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4 1 Organic matter sources and size structuring in stream invertebrate food
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7 2 webs across a tropical to temperate gradient
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33 13 Key words: periphyton, leaf litter, trophic level, stable isotopes, omnivorous crustaceans
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38 15 **Summary**
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40 16 1. Given large differences in species diversity across latitudinal gradients, it has been suggested
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42 17 that certain structural and functional attributes of freshwaters may differ over a latitudinal range,
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44 18 an important consideration for knowledge-poor tropical systems that are undergoing major
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46 19 human-induced changes.
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50 20 2. Two key features of aquatic ecosystems, the contribution of *in situ* production (periphyton) to
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52 21 consumer biomass and size structuring (body size versus trophic level), were measured in stream
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54 22 invertebrate food webs across a gradient of 15 degrees of latitude in Australia from the wet
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56 23 tropics to a temperate island.
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3. Consistent with earlier work, crustaceans (Atyidae and Palaemonidae) were responsible for a large fraction of the community biomass at tropical and subtropical sites. The periphyton food source pathway accounted for a large proportion of the biomass at intermediate latitudes (79% in the subtropics and 95% at temperate mainland sites), with lower contributions in the most distal regions (51% in the tropics and 31% in temperate island sites). These lower contributions were driven by the biomass dominance of large crustaceans in the tropical region and by shredding and filtering insects in the temperate island region.

4. Though these differences in taxonomic composition did not translate into a latitudinal gradient in size-structuring, the larger individual body size of crustaceans compared to insects for a given trophic level suggests that food webs dominated by the former, as is expected in tropical river food webs, are likely to exhibit altered biomass distributions with implications for higher predators.

5. These results suggest that stream food webs can share common features despite differences in species composition, and that local factors are likely as important as broad-scale biogeography and climate in driving food web patterns.

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Introduction

Early ecologists recognized strong latitudinal gradients in productivity and diversity (Hawkins 2001). Tropical systems with their higher temperatures and greater insolation supported greater species diversity than temperate and arctic systems, leading to decades of research on the possible links between these factors (Pianca 1966; Huston 1979; Partel, Laanisto & Zobel 2007). Less studied are patterns of ecological structure and function across the gradient (Mullholland *et al.* 2001; Olesen & Jordano 2002; Liski *et al.* 2003), most notably those for aquatic food webs (Paine 1966; Hecky & Hesslein 1995; Boyero *et al.* 2011). The same environmental factors that may be responsible for the diversity gradient could influence the relative importance of different primary food sources (leaf litter, periphyton) to overall secondary production in rivers and streams and the predator-prey relationships that occur therein. If ecological organization differs in the tropics and certain ecological processes are unique, there are potential management implications for systems that are likely to be placed under greater human-induced pressures in the 21st century (Dudgeon 2000). This has led to a call for greater study of tropical freshwaters, and a particular need for direct comparisons between the tropics and other biomes (Boulton *et al.* 2008; Boyero *et al.* 2009).

Recent investigations using stable isotope analysis of carbon (¹³C/¹²C) have found a disproportionately large importance of periphyton in supporting food webs in tropical and subtropical rivers. Traditional views of organic matter processing in temperate systems, particularly small heterotrophic streams, held that food webs were supported almost exclusively by inputs of terrestrial leaf litter (Fisher & Likens 1973; Vannote *et al.* 1980). While these predictions have been reasonably well-supported by isotopic evidence in temperate systems (*e.g.* Doucett *et al.* 1996; Reid *et al.* 2008), early isotope work in the Neotropics suggested a strong

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3 70 role for algae (Hamilton, Lewis & Sippel 1992), and more recent investigations in the northern
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5 71 half of Australia (Bunn, Davies & Kellaway 1997; Bunn, Davies & Mosisch 1999; Hadwen,
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7 72 Spears & Kennard 2010), Puerto Rico (March & Pringle 2003), Hong Kong (Lau, Leung &
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9 73 Dudgeon 2009) and Brazil (Brito *et al.* 2006) have shown a dominance of aquatic sources,
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11 74 largely periphyton, in supporting these food webs. Some of these latter studies were conducted
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13 75 in small, canopied streams (Lau *et al.* 2009), suggesting that climatic or biogeographic factors
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15 76 unique to warm regions may override the importance of local factors such as shade and nutrients
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17 77 on energy pathways for food webs. Thus, separate food web models for the tropics have been
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19 78 proposed that include a reduced emphasis on the importance of detritus and a greater role for
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21 79 autochthonous or in-stream sources (Dudgeon, Cheung & Mantel 2010).
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27 80 Size-structuring, a key attribute of food webs that dictates metabolism and energy
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29 81 requirements (Peters 1986) and predator-prey interactions (Cohen *et al.* 1993) may also vary
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31 82 across climatic gradients. Gape limitation in many aquatic communities leads to predators
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33 83 necessarily being larger than their prey (Brose *et al.* 2006), resulting in a progressive increase in
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35 84 body size with each increase in trophic level with some exceptions (Riede *et al.* 2011). For
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37 85 example, fish food webs in tropical freshwaters are only weakly size-structured because of the
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39 86 presence of large-bodied herbivores (Layman *et al.* 2005). In addition, large-bodied crustaceans
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41 87 such as prawns (*Macrobrachium* spp.), shrimps (Atyidae) and crayfish (*Cherax* spp.) often
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43 88 dominate invertebrate biomass in the tropics (Jacobsen, Schultz & Encalada 2008) and therefore
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45 89 play a central role in organic matter cycling. These taxa are unlikely to be preyed upon by other
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47 90 invertebrates, particularly insects, which may weaken overall body size-trophic level
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49 91 relationships if the crustaceans occupy low trophic levels (Burns & Walker 2000). Given these
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92 observations, invertebrate food webs in the tropics, like fish food webs, may also be less size-
93 structured than those in temperate systems.

