Can C₄ plants contribute to the aquatic food webs of subtropical streams?

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03 October 2002

keywords: stable isotopes, riparian vegetation, benthic fauna, shredders

SUMMARY

1. Recent stable isotope studies have revealed that C₄ plants play a minor role in aquatic food webs, despite their often widespread distribution and production. We compared the breakdown of C₃ (*Eucalyptus*) and C₄ (*Saccharum* and *Urochloa*) plant litter in a small rainforest stream and used laboratory feeding trials to determine their potential contribution to the aquatic food web.
2. All species of litter broke down at a fast rate in the stream, though *Urochloa* was significantly faster than *Eucalyptus* and *Saccharum*. This was consistent with the observed higher total organic nitrogen of *Urochloa* compared with the other two species.
3. The breakdown of *Urochloa* and *Saccharum* was, however, not associated with shredding invertebrates, which were poorly represented in leaf packs compared with the native *Eucalyptus*. The composition of the invertebrate fauna in packs of *Urochloa* quickly diverged from that of the other two species.
4. Feeding trials using a common stream shredder *Anisocentropus kirramus* (Trichoptera: Calamoceratidae) showed a distinct preference for *Eucalyptus* over both C₄ species. *Anisocentropus* was observed to ingest C₄ plant litter, particularly in the absence of other choice, and faecal material collected was clearly of C₄ origin, as determined by stable isotope analysis. However, the stable carbon isotope values of the larvae did not shift away from their C₃ signature in any of the feeding trials.
5. These data suggest that shredders avoid the consumption of C₄ plants, in favour of native C₃ species that may even appear to be of lower food quality (based on C:N ratios). Lower rates of consumption and lack of assimilation of C₄ carbon also suggest that shredders may have a limited ability to process this material, even in the absence of alternative litter sources. Large scale clearing of forest and vegetation for C₄ crops such as sugarcane, will undoubtedly have important consequences for stream ecosystem function.

Introduction

Only a small fraction of the total carbon present in a river or wetland is likely to enter the aquatic food web because most is either of poor quality for animal consumers or is not truly available and other factors prevent access to it (e.g. Lewis *et al.*, 2001; Thorp & Delong, 2002). A fundamental question in the study of aquatic food webs is to identify which source or sources of organic carbon are actually used by metazoan consumers, such as invertebrates and fish. This difficult task has been simplified in recent years with the widespread use of stable isotope tracing techniques (Peterson & Fry, 1987; Lajtha & Michener, 1994). This approach has several advantages over traditional methods for determining the diet of consumers. In particular, the isotope signature of a consumer reflects material assimilated rather than merely ingested and provides integration over time based on body tissue turnover rates (i.e. weeks to months), rather than a snapshot of recently ingested food (Peterson & Fry, 1987; Hesslein, Hallard & Ramlal, 1993; Pinnegar & Polunin, 2000). Stable isotope analysis of carbon has proved particularly effective in the study of aquatic food webs where there are often marked differences in the isotope signatures of the major primary sources (Rosenfeld & Roff, 1992; Boon & Bunn, 1994; Huryn *et al.*, 2001).

One intriguing finding of recent stable isotope studies is the apparent lack of transfer of C₄ plant carbon into stream and river food webs (Hamilton, Lewis & Sippel, 1992; Bunn & Boon, 1993; Forsberg *et al.*, 1993; Bunn, Davies & Kellaway, 1997). This implies that aquatic consumers have not assimilated C₄ carbon directly, as living or dead matter, or indirectly via a microbial loop. This is despite the fact that C₄ plants are often the most productive and conspicuous components of aquatic systems. For example, Forsberg *et al.* (1993) have shown that while C₄ plants accounted for over half of the annual primary production of floodplains in the Central Amazon, they contributed only a small percentage of the carbon source for aquatic food webs. Similarly, Thorp *et al.* (1998) found little, if any, contribution of C₄ plants to the food web of a regulated river channel. The only contribution of C₄ plants to aquatic food webs appears to be through

the consumption of terrestrial animals by fish and other large predators (Hamilton *et al.*, 1992; Forsberg *et al.*, 1993; Bunn *et al.*, 1997).

C₄ plants not only differ fundamentally from C₃ plants in their photosynthetic pathways (Hatch & Slack, 1970; Raven, Evert & Eichhorn, 1998) but also show marked physiological and anatomical differences. The observed avoidance of C₄ plants by some insects in terrestrial systems is suggested to be a consequence of lower nutritional quality (Caswell *et al.*, 1973, Barbehenn & Bernays, 1992), lower nutrient availability (Caswell & Reed, 1975), and physical constraint due to C₄ plant anatomy (Schiers, Bruyn & Verhagen, 2001). In freshwater ecosystems, the chemical and physical characteristics of allochthonous inputs are also known to influence invertebrate palatability and consumption (Peterson & Cummins, 1974; Bunn, 1986; Boulton & Boon, 1991; Benfield, 1996). The observed lack of C₄ plants in aquatic food webs suggests that C₄ plant litter either has physical or chemical properties that prevent consumption by aquatic invertebrates or that aquatic metazoans are able to select sources of organic matter of higher quality.

