the low flow streams (SC and WC), for which the relative contribution of algal carbon sources to consumers rose between T4 and T6 by 11% (SC) and 6% (WC). In contrast, for sites with less flow variability (LHB and LW) and consequently low variation in algal $\delta^{13}C$ signatures, IsoSource predictions of algal contributions were not substantially altered. Indeed, over the same period (between T4 and T6), percent contribution of autochthonous sources to consumer tissues did not change for LHB and decreased by just 1% for LW (Table 3).

Discussion

Variation in algal $\delta^{13}C$ signatures

We observed a considerable range of variation in $\delta^{13}C$ signatures of rock biofilm and filamentous algae between our study sites. On any one sampling occasion, the rock biofilm $\delta^{13}C$ signatures varied up to 11.6‰ between sites (T4) and filamentous algae varied up to 8‰ between sites (T9) (Figure 2). These results are within the range of the spatial variation observed between the five study streams and those reported in other studies. For example, France & Cattaneo (1998) found that rock biofilm varied 16.1‰ in $\delta^{13}C$ between streams in the Laurentian mountains of Quebec. Similarly, Trudeau & Rasmussen (2003) found that rock biofilm varied 9‰ between –16.7‰ and –25.7‰ under variable conditions in artificial streams. Although we were unable to isolate the causes of variation in $\delta^{13}C$ signatures in this study, it is apparent that the variation between sites is considerable and it is likely that a complex combination of

processes, including variation in local hydraulic conditions (e.g. water velocity and turbulence), light intensity and temperature (Boon & Bunn, 1994; Finlay et al., 1999; Hill & Middleton, 2006) might influence algal $\delta^{13}C$ signatures at these sites.

Over the eight-week sampling period of this study, algal $\delta^{13}C$ signatures were highly variable, although the extent of this temporal variation differed between sites. It was clear that for most of the sites, substantial differences in algal $\delta^{13}C$ occurred after T5. Immediately before, and soon after T5, several rainfall events occurred in the study region (Figure 1) that resulted in visibly elevated stream flows at all five sites. For both rock biofilm and filamentous algae, significant and rapid changes in $\delta^{13}C$ signatures between T5 and T6, in addition to substantial changes in algal biomass at some sites, are likely to be attributable to these flow events. Other authors have also found that increased flow causes depletion in the $\delta^{13}C$ signatures of rock biofilm owing to changes in boundary layers and CO_2 diffusion (Trudeau & Rasmussen, 2003; Singer et al., 2005). Furthermore, Hill & Middleton (2006) state that flow events result in scouring which can influence thickness of the layers of rock biofilm which in turn influences $\delta^{13}C$ signatures. Whilst determining the factors responsible for the algal $\delta^{13}C$ variation observed in this study was not an aim of the work outlined in this paper, it is clear that understanding the factors that drive spatial and temporal variation in algal $\delta^{13}C$ signatures deserves further investigation (Trudeau & Rasmussen, 2003).

Food webs at T1

Autochthonous sources of carbon were most important to consumers in the food webs of all the five streams at T1. This finding is in accordance with other studies of

Australian sub-tropical systems (e.g. Bunn & Boon, 1993; Clapcott & Bunn, 2003; Cheshire et al., 2005) and tropical systems elsewhere (e.g. Lau et al., 2008). Our analyses revealed that the only site with substantial contributions of allochthonous carbon to consumer tissues was Stockyard Creek (32% at T1). For this site, the importance of allochthonous materials may be a consequence of the prevailing environmental conditions. For example, this site experienced little or no flow for the majority of the study period, such that the residence time of allochthonous contributions in the large pool at this site is likely to have relatively long compared with the other study sites. In addition, the reach at Stockyard Creek was heavily shaded, with no filamentous algae present and only small amounts of rock biofilm. Together with high riparian contributions, this low biomass of autotrophs may contribute to the comparatively greater reliance of consumers on allochthonous sources at this site than at the other four sites examined in this study.

Effects of algal $\delta^{13}C$ variability on food web interpretation

Many authors investigating freshwater food webs using stable isotope analysis sample the food webs on only one occasion (e.g. James et al., 2000; Moulton et al., 2004) and draw conclusions regarding the relative importance of carbon flow based on this information. However, this study and others (e.g. Singer et al., 2005; Hill & Middleton, 2006; Syvaranta et al., 2006) have clearly shown that algal $\delta^{13}C$ signatures vary substantially in space and time. Therefore, a food web study conducted on a single sampling occasion represents only a "snapshot" view in time (and space), and does not adequately encompass potential fluctuations of carbon stable isotope signatures. Given that algae contributes substantially to the carbon composition of consumers in streams of southeast Queensland, our study demonstrates the importance

of quantifying the extent of temporal variation in $\delta^{13}C$ algal signatures to aid assessment of the relative importance of algal carbon to consumers.