94 Much of the difficulty in comparing the structure and function of tropical and temperate
95 streams comes from synthesizing data across studies that vary widely in local factors such as
96 stream size (Finlay 2001; Sabo *et al.* 2010; Kobayashi *et al.* 2011). In this study, invertebrate
97 food webs were sampled in similar-sized streams and rivers from tropical North Queensland to
98 temperate Tasmania, Australia. Sources (periphyton and leaf litter) and consumers were
99 analysed for stable C isotopes that are known to vary from site to site, and the gradient approach
100 was used to calculate the contribution to the food web from periphyton (Rasmussen 2010). Body
101 mass of all organisms was measured and compared to trophic level (calculated from stable N
102 isotopes) to assess size-structuring. I hypothesized that invertebrates from tropical streams
103 would exhibit stronger links to periphyton and show weaker size-structuring than those from
104 temperate streams.

106 **Methods**

107 A total of 43 sites were sampled from four regions along the east coast of Australia
108 (Table 1). The Great Dividing Range runs in a north-south direction along this coast, limiting
109 the size of east-flowing coastal streams and rivers that drain to the Coral Sea in the north and the
110 Tasman Sea in the south and thus constraining stream size as a contributing variable in dictating
111 food web structure (Finlay 2001; Sabo *et al.* 2010; Kobayashi *et al.* 2011). The four regions
112 were chosen to make latitudinal comparisons, and sites were largely upstream of road crossings
113 to facilitate access. The most northerly region was tropical north Queensland, where nine sites
114 were located north of the city of Cairns, ranging in latitude from 15.4° S to 16.9° S. Southeast

Queensland served as the subtropical region, with six sites sampled south of Brisbane from 27.8° S to 28.1° S. There were two regions sampled in the temperate zone. The first was in the state of Victoria, east of the city of Melbourne in the Gippsland Lakes region. These 16 sites had a narrow latitudinal range from 37.7° S to 38.0° S and are hereafter referred to as “temperate mainland”. The second temperate region was the island of Tasmania. East-flowing streams and rivers were sampled and ranged in latitude from 40.9° S to 42.3° S. These 12 sites are hereafter referred to as “temperate island”.

Sites in all regions were wadeable streams and rivers that typically had gravel/cobble bottoms. Stream order ranged from 2 to 5, and upstream drainage area ranged from 9 to 2112 km² (Table 1). Overall, sites were typically circumneutral and well-oxygenated with low nutrient concentrations and benthic algal biomass, indicative of oligotrophic conditions (Smith, Tilman & Nekola 1999) (Table 1). Riparian disturbance was generally minimal with the exception of some sites in the temperate mainland region where the dominant land use was pasture for dairy production, leading to slightly elevated turbidity (Table 1). While mean canopy cover at sites (estimated from satellite imagery) was similar among regions ($F_{3,39} = 1.278$, $p = 0.295$), mean site elevations did differ ($F_{3,39} = 6.655$, $p = 0.001$), with subtropical sites significantly higher in elevation than temperate island or tropical sites (Table 1). Combining all environmental variables into a principal component analysis following log-transformation and normalization (subtracting the mean and dividing by the standard deviation) separated regions but with low explanatory power (Figure S1). Only 46.3% of total variation was explained by PC1 (25.2%) and PC2 (21.1%). Temperate mainland sites separated from other regions based on larger stream order and catchment areas (PC1), and tropical sites separated from other regions based on lower total phosphorus, pH and turbidity (PC2) (Figure S1).

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At each site, three replicate samples of benthic invertebrates were collected with a 676 cm² surber sampler with 500 µm mesh in representative habitats (pools, rifles and runs). Sampling methods were not optimized to capture crustaceans; thus, their absolute contribution to community biomass is likely an underestimate. However, the same individual (TDJ) was responsible for all collections so their relative contribution across sites and regions is likely comparable. Samples were stored on ice and frozen for transport to the laboratory. To characterize isotope ratios of food sources, three composite samples of biofilm were scrubbed from submerged surfaces (almost exclusively rocks) and stored frozen in ziplock bags. A single pooled sample of leaf litter was also collected at each site to verify that isotopic variation across sites was minimal (Finlay 2001).

In the laboratory, the biomass of invertebrates was quantified while preparing samples for isotope analysis. Samples were thawed, identified to family level, counted, tamped dry on a paper towel (kimwipe), and individuals from a given family placed together in a pre-weighed plastic vial. The total wet mass of each sample was then determined before being dried. Because the number of individuals in a sample was counted, wet mass per individual could also be estimated by division. After drying (60° C for 48 hr), samples were re-weighed to estimate dry mass then ground into a powder and a sub-sample weighed out at approximately 0.8 mg for C&N isotope analysis.

Samples of biofilm were thawed, allowed to settle and the top layer and overlying water pipetted into a plastic tube. The bottom (heavy), inorganic/detrital-dominated layer was discarded. Algal-dominated top layer samples were dried for 48 hr at 60° C, ground to a powder and weighed at approximately 3 mg for C&N isotopes. Leaf litter samples were dried under the same conditions, ground to a powder and weighed at approximately 4 mg for C&N isotopes.

Samples were analysed for stable isotope ratios at two laboratories. The majority of the samples were analysed by combustion in a PDZ Europa ANCA-GSL elemental analyser followed by delivery of gases to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Ltd., Cheshire, UK). Duplicate samples yielded a mean absolute difference of 0.3‰ for $\delta^{13}\text{C}$ and 0.5‰ for $\delta^{15}\text{N}$ ($n = 20$), while five working standards analysed alongside samples had 1 S.D. that ranged from 0.1‰ to 0.3‰ for sample sizes ranging from 12 to 119. A small number of samples was analyzed by combustion in a Eurovector EA 3000 followed by delivery of gases to an Isoprime mass spectrometer (GV Instruments, Manchester, UK). Duplicate samples yielded a mean absolute difference of 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$ ($n = 7$), while five working standards analysed alongside samples had 1 S.D. that ranged from 0.1‰ to 0.2‰ for sample sizes ranging from 4 to 9. To ensure comparability among labs, two samples that had been analysed at the first lab ($\delta^{13}\text{C} = -19.3 \pm 0.1$ ‰ S.D., $\delta^{15}\text{N} = 9.1 \pm 0.2$ ‰ S.D. and $\delta^{13}\text{C} = -25.7$ ‰, $\delta^{15}\text{N} = 0.0$ ‰) were also analysed once at the second lab, yielding comparable values ($\delta^{13}\text{C} = -19.3$ ‰, $\delta^{15}\text{N} = 8.3$ ‰ and $\delta^{13}\text{C} = -25.9$ ‰, $\delta^{15}\text{N} = 0.5$ ‰).

