Carbon and nitrogen ratios of aquatic and terrestrial prey for freshwater fishes

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Ecological subsidies are materials and energy that cross a boundary between two ecosystems. An example is terrestrial leaf litter that falls into a stream to ultimately become the basis of the stream food web. Terrestrial arthropods that fall into streams are often an important food resource for fishes, but variation in the nutritional quality of aquatic versus terrestrial items has not been fully assessed. Carbon and nitrogen ratios in invertebrates roughly reflect relative amounts of structural chitin and since chitin is mostly indigestible to fishes, it can be an indicator of food quality, where a greater amount of chitin (or greater C:N) indicates lower quality. To better understand potential differences in the quality of terrestrial versus aquatic arthropods, we sampled terrestrial and aquatic arthropods during winter, spring, and summer, and measured their molar C:N values. We tested arthropod C:N values for origin (terrestrial vs. aquatic), taxonomy (order level), and time of year (winter, spring, and summer). We did not detect significant differences in any of these comparisons. The average molar C:N (±1 SD) for aquatic arthropods (n = 81 samples) was 5.0 ± 0.6 and ranged from 4.7 to 5.6 and that for terrestrial arthropods (n = 42 samples) was 5.1 ± 0.7 and ranged from 4.4 to 5.6. Molar C:N values were not different among aquatic and terrestrial arthropods (df = 74.3, t = −0.995, p = 0.418) and did not differ across arthropod taxa (F7,50 = 1.9, p = 0.087). Any trends in molar C:N variability in arthropods, whether they be aquatic or terrestrial, are probably due to relative amounts of structural chitin. Since chitin is largely undigested in fish diets, molar C:N might be important in considering the true benefits of terrestrial subsidies on fish life histories.

Keywords: subsidies; nutritional ecology; food quality; C:N ratio; fish foraging

Introduction

The nutritional quality of a food item is related to the ratios of carbon, nitrogen, and phosphorus, and all organisms must balance their nutritional requirements with what is available in the environment, evolutionary constraints on life history, and the need to maintain support structures and life systems (Fagan et al. 2002; Sterner & Elser 2002). Fecundity and growth rates increased in invertebrate taxa that were fed diets balanced in carbon, nitrogen, and phosphorus (Twombly & Burns 1996; Edwards et al. 1999; Frost & Elser 2002), and the ratios of protein and fiber in plant forage have important life history implications for grazing ungulates (White 1981). Many stream fishes rely on both aquatic and terrestrial invertebrates as a primary food supply (Goldstein & Simon 1999), but similar to grazing ungulates, they must overcome that their primary food resource contains relatively high proportions of mostly indigestible carbohydrates – chitin in the case of the invertebrates that fishes consume and plant fibers in the case of grazing ungulates.

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Some fishes are selective foragers (Gardner 1981), but diet selectivity interacts with ontogeny, morphology, habitat selection, prey size, and prey abundance (Mittelbach 1981; Newman 1987; Osenberg & Mittelbach 1989; Mittelbach et al. 1992); therefore, the consumption of terrestrial arthropods by cyprinid fishes is attributed to multiple factors. In temperate zones, terrestrial arthropod influx into fish diets peaks in summer, coinciding with peak terrestrial vegetative production, whereas aquatic insect availability is at an annual low because aquatic larvae are hatching and being exported to the terrestrial system (Wipfli 1997; Nakano & Murakami 2001; Sweka & Hartman 2008). The asynchronous pattern in the abundance and use of aquatic and terrestrial food items is predicted to stabilize stream-fish biomass (Takimoto et al. 2002) and the nutritional quality of these food items may also vary seasonally, perhaps enhancing the benefits to fishes.

