

Stable isotope analyses of web-spinning spider assemblages along a headwater stream in Puerto Rico

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ABSTRACT

Web-spinning spiders that inhabit stream channels are considered specialists of aquatic ecosystems and are major consumers of emerging aquatic insects, while other spider taxa are more commonly found in riparian forests and as a result may consume more terrestrial insects. To determine if there was a difference in spider taxa abundance between riverine web-spinning spider assemblages within the stream channel and the assemblages 10 m into the riparian forest, we compared abundances for all web-spinning spiders along a headwater stream in El Yunque National Forest in northeast Puerto Rico. By using a nonmetric dimensional scaling (NMDS) abundance analysis we were able to see a clear separation of the two spider assemblages. The second objective of the study was to determine if aquatic insects contributed more to the diet of the spider assemblages closest to the stream channel and therefore stable isotope analyses of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for web-spinning spiders along with their possible prey were utilized. The results of the Bayesian mixing model (SIAR) however showed little difference in the diets of riverine (0 m), riparian (10 m) and upland (25 m) spiders. We found that aquatic insects made up ~50% of the diet for web-spinning spiders collected at 0 m, 10 m, and 25 m from the stream. This study highlights the importance of aquatic insects as a food source for web-spinning spiders despite the taxonomic differences in assemblages at different distances from the stream.

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INTRODUCTION

Riparian zones have been identified as areas of high importance for maintaining biodiversity in aquatic and terrestrial habitats along with being an important interface for the exchange of resources, resulting in an ecosystem with unique environmental dynamics (*Naiman & Decamps, 1997; Naiman, Decamps & Pollock, 1993; Nakano & Murakami, 2001*). The importance of terrestrial subsidies (i.e., resources that are transported across ecosystem boundaries) as an energy source in the food webs of headwater streams has long been recognized (*Vannote et al., 1980*), but only more recently has it become evident that aquatic subsidies can be equally important in terrestrial food webs

(Kato et al., 2003; Nakano & Murakami, 2001; Polis, Anderson & Holt, 1997; Sanzone, 2001; Sanzone et al., 2003). Emerging aquatic insects have been shown to be an important food source for a variety of terrestrial predators (Nakano & Murakami, 2001; Polis, Anderson & Holt, 1997) and the abundance of aquatic insects can affect the distribution of generalist predators such as insectivorous bats, reptiles, birds and spiders (Chan et al., 2008; Fukui et al., 2006; Iwata, Nakano & Murakami, 2003; Kato et al., 2003; Marczak & Richardson, 2007; Sabo & Power, 2002).

Web-spinning spiders are a particularly good model organism for studying the exchange of subsidies across riparian ecotones due to the fact that they are major consumers of emerging aquatic insects, and some taxa of web-spinning spiders have been associated exclusively with fresh water ecosystems (e.g., *Tetragnatha* (Tetragnathidae) and *Wendilgarda* (Theridiosomatidae) (Coddington, 1986; Eberhard-Crabtree, 1989; Gillespie, 1987). The distribution of these spiders has been correlated with aquatic insect abundances and for this reason these taxa of spiders are disproportionately more abundant within the first few meters from the stream channel where emerging insects tend to aggregate (Muehlbauer et al., 2014). The genus *Tetragnatha* has a worldwide distribution and can be found on all continents (except Antarctica) (Aiken & Coyle, 2000). Juvenile and female *Tetragnatha* typically construct relatively large, horizontal orb-webs directly above the surface of lentic and lotic bodies of freshwater (Gillespie, 1987). *Wendilgarda* is another genus of spider known to be associated with freshwater ecosystems; however, they are quite different from *Tetragnatha* in the sense that they are only found in tropical regions and the majority of taxa build a very reduced web structure that consists of one or two structural silk lines attached to rocks or vegetation along the stream with additional lines being attached to the water surface to snag drifting insects (Eberhard-Crabtree, 1989; Eberhard, 2001). Along with these aquatic specialists there has also been evidence that a variety of other taxa of web-spinning spiders (Araneidae, Lynphiidae and Theridiidae) are also more abundant along streams where there are greater densities of aquatic insects (Marczak & Richardson, 2007).

Originally food web studies were generally conducted using observations in the field and gut content analyses, but recently the use of stable isotopes has become a preferred method for several reasons. One benefit of using stable isotopes is that gut content analyses are not viable methods for some organisms due to their feeding habits (e.g., spiders who feed on liquefied tissue) (Foelix, 2011). Another advantage of stable isotopes is that it is able to infer relatively long term feeding habits due to the bioaccumulation of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ into the tissue of the consumer. A third advantage is that naturally occurring stable isotopes have been shown to be effective at identifying the contribution of different prey items in the diets of consumers through the use of mixing models (Parnell & Jackson, 2013; Peterson & Fry, 1987; Phillips & Gregg, 2003). This final aspect of stable isotope analyses is especially useful in aquatic and riparian food webs when determining the importance of subsidies that cross ecosystem boundaries, such as leaf litter falling into streams or emerging aquatic insects becoming food for terrestrial predators (Akamatsu, Toda & Okino, 2004;

Burdon & Harding, 2008; Davis, Rosemond & Small, 2011; Hicks, 1997; Lau, Leung & Dudgeon, 2009; Sanzone, 2001; Sanzone et al., 2003; Walters, Fritz & Phillips, 2007).