The taxonomic composition of the biomass and average body sizes were compared among regions. Taxonomic composition was qualitatively compared by plotting average percent biomass contribution within sites. The average body size of consumers among regions was tested using analysis of co-variance (ANCOVA), with $\log_{10}$ of individual wet mass as the dependent variable, region as the fixed factor, and $\log_{10}$ of the proportion of site biomass as the co-variate. The ANCOVA was used because few large individuals can dominate biomass and thus bias comparisons among regions if only mean values are used; by having proportion of site biomass as the co-variate direct comparisons can be made more readily. The interaction term in this model was first tested, found to be non-significant ($F_{3,302} = 1.004$, $p = 0.391$) and the model

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re-run after its removal. For taxon-specific comparisons of individual wet mass, a GLM-ANOVA was used; the ANCOVA was not necessary because the range of body sizes within taxa and their contribution to biomass was far less variable.

To quantify the reliance of consumers on periphyton as a food resource in the different regions, the gradient approach was used (Rasmussen 2010). The site-specific $\delta^{13}\text{C}$ of a given taxon (family or functional feeding group) was regressed against the $\delta^{13}\text{C}$ of biofilm (periphyton-dominated) at the site. Because the other potential food source pathway begins with terrestrial leaf litter that exhibits invariant $\delta^{13}\text{C}$ across sites (Finlay 2001), the slope of the consumer-biofilm regression is directly proportional to the percent contribution of the periphyton/biofilm food source pathway to the diet (Rasmussen 2010). While this approach assumes similarity in feeding within taxa across sites, it largely circumvents problems that originate from overlapping $\delta^{13}\text{C}$ values in the center of the wide $\delta^{13}\text{C}$ distribution of periphyton (Finlay 2001). Using the gradient approach, confidence intervals around slope estimates can be used to compare periphyton reliance among taxa and regions (Jardine *et al.* 2012a). When 95% confidence intervals include one but not zero, periphyton can be considered to contribute roughly 100% to the diet. When the 95% C.I. includes zero but not one, periphyton is assumed to contribute nil to the diet. A 95% C.I. that includes neither zero nor one suggests a mixed contribution from the two source pathways, and a 95% C.I. that includes both zero and one results from an unsolved model (Jardine *et al.* 2012a). Though both x and y in these regressions are measured with error suggesting model II regression would be more appropriate, results reported here are for ordinary least squares (OLS) regressions because model II regressions commonly result in slopes that are higher than one or lower than zero (Jardine *et al.* 2012a), and proportions derived from OLS

slopes provide values that are closer to those estimated directly from mixing models at subsets of sites where sources differ (Jardine, Kidd & Rasmussen 2012b; T.D. Jardine, unpublished data).

By grouping organisms into functional feeding groups and using the resulting consumer-biofilm $\delta^{13}\text{C}$ slopes as estimates of % periphyton in the diet, coarse comparisons can be made among regions of the biomass-specific contribution from periphyton to the invertebrate community (Jardine *et al.* 2013). This is achieved by multiplying the total biomass of a taxon at a site by its estimated dietary contribution from periphyton and then summing the totals for the site. The result is the total amount of biomass that can be attributed to periphyton production, which can then be divided by the total biomass to estimate the overall percent contribution from periphyton. Error terms can be derived from slope estimates (e.g. 1 S.E.) and also multiplied by biomass to obtain error around final totals. While this does not allow formal statistical testing among regions, it provides a relative indicator of the importance of periphyton and associated uncertainty along the tropical to temperate gradient.

To estimate trophic level for individual taxa within sites, $\delta^{15}\text{N}$ data were used (Post 2002). Trophic level was calculated according to: $(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}})/\Delta^{15}\text{N} + 2$, where $\Delta^{15}\text{N}$ is an average diet-tissue $\delta^{15}\text{N}$ fractionation (2.5‰, Vanderklift & Ponsard 2003) and $\delta^{15}\text{N}_{\text{baseline}}$ is the average value within a site for primary consumers. Primary consumers were classified according to Gooderham and Tsyrlin (2002) and included various combinations of Leptophlebiidae, Psephenidae, Baetidae, Caenidae, Elmidae, Gripopterygidae, Pyralidae, Oligochaeta, Simuliidae, Tipulidae, Scirtidae, Corbiculidae, Calocidae, Gastropoda, and Notonemouridae. Trophic level measurements were then linked to wet weights per individual by regression. Slopes of these regressions in the four regions were compared by testing for overlap among 95% confidence intervals, using model II regression with the lmodel2 package in R

(Legendre 2001) to facilitate direct comparisons with prior work (Riede *et al.* 2011). Initial screening removed data from two sites. The entire Endeavour River site (tropical region) was removed because unlike all other sites in the study, predators at that site had lower $\delta^{15}\text{N}$ than primary consumers, suggesting an inappropriate selection of baseline organism. A single water penny sample (Family Psephenidae) at a site in the temperate mainland region (Thomson River @ Trevor Platt's) was also removed from the analysis as an outlier because it had $\delta^{13}\text{C}$ (-27.3‰) and $\delta^{15}\text{N}$ (-0.3‰) that greatly differed from the remainder of the food web ($\delta^{13}\text{C}$ = -39.9 to -33.0 and $\delta^{15}\text{N}$ = 6.3 to 9.3‰, $n = 4$) resulting in a clearly incorrect trophic level (-0.4).