When the consumer diets of the food webs were re-analysed using $\delta^{13}C$ of algae collected on different sampling occasions, the variations in relative contribution of autochthonous materials to consumer diets changed substantially, resulting in consequent changes in the interpretation of carbon flows through these stream food webs. Of our five study streams, Lost World, Left Hand Branch and Christmas Creek reflected only subtle changes in percent autochthonous contribution to consumer diets (Table 3). This is probably due to the fact that with constant and relatively uniform discharge, algal $\delta^{13}C$ signatures are not likely to change as much as sites with lower discharge (France, 1995), as reflected in the greater variations in percent autochthonous contribution to consumer diets in the more hydrologically variable sites (e.g. Stockyard Creek and Widgee Creek).

In addition to moderate changes in the calculated percent contribution of autochthonous carbon sources to food webs, it was evident that source signature variability may result in the $\delta^{13}C$ signatures of many consumers falling outside the range of source $\delta^{13}C$ signatures. In these instances, the diets of these consumers could not be determined using stable isotope approaches. Given that it is unlikely that these consumers were feeding outside the system, it is probable that temporal variations in algal $\delta^{13}C$ signatures and the lag time associated with the assimilation of source $\delta^{13}C$ signatures into consumer tissues contributed to the difficulty in interpretation of consumer feeding patterns and therefore carbon flow for some sampling occasions (O'Reilly et al., 2002; Gratton & Forbes, 2006).

Recommendations for future studies

Accounting for the temporal fluctuations of algal $\delta^{13}C$, like those observed in this study, is essential if researchers are to overcome some of the limitations of using stable isotopes to investigate food webs (Gannes et al., 1997; Fry, 2006). Failure to account for temporal variation in source signatures may lead to conclusions about carbon flows that may not be able to be generalised over time. Indeed, our findings suggest that all food web studies should be conducted in the context of characterising source $\delta^{13}C$ variability to best account for temporal fluctuations that can influence assessments of source importance to consumer diets. Ideally, researchers could do this by instituting a regular sampling regime of primary carbon sources prior to food web sampling. The length of time for which this source sampling would need to be conducted is likely to be dependent on the ecosystem and the time of year, especially if sampling occurs during a hydrologically variable or biologically active period. At the end of the temporal sampling period, the entire food web should then be sampled, with the end member values to be used in mixing models informed by the temporal data collected prior to animal sampling. Whilst we did not do this in our study, the advantages of conducting the food web after the temporal algal sampling is that studies can potentially capture the lag effect caused by slower tissue turnover time in consumers compared to algae (Bunn & Boon, 1993) and to ensure that sampled sources are more ecologically relevant to the signatures of consumers captured at the end of the sampling period. We suggest that adding a temporal dimension to food web studies can help to minimise uncertainty in estimates of stable isotope source signatures and their relevance to consumers, and provide a more accurate understanding of food web structure and function.

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Figure 1. Daily variation in rainfall during the study period at A) Darlington TM weather station and B) Foxley Alert weather station. The timing of sampling occasions T1-T9 are indicated by arrows at the top of the figure. Data source: Australian Bureau of Meteorology.

Figure 2. Temporal variation in mean (±SE) $\delta^{13}C$ signatures for A) rock biofilm and B) filamentous algae at each study site over the eight week sampling period. Symbols for Stockyard Creek algal samples are marked as solid circles; Left Hand Branch samples are solid diamonds, Lost World samples are empty triangles and Widgee Creek samples are empty squares.

Figure 3. Stable isotope signatures ($\delta^{13}C$ *versus* $\delta^{15}N$) of all sampled food web components at (A) Stockyard Creek, (B) Left Hand Branch, (C) Lost World, (D) Widgee Creek and (E) Christmas Creek. Riparian vegetation (solid black boxes) and algal (solid grey boxes) sources collected at T1 are represented as mean (±SE) of $\delta^{13}C$ and $\delta^{15}N$ signatures. Points with dotted error bars represent the mean (±SE) of total variation in algal signatures over the eight week sampling period. For animals collected at T1, solid triangles represent aquatic macroinvertebrates, open diamonds represent fish and open circles represent tadpoles.