Recent studies indicate that terrestrial arthropods can be a major component of fish diets, both in terms of amounts in diets (Sullivan et al. 2012) and energy densities (Francis & Schindler 2009). However, direct measurements of the differences in nutritional quality between aquatic and terrestrial arthropods commonly found in fish diets are lacking; therefore, direct implications for terrestrial food subsidies in life history benefits for fishes are speculative. While the addition of terrestrial arthropods to fishes might provide more available energy, it is unclear whether or not this excess energy is utilized since insects are mostly comprised of chitin, which is mostly indigestible in the gut tract of fishes (Lindsay et al. 1984). The addition of chitin in the diets of some salmonids (Lindsay et al. 1984; Olson et al. 2006) and tilapia (Shiau & Yu 1999) depressed growth rates. However, in common carp (Cyprinus carpio), addition of chitin increased growth, although the increase in growth was less compared to supplementation of chitosan and levamisole (Gopalakannan & Arul 2006). Aquatic arthropod taxa tend to have less chitin than terrestrial arthropods because of a decreased influence of gravity in the evolution of their exoskeletal systems (Cauchie 2002) and therefore may be a more quality food resource, even if they are not as energy dense as terrestrial arthropods as indicated in Francis and Schindler (2009).

The objective of this study was to determine the nutritional quality of aquatic and terrestrial arthropods frequently consumed by fishes using mean molar C:N values. The ratio of carbon to nitrogen is indicative of the relative amounts of carbohydrates and proteins and is commonly used as a measure of nutritional quality in grazing herbivores in terrestrial ecosystems (Van der Wal et al. 2000; Alldredge et al. 2002), but for this study, C:N values are used as an indirect measure of the relative amounts of chitin among arthropod taxa. We assume that the C:N ratios of invertebrates reflect relative amounts of chitin because the range of %C and %N values reported for structural chitin in other invertebrates (41.1% C and 5.3% N from Yen et al. 2009 and 26.2% C and 4.0% N from Abdulkarim et al. 2013) would translate to similar C:N ratios reported in Cross et al. (2003) for aquatic and terrestrial invertebrates. The results from Cross et al. (2003) also suggest that aquatic invertebrates will have slightly less chitin or a lower C:N than terrestrial invertebrates, but they did not directly test this. Since we have support for the notion that aquatic insects have less chitin than terrestrial counterparts, we predict that aquatic insects will have a lower C:N ratio which indicates a greater quality. If this is true, the quality aspect of aquatic and terrestrial arthropods is not interchangeable in fish diets and determinations of the importance of terrestrial food items to fishes should be followed up with determinations of the energetic benefits between aquatic and terrestrial food items separately. Conversely, if the C:N ratios of aquatic and terrestrial food items do not significantly differ, the effects of chitin upon energy assimilation is interchangeable between aquatic and terrestrial invertebrates, and it can be assumed that whatever effects chitin has on the
digestion and energy assimilation in insectivorous fishes will be consistent and that terrestrial food items are not necessarily enhancing fish fitness beyond what they might get from aquatic invertebrates alone.

**Methods**

Terrestrial and aquatic arthropod samples were taken from three sites within the Llano River in Central Texas, USA, during December 2010, March 2011, and June 2011. The sample sites lay within a reach that includes the South Llano River (30° 23.759' N, 99° 52.930’ W) and the main stem Llano (between 30° 32.241’ N, 99° 37.599 and 30° 42.210’ N, 98° 57.542’ W). These sites were chosen because they are spaced appropriately to encompass a longitudinal gradient of substrates and a riparian cover where coarser substrates and denser riparian vegetation were observed in the most upstream site (602 m above sea level) relative to downstream sites (478–352 m above sea level).

We collected aquatic arthropods using D-frame kick-nets (KNs) and Surber samplers (SSs). Terrestrial arthropods were sampled using a combination of sweep nets (SNs) and pan-traps (PTs). Because of variability in stream flow during the sample period, SSs were sometimes not possible and sampling consisted only of KNs within all types of mesohabitats (i.e., riffles, runs, and pools). Effort among seasons was 18 SSs and 24 KNs in December, 24 SSs and 12 KNs in March, and 12 SSs and 22 KNs in June. The number of aquatic samples at each site varied with the stream flow and available mesohabitats. Surber and KN sampling was performed for one minute within each mesohabitat, in order to standardize by time, and combined to obtain an estimate of community composition. Every month at each site, eight SNs were performed and eight PTs were deployed in the terrestrial habitats. Sweep-net samples were standardized by time and area (two minutes within a two square meter area) and PT samples were standardized by time (24 hours).