Results

Taxonomic composition of biomass

The four regions differed in taxonomic composition and individual body sizes. Temperate island sites had communities whose biomass was dominated by small-bodied taxa, including Leptophlebiid mayflies (mean individual wet mass = 3.5 mg) that made up an average of $38 \pm 28\%$ of the biomass (Fig. 1). Omnivorous shrimps (Atyidae, $19 \pm 29\%$, mean mass = 70.3 mg) and net-spinning caddisflies (Hydropsychidae, $11 \pm 16\%$, mean mass = 5.9 mg) were also important in this region. This latter taxon also dominated the biomass at temperate mainland sites, accounting for $30 \pm 23\%$ of the biomass on average (Fig. 1) at an average size of 5.3 mg. No other taxon contributed more than 10% in this region. In the subtropical region, large-bodied freshwater prawns (Palaemonidae, mean mass = 395.3 mg) were the dominant taxa ($29 \pm 33\%$). Leptophlebiids ($17 \pm 10\%$, mean mass = 3.5 mg) and two predatory taxa (Megaloptera dobsonflies $19 \pm 21\%$, mean mass = 42.5 mg, and dragonflies Odonata $11 \pm 14\%$, mean mass = 55.9 mg) also contributed more than 10%. In the tropics, there were five taxa with

considerable biomass that were also important in the other regions. The crustaceans Atyidae (mean mass = 48.8 mg) and Palaemonidae (mean mass = 138.8 mg) made up $24 \pm 24\%$ and $13 \pm 34\%$ of the biomass, respectively. Other taxa included Hydropsychidae ($15 \pm 15\%$, mean mass = 2.4 mg), Odonata ($18 \pm 19\%$, mean mass = 39.8 mg) and Leptophlebiidae ($10 \pm 13\%$, mean mass = 1.0 mg). In all regions, the largest individuals at a given site were responsible for the greatest fraction of the total biomass ($F_{1,305} = 181.139$, $p < 0.001$). There were significant differences among regions in individual wet mass ($F_{3,305} = 21.484$, $p < 0.001$). On a community-wide basis, temperate island sites had significantly lower average individual wet mass compared to the other three regions. However, when comparing the wet mass of individuals within taxa across regions, the tropics had significantly smaller individuals compared to the other three regions: Baetidae [$F_{3,18} = 4.351$, $p = 0.018$; tropical < temperate mainland ($p = 0.003$); Hydropsychidae [$F_{3,29} = 4.149$, $p = 0.015$; tropical < temperate mainland ($p = 0.002$), temperate island ($p = 0.018$) and subtropical ($p = 0.045$); and Leptophlebiidae [$F_{3,29} = 2.837$, $p = 0.055$; tropical < subtropical ($p = 0.019$), temperate mainland ($p = 0.022$) and temperate island ($p = 0.028$)]. There were no differences among the other three regions except for Baetidae, where individuals from temperate mainland sites were significantly larger than those from temperate island sites ($p = 0.015$).

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269 *Carbon sources and $\delta^{13}C$*

270 As expected, $\delta^{13}C$ was far more variable in biofilm (periphyton) compared with leaf litter
 271 (Fig. S2). Values for biofilm ranged from -37.1‰ to -17.9‰ across all sites in all regions (mean
 272 $= -28.3 \pm 3.7\text{‰}$ S.D., $n = 44$). Ranges in biofilm $\delta^{13}C$ were smaller in subtropical (-27.9‰ to -
 273 21.3‰) and temperate mainland (-32.0‰ to -23.0‰) sites compared to tropical (-37.1‰ to -
 274 17.9‰) and temperate island (-34.0‰ to -22.8‰) sites. Leaf litter was analyzed at fewer sites

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than biofilm but yielded a similar mean value as that reported elsewhere (-29.9‰) and unsurprisingly, had limited variation across sites (range = -31.6‰ to -28.2‰, 1 S.D. = 1.0, n = 28).

The combination of the taxonomic composition of the biomass and use of food source pathways led to differences in the importance of periphyton in the four regions. In the tropics, primary consumers were strongly linked to the periphyton source pathway (Table 2, Fig. 2a), but organisms classed as omnivores (Atyidae, Palaemonidae, Hydropsychidae) derived less of their diet from this pathway (Fig. 2b) and dominated the biomass (Fig. 1). This caused the overall community biomass to be roughly equally reliant on the two food sources in this region (Fig. 3). Both the subtropical and temperate mainland regions had invertebrate communities that derived the vast majority of their biomass from the periphyton pathway (Table 2, Fig. 3). All three functional feeding groups in these regions were strongly reliant on periphyton, with consumer-biofilm $\delta^{13}\text{C}$ slopes > 0.59 for all taxonomic and functional feeding groups (Table 2, Fig. 2). In the temperate island region, consumers were less strongly linked to biofilm, with slopes ranging from 0.22 to 0.60 (Table 2). As a result, the average contribution to community biomass was only 31% in this region (Fig. 3).

Size structuring and $\delta^{15}\text{N}$

Average trophic level across all regions for those taxa not classed as primary consumers ranged from 1.8 (Ceratopogonidae) to 3.3 (Tabanidae). Large-bodied, biomass-dominant omnivores had intermediate trophic levels, including Hydropsychidae (TL = 2.3), Atyidae (TL = 2.7) and Palaemonidae (TL = 2.5). Two predatory taxa that had relatively high biomass [Megaloptera (Corydalidae) and Odonata] both had an average TL = 2.7.

Body size increased with trophic level in these stream invertebrate food webs (Fig. 4). Though much variability remained unexplained, regressions of log body size vs. trophic level were significant in all regions (Tropical: $r^2 = 0.19$, $F_{1,41} = 9.371$, $p = 0.004$; Subtropical: $r^2 = 0.41$, $F_{1,46} = 31.838$, $p < 0.001$; Temperate mainland: $r^2 = 0.16$, $F_{1,82} = 16.157$, $p < 0.001$; Temperate island: $r^2 = 0.18$, $F_{1,57} = 12.687$, $p = 0.001$). As estimated from the slopes of body size vs. trophic level regressions, there were slight differences in the size-structuring of invertebrate food webs among the regions (Fig. 4). Despite the biomass dominance of large-bodied crustaceans in the tropics and the dominance of small-bodied insects at temperate island sites (Fig. 1), there was no evidence for altered size-structuring between these regions. Only the temperate mainland region had sites with 95% confidence intervals around slope estimates that did not overlap with all other regions (Table 2), a pattern that was repeated when a more liberal comparison with 85% confidence intervals was used. The minor difference in the temperate mainland region was driven by the presence of non-insect taxa in other regions; crustaceans were generally larger than insects for a given trophic level, exhibiting positive residuals in the body size-trophic level regressions (Fig. 4). Removal of non-insects from the regressions resulted in overlap in 95% C.I.'s among regions and allowed the consolidation of all insect data into a single common slope estimate (Table 2).