This study had two main objectives. The first was to determine if there were differences in the composition of taxa in the assemblages of web-spinning spiders that were found over the stream channel compared to those 10 m from the stream. Because some spiders are specialists of aquatic ecosystems we predicted that these taxa would be in far greater abundance over the stream channel. The second objective was to determine if there were differences in the diets of these two assemblages using stable isotopic analyses. Most emerging aquatic insects remain very close to the stream channel and their abundance can drop exponentially only a few meters into the riparian area (*Muehlbauer et al., 2014*), so we predicted that the assemblage of web-spinning spiders in the stream channel would have a diet that reflects a greater dependence on aquatic insects while the assemblage in the riparian area would be feeding on a greater number of terrestrial insects.

MATERIAL AND METHODS

Study area

This study was conducted along the small headwater stream Quebrada Prieta within El Yunque National Forest in northeastern Puerto Rico at latitude 18°18'N and longitude 65°47'W (*Masteller, 1993*). The stream begins at around 600 m above sea level and runs into the Quebrada Sonadora at around 310 m above sea level with an average slope of 20% (*Masteller, 1993*). The stream ranges from 2–4 m in width and is mainly composed of large boulders and cobble with intermittent small pools with finer sediments of sand and silt. In 2012 total rainfall was 397.7 cm and the mean temperature was 23.93 (± 2.94) °C (Luquillo-LTER). The stream is surrounded by a mainly closed canopy of tabonuco (*Dacryodes excels* Vahl) forest which is the dominant tree species in the Luquillo Mountains up to 600 m in elevation (*Masteller, 1993*). Other common plant species include bullwood (*Sloanea berteriana* Choisy) and palms (*Prestoea montana* Graham and Nicolson)(*Masteller, 1993*).

The macroinvertebrate community of Quebrada Prieta is diverse and is composed of a variety of aquatic insects with the most abundant being trichopterans and ephemeropterans (*Masteller, 1993*). During a 52 week sampling period from 1990 to 1991, the total number of specimens collected in an emergence trap above Quebrada Prieta found 35% to be ephemeropterans, 24% trichopterans, 21% chironomids and 20% other dipterans (*Masteller & Buzby, 1993a*). In this study, emergence patterns varied somewhat between taxa, although as with most tropical streams, all taxa were present throughout the year (*Ferrington, Buzby & Masteller, 1993; Flint & Masteller, 1993; Masteller & Buzby, 1993b; Pescador, Masteller & Buzby, 1993; Wagner & Masteller, 1993*). For all taxa, the abundances were generally lowest during the summer months and highest during late fall to early spring (*Ferrington, Buzby & Masteller, 1993; Flint & Masteller, 1993; Masteller & Buzby, 1993b; Pescador, Masteller & Buzby, 1993; Wagner & Masteller, 1993*). For an example, ephemeropterans, trichopterans and chironomids were found to be the most abundant during November, March and January, respectively (*Ferrington, Buzby & Masteller, 1993*;

Flint & Masteller, 1993; Pescador, Masteller & Buzby, 1993). All three taxa were found to be the least abundant during June and July (*Ferrington, Buzby & Masteller, 1993; Flint & Masteller, 1993; Pescador, Masteller & Buzby, 1993*).

Web-spinning spider assemblages

A 100 m reach of Quebrada Prieta was selected and then divided into four 25 m subsections. Field work for this portion of the project was conducted from April to August 2012, with at least one week between sampling dates to minimize the possibility of impacting the study area. A 3 m × 3 m riverine quadrat was selected within the stream channel in the first 25 m section of stream, measuring 3 m from the stream edge into the stream channel. Each quadrat was selected to contain a random mixture of available substrates, such as boulders, vegetation and deadwood which may affect web-spinning spider distribution. All web-spinning spiders within the quadrat up to 2.5 m in height were hand collected and preserved in 70% ethanol for later identification. This process was then repeated for a riparian site 10 m laterally from the stream edge into the riparian forest from the riverine sampling site. The same sampling procedure was then repeated for the next 25 m section of the stream. On the following sampling date we would sample the remaining two 25 m sections of the stream not sampled during the previous visit.

Each sampling date consisted of two riverine and two riparian quadrats. We conducted both diurnal and nocturnal sampling because some taxa of web-spinning spiders (e.g., *Tetragnatha* and *Chrysometa*) are more active at night and rarely build webs during the day. Nocturnal sampling on average was conducted from 19:00–23:00, while diurnal sampling on average was from 10:00–14:00. Sampling was only conducted during favorable weather conditions, because spider webs are many times easily destroyed by wind and rain (*Foelix, 2011*). Diurnal and nocturnal samplings were combined and therefore a total of eight riverine and eight riparian quadrats were analyzed for differences in web-spinning spider assemblages.