In many tropical and subtropical catchments along the east coast of Australia, forest (C₃) vegetation has been extensively cleared for sugarcane (*Saccharum sp.*) cultivation. Furthermore, riparian zones and in some cases the stream channels in many systems are choked by an invasive pasture grass, para grass (*Urochloa mutica*), another C₄ plant (Bunn *et al.*, 1998). This shift in catchment and riparian plant composition is likely to have a marked influence on the fluxes of organic carbon into streams and rivers and ultimately coastal ecosystems. Bunn *et al.* (1997) showed that very little C₄ carbon from para grass and sugarcane contributed to the food web of a disturbed tropical lowland stream, even though more than 50% of the detritus was of C₄ origin. Similarly, Martinelli *et al.* (1999) showed how a change from C₃ to C₄ vegetation in a Brazilian river basin contributed a proportional change in the composition of C₃ and C₄ particulate organic matter instream.

In this study, we used field and laboratory experiments to test whether the observed lack of C₄ carbon in aquatic food webs is a consequence of simple preference for higher quality carbon sources or due to chemical or physical constraints on consumption and assimilation. Breakdown rates of packs of C₄ leaf litter (*Urochloa* and *Saccharum*) were compared with that of native C₃ litter (*Eucalyptus grandis*) in a small subtropical stream. The fauna associated with these packs was also sampled to determine any apparent preference of invertebrates in the field. Laboratory feeding trials using a common stream shredder were also conducted to determine preference of leaf litter type and to measure rates of consumption.

Methods

Breakdown of C₃ and C₄ litter in situ

A leaf litter breakdown experiment was conducted from March to June 2000 in Bundaroo Creek (152°36'E, 26°42'S), a low order upland stream (490 m elevation, 5.45 km² catchment area) in the Conondale Ranges, approximately 200 km north of Brisbane, southeast Queensland. The climate of the area is subtropical and seasonal with an average annual rainfall of 1500 mm (Young & McDonald, 1987). The catchment vegetation is dominated by dense wet sclerophyll rainforest characterised by *Eucalyptus* species on ridges and gullies. Further details of the study area and streams can be found in Hughes *et al.* (1995), Hancock & Bunn (1997) and Mosisch & Bunn (1997). Rainfall recorded at Kenilworth varied from 398 mm in March 2000 to 563 mm in May. Water temperature varied from 17.5/20°C min/max in March 2000 to 9.5/15.5°C min/max in June 2000. The channel was 3 m to 11 m wide at the study site, with a substrate consisting of 40% gravel and 30 % cobble, 10 % boulders and 20 % bedrock.

Para grass (*Urochloa mutica*) and sugarcane (*Saccharum sp.*) leaf samples were collected from approximately 50 km east of the study site, and eucalypt (*Eucalyptus grandis*) samples were collected at the study site. The age of the leaves were chosen to

coincide with the state in which they would naturally enter streams. Therefore, para grass and sugarcane samples were collected by harvesting mature-aged leaves, and eucalypt samples consisted of senescent leaf litter ranging in colour from green to yellow to red, but not brown. All leaves were placed in drying ovens for 72 h at a low temperature (45°C) to minimise heat damage (Boulton & Boon, 1991), and yet sufficient to allow for the accurate weighing of packs. Forty leaf packs of each species were constructed by threading 10 g of leaf litter onto monofilament line and tethering into bundled packs. Aluminium labels were attached to each pack using copper wire. Eucalypt packs consisted on average of 20 leaves with a total approximate surface area of 1552 cm². Sugarcane packs were constructed using strips cut at 10-20 cm in length. They produced packs of uniform appearance, averaging 20 strips with a total approximate surface area of 1416 cm². Para grass packs contained on average 45 leaves with a total approximate surface area of 1068 cm².

Leaf packs were placed randomly in riffles in Bundaroo Ck at least 1.0 m apart in early March 2000. Packs were tethered to small boulders using monofilament line and positioned facing upstream to simulate natural leaf accumulation. Day 0 samples were transported to the site and returned to the laboratory without immersion in the stream to provide an estimate of weight loss due to handling. Recovery of remaining packs occurred on Days 1, 7, 14, 28, 41, 64, 85, and 112. This collection regime was chosen as the variability of packs usually increases with time (Boulton & Boon, 1991), and leaf breakdown has been shown to correspond generally to a negative exponential model (Webster & Benfield, 1986). A spate resulting from a 90 mm rain event on Days 58-59 did not allow for the anticipated number of samples (5 per treatment and time) to be collected during the latter stage of the experiment. Leaf packs were randomly chosen and collected by carefully lifting the attached boulder into a hand net (250µm) in-stream and then cutting the monofilament line. The pack and any attached invertebrates were then transferred to a plastic bag and kept on ice during transportation.

Leaf packs were washed over a sieve (100µm) within 24 h of recovery and any invertebrates were removed and preserved in 70% ethanol. Leaves were then placed in

paper bags and dried at 45°C for 72 h. Oven dry weight of packs was recorded prior to the samples being ground in a ring grinder (Rocklabs, NZ). AFDW was obtained by ashing 0.5-1.0 gm of each sample at 450°C for 4 h. Samples were then allowed to cool in a desiccator and re-weighed. AFDW was calculated as the difference between dry weight and ashed weight, and the mean percentage AFDW remaining for each pack was calculated using the mean AFDW of the handling-loss leaf packs as an estimate of the initial AFDW of all leaf packs. Ground leaf samples were also oxidised at high temperatures in a EuroEA 2000 (EuroVector, Italy) elemental analyser and the resulting N₂ (and CO₂) gas analysed with an IsoPrime (Micromass, UK) continuous-flow isotope ratio mass spectrometer to obtain total organic nitrogen (TON), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each litter pack.