All samples were placed in sample containers and kept on ice for transfer back to the laboratory for analyses. Once in the laboratory, terrestrial arthropods were frozen until processed, whereas aquatic samples were processed within 48 hours of capture. Each sample was sorted to the lowest taxonomic resolution practical but because of frequently low sample sizes within the families, analyses were performed at the order level. Arthropods were grouped by origin based on whether the organism began its life-cycle in the aquatic or terrestrial environment. For example, emergent-adult aquatic insects captured in PTs or SNs were still recorded as aquatic. Because our sampling occurred within the edge of the stream, we assumed all adult dipterans aquatic unless the origin is well established at the family level (i.e., Muscidae and Calliphoridae which lay eggs in carcasses and other decaying organic matter). Once separated by taxon, samples were desiccated for 48 hours at 60°C and weighed to the nearest 0.001 mg.

To measure %C and %N by dry mass (mg), we used a CN analyzer (Thermo Flash EA 1112 series, http://www.thermoscientific.com) calibrated with aspartic acid and marine sediments as a certified reference material. All statistical analyses were performed in SPSS and we used t-tests to determine differences in molar C:N values between terrestrial and aquatic taxa across seasons and used a one-way analysis of variance (ANOVA) to test for differences in molar C:N values between arthropod orders that were most frequently consumed in Sullivan et al. (2012). A two-way ANOVA was used to assess the variation in molar C:N values between aquatic and terrestrial arthropods across the sample month. We used $\alpha = 0.05$ as significance, tested homogeneity of variances for all hypothesis tests, and used an LSD post hoc test on ANOVAs to assess pair-wise differences.
Results
Mean molar C:N values (±1 SD), pooled across sample months, life stages, and taxa, were 5.0 ± 0.6 for aquatic arthropods \((n = 85\) samples) and 5.1 ± 0.7 for terrestrial arthropods \((n = 42\) samples). Maximum molar C:N values were similar for both aquatic (range: 4.7–5.6) and terrestrial taxa (range: 4.4–5.6) across seasons. Greatest molar C:N values were observed in Trichoptera larvae (5.6) among the aquatic arthropods and in Coleoptera larvae (5.2) among the terrestrial arthropods (Table 1). Differences in molar C:N values were not detected \((p = 0.42)\) between aquatic and terrestrial arthropods, nor were differences detected among arthropod taxa where life stages were pooled in order to preserve the sample size \(F_{7,50} = 1.9, p = 0.09\).

Numbers of terrestrial arthropods \(n_{\text{total}} = 215\) were less and more variable (coefficient of variation = 0.48) in availability across seasons than those of aquatic arthropods \(n_{\text{total}} = 3,032;\) coefficient of variation = 0.05; Table 1). Terrestrial arthropod taxa most frequently consumed by fishes were the greatest in relative abundance (Arachnida = 28.4%, Coleoptera = 21.4%, and Hymenoptera = 19.1%). An interaction was detected for mean molar C:N values between sample month and origin \(F_{4,117} = 3.5, p < 0.01;\) Figure 1). When mean molar C:N values for aquatic and terrestrial arthropods were separately analyzed across month, mean molar C:N values for aquatic arthropods were lower in December than in March \(F_{2,78} = 8.0, p < 0.01\) and June \(F_{2,78} = 8.0, p < 0.01\), but no monthly differences were found for terrestrial arthropods \(F_{2,39} = 0.88, p = 0.47\).