A Nonmetric Dimensional Scaling (NMDS) analysis along with a post-hoc Analysis of Similarity (ANOSIM) were used to determine if there were differences in taxa composition of the two web-spinning spider assemblages. A secondary post-hoc analysis, Similarity Percentages (SIMPER), was used to determine which particular taxa of spiders were causing a difference in the composition of the two assemblages. All of these analyses were conducted with the statistical program PAST (*Hammer, Harper & Ryan, 2001*).

Stable isotopes

To verify that the spider assemblages and their prey had stable isotope signatures that fell within realistic ranges of the basal C sources we sampled the three principal energy sources for aquatic and terrestrial arthropods. The three C sources sampled were stream leaf litter, periphyton and terrestrial vegetation. Stream leaf litter was collected at random throughout the 100 m stream transect and was gently rinsed to remove any macroinvertebrates. Periphyton was also sampled randomly by collecting rocks from the stream, gently rinsing them to remove any macroinvertebrates, and then scrubbing them with a small wire brush. The resultant slurry was then collected into glass vials to be dried

later. For terrestrial vegetation samples, green leaves were collected at random from C3 plants within the riparian forest.

Possible insect prey of the spider assemblages were collected for isotope analysis using two methods. Flying insects were collected using a passive sampling method with three Malaise traps that were placed within the stream channel for approximately four hours during the diurnal and nocturnal spider sampling. Traps were placed within three different sections of the stream reach (0–25 m, 25–75 m and 75–100 m) during each sampling period. This was done in order to have a representation of the available prey of web-spinning spiders flying along the stream channel. Aquatic insect larvae were collected using hand nets throughout the 100 m stream reach. Sampling was conducted in pools, riffles, and cascades to ensure that all major microhabitats were sampled. The larval stages of Ephemeroptera, Trichoptera and Chironomidae were used for isotopic analysis because they no longer feed as adults and thus their isotopic signature is fixed during the aquatic larval stage.

To compare the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope signals for the different spider assemblages, individuals were collected from a riverine transect within the stream channel, from a riparian transect 10 m parallel from the stream edge and from an upland transect 25 m parallel from the stream edge. In each transect, web-spinning spiders were collected from the four most abundant families: Tetragnathidae, Theridiosomatidae, Pholcidae and Uloboridae. Spiders were collected and maintained live in small containers for a day, to allow for the digestion of prey that may have been recently consumed to reduce the influence of the isotopic signal from what they were consuming.

Specimens were frozen ($-20\text{ }^{\circ}\text{C}$) for a minimum of 24 h, then placed in a drying oven for a minimum of 48 h ($70\text{ }^{\circ}\text{C}$) and finally ground to a fine powder for isotopic analysis. Insects were identified to family (except for Lepidoptera identified to order) and spiders were identified to genus (except for *Wendilgarda clara* Keyserling 1886, identified to species). Composite taxa samples of a minimum of four individuals for spiders 1 ± 0.05 mg of animal tissue and 5 ± 0.05 mg of plant tissue was measured for the natural abundances of ^{15}N and ^{13}C using ratio mass spectrometry at the Miami Stable Isotope Ecology Lab at the University of Miami in Florida. Natural abundances of stable isotopes for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were calculated as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000$$

where, $R_{\text{sample}} = ^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$ ratio in the sample and $R_{\text{standard}} = ^{13}\text{C}:^{12}\text{C}$ ratio in Pee Dee Belemnite for $\delta^{13}\text{C}$ and $R_{\text{standard}} = ^{15}\text{N}:^{14}\text{N}$ ratio in the atmosphere for $\delta^{15}\text{N}$ (Peterson & Fry, 1987).

The stable isotopes ^{15}N and ^{13}C of insects were analyzed as composite samples with aquatic insect taxa compiled first by family into one of five functional feeding groups: collector-gatherers ($n = 1$), filterers ($n = 2$), predators ($n = 3$), scrapers ($n = 2$) and shredders ($n = 2$) (Ramirez & Gutierrez-Fonseca, 2014). Terrestrial insects were grouped as either herbivorous ($n = 3$) or predacious ($n = 2$). Terrestrial dipterans were only identified to order, and due to their varied feeding behaviors they were not placed into

Table 1 Web-spinning spider abundance. Average number of individuals for each spider taxa collected from the eight riverine and riparian quadrats.