Invertebrates were sorted to family level, except for Leptoceridae (Trichoptera) and Leptophlebiidae (Ephemoptera), which were further sorted to genus level to determine functional feeding categories. Invertebrates were classified into functional feeding groups as determined by Cummins & Klug (1979) and Boulton & Brock (1999), where possible.

Weight loss of leaf packs was expressed as the percentage of AFDW remaining. Samples collected from Day 0 through to Day 85 were used to calculate processing rates for each species. By Day 112, only two eucalypt samples remained in stream and the data from these packs were not used in future calculations. Processing rates were obtained by linear regression of the natural log (ln) of data with time to obtain a processing coefficient (*k*). Data were also fitted to this negative exponential model using degree-days, and T₅₀ calculated. Significant differences between processing rates were examined using ANCOVA with time as the covariate, with t-tests to determine where differences occurred. Linear regression was also performed on the percentage of TON remaining.

Faunal density of leaf bundles was expressed as the mean density of total taxa per pack and per gram remaining. These means were compared using ANCOVA and t-tests

to examine where differences occurred. The fauna was then grouped into functional feeding categories and the mean density of shredders per pack and per gram remaining compared using ANCOVA and t-tests. Semi Strong Hybrid Multi Dimensional Scaling, based on the Bray-Curtis dissimilarity matrix, was used to graphically depict faunal composition relationships amongst leaf packs (PATN) (Faith, Minchin & Belbin, 1987).

Consumption and assimilation of C₃ and C₄ litter and growth of a common shredder

Anisocentropus kirramus (Trichoptera: Calamoceratidae) larvae are an abundant shredder in Bundaroo Creek and were common in the leaf packs in the experiment. *A. kirramus* larvae were collected from natural leaf packs within Bundaroo Ck at the end of May 2000. Specimens were kept in aquaria for two weeks and fed a combination of leaf litter from riparian tree species collected from the stream. Larvae were grouped into growth instars using the approach of Nolen & Pearson (1992). This involved calculating a head capsule width-to-case length ratio so as to enable subsequent determination of instars by case length only. Fourth instar larvae were mostly used in all experiments.

Uniform discs were cut from previously dried eucalypt, sugarcane and para grass leaves using an 11-mm-diameter metal stamp. All discs were cut to include a similar degree of leaf venation. Discs were conditioned by placement in a 50:50 combination of stream and distilled water for 48 h to allow for the leaching of potential inhibitory substances and to improve processing rates by *A. kirramus* (Nolen & Pearson, 1993). Discs were re-dried (45°C) and the dry weight recorded prior to experiments. Three feeding experiments were conducted within an air-conditioned laboratory at the constant temperature of 17°C ($\pm 2.5^\circ\text{C}$). Each treatment included 200ml of a 50:50 combination of Bundaroo Ck and distilled water contained within aerated plastic containers. Leaf discs were weighted down using several clean grains of coarse sand.

Experiment 1: Leaf processing with multiple larvae

A preliminary experiment was conducted to investigate the processing rates of leaf litter by *A. kirramus* larvae. Six replicates of each single leaf species were used (n = 18). Five

replicates each included five leaf discs and five *A. kirramus* larvae and a sixth replicate served as a control with larvae. Leaf discs were replaced with new discs during the experiment when it was determined more than 50% had been consumed. *A. kirramus* larvae were also replaced if more than 75% of their casing had disappeared (8 larvae were replaced in the sugarcane treatments, 16 in the para grass treatments and 17 in the eucalypt treatments). The main cause for replacement in sugarcane and para grass treatments was the disintegration of casing due to feeding by other larvae. In contrast, larvae from eucalypt treatments were primarily replaced due to the cutting of new cases. When new cases were cut the new 'leaf disc' case was returned to the respective container. On completion of the experiment (21 days) *A. kirramus* larvae were removed from their cases and together with all leaf discs were oven-dried and weighed.

Experiment 2: Leaf processing, with a single larva

To overcome the problem of case consumption by other caddis larvae in Experiment 1, a second feeding trial was conducted using only one *A. kirramus* larva in each container. Ten containers, each with a single *A. kirramus* and five leaf discs, and an eleventh serving as a control with leaf discs only, were used for each leaf species over a 21 day period (n = 33). Water was changed once on day 6 and discarded as it was thought that any FPOM collected might contain material ingested prior to the experiment. Water collected on day 16 was evaporated at 60°C and FPOM collected for weighing and stable isotope analysis. The gut contents of *A. kirramus* larvae were voided by keeping them in containers without food and then specimens dried and prepared for stable isotope analysis. By the end of the experiment, one *A. kirramus* larvae had died and a further six had begun to pupate (two within each treatment). These specimens were not included in the analysis. The remaining larvae had suffered a small amount of case fragmentation but otherwise appeared intact. Leaf discs were dried and weighed as in the first experiment.