Discussion

Contrary to predictions, energy pathways and size structuring of invertebrate food webs in tropical streams were not particularly different from those in temperate regions. Despite considerable differences in the community composition at the extreme ends of the range (tropical and temperate island), overall food webs in these two distal regions exhibited a similar

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contribution from periphyton and equal slopes of trophic level vs. body size regressions. These observations suggest that there may be little that is unique about lower trophic levels in tropical river food webs (Boulton *et al.* 2008). However, the potential role of crustaceans in stream food webs in general and their greater prominence in the tropics require further investigation because they exert strong controls on both energy pathways and size-structuring examined here.

Large-bodied omnivores that dominated biomass in the tropics exhibited mixed diets originating from both periphyton and leaf litter, a product of their generalist nature (Covich 1988). Both crustacean taxa (Atyidae and Palaemonidae) likely play a role in breaking down leaf litter in these streams while supplementing their diet with insect prey of aquatic origin (March & Pringle 2003), thereby filling similar niches and performing similar functions as omnivorous crayfish (Parkyn, Collier & Hicks 2001). Their dietary contribution from the periphyton source pathway measured in this study (Atyidae slope = 0.48, corresponding to approximately equal contributions from periphyton and detritus) is consistent with values for streams, rivers and waterholes in the wet-dry tropics, where local biofilm contributions ranged from 39 to 70% for these taxa (Jardine *et al.* 2012a; Jardine *et al.* 2013). The biomass of palaemonid prawns can also rival that of fish in tropical waterholes (Jardine *et al.* 2013), and thus their consumption of detrital and algal material will exert a strong role in transferring this energy to fish and other predators at higher trophic levels.

The approximate 50% contribution from terrestrial inputs to invertebrate food webs in the tropics likely represents a maximum possible value for this region because samples were largely collected from forested, lowland streams (Kobayashi *et al.* 2011). Allochthonous inputs can dominate tropical food webs in heavily shaded, low productivity systems (Yule 1996). Though site-level shredder species diversity is lower in equatorial regions (Boyero *et al.* 2011), some

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3 344 taxa such as leptophlebiid mayflies and crayfish (*Cherax* spp.) can be common (Cheshire,
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5 345 Boyero & Pearson 2005; Coughlan, Pearson & Boyero 2010) and can break down leaf litter at a
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8 346 rate comparable to that found in temperate streams (Covich 1988), likely aided by microbial
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10 347 degradation at higher temperatures. The sites from the tropical region in this study had low
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12 348 nutrient concentrations (TP < 10 µg/L, TN < 330 µg/L, Table 1) suggestive of oligotrophic
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14 349 conditions (Smith, Tilman & Nekola 1999). Despite these limitations to in-stream primary
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16 350 productivity, periphyton still accounted for half of the invertebrate biomass. Subtropical and
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18 351 temperate mainland regions both had high contributions from periphyton but were arguably the
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20 352 most dissimilar in terms of environmental variables (Figure S1). One feature these two regions
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22 353 had in common was higher phosphorus concentrations (Table 1); therefore, the high
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24 354 contributions from periphyton observed at those sites may be a function of their mesotrophic
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26 355 status (Bunn *et al.* 1999). Subtropical sites also were at significantly higher elevations than those
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28 356 in other regions, and food web contributions from periphyton have been observed to peak at mid-
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30 357 order, upland sites in stream continua (Kobayashi *et al.* 2011).
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36 358 The dominance of Leptophlebiidae at the temperate island sites is consistent with prior
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38 359 work in this region (Read & Barmuta 1999) where this taxon is known to shred leaf litter (Yeates
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40 360 & Barmuta 1999). The Tasmanian fauna is generally depauperate relative to other parts of the
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42 361 continent due to its isolation and history of glaciation, and insect taxa dominate stream
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44 362 invertebrate assemblages (Magierowski *et al.* 2012). The low contribution of periphyton to the
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46 363 invertebrate community biomass was driven largely by Leptophlebiidae and Hydropsychidae that
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48 364 had lower periphyton-consumer $\delta^{13}\text{C}$ slopes compared to other regions. Although crustaceans
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50 365 are present in Tasmanian freshwaters, they include small-bodied amphipods (Fig. 4D), and large
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52 366 bodied forms are less common (D.M. Warfe, University of Western Australia, pers. comm.).
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Running waters in temperate systems worldwide are often dominated by insects (Hall, Likens & Malcolm 2001) that exhibit a wide variety of feeding habits. As a consequence, the relative biomass and secondary production of different functional feeding groups will dictate the relative contribution to overall food web biomass from the two food source pathways (Rasmussen 2010; Jardine *et al.* 2012b). Because larger animals have lower ratios of annual production to biomass (Banse & Mosher 1980), stream food webs with biomass dominated by terrestrially-subsidized, large-bodied crustaceans such as those in the tropics may underestimate a high-production, rapid-turnover fraction of small-bodied insects that are instead more heavily reliant on periphyton. While outside the scope of this study, the smaller average body mass of insect taxa in the tropics (Baetidae, Hydropsychidae, Leptophlebiidae) suggests that a similar investigation with secondary production as the endpoint could produce a different result than using community biomass as shown here.

Differences among seasons may also have played a role in observed patterns of carbon source pathways. The majority of sites were sampled between March and May, corresponding to the end of the Austral growing season. Two sites that were sampled in both April and December exhibited similar patterns in the dominant taxa despite differences in community developmental stage. Leptophlebiid mayflies accounted for 59% (April) and 66% (December) of the community biomass at the Douglas River bridge site despite this taxa having an average wet mass that was approximately ten times smaller in December (1.4 mg versus 13 mg). At the Meredith River site, Leptophlebiids were approximately the same size (3 mg versus 5 mg) and Atyid shrimps were larger in December (157 mg versus 70 mg) yet their contribution to community biomass was similar in both seasons (54% and 22% in April and 42% and 46% in December, respectively). Short-lived producers and consumers such as algae and insects can

also exhibit wide temporal variation in isotope ratios; however, the gradient approach is robust to these seasonal changes because spatial variation in $\delta^{13}\text{C}$ across sites exceeds temporal variation within sites (Jardine *et al. in press*).