<table>
<thead>
<tr>
<th>Order</th>
<th>March</th>
<th>June</th>
<th>December</th>
<th>Total</th>
<th>n</th>
<th>%RA</th>
<th>Molar C:N</th>
<th>%N</th>
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<td></td>
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<td>Coleoptera (A)</td>
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<td>456 (3)</td>
<td>456 (5)</td>
<td>1346</td>
<td>10</td>
<td>44.3</td>
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<td>Diptera (A)</td>
<td>27 (4)</td>
<td>37 (4)</td>
<td>36 (3)</td>
<td>100</td>
<td>11</td>
<td>3.3</td>
<td>5.2 (0.6)</td>
<td>11.1 (1.9)</td>
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<td>Diptera (L)</td>
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<td>71 (2)</td>
<td>74 (2)</td>
<td>386</td>
<td>9</td>
<td>12.7</td>
<td>4.7 (0.9)</td>
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<td>8 (0)</td>
<td>21</td>
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<td>0.7</td>
<td>5.0 (-)</td>
<td>11.9 (-)</td>
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<tr>
<td>Ephemeroptera (L)</td>
<td>58 (2)</td>
<td>92 (0)</td>
<td>128 (3)</td>
<td>278</td>
<td>5</td>
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<td>4.9 (0.1)</td>
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<td>29 (3)</td>
<td>75</td>
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<td>2.5</td>
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<td>11.1 (0.5)</td>
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<td>Megaloptera (L)</td>
<td>13 (2)</td>
<td>4 (6)</td>
<td>14 (8)</td>
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<td>16</td>
<td>1.0</td>
<td>4.8 (0.6)</td>
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<td>4.8 (0.2)</td>
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<td>Odonata (L)</td>
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<td>30 (4)</td>
<td>108</td>
<td>9</td>
<td>3.6</td>
<td>4.7 (0.4)</td>
<td>11.3 (0.5)</td>
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<td>Plecoptera (L)</td>
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<td>2</td>
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<td>10.7 (-)</td>
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<td>4 (0)</td>
<td>12 (0)</td>
<td>32</td>
<td>1</td>
<td>1.1</td>
<td>4.8 (-)</td>
<td>11.7 (-)</td>
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<td>69 (1)</td>
<td>196</td>
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<td>1015</td>
<td>3032</td>
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<td>Arachnida</td>
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<td>27 (9)</td>
<td>22 (3)</td>
<td>61</td>
<td>15</td>
<td>28.4</td>
<td>4.8 (0.4)</td>
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<td>Coleoptera (A)</td>
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<td>21 (5)</td>
<td>9 (1)</td>
<td>46</td>
<td>9</td>
<td>21.4</td>
<td>5.6 (0.8)</td>
<td>10.4 (1.0)</td>
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<tr>
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<td>13 (1)</td>
<td>13 (1)</td>
<td>32</td>
<td>2</td>
<td>14.9</td>
<td>5.6 (-)</td>
<td>10.9 (-)</td>
</tr>
<tr>
<td>Hymenoptera</td>
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<td>9 (0)</td>
<td>41</td>
<td>4</td>
<td>19.1</td>
<td>5.3 (0.7)</td>
<td>13.1 (0.5)</td>
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<tr>
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<td>9 (3)</td>
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<td>4</td>
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<td>Orthoptera</td>
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<td>1</td>
<td>19</td>
<td>8</td>
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<td>5.3 (0.3)</td>
<td>10.7</td>
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<tr>
<td>Total</td>
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<td>111</td>
<td>56</td>
<td>215</td>
<td>42</td>
<td>100.0</td>
<td></td>
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</tbody>
</table>
Discussion

Molar C:N values did not differ between aquatic and terrestrial arthropods pooled across seasons and taxa and the arthropods most frequently consumed by fishes did not differ in their mean molar C:N values. Our molar C:N values for both aquatic and terrestrial arthropods are within the range of those reported in Cross et al. (2003). Our results do not support the hypothesis that terrestrial arthropods are of a greater nutritional quality than aquatic arthropods. Differences were detected in the mean molar C:N values across sample months within aquatic arthropods only where their mean molar C:N values were lower in December than in March or June. Aquatic and terrestrial arthropods might not vary in their overall molar C:N values because of compensation in their foraging habits. Herbivorous insects can alter their foraging patterns in order to balance their carbohydrate and protein intake (Raubenheimer & Simpson 1999, 2004; Behmer 2009) and the result would be similar nutrient concentrations of C and N, despite the varied quality of their food resources. For this paper, the variation in carbon resources available to aquatic and terrestrial arthropod consumers is reflected in the aquatic versus terrestrial origin.