Experiment 3: Leaf preference

This experiment was conducted to determine processing rates for each leaf type when larvae of *A. kirramus* were offered a selection. Eleven replicates were established, each

with one leaf disc of para grass, sugarcane and eucalypt. To ten containers, a single *A. kirramus* larva was introduced and the eleventh container served as a control. Water was collected on completion of the experiment (16 days) and filtered through pre-combusted carbon-fibre filters to collect fine particulate organic matter (FPOM). The filtered samples were oven-dried and stored in airtight containers for stable isotope analysis. *A. kirramus* larvae were dried intact and retained for stable isotope analysis. Leaf discs were dried and weighed as above.

Leaf disc dry weight (W_2) was used to calculate a consumption rate (C). $C = (W_1 - W_2)/t$, where W_1 is the initial leaf disc weight adjusted using losses from control treatments (due to leaching). Significant differences between leaf discs dry weights and controls were examined using ANOVA and pairwise comparisons. *A. kirramus* final dry weights were compared using ANOVA. Feeding observations were expressed in proportional time budgets. The dry weight of FPOM was recorded from Experiment 2 and compared using ANOVA and t-tests to confirm where differences occurred.

Stable isotope analysis

Stable carbon isotope analysis provides an effective means of distinguishing the relative importance of C_3 and C_4 plants in trophic studies because they exhibit distinctly different $\delta^{13}C$ signatures (DeNiro, 1987; Lajtha & Michener, 1994). Stable carbon isotope values of C_3 plants typically range from -21 to -35 ‰, while those of C_4 plants range from -10 to -14 ‰ (Ehleringer, 1991), however, the isotope signatures for a plant species at any specific site or time show a much narrower range. This large difference in $\delta^{13}C$ values allows for the tracing of their isotopic signatures throughout food webs because the isotopic composition of animals reflects those of their diets to within about 1 ‰ (Michener & Schell, 1994; Kelly, 2000; Post, 2002).

Stable carbon and nitrogen isotope ratios were measured using an Isoprime (Micromass, UK) mass spectrometer with an average analytical variability of (0.2 ‰). Samples were dried immediately and stored in airtight containers until ground and

weighed for analysis. Isotope measurements were expressed as $\delta^{13}\text{C}$ values relative to the Pee Dee Belemnite (PDB) isotopic standard: $\delta^{13}\text{C} \text{ ‰} = (R_{\text{sample}}/R_{\text{std}}) - 1 \times 1000$, where R_{sample} and R_{std} are the $^{13}\text{C}: ^{12}\text{C}$ of the sample and standard, respectively. Similarly, $\delta^{15}\text{N}$ values were expressed relative to the isotopic standard of N_2 in air (Peterson & Fry 1987). A simple mixing model was used to ascertain the potential source of assimilated carbon in *A. kirramus* larvae from feeding trial experiments:

$$P_A = \frac{\delta^{13}\text{C}_{\text{consumer}} - f - \delta^{13}\text{C}_{\text{sourceB}}}{\delta^{13}\text{C}_{\text{sourceA}} - \delta^{13}\text{C}_{\text{sourceB}}}$$

Where P_A is the proportion of Source A and f is the isotopic fractionation (‰). An isotopic fractionation of 1 ‰ was used (Peterson & Fry, 1987).

Results

Litter processing in situ

Weight loss

All species of leaves showed a rapid weight loss within the first 24 hours (between 9 and 22%) due to initial leaching. Processing rate coefficients (k) for all species were quite high, with para grass the most rapid (0.026 ± 0.003) followed by eucalypt (0.017 ± 0.002) then sugarcane (0.012 ± 0.007) (Figure 1). Weight loss of leaf packs was significantly different among treatments and over time (Table 1). On all but one occasion sugarcane packs weighed more than eucalypt packs, which in turn were consistently heavier than para grass packs (Figure 1). Comparison of k between species showed no significant difference between eucalypt and sugarcane, but both sugarcane and eucalypt were significantly slower than para grass ($t = 3.5$, $p = 0.01$) and ($t = 3.7$, $p = 0.008$). A spate on days 58-59 appeared to result in a more rapid loss of leaf material, especially for the eucalypt packs.

The physical appearance of leaves during the course of the experiment was markedly different across species. Eucalypt leaf packs were the least uniform throughout the experiment and appeared to suffer from a high level of physical abrasion, becoming increasingly brittle with time. Para grass leaf packs were quickly covered with a brown biofilm and rapidly lost their original shape and texture. Sugarcane leaf packs maintained their shape throughout the experiment but did discolour more than other species and their weight loss seemed to be largely due to the physical abrasion of softer leaf parts.

Total organic nitrogen

The initial total organic nitrogen (TON) contents of sugarcane and eucalypt were very similar (1.00 mg. g⁻¹ and 0.86 mg. g⁻¹, respectively) (Figure 2). Para grass had a much higher initial TON content (2.33 mg. g⁻¹). The mean TON content for each species was significantly different throughout the experiment ($F = 46.0$, $p < 0.001$) and there was no obvious change over time, except perhaps in the first 40 days for eucalypt leaves (Figure 2).