Despite the presence of large-bodied crustaceans, food webs in the tropics were not less size-structured than subtropical or temperate streams. This was likely driven by the fact that trophic levels for these crustaceans were higher than expected, ranging from 2.3 to 3.7 for Atyidae and 2.5 to 3.6 for Palaemonidae rather than $\text{TL} \approx 2$ for both taxa if their diet had been dominated by plant material (Burns & Walker 2000). Because crustaceans were larger than insects for a given TL (Fig. 4), their higher TLs tended to drive regressions towards steeper slopes rather than shallower slopes as was predicted. Tropical streams that are dominated by these taxa (as has been observed previously, Bright 1982; Jacobsen *et al.* 2008), will therefore have altered biomass spectra (Morin, Rodriguez & Nadon 1995), with a larger fraction of the community biomass concentrated at trophic levels 2.5 to 3.0 despite similar slopes of individual body size versus trophic level. Invertebrates generally exhibit weaker size structuring than other taxa, with predator-prey body mass ratios less than 1 (Brose *et al.* 2006; Riede *et al.* 2011), and insects in the current study had shallower slopes when crustaceans and other taxa (bivalves, gastropods, oligochaetes) were excluded from regressions. As a result, streams dominated by insects will require longer food chains to support top predators that only forage on prey above a certain size threshold (Pyke, Pulliam & Nekola 1977).

Stable N isotopes are a promising tool for further exploring body mass vs. trophic level relationships. Regression slopes using TL derived from $\delta^{15}\text{N}$ have been proposed as a measure of the impacts of human activities on lotic systems, with positive relationships observed between slopes and stream productivity owing to a greater proportion of predators in larger size classes in

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productive streams (Anderson & Cabana 2009). These slopes are, however, sensitive to trophic fractionation that is known to exhibit variation according to animal physiology and diet (Vanderklift & Ponsard 2003). To illustrate this sensitivity, trophic level estimates were adjusted using a lower $\Delta^{15}\text{N}$ value that was empirically determined for algae to predators in riverine systems such as these (1.8‰, Bunn, Leigh & Jardine 2013). With this lower value, the regression slope of body mass versus trophic level for all insects combined (0.82, 95% C.I. = 0.73-0.93) was almost identical to the slope of 0.81 (95% C.I. = 0.71-0.91) obtained for stream invertebrates across a series of food webs worldwide (Riede *et al.* 2011). However, using a higher, commonly used estimate of $\Delta^{15}\text{N}$ (3.4‰, Post 2002) would have resulted in slopes that greatly exceeded those reported from prior studies.

Overall these results suggest that stream invertebrate communities can converge on common structural attributes despite being separated by a large latitudinal gradient (Boulton *et al.* 2008). The similarity in stream food web structure occurred despite differences in the types of organisms responsible for the transfer of energy through food webs. Though sites were selected to minimize variation in size (width/depth) and human disturbance to allow fair comparisons among geographic regions, subtle differences in elevation, land use and nutrient status may have driven the observed patterns. This further supports the view that local factors may be as responsible as broad-scale geographic factors in dictating freshwater food web structure and function (Boulton *et al.* 2008). Evidence presented here does not support a need for separate food web models for tropical streams (Dudgeon *et al.* 2010), though continued comparisons across latitudinal gradients (*e.g.* Boyero *et al.* 2011) with a particular focus on the role of crustaceans will enable a fuller understanding of freshwater food web patterns and processes.

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Table 1. Limnological information for study streams in eastern Australia, where invertebrates were collected to estimate importance of organic matter sources to the food web and size-structuring.