| Family | Genus | Riverine spiders (mean \pm STDEV) | Riparian spiders (mean \pm STDEV) |
|-------------------|---------------------------------|--|--|
| Araneidae | | 0.1 \pm 0.4 | 0.1 \pm 0.4 |
| Pholcidae | <i>Modisimus</i> | 2.5 \pm 1.7 | 1.0 \pm 0.9 |
| Tetragnathidae | <i>Chrysometa</i> | 2.6 \pm 4.7 | 0.9 \pm 1.5 |
| | <i>Leucauge</i> | 6.3 \pm 6.3 | 7.8 \pm 7.5 |
| | <i>Tetragnatha</i> | 0.8 \pm 0.9 | 0.0 \pm 0.0 |
| Theridiosomatidae | <i>Theridiosoma</i> | 0.6 \pm 0.7 | 2.8 \pm 2.1 |
| | <i>Wendilgarda</i> ^a | 19.4 \pm 9.0 | 2.1 \pm 2.2 |
| Uloboridae | <i>Miagrammopes</i> | 0.9 \pm 0.6 | 3.1 \pm 3.1 |

Notes.

^a Identified to species, *Wendilgarda clara* (Keyserling, 1886).

a particular feeding group. The isotopic values for the five aquatic functional groups were then combined into a single aquatic insect group and the values for the three terrestrial insect groups (predators, herbivores and dipterans) were combined as well. Spider taxa were identified to genus and were grouped as either having been collected in riverine ($n = 7$), riparian ($n = 5$) or upland ($n = 5$) transects. Mean averages of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for each group (aquatic insects, terrestrial insects, riverine spiders, riparian spiders and upland spiders) were used in subsequent biplots and dietary analyses.

Dietary analyses were conducted utilizing Bayesian mixing models in the SIAR package version 4.2 with Stable Isotope Analysis in R (SIAR) (Parnell & Jackson, 2013) for R version 3.0.3 (R Core Team, 2012). Consumers were the three spider groups (riverine, riparian and upland) and the sources were the two insect groups (aquatic and terrestrial). Fractionation factors between consumers and sources ($\delta^{13}\text{C}$: $0.08 \pm \text{SD } 1.90$ and $\delta^{15}\text{N}$: $2.75 \pm \text{SD } 2.20$) were adopted from the work by Yuen & Dudgeon (in press) in which they had reviewed fractionation values for arthropod consumers from a previous comprehensive study (Caut, Angulo & Courchamp, 2009). The proportion of aquatic insects in the diets of the three spider groups were determined from the SIAR package that provides 5, 25, 75 and 95% credibility intervals from the Bayesian mixing models.

RESULTS

Web-spinning spider assemblages

Four diurnal and four nocturnal samplings were conducted for both riverine and riparian habitats. Five families of web-spinning spiders (Araneidae, Pholcidae, Tetragnathidae, Theridiosomatidae and Uloboridae) were collected in varying abundances from riverine and riparian quadrats (Table 1). The least abundant family was Araneidae with only two individuals collected, while the family Theridiosomatidae was the most abundant with 199

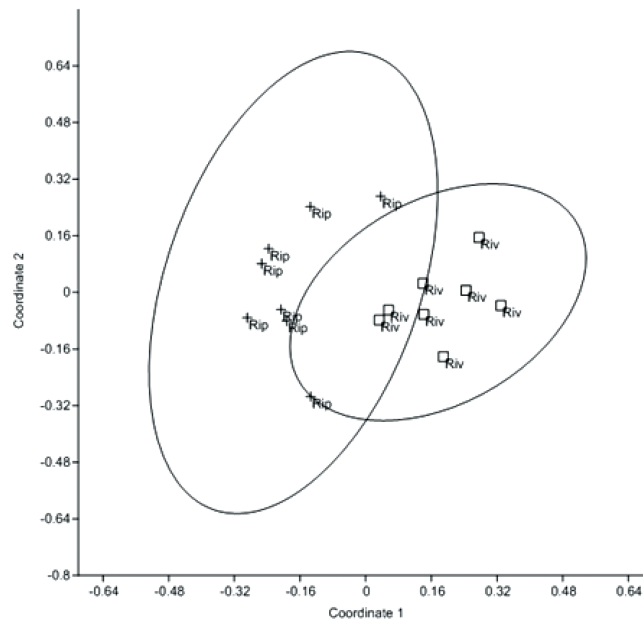


Figure 1 NMDS analysis. NMDS of web-spinning spider abundances for each sampling date. Riparian (Rip) quadrats and riverine (Riv) quadrats. Bray-Curtis 95% ellipses. ANOSIM Bonferroni-corrected $p = 0.002$, $R = 0.7224$.

individuals collected from two taxa, *Theridiosoma sp.* and *Wendilgarda clara* (Keyserling) (Table 1). The second most abundant family was Tetragnathidae with 146 individuals collected from three genera, *Chrysometa*, *Leucauge* and *Tetragnatha* (Table 1). Uloboridae was the third most abundant family with 32 individuals collected from the *Miagrammopes* genus (Table 1). Pholcidae was the second to least abundant family with 28 individuals collected from the *Modisimus* genus (Table 1). There were 265 spiders collected from the riverine habitat while in the riparian habitat 142 spiders were collected.