Invertebrate density

Over 25 different family-level taxa were found in the leaf packs throughout the duration of the experiment (Table 2). By far the most abundant taxon was *Nousia* (Leptophebiidae), comprising 44.5 to 51.2 % of the total individuals. All packs were colonised on Day 1, with a rapid increase in density to Day 7. A peak in invertebrate density per pack was observed on Day 14 in all treatments before a rapid decline (Figure 3a). The spate on Days 58-59 appeared to have had little effect on invertebrates, though densities increased in sugarcane and eucalypt packs from Day 64 to Day 85. A two-way ANCOVA of total invertebrate densities per pack showed no difference between vegetation types over time (Table 3a). A similar trend over time was observed when invertebrate densities were plotted per gram of litter remaining, with the exception of para grass packs, which showed a peak in invertebrate numbers on Day 28 (Figure 3b). An ANCOVA on total invertebrate densities per gram remaining showed a significant difference between leaf species (Table 3b). Pairwise comparisons revealed invertebrate densities on para grass were significantly higher than on sugarcane ($t = 2.8$, $p = 0.033$),

and marginally higher than on eucalypt ($t = 2.0$, $p = 0.087$). However, no difference in invertebrate densities per gram was observed between sugarcane and eucalypt packs ($t = 1.0$, $p = 0.364$).

Composition of the leaf pack fauna

All leaf pack treatments started out with similar faunas and the temporal trajectories of invertebrate assemblage composition remained similar up to and including Day 14 (Figure 4). By Day 28, however, the fauna in para grass had diverged from that in both sugarcane and eucalypt, especially evident on Vectors 1 and 2. The spate resulted in a shift in invertebrate composition between Days 42 and 64, though composition for all leaf species seemed to become similar to pre-spate again by Day 85.

Almost twice as many shredders were found on eucalypt packs than on sugarcane or para grass packs. The most abundant shredder was *Anisocentropus kirramus*. Over time, para grass and eucalypt packs showed a general increase in shredder numbers until a peak at Day 28. In contrast the highest numbers of shredders found on sugarcane occurred at Day 85 (Figure 5a). Shredder densities per pack were significantly different among leaf species and over time (Table 4a). Eucalypt packs had significantly more shredders than sugarcane ($t = 2.4$, $p = 0.053$) or para grass leaf packs ($t = 2.8$, $p = 0.032$), however, there was no difference between para grass and sugarcane densities ($t = 0.1$, $p = 0.952$). This pattern was less obvious when shredder densities were plotted per gram of leaf litter remaining over time (Figure 6b). ANCOVA of shredder densities per gram remaining showed only a marginal difference between species (Table 4b). However, on all but one occasion eucalypt leaf packs consistently had more shredders per gram than other leaf packs.

Feeding trials

General observations

Anisocentropus kirramus larvae were examined under a dissection microscope as they fed on offered vegetation types. Larvae rapidly processed the upper epidermal layer of

eucalypt discs with a scraping motion (approximately 1 - 2 mm² per minute) leaving a skeletonised leaf. Specimens also chewed at the edges of eucalypt leaf discs. Para grass discs were not consumed during 30 minutes of observations of five *A. kirramus* specimens. Larvae passed over the surface of the discs stopping only at the edges, but no chewing was observed. Likewise, larvae passed over the surface of sugarcane discs stopping only at the edges, though two specimens did appear to chew at the edges. This process appeared to generate a lot of mucous and it was difficult to tell if anything was ingested.

Leaf processing with multiple consumers

Experimental containers were examined irregularly throughout the experiment at which time the locations of all *A. kirramus* larvae were noted. Larvae were observed positioned more often on leaf discs in eucalypt and sugarcane treatments than on another *A. kirramus* specimen ($t = 4.4$, $p = 0.003$) and ($t = 2.1$, $p = 0.038$). However, there was no difference between the time spent on leaf discs and another *A. kirramus* specimen in para grass treatments. Processing rates of both eucalypt and sugarcane were significantly higher than that of para grass ($t = 6.1$, $p < 0.001$; $t = 10.6$, $p < 0.001$, respectively), but there was no difference between eucalypt and sugarcane processing ($t = 1.2$, $p = 0.254$) (Table 5). The low para grass processing rate was significantly different to the weight loss due to leaching ($p < 0.001$). Comparison of all *A. kirramus* dry weights after the feeding trial showed no difference between leaf disc treatments. Because larvae spent variable amounts of time in the experiment, weights of specimens of known experimental participation duration were also compared and again no differences were observed between different leaf disc treatments ($t = 1.9$, $p = 0.166$).

Leaf processing with a single consumer

The processing of leaf discs was clearly different between treatments (Table 5). Eucalypt leaf discs were processed significantly faster than sugarcane leaf discs ($t = 16.3$, $p < 0.001$), and sugarcane leaf discs were processed considerably faster than para grass leaf discs ($t = 14.2$, $p < 0.01$). Whilst some para grass replicates displayed visible attempts at leaf consumption, no difference were observed between the weight loss due to leaching

and the weight loss due to consumption. The weight of FPOM generated by shredder feeding was also significantly different among treatments ($F = 80.1$, $p < 0.001$). The amount of FPOM generated in each treatment displayed a similar trend to leaf disc processing, with larvae in eucalypt replicates generating significantly more FPOM than sugarcane replicates ($t = 11.3$, $p < 0.001$). Furthermore, those on sugarcane replicates produced significantly more FPOM than those on para grass replicates ($t = 2.838$, $p = 0.025$).