Table 1. Temporal range (most depleted minus least depleted) of $\delta^{13}C$ signatures in rock biofilm, filamentous algae, rock algae and stringy algae sampled from five streams in southeast Queensland on nine occasions between 28 January and 5 April 2007.

Algal type	Site	Sample	$\delta^{13}C$ (‰)	Sample Trip	Range
Rock Biofilm	Stockyard Creek (SC)	most depleted	-23.85	T5	
		least depleted	-15.78	T2	8.07
	Left Hand Branch (LHB)	most depleted	-27.38	T8	
		least depleted	-22.01	T4	5.37
	Lost World (LW)	most depleted	-27.84	T4	
		least depleted	-24.93	T6	2.92
	Widgee Creek (WC)	most depleted	-22.31	T6	
		least depleted	-14.56	T3	7.75
Filamentous Algae	Stockyard Creek (SC)	most depleted	-32.80	T5	
		least depleted	-15.80	T2	17.00
	Left Hand Branch (LHB)	most depleted	-30.48	T3	
		least depleted	-18.69	T9	11.79
	Lost World (LW)	most depleted	-37.25	T8	
		least depleted	-28.55	T1	8.79
	Christmas Creek (CC)	most depleted	-31.32	T2	
		least depleted	-20.24	T5	11.08

	Widgee Creek (WC)	most depleted	-30.46	T2	
		least depleted	-10.75	T1	19.71
Rock Algae	Left Hand Branch (LHB)	most depleted	-24.06	T5	
		least depleted	-16.60	T5	7.46
	Lost World (LW)	most depleted	-27.71	T9	
		least depleted	-24.78	T8	2.93
	Christmas Creek (CC)	most depleted	-29.99	T7	
		least depleted	-26.02	T5	3.96
Stringy Algae	Stockyard Creek (SC)	most depleted	-32.83	T6	
		least depleted	-26.20	T8	6.63
	Widgee Creek (WC)	most depleted	-25.47	T7	
		least depleted	-23.00	T6	2.48

Table 2. *F* values and their associated significance levels (*p*) for repeated measures analysis of variance testing within subject (time) and between subject (site) variation in $\delta^{13}C$ signatures for rock biofilm and filamentous algae. Significance levels for pre-planned orthogonal contrasts between each successive time period are also shown.

Rock biofilm				**Filamentous algae**			
	Within subjects		**Between subjects**		**Within subjects**		**Between subjects**
Source	Time	Time x Site	Site	**Source**	Time	Time x Site	Site
d.f	6,48	18,48	3,8	d.f	6,24	6,24	1,4
F	10.26	4.03	79.74	*F*	11.76	5	0.954
p	<0.001	<0.001	<0.001	*p*	<0.001	0.003	0.384
Contrast				**Contrast**			
T2vT3				T2vT3	0.052	0.012	
T3vT4	0.155	0.614		T3vT4	0.042	0.282	
T4vT5	0.013	0.284		T4vT5	0.978	0.300	
T5vT6	<0.001	0.001		T5vT6	0.001	0.219	
T6vT7	0.029	0.079		T6vT7	0.210	0.323	
T7vT8	0.764	0.267					
T8vT9	0.246	0.249		T7vT9	0.746	0.193	

Table 3. Temporal change in food web analyses on the basis of variable algal $\delta^{13}C$ signatures. Data represent the percent contribution of autochthonous carbon (Mean % autochthonous ± SE) to consumer diets and the percent of consumers with $\delta^{13}C$ signatures that fell within the range of source $\delta^{13}C$ signatures at each study site during each sampling period.

Site	Mean % autochthonous ± SE	% consumers within source signature range
Stockyard Creek (SC)		
T1	68 ±8	100
T3	67 ±8	100
T4	66 ±8	100
T6	77 ±6	100
T8	N/A	N/A
Total range	11%	
Left Hand Branch (LHB)		
T1	81 ±10	91
T3	88 ±7	73
T4	84 ±5	91
T6	84 ±11	73
T8	83 ±13	64
Total range	7%	
Lost World (LW)		
T1	86 ±6	71
T3	89 ±7	47
T4	85 ±6	67
T6	84 ±7	67
T8	86 ±14	27
Total range	5%	
Widgee Creek (WC)		
T1	78 ±6	100
T3	68 ±8	90
T4	84 ±6	100
T6	90 ±13	70
T8	92 ±8	60
Total range	22%	
Christmas Creek (CC)		