Location	Date	S	E	Order	Catch area	Elevation (m)	Canopy (%)	pH	turb	DO	temp	TP (mg/L)	TN (mg/L)	benthic chlor
TROPICAL - NORTH QUEENSLAND														
Endeavour R.	26-May-10	15.429	145.118	4	372.8	22	40	6.8	5.1	7.7	24.9	0.009	0.19	32.4
Trevathan Cr.	26-May-10	15.620	145.221	3	75.9	47	70	6.6	6.6	7.4	24.8	0.002	0.17	3.6
Bloomfield R.	27-May-10	15.960	145.319	4	314.4	18	10	6.9	1.6	8.1	23.7	0.002	0.11	3.2
Thompson Cr.	27-May-10	16.102	145.459	2	8.6	12	90	5.9	0.1	7.6	23.3	0.002	0.10	11.6
Douglas Cr.	28-May-10	16.280	145.297	3	60.3	13	10	6.7	1.1	7.9	22.5	0.006	0.14	0.7
Cooper Cr.	28-May-10	16.166	145.416	2	9.5	29	90	6.5	0.5	7.0	23.7	0.002	0.06	1.4
Saltwater Cr.	29-May-10	16.417	145.356	2	33.4	25	50	6.6	0.0	8.4	21.8	0.002	0.21	1.1
Mossman R.	29-May-10	16.450	145.360	4	87.5	21	80	6.7	0.0	8.3	22.2	0.002	0.20	2
Freshwater Cr.	30-May-10	16.907	145.704	4	93.2	19	80	6.7	2.7	7.6	23.8	0.008	0.32	6.7
SUBTROPICAL - SOUTHEAST QUEENSLAND														
Coomera R. upper	23-Apr-10	28.047	153.190	3	84.7	119	70	7.6	5.8	8.5	21.0	0.077	0.05	27.3
Coomera R. @ Durston's	23-Apr-10	28.056	153.179	3	84.7	125	70	7.6	1.9	8.4	21.3	0.067	0.05	16.5
Canungra Cr. @ Sarabah Road	06-Oct-11	28.095	153.115	2	74.6	165	30	6.4	2.5	8.7	16.5	0.047	0.16	12.2
Canungra Cr. @ Double crossing	06-Oct-11	28.037	153.141	2	99.5	115	60	6.4	3.0	7.2	17.1	0.053	0.18	2.6
Albert R. @ Ward Bridge	07-Oct-11	28.172	153.042	3	92.8	155	30	6.4	7.8	8.9	17.5	0.060	0.18	2.4
Cedar Cr.	07-Oct-11	27.840	153.179	2	26.9	28	60	6.3	2.7	7.2	17.1	0.012	0.14	1.6
TEMPERATE MAINLAND - VICTORIA														
Avon R. @ Stratford	15-Mar-10	37.973	147.078	5	1513.5	12	10	6.5	3.0	12.1	22.6	0.009	0.14	1.4
Avon R. @ Weir's Crossing	15-Mar-10	37.951	147.055	5	1432.5	17	10	6.8	8.7	11.4	24.2	0.011	0.13	1.8
Avon R. @ Bushy Park	15-Mar-10	37.863	147.008	5	1326.1	34	30	6.6	4.8	11.9	27.5	0.008	0.12	1.4
Avon R. @ Huggett's Crossing	16-Mar-10	37.811	146.855	3	49.4	103	20	.	.	.	.	0.008	0.11	1.5
Avon R. @ Avon-Turton Track	16-Mar-10	37.722	146.880	4	256.7	195	30	7.3	0.1	8.5	20.8	0.007	0.08	1
Avon R. @ 3rd Crossing	16-Mar-10	37.712	146.886	4	256.7	211	30	7.3	0.4	9.3	22.4	0.007	0.08	1
Avon R. @ Wombat Road	16-Mar-10	37.817	146.945	5	589.3	59	20	7.1	0.6	13.0	26.5	0.007	0.09	2.1
McAllister R. @ Trevor Platt's	17-Mar-10	38.035	146.982	5	2111.9	22	60	7.0	16.2	6.9	21.8	0.150	0.50	
Thomson R. @ Trevor Platt's	17-Mar-10	38.034	146.972	5	1480.4	19	60	6.8	18.3	9.5	22.4	0.076	0.32	
McAllister R. @ Davis Bridge	17-Mar-10	38.022	146.980	5	2111.9	23	70	6.7	64.0	10.0	23.7	0.120	0.38	
Thomson R. @ Riverview Road	17-Mar-10	38.007	146.871	5	1326	28	70	7.0	15.0	6.8	23.8	0.011	0.11	
Thomson R. @ Denison	18-Mar-10	38.003	146.794	5	1312.7	41	20	6.8	10.9	8.4	22.2	0.017	0.25	21.2
Thomson R. @ Reilly's Bridge	18-Mar-10	37.986	146.694	5	1175	60	30	7.1	5.0	8.7	22.4	0.011	0.18	1.8
Thomson R. @ Higgins Road	18-Mar-10	37.986	146.757	5	1242.5	47	80	6.9	10.0	7.5	24.2	0.017	0.19	16.7
Avon R. @ Valencia Creek Road	19-Mar-10	37.825	146.986	5	869.8	52	20	6.8	1.1	7.3	23.4	0.039	0.30	2.1
Rainbow Creek @ Neilson's Road	19-Mar-10	38.013	146.715	5	1107.4	53	50	6.9	4.0	7.4	22.4	0.017	0.22	0.9
TEMPERATE ISLAND - TASMANIA														
Douglas R. @ Bridge	14-Apr-10	41.781	148.256	3	72.2	9	50	7.5	1.0	10.2	14.6	0.020	1.10	11.3
Douglas R. @ Bridge	08-Dec-11	41.781	148.256	3	72.2	9	50	7.8	.	9.8	21.0	<0.005	0.19	6.3
Meredith R.	15-Apr-10	42.116	148.061	3	97.9	10	50	6.8	0.2	7.9	14.6	0.012	0.34	4.8
Meredith R.	10-Dec-11	42.116	148.061	3	97.9	10	50	7.3	.	6.5	18.4	0.008	0.46	3.8
Swan R.	15-Apr-10	41.996	148.079	4	251	19	30	7.0	1.2	9.6	14.6	0.010	0.21	0.6
Apsley R.	15-Apr-10	41.863	148.186	3	83.2	89	10	7.2	1.1	9.7	14.0	0.009	0.10	0.3
Scamander R.	16-Apr-10	41.450	148.162	3	147.3	38	50	7.3	0.6	9.4	15.1	0.008	0.19	0.4
Boobyalla R.	17-Apr-10	40.924	147.861	4	242.9	13	90	7.0	7.3	9.0	14.3	0.051	0.66	22.5
Groom R.	17-Apr-10	41.257	148.054	2	35.1	120	90	7.0	1.9	9.8	13.7	0.014	0.39	6
Trout Cr.	09-Dec-11	41.443	148.194	4	272.6	15	50	7.1	.	9.4	17.8	<0.005	0.45	0.7
Swan R.	09-Dec-11	42.007	148.076	4	256.9	17	20	7.5	.	9.5	23.3	<0.005	0.20	2.7
Buxton R.	10-Dec-11	42.261	148.001	3	60.11	11	60	7.4	.	8.8	20.0	0.005	0.26	1.4
Douglas R. upstream	10-Dec-11	41.776	148.220	3	72.2	88	10	7.6	.	9.7	18.4	<0.005	0.18	3.9
Wye R.	11-Dec-11	42.068	148.064	3	162.13	8	70	7.3	.	6.8	19.2	0.469*	0.51	1.5
*this sample was re-analyzed to confirm the high value														

Table 2. Estimated contributions of the biofilm/periphyton source pathway to the diet of stream invertebrates, based on $\delta^{13}\text{C}$ consumer versus $\delta^{13}\text{C}$ biofilm regressions. Slopes indicate mean biofilm contribution to the diet and 95% confidence intervals around slopes (not shown) indicate dietary source categories (superscripts, see Methods).