The isotope values of *A. kirramus* larvae from all treatments ranged from -26.37 to -29.29 ‰ for $\delta^{13}\text{C}$ and from 1.38 to 3.43 ‰ for $\delta^{15}\text{N}$. These values were not significantly different to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *A. kirramus* specimens collected from the study stream (Figure 6). Isotope values of the FPOM generated from the eucalypt treatment were very similar to that of the leaves themselves. FPOM generated from the sugarcane and para grass treatments had isotope values indicative of a significant proportion of C_4 carbon, particularly so in the latter (Figure 6). Of interest is the high mean $\delta^{15}\text{N}$ value of FPOM in the para grass treatment, compared with the value of its source.

Leaf preference

The location of each *A. kirramus* larva was randomly observed throughout this experiment. Specimens were observed to spend 32 ± 4 % of their time attached to eucalypt discs, 16 ± 1 % of their time attached to sugarcane discs, and 12 ± 1 % of their time attached to para grass discs. The proportion of time spent on each leaf disc was not reflected by the respective processing rates, which were significantly different for all vegetation types (Table 5). Consumption of eucalypt leaf discs was faster than sugarcane discs, which was faster than para grass discs. Processing of the latter was significantly different to the weight loss due to leaching ($p = 0.003$).

The $\delta^{13}\text{C}$ values of all larvae ranged between -25.97 and -27.28 ‰ (mean $\pm 1\text{SE} = -27.06 \pm 0.14$ ‰). The $\delta^{15}\text{N}$ values ranged between 1.67 and 3.69 ‰ (mean $\pm 1\text{SE} = 2.47 \pm 0.21$ ‰). After 21 days, the specimens had clearly ingested or assimilated very little of

either C₄ plant. Mixing models suggest eucalypt contributed at least 79-88% to the larval diet (average = 85%), leaving a maximum 21% contribution for sugarcane or 20% contribution for para grass. It is worth noting, however, that the larval signatures were all similar to values obtained from field specimens that could only have been feeding on C₃ detritus (Figure 6; Field values $\delta^{13}\text{C} = -27.68 \pm 0.44 \text{ ‰}$; $\delta^{15}\text{N} = 3.26 \pm 0.15 \text{ ‰}$).

Discussion

Processing of C₄ plants in streams

Despite the fact that C₄ plants are often conspicuous and highly productive components of freshwater systems, previous field studies have shown that very little of the organic carbon from C₄ plants appears to be incorporated into aquatic food webs (Hamilton *et al.*, 1992; Forsberg *et al.*, 1993; Bunn *et al.*, 1997; Thorp *et al.*, 1998). Two plausible explanations have arisen: that C₄ plants (like other refractory macrophytes) do not contribute to aquatic food webs due to the presence of an alternative (and perhaps less conspicuous) favourable carbon source; or that C₄ plants have inherent characteristics that deter consumption or assimilation.

Both C₄ and C₃ plant species used in this study exhibited ‘fast’ breakdown rates *in situ* (Peterson & Cummins, 1974). In part, this is likely to be associated with the use of leaf bundles, which have been shown to allow for faster breakdown rates than mesh packs (Boulton & Boon, 1991; Benfield, 1996). Physical abrasion of leaf parts is unlikely to be the main cause of weight loss, however, because flows were low throughout the study period, except for a spate on Days 58-59 that only slightly increased processing rates. Initial rapid weight loss in the first 24 hours, followed by a gradual decline in leaf pack weight was consistent with the literature where the rapid leaching of labile components occurs, followed by a period of microbial conditioning and the gradual breakdown of more refractory components (Bowen, 1987; Boulton & Boon, 1991; Lake, 1995; Benfield, 1996). Although both C₄ species used in the experiment were not native to the region, one of them (*Urochloa*) had a faster breakdown rate than the native species.

This suggests that there is nothing preventing the conversion of C₄ plant litter into FPOM or DOM, and subsequently serving as a potential food source for aquatic invertebrates. However, previous stable isotope studies suggest that this cannot occur to any great degree.

Role of macroinvertebrates in processing C₄ plants

Many previous litter breakdown studies have shown that processing rates of litter material are related to the consumption of litter by detritivores (Webster & Benfield, 1986; Young, Huryn & Townsend, 1994). Although total invertebrate densities were similar for C₃ and C₄ litter packs, there were clear differences in species composition and functional feeding groups. Shredders are generally recognised as contributing most to the breakdown of leaf litter (Webster & Benfield, 1986) and were more abundant in eucalypt leaf packs than in C₄ leaf packs. Collector/gatherers were the main feeding group represented in C₄ packs and it is likely that they were utilising the litter as a substrate for accumulated fine particulate organic matter, and not directly consuming the plant litter. This suggests that, although shredders may play an important role in the breakdown of C₃ leaf litter, they are unlikely to be responsible for the observed fast processing rates of C₄ plant material.

Anisocentropus kirramus is an important shredder of leaf litter in subtropical and tropical forest streams in eastern Australia (Pearson *et al.*, 1989; this study). They have been observed to show distinct preferences in litter choice, despite the ability to cut a wide range of vegetation for the construction of leaf cases (Pearson & Tobin, 1989; Nolen & Pearson, 1993). Although the life history of many Northern Hemisphere shredder species is thought to be strongly linked to the seasonal input of riparian litter (Anderson & Cummins, 1979), *A. kirramus* adults and all larval instars can be found year round (Nolen & Pearson, 1992). This means *A. kirramus* is able to play a continuous functional role in rainforest stream food webs with the year-round processing of coarse particulate organic matter.