A NMDS analysis of the two web-spinning spider assemblages shows a clear spatial separation of the eight riparian and eight riverine groups (Fig. 1). This was statistically verified with the post-hoc test ANOSIM, which showed a significant difference in the degree of separation between the two assemblages (Bonferroni-corrected, $p < 0.002$, $R = 0.722$) (Fig. 1). An additional post-hoc analysis, SIMPER, found that around 48% of the dissimilarity between the assemblages was attributed to the abundance of *Wendilgarda clara*.

Basal carbon sources and prey taxa

Stable isotope analyses of the basal C sources showed a difference of $\delta^{13}\text{C}$ in terrestrial vegetation, periphyton and stream leaf litter. Terrestrial vegetation (-34.90‰) was more depleted in $\delta^{13}\text{C}$ than aquatic periphyton (-32.40‰) and stream leaf litter (-25.50‰) (Table 2). $\delta^{15}\text{N}$ values were very similar for C3 vegetation (-1.30‰) and periphyton (-0.80‰) while stream leaf litter had the highest $\delta^{15}\text{N}$ value (0.80‰). Despite these differences in $\delta^{13}\text{C}$, there was no clear separation between aquatic and terrestrial C signatures.

Table 2 Stable isotope values. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of all samples used in subsequent analyses.

| | Functional feeding group (FFG) | Order | Family | Genus | $\delta^{13}\text{C}$ | $\delta^{15}\text{N}$ |
|---------------------|--------------------------------|---------------|-------------------|---------------------------------|-----------------------|-----------------------|
| Stream leaf litter | | | | | -25.50 | 0.80 |
| Forest vegetation | | | | | -34.90 | -1.30 |
| Stream periphyton | | | | | -32.40 | -0.80 |
| Aquatic insects | Collector-gatherer | Diptera | Chironomidae | | -26.63 | 2.63 |
| | Filterer | Diptera | Simuliidae | | -27.76 | 2.62 |
| | Scraper | Ephemeroptera | Leptophlebiidae | | -28.15 | 2.59 |
| | Predator | Odonata | Coenagrionidae | | -27.42 | 4.98 |
| | Shredder | Trichoptera | Calamoceratidae | | -28.55 | 0.78 |
| | Scraper | Trichoptera | Helicopsychidae | | -34.88 | 1.96 |
| | Predator | Trichoptera | Hydrobiosidae | | -26.51 | 5.09 |
| | Filterer | Trichoptera | Hydropsychidae | | -29.41 | 3.69 |
| Terrestrial insects | Predator | Coleoptera | Lampyridae | | -25.30 | 6.31 |
| | | Diptera | | | -26.75 | 4.32 |
| | Herbivore | Hemiptera | Cicadoidea | | -28.69 | -0.55 |
| | Predator | Hymenoptera | Evaniidae | | -26.82 | 3.83 |
| | Herbivore | Lepidoptera | | | -27.40 | 1.94 |
| Riverine spiders | | Aranea | Pholcidae | <i>Modisimus</i> | -26.65 | 3.40 |
| | | Aranea | Tetragnathidae | <i>Chrysometa</i> | -26.72 | 5.19 |
| | | Aranea | Tetragnathidae | <i>Leucauge</i> | -26.66 | 4.53 |
| | | Aranea | Tetragnathidae | <i>Tetragnatha</i> | -27.54 | 3.87 |
| | | Aranea | Theridiosomatidae | <i>Theridiosoma</i> | -27.34 | 3.84 |
| | | Aranea | Theridiosomatidae | <i>Wendilgarda</i> ^a | -27.24 | 4.24 |
| | | Aranea | Uloboridae | <i>Miagrammopes</i> | -27.35 | 2.90 |
| Riparian spiders | | Aranea | Pholcidae | <i>Modisimus</i> | -28.49 | 3.44 |
| | | Aranea | Tetragnathidae | <i>Chrysometa</i> | -27.40 | 4.51 |
| | | Aranea | Tetragnathidae | <i>Leucauge</i> | -27.25 | 4.76 |
| | | Aranea | Theridiosomatidae | <i>Theridiosoma</i> | -27.74 | 3.55 |
| | | Aranea | Uloboridae | <i>Miagrammopes</i> | -27.24 | 2.54 |
| Upland spiders | | Aranea | Pholcidae | <i>Modisimus</i> | -27.50 | 3.80 |
| | | Aranea | Tetragnathidae | <i>Chrysometa</i> | -26.90 | 5.00 |
| | | Aranea | Tetragnathidae | <i>Leucauge</i> | -27.30 | 3.80 |
| | | Aranea | Theridiosomatidae | <i>Theridiosoma</i> | -26.50 | 1.90 |
| | | Aranea | Uloboridae | <i>Miagrammopes</i> | -33.30 | 0.10 |

Notes.