Three conclusions in the literature suggest that terrestrial arthropod prey items will be important for maintenance and growth in fishes: (1) terrestrial arthropod prey items consumed by fishes were more energy dense than aquatic prey items (Francis & Schindler 2009), (2) fishes are more often energy-limited than nutrient-limited (Schindler & Eby 1997), and (3) there is ubiquity of terrestrial food consumption within the families of Salmonidae and Cyprinidae (Sullivan et al. 2012). Although terrestrial

Figure 1. Mean molar C:N values for aquatic and terrestrial arthropods by season. The interaction between origin (aquatic vs. terrestrial) and season was significant ($F_{4,117} = 3.5, p = 0.007$). Aquatic and terrestrial molar C:N values were analyzed separately across seasons.
arthropods are frequently eaten by fishes (Sullivan et al. 2012), Kawaguchi et al. (2003) found the experimental addition of terrestrial food items did not increase growth rates in stream-dwelling salmonids. The ability of any organism to assimilate energy from a food resource is related to its quality and digestibility. Given that the digestibility of chitin within post-larval fishes is not fully understood (Krodahl et al. 2005), the relationship between chitin ingestion and energy assimilation within fishes and insectivores in general is necessary when assessing the importance of terrestrial arthropods in the diets of fishes. For example, scarab beetles (Coleoptera) are frequent diet items in brook trout (Salvelinus fontinalis) inhabiting Appalachian streams (Utz et al. 2007) and are relatively energy dense when compared to available aquatic prey items. However, coleopterans also invest some of the greatest amounts of chitin per unit body mass into their exoskeleton (Lease & Wolf 2010), which might equate to coleopterans not being readily digestible, still leaving the question of how much of the energy within these scarab beetles is being assimilated into growth and other measures of fitness. Furthermore, the results of Francis and Schindler (2009) suggest that terrestrial food items consumed by fishes are more energy dense than the available aquatic insect prey items, but there are three factors that we feel warrant further investigation into this finding. First, energy densities of terrestrial prey items were taken from other published resources and not directly measured. Second, final energy densities were based upon J per unit wet mass and since terrestrial arthropods have a wider range of potential body sizes and a greater unit dry mass per unit length, the conversion of dry to wet mass energy densities might be misleading. Third, there is no account of how digestibility will influence energy assimilation. This is not to say that the present study is all encompassing with respect to the nutritional quality of terrestrial versus aquatic taxa. For example, we only measured molar carbon and nitrogen ratios but did not consider other important nutrients such as phosphorus, and this study represents only one segment of stream within a sub-tropical, semi-arid region. It might be that in regions where there is greater productivity in the riparian area, for example, in the temperate rainforests, the great abundance of terrestrial food items and energy available overcomes the counterbalance of digestibility.

Given the body of literature available that documents terrestrial arthropods as contributing large proportions of fish diets, our findings indicate that a more comprehensive model for the assimilation of terrestrial arthropods is needed in order to accurately predict the amounts of terrestrial arthropods required for maintaining fish communities and how fishes assimilate energy between aquatic and terrestrial prey items. For example, with more information about the relationship between nutritional quality, digestibility, and energy assimilation of terrestrial and aquatic arthropod taxa commonly consumed by fishes coupled with the information from studies such as Utz et al. (2007), Sweka and Hartman (2008), and Francis and Schindler (2009), ecologists concerned with terrestrial subsidies and fish communities would be able to better quantify how much energy is being exported to fishes and will provide some threshold value for desired energy fluxes in terms of terrestrial arthropod inputs from adjacent riparian zones.

Acknowledgements

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