Feeding trials with *A. kirramus* larvae clearly demonstrated their ability to consume C₄ plants and weight loss of leaf discs was significantly different to leaching losses. Furthermore, the FPOM or frass produced in C₄ plant treatments displayed $\delta^{13}\text{C}$ values of C₄ origin. However, despite the ability to consume C₄ plant material, it is apparent from this study that this species of shredder prefers not to. In the field, consistently more shredders (predominantly *A. kirramus*) were observed on eucalypt packs than on C₄ plant litter, suggesting preferential colonisation. There appears to be an obvious preference for C₃ plants over C₄ plants by shredders in the stream. Cummins & Klug (1979) suggest shredders prefer 'fast' conditioned leaf litter. In this study, litter processing rate was clearly not a function of shredder preference *in situ* as para grass packs harbored few shredders yet broke down faster than eucalypt packs. In feeding trials, C₃ plant discs were processed at a much faster rate than C₄ plant discs, especially when *A. kirramus* larvae were offered a choice. Similarly the production of FPOM in C₃ treatments was much higher than that in C₄ treatments. Furthermore, *A. kirramus* larvae were observed to spend more time on C₃ leaf discs when offered a choice between leaf types. In fact, when there only para grass was available, *A. kirramus* seemed to prefer the cases of another caddis (made of C₃ material).

These data imply that shredders can consume C₄ plant material, but prefer not to. Furthermore, they do not appear to assimilate C₄ carbon, as illustrated by the lack of shift in larval $\delta^{13}\text{C}$ values in feeding trials. Larval development time for *A. kirramus* instars are not known, although Nolen & Pearson (1992) suggest that several months pass between egg hatching and emergence. Feeding trials in this study lasted for up to three weeks and, although few changes in instars were observed, we would have expected to see a shift in larval $\delta^{13}\text{C}$ values if any assimilation of C₄ carbon had occurred. This is most likely due in part to reduced consumption, as illustrated by lower disc processing rates and lower FPOM production in the C₄ treatments.

Contribution of C₄ plants to aquatic food webs

The results of this study suggest that the limited contribution of C₄ carbon to aquatic food webs observed in previous studies may be due to the preference of aquatic invertebrates for C₃ plant litter, and limited ability to assimilate C₄ plants (although the former may be related to the latter). A variety of factors may contribute to this preference, including (evolutionary) familiarity of food source, ability to digest, nutritional value of leaf litter, and the degree of microbial conditioning. In this study, *A. kirramus* larvae were observed to exhibit two distinct feeding patterns. They chewed at the edges of sugarcane and para grass discs and skeletonised eucalypt discs. A similar differential feeding approach to vegetation types was also observed by Nolen & Pearson (1993), where *A. kirramus* larvae were fed a selection of five vegetation types. Their ability to cut leaf discs from a wide variety of plant species, suggests that leaf consumption is not inhibited by physical leaf properties. In fact, *A. kirramus* were regularly observed to construct new casings from all three vegetation types used in this study. Other possible physiological limitations on processing may include the lack of digestive or symbiotic enzymes present in terrestrial invertebrates (Cummins & Klug, 1979). Examination of *A. kirramus* specimens by Chessman (1986) showed a digestive tract dominated by fine and ultrafine detritus with a complete lack of bacteria, fungi, or algae (although a limited sample size was examined). Total organic nitrogen usually indicates the nutritional value of leaf litter as a potential food source for invertebrate consumers (Irons, Oswood & Bryant, 1988). However, the high TON content of para grass appeared to have little influence on the processing by shredders in this study. Other leaf properties have also been offered as factors potentially inhibiting consumption of plant litter by shredders. Most often, defensive or deterrent chemicals such as phenolic compounds are implicated (Bunn, 1988; Newman, 1991; Shulze & Walker, 1997). Phenolic compounds are known to bind cellulose, inhibiting not only direct digestion, but also the colonisation of microorganisms that make litter more attractive to shredders.

An alternative explanation is that the limited consumption of C₄ plants by aquatic invertebrates may simply be a function of evolution (*i.e.* species select familiar food sources rather than unfamiliar ones). Although this might explain the preference for the native eucalypt over introduced species in this study, it cannot explain the apparent

avoidance of C₄ plants in other systems where such species are prevalent (Hamilton *et al.*, 1992; Forsberg *et al.*, 1993).

Conversion of catchments and riparian zones from C₃ to C₄ vegetation will undoubtedly lead to major changes in carbon flux in streams and rivers, due in part to the reduced processing of coarse particulate organic matter by shredding invertebrates. Although the breakdown of such material may occur via physical or microbial mechanisms, it is clear from isotopic studies that the resulting fine and dissolved organic matter does not contribute in any major way to the aquatic food web. The loss of preferred food types will undoubtedly affect the functional organization and structure of benthic invertebrate assemblages and lead to significant changes in stream ecosystem function.