	Tropical			Subtropical			Temperate mainland			Temperate island			All regions		
Group	Slope	r	n	Slope	r	n	Slope	r	n	Slope	r	n	Slope	r	n
<i>Functional feeding groups</i>															
Primary consumers	1.12 ^a	0.83	8	0.76 ^a	0.86	6	1.56 ^a	0.76	15	0.37 ^c	0.48	11	0.90 ^a	0.65	40
Omnivores	0.47 ^d	0.57	8	0.82 ^a	0.92	6	0.95 ^a	0.80	16	0.22 ^c	0.33	10	0.55 ^b	0.58	40
Predators	0.19 ^c	0.22	8	0.78 ^a	0.93	6	0.81 ^a	0.77	7	N/A			0.43 ^b	0.57	24
<i>Families</i>															
Baetidae	N/A			N/A			2.27 ^a	0.80	7	0.44 ^d	0.42	5	1.44 ^a	0.72	17
Leptophlebiidae	1.13 ^a	0.96	6	0.75 ^a	0.88	6	N/A			0.60 ^a	0.62	11	0.86 ^a	0.76	27
Psephenidae	N/A			1.34 ^d	0.85	5	1.24 ^a	0.85	6	N/A			1.25 ^a	0.92	14
Simuliidae	N/A			N/A			N/A			N/A			0.84 ^d	0.52	10
Hydropsychidae	0.47 ^b	0.95	6	0.59 ^a	0.88	6	1.07 ^a	0.88	13	0.43 ^d	0.53	6	0.64 ^b	0.62	32
Atyidae	0.48 ^d	0.50	6	N/A			1.26 ^a	0.96	5	N/A			0.51 ^d	0.43	15
Corydalidae	N/A			0.81 ^a	0.96	6	N/A			N/A			0.80 ^a	0.88	10
Odonata	0.18 ^c	0.22	8	N/A			1.14 ^a	0.89	5	N/A			0.34 ^c	0.45	18
^a biofilm dominant, ^b mixed diet, ^c terrestrial dominant, ^d unresolved															

Table 3. Slopes of regressions of log wet weight per individual (Wgt) versus trophic level (TL) for invertebrates in streams from four regions of eastern Australia. Two different values for $\delta^{15}\text{N}$ trophic fractionation ($\Delta^{15}\text{N}$), or the expected increase in $\delta^{15}\text{N}$ per TL, are shown because this variable is uncertain (Bunn et al. 2013).

Taxa	Region	$\Delta^{15}\text{N} = 2.5\text{‰}$	$\Delta^{15}\text{N} = 1.8\text{‰}$	r^2	n	p
		Wgt-TL Slope (95% C.I.)	Wgt-TL Slope (95% C.I.)			
All invertebrates	Tropical	1.44 (1.09-1.90)	1.04 (0.78-1.37)	0.19	42	0.004
	Subtropical	1.53 (1.22-1.92)	1.10 (0.88-1.38)	0.41	47	<0.001
	Temperate mainland	0.98 (0.80-1.19)	0.70 (0.58-0.86)	0.16	83	<0.001
	Temperate island	1.45 (1.14-1.83)	0.96 (0.78-1.18)	0.18	58	0.001
Insects only	Tropical	1.30 (0.98-1.73)	0.93 (0.70-1.24)	0.29	37	<0.001
	Subtropical	1.25 (0.98-1.59)	0.90 (0.71-1.15)	0.41	43	<0.001
	Temperate mainland	0.90 (0.71-1.13)	0.65 (0.51-0.81)	0.15	65	<0.001
	Temperate island	1.15 (0.88-1.50)	0.83 (0.63-1.08)	0.11	51	0.008
Insects only	All regions	1.14 (1.01-1.30)	0.82 (0.73-0.93)	0.20	196	<0.001

Figure legends

Figure 1. Average percent contribution to the standing biomass of invertebrates in rivers in four regions of eastern Australia: (A) Tropical (North Queensland; 7 sites), (B) Subtropical (southeast Queensland; 6 sites), (C) Temperate mainland (Victoria; 16 sites), (D) Temperate island (Tasmania; 11 sites). Taxon identifiers are: Am = Amelotopsidae, Ap = Amphipoda, At = Atyidae, Ba = Baetidae, Ca = Caenidae, Cb = Corbiculiidae, Ce = Ceratopogonidae, Co = Corydalidae, Di = Diptera, Ec = Ecnomidae, El = Elmidae, Ep = Ephemeroptera, Ga = Gastropoda, Gr = Gripopterygidae, Gy = Gyrinidae, Hb = Hydrobiosidae, Hy = Hydropsychidae, Le = Leptophlebiidae, No = Notonemouridae, Od = Odonata, Ol = Oligochaeta, Ot = Other, Pa = Palaemonidae, Ph = Philopotamidae, Ps = Psephenidae, Py = Pyralidae, Sc = Scirtidae, Si = Simuliidae, Ta = Tabanidae, Ti = Tipulidae, Zy = Zygoptera

Figure 2. Regressions of consumer $\delta^{13}\text{C}$ vs biofilm $\delta^{13}\text{C}$ as a measure of the contribution of the periphyton food source pathway to the diet of primary consumers (A), omnivores (B) and predators (C) in tropical (open circles), subtropical (shaded triangles), temperate mainland (shaded squares) and temperate island (solid diamonds) regions of Australia. Slopes significantly >0 are shown with best-fit lines, and correspond to values in Table 2.

Figure 3. Site-specific invertebrate biomass from four regions in eastern Australia showing the percent contribution from periphyton (solid) and terrestrial (open) source pathways. % periphyton for a site is calculated by multiplying the estimated % periphyton contribution to a given feeding group by its biomass and summing the totals. The remaining biomass is assumed to be contributed by terrestrial detrital pathways.

Figure 4. Regressions of the $\log_{10}$ of invertebrate individual wet mass vs. trophic level (calculated from $\delta^{15}\text{N}$) for riverine sites in tropical (A), subtropical (B), temperate mainland (C)

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3 651 and temperate island (D) sites in Australia. Open symbols represent insects, solid symbols are
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6 652 crustaceans (Atyidae and Palaemonidae with the exception of a single amphipod sample with TL
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8 653 = 1.1 in D), and shaded symbols are other taxa (Oligochaeta, Gastropoda, Bivalvia). Best-fit
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10 654 lines are for all taxa combined.
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Figure 1.

Figure 2.

Figure 4.

Supplementary material for Jardine, T.D. In review. Organic matter sources and size structuring in stream invertebrate food webs across a tropical to temperate gradient. Freshwater Biology.

Figure S1. Principal component analysis of environmental variables for streams in four regions of eastern Australia. Variable codes as follows: temp = temperature, DO = dissolved oxygen, Order = stream order, Catch area = upstream catchment area, turb = turbidity, TN = total nitrogen, benthic chlor = benthic chlorophyll *a*, cond = conductivity, TP = total phosphorus.

Figure S2. Frequency histogram for stable carbon isotope ratios ($\delta^{13}\text{C}$) of biofilm and leaf litter in streams of eastern Australia.