T1	88 ±7	100
T3	N/A	N/A
T4	82 ±7	100
T6	N/A	N/A
T8	84 ±5	50
Total range	5%	

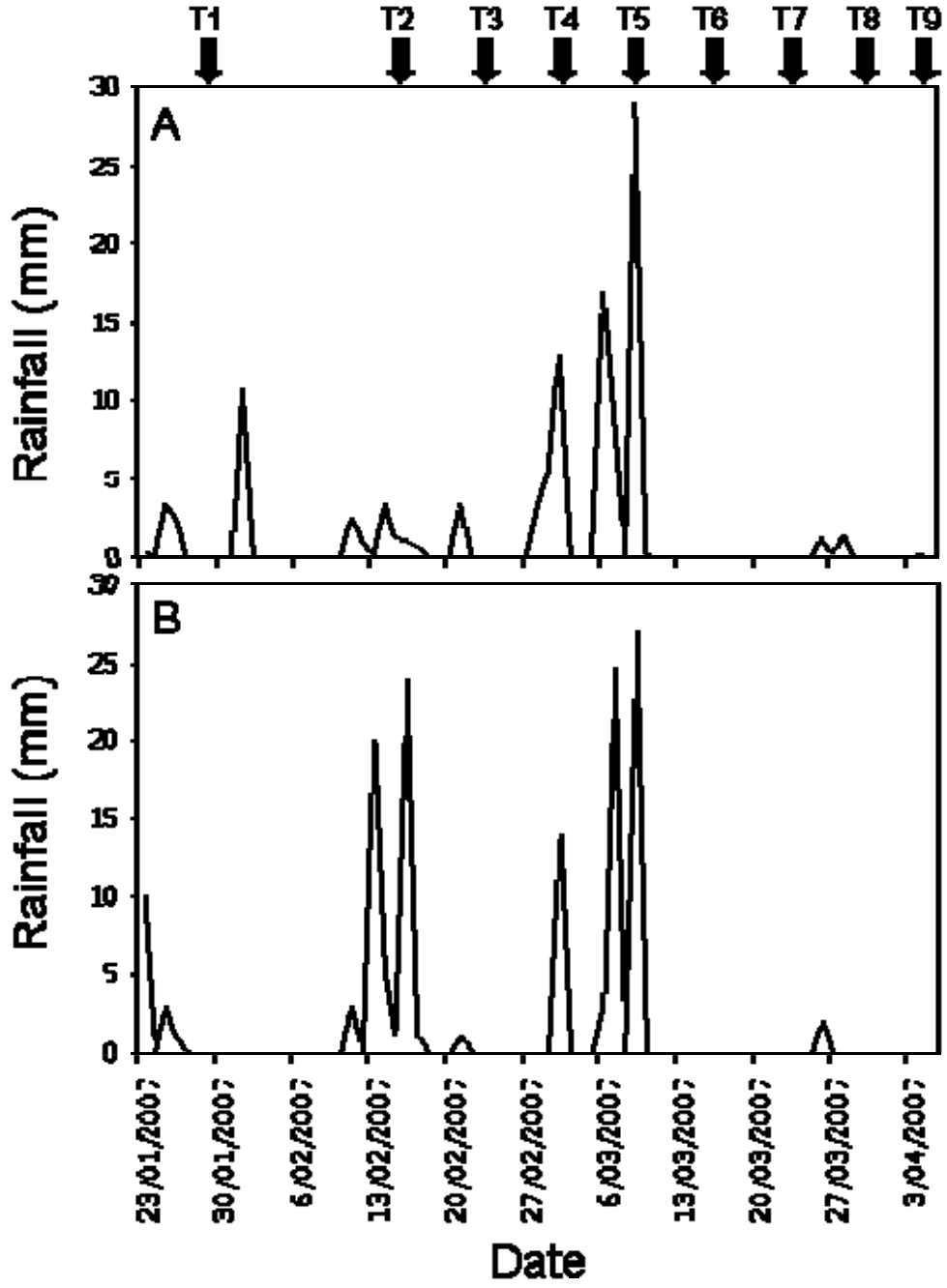
T1
T2
T3
T4
T5
T6
T7
T8
T9
A
B
Rainfall (mm)
Rainfall (mm)
0
5
10
15
20
25
30
23/01/2007
30/01/2007
6/02/2007
13/02/2007
20/02/2007
27/02/2007
6/03/2007
13/03/2007
20/03/2007
27/03/2007
3/04/2007
Date

Figure 1.