^a Identified to species, *Wendilgarda clara* (Keyserling).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for individual insect groups varied among taxa. The family Helicopsychidae (Trichoptera) was the most depleted in $\delta^{13}\text{C}$ (-34.88‰), while the family Lampyridae (Coleoptera) was the most enriched in $\delta^{13}\text{C}$ (-25.30‰) (Table 2). The family Cicadoidea (Hemiptera) had the lowest $\delta^{15}\text{N}$ value (-0.55‰), while Lampyridae, a terrestrial predator, was not only the most enriched in $\delta^{13}\text{C}$ but was also the most enriched in $\delta^{15}\text{N}$ (6.31‰) (Table 2). There was also a large amount of variation seen in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values when insects were analyzed according to their functional

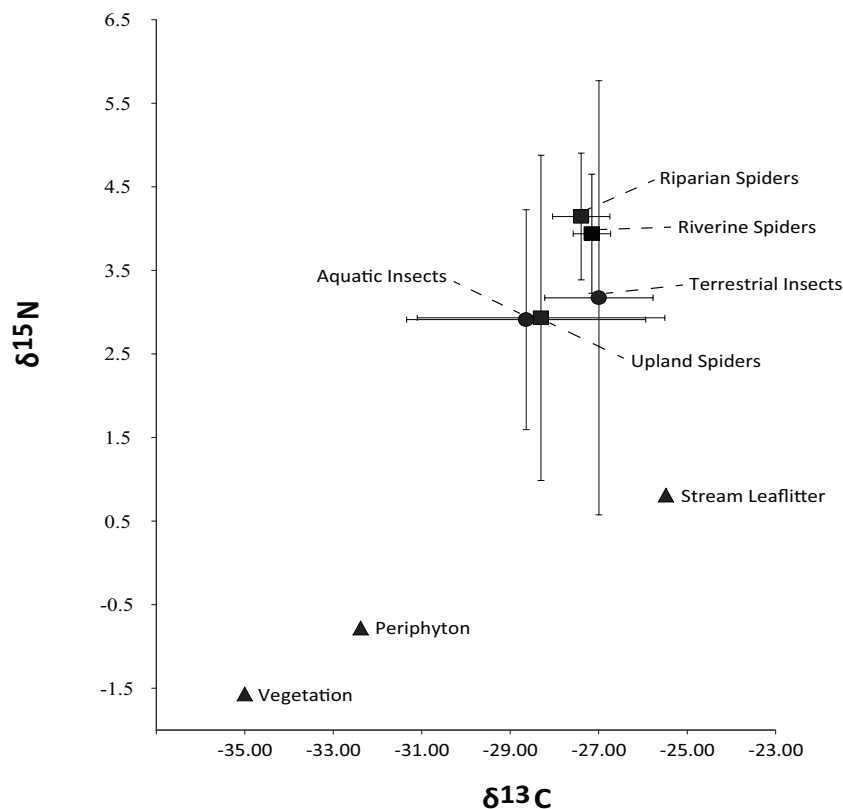


Figure 2 Biplot of stable isotope values. Biplot of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of basal resources and consumers. Points are mean values with error bars representing standard deviation. Basal resources are single samples and therefore without error bars. Spiders (squares), insects (circles), basal resources (triangles).

feeding groups. The terrestrial predator group of insects was the most enriched in $\delta^{13}\text{C}$ ($-26.06 \pm \text{SD}1.75\text{‰}$), followed by collector-gatherers (-26.63‰), aquatic predators ($-27.26 \pm \text{SD}0.68\text{‰}$), terrestrial herbivores ($-28.05 \pm \text{SD}0.91\text{‰}$), shredders (-28.55‰), filterers ($-28.59 \pm \text{SD}1.17\text{‰}$) and scrapers ($-31.52 \pm \text{SD}4.75\text{‰}$). Terrestrial predators ($5.07 \pm \text{SD}1.75\text{‰}$) were the most enriched in $\delta^{15}\text{N}$, followed by aquatic predators ($4.35 \pm \text{SD}1.20\text{‰}$), filterers ($3.16 \pm \text{SD}0.76\text{‰}$), collector-gatherers (2.63‰), scrapers ($2.27 \pm \text{SD}0.45\text{‰}$), shredders (0.78‰) and terrestrial herbivores ($0.69 \pm \text{SD}1.76\text{‰}$). When insect taxa were grouped together and analyzed as either terrestrial ($n = 5$) or aquatic ($n = 10$), no significant difference was found in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the two groups (Fig. 2). Although there was no significant difference in $\delta^{13}\text{C}$, terrestrial insects ($-26.99 \pm \text{SD}1.22\text{‰}$) were overall more enriched than aquatic insects ($-28.66 \pm \text{SD}2.69\text{‰}$). Terrestrial insects ($3.17 \pm \text{SD}2.60\text{‰}$) were also more enriched in $\delta^{15}\text{N}$ although they also showed greater variation than aquatic insects ($3.04 \pm \text{SD}1.47\text{‰}$).