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Table 1: ANCOVA comparing the AFDW remaining of leaf packs among treatments over time in Bundaroo Creek.

	df	SS	F	P
Time	7	33.4	133.5	<0.001
Leaf type	2	6.5	90.6	<0.001
Interaction	14	3.4	6.8	<0.001

Table 2: Composition of abundant invertebrate taxa in leaf packs from Bundaroo Creek collected between March and June 2000

Taxon	Para grass	Sugarcane	Eucalyptus	Feeding group
Leptophlebiidae	51.8	59.4	52.9	
(<i>Nousia</i>)	(44.5)	(51.2)	(50.8)	Co/Ga
(<i>Atalophlebia</i>)	(3.2)	(0.9)	(0)	Sh
(<i>Atalomicria</i>)	(3.9)	(5.7)	(2.1)	Co/Ga
Chironomidae	22.9	10.4	6.6	Co/Ga
Syrphidae	0.6	1.4	0	Co/Ga
Calamoceratidae	9.3	12.5	30.9	Sh
Leptoceridae	5.8	5.7	4.3	
(<i>Triplectides</i>)	(5.6)	(5.4)	(4.3)	Sh
Gryopterigidae	1.1	3.9	2.3	Sh
Gyrinidae	2.2	1.1	0.9	Pr
Scirtidae	0.2	0.4	0.2	Co/Fi
Megaloptera	0.9	0.9	0.2	Pr
Zygoptera	0.2	0.4	0.2	Pr
Anisoptera	0.9	0.7	0	Pr
Gordioidea	0.4	0.2	0	Para
Mollusca	1.5	0.4	0.2	Gr
% Total	97.4	97.4	98.7	
Additional taxa	3	8	4	
Total individuals	463	441	437	

Table 3: ANCOVA comparing invertebrate density among treatments (a) per leaf pack and (b) per gram AFDW remaining.

	df	SS	F	P
(a)				
Time	6	11806.3	34.8	<0.001
Leaf type	2	61.2	0.5	0.585
Interaction	12	1932.1	2.8	<0.003
(b)				
Time	6	342.3	12.1	<0.001
Leaf type	2	143.3	15.2	<0.001
Interaction	12	167.3	3.0	<0.002

Table 4: ANCOVA comparing shredder density among treatments (a) per leaf pack and (b) per gram AFDW remaining.

	df	SS	F	P
(a)				
Time	6	482.2	7.6	<0.001
Leaf type	2	195.0	9.2	<0.001
Interaction	12	299.0	2.4	0.014
(b)				
Time	6	56.9	2.1	0.067
Leaf type	2	19.2	2.1	0.129
Interaction	12	40.4	0.7	0.707

Table 5: Mean rates ($1 \pm \text{SE}$) of processing of leaf discs ($\text{mg individual}^{-1} \text{ day}^{-1}$) of three leaf types by *Anisocentropus* larvae during three laboratory feeding trials. Results of an ANOVA comparing processing rates are presented for each trial. Mean losses due to leaching and amounts of FPOM generated ($\text{mg individual}^{-1} \text{ day}^{-1}$) are also presented.

Treatment:	Eucalyptus	Sugarcane	Para grass	ANOVA of disc processing rates			
				df	SS	F	P
Experiment 1:							
Disc losses	0.326 ± 0.046	0.275 ± 0.018	0.082 ± 0.007	2	1.1	29.8	<0.001
Leaching losses	0.217 ± 0.037	0.129 ± 0.026	0.024 ± 0.011				
FPOM	-	-	-				
Experiment 2:							
Disc losses	0.939 ± 0.043	0.134 ± 0.024	0.047 ± 0.028	2	3.9	211.4	<0.001
Leaching losses	0.041	0.058	0.033				
FPOM	1.056 ± 0.091	0.280 ± 0.039	0.171 ± 0.010				
Experiment 3:							
Disc losses	0.271 ± 0.062	0.050 ± 0.009	0.004 ± 0.003	2	0.4	15.5	<0.001
Leaching losses	0.096 ± 0.061	0.073 ± 0.003	0.023 ± 0.000				
FPOM	-	-	-				

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Figure 1: Weight (AFDW) loss of leaf packs in Bundaroo Creek over time (degree days °C), where σ = eucalypt, λ = sugarcane, ν = para grass (Mean $\pm$ 1SE, $n = 4$ in most cases). Arrow indicates the timing of a spate on Days 58-59 associated with a 90 mm rainfall event.

Figure 2: Total organic nitrogen (TON) content (mg g^{-1}) of leaf pack samples collected from Bundaroo Creek, where σ = eucalypt, λ = sugarcane, ν = para grass. (Other details as per Figure 1).

Figure 3: Invertebrate densities (a) per pack and (b) per gram remaining in eucalypt (σ), sugarcane (λ) and para grass (ν) leaf packs. (Other details as per Figure 1).

Figure 4: Vectors 1 and 2 (a) and 2 and 3 (b) of an ordination (SSHMDs) of invertebrate assemblages from leaf packs in Bundaroo Creek, where σ = eucalypt, λ = sugarcane, ν = para grass. Numbers represent time of incubation in the stream (days).

Figure 5: Shredder densities (a) per pack and (b) per gram remaining in eucalypt (σ), sugarcane (λ) and para grass (ν) leaf packs. (Other details as per Figure 1).

Figure 6: Stable carbon and nitrogen isotope signatures from eucalypt (σ), sugarcane (λ) and para grass (ν) treatments. Open symbols in (a) represent leaf litter and closed symbols FPOM. Signatures of *Anisocentropus kirramus* larvae from Experiment 2 are expanded in (b). (Means $\pm$ 1SE are presented; $n = 10$ for leaf samples, 10 for FPOM and 7-8 for larvae).

a.

b.