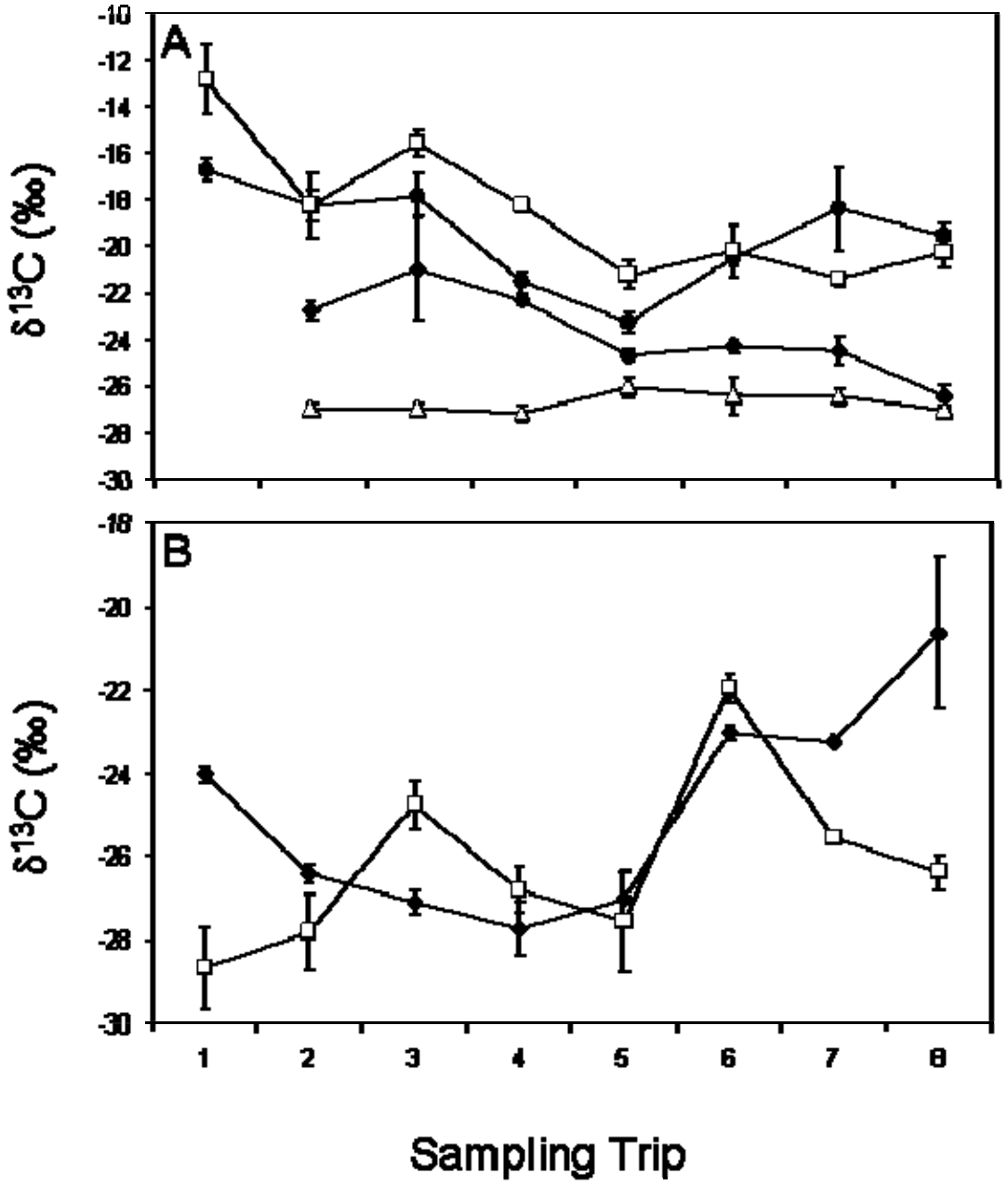
A
B
δ13C (‰)
δ13C (‰)
-10
-12
-14
-16
-18
-20
-22
-24
-26
-28
-30
-18
-20
-22
-24
-26
-28
-30
1
2
3
4
5
6
7
8
Sampling Trip

Figure 2.

Figure 3.