Web-spinning spiders

Stable isotope analyses of the individual spider taxa showed less variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than was seen in the insect taxa. The genus *Miagrammopes* (Uloboridae) along

the upland transect was the most depleted in $\delta^{13}\text{C}$ (-33.30‰), while *Theridiosoma* (Theridiosomatidae) also from the upland transect was the most enriched in $\delta^{13}\text{C}$ (-26.50‰) (Table 2). The genus *Chrysometa* (Tetragnathidae) from the riverine transect had the highest $\delta^{15}\text{N}$ value (5.19‰), while upland *Miagrammopes* (Uloboridae) had the lowest $\delta^{15}\text{N}$ value (0.10‰) (Table 2). There were no significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the three spider groups. The group the most enriched in $\delta^{13}\text{C}$ were riverine spiders ($-27.07 \pm \text{SD}0.38\text{‰}$), followed by riparian spiders ($-27.62 \pm \text{SD}0.52\text{‰}$) and finally upland spiders ($-28.30 \pm \text{SD}2.82\text{‰}$) (Fig. 2). Similarly, the group with the highest $\delta^{15}\text{N}$ values were riverine spiders ($4.00 \pm \text{SD}0.75\text{‰}$), followed by riparian spiders ($3.76 \pm \text{SD}0.89\text{‰}$) and finally upland spiders ($2.92 \pm \text{SD}1.93\text{‰}$) (Fig. 2). The greatest amount of variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values was seen in upland spiders (Fig. 2).

Bayesian mixing model analyses

Bayesian mixing models determined that the proportion of aquatic insects in the diets of the three spider groups was relatively similar. However, the proportion of aquatic insects in the diets of the spiders increased slightly in the groups further away from the stream channel (Fig. 3). The analyses revealed that riverine spiders had the least amount of aquatic insects in their diet (45–47%) although there was considerable variation (12–71%) (Fig. 3). In the riparian spider group, aquatic insects made up a slightly greater proportion (47–49%), but again a great deal of variation was seen (10–80%) (Fig. 3). The proportion of aquatic insects was greatest in the upland spiders (50–53%) although this group also had the greatest amount of variation (10–98%) (Fig. 3).

DISCUSSION

The influence of emerging aquatic insects has been shown to affect web-spinning spider distributions in riparian areas, especially within the first 10 m from the stream edge (Collier, Bury & Gibbs, 2002; Kato et al., 2003; Kato, Iwata & Wada, 2004; Sanzone et al., 2003). Most emerging aquatic insects follow a negative power function abundance curve and over 50% of their “signature” has been found to be within only 1.5 m from the stream, although some variation has been found depending on the taxa of aquatic insect (Muehlbauer et al., 2014). We established our working hypotheses based on the strong link between web-spinning spiders and emerging aquatic insects and the fact that the majority of the insects congregate within only a few meters of the stream edge. First we proposed that there would be a different assemblage of web-spinning spiders, due to the presence of aquatic specialists (*Tetragnatha* and *Wendilgarda*), within the stream corridor compared to 10 m into the riparian forest. We then proposed that because the majority of aquatic insects congregate within only a few meters of the stream, that the riverine spider assemblage in the stream corridor would be consuming more aquatic insects than riparian and upland spiders. We found that there was indeed a significant difference between the riverine and riparian assemblages and that around 48% of the dissimilarity between the assemblages was attributed to the abundance of *Wendilgarda*, a specialist of aquatic habitats. The results did not entirely support our second hypothesis. The analyses of stable isotopes showed no clear separation between the $\delta^{13}\text{C}$ signature for aquatic and terrestrial prey due to

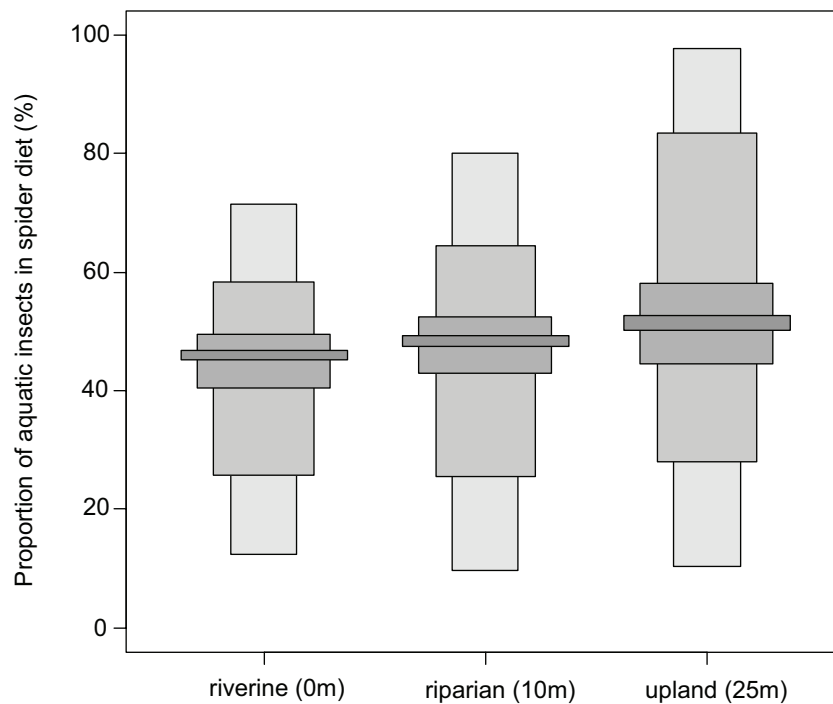


Figure 3 Bayesian mixing model dietary analysis. Boxplots with 5, 25, 75 and 95% credibility intervals representing the proportion of aquatic insects in the diets of riverine, riparian and upland spiders.

the fact that the aquatic food web was driven by leaf litter inputs from the terrestrial vegetation that resulted in similar $\delta^{13}\text{C}$ ranges for both terrestrial and aquatic primary consumers. As a result, the biplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ showed significant overlapping of the three spider groups along with the aquatic and terrestrial insects. This resulted in the inability to visually separate the consumer groups or their prey. The Bayesian SIAR dietary analysis showed that the upland group of spiders relied the most heavily on aquatic insects (50–53%) although only slightly more so than riverine (45–47%) and riparian (47–49%) spiders (Fig. 3). Overall, aquatic insects were found to be an important food source for web-spinning spiders even up to 25 m from the stream channel.

The difference in assemblage composition between the stream channel and the riparian forest was found to be driven mainly by an aquatic specialist, *Wendilgarda*, which snare their prey directly from the water surface (Coddington, 1986; Eberhard-Crabtree, 1989). *Tetragnatha*, another aquatic specialist (Aiken & Coyle, 2000; Alvarez-padilla & Hormiga, 2011; Gillespie, 1987), was also only found only in riverine quadrats however there were too few individuals to have any statistical significance. Studies of riparian spider assemblages in other parts of the world have found similar shifts in taxa composition, in which the abundance of some spiders was directly related to the distance from the stream edge and that significant differences could be found within only 10 m into the riparian zone (Sanzone, 2001; Sanzone et al., 2003). However, most studies have only been conducted in temperate regions and so far few studies that have investigated whether this distribution of spider taxa also occurs along tropical streams. Some of the proposed biotic and

abiotic factors that could explain the shift in spider distributions range from differences in vegetative complexity and structure (*Chan, Zhang & Dudgeon, 2009*) to changes in humidity and temperature, but the most common factor associated with the distribution of web spinning spiders has been associated with the abundance of aquatic insects (*Kato et al., 2003; Kato, Iwata & Wada, 2004; Sanzone, 2001; Sanzone et al., 2003*).

Basal carbon sources (stream leaf litter, periphyton and C3 vegetation), prey items (terrestrial and aquatic insects) and web spinning spiders (riverine, riparian and upland) (*Table 2*) were all found to have isotopic signals within the range of reported values from other studies (*Fry, 1991; Kato, Iwata & Wada, 2004; Lau, Leung & Dudgeon, 2009; March & Pringle, 2003; Ometto et al., 2006; Trudeau, 2003*). Terrestrial vegetation was the most depleted in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, stream leaf litter was the most enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and periphyton was the intermediate of the two (*Table 2*). The difference between the stable isotopic signals of the stream leaf litter and terrestrial vegetation could be a result of the stream leaf litter having been derived from other vegetation found upstream that were not necessarily present long the section of stream that was sampled during the study. The differences in the type of terrestrial vegetation found upstream could account for some of the difference in isotopic values. Allochthonous and autochthonous C sources in riparian food webs can vary considerably in their $\delta^{13}\text{C}$ signature ($\pm 10\text{‰}$) depending on several factors such as plant taxa, water velocity, and canopy cover (*Lau, Leung & Dudgeon, 2009; March & Pringle, 2003; Ometto et al., 2006; Trudeau, 2003*). Basal carbon sources were utilized in determining a reasonable range in which subsequent consumers should be found.

Isotopic values of insect taxa were all found to be within the range of basal C sources; however there was no clear separation in the isotopic signals between terrestrial and aquatic insects (*Fig. 2*). Terrestrial predators and herbivores showed little variation in their $\delta^{13}\text{C}$ signal, $-28.05 \pm 0.91\text{‰}$ and $-26.06 \pm 1.08\text{‰}$ respectively. The enriched $\delta^{13}\text{C}$ signal in the predators is most likely associated with bioaccumulation more so than a change in C sources. Of all the insect groups, terrestrial herbivores and predators had the lowest and highest $\delta^{15}\text{N}$ values, respectively, similar to what was reported in a study done by *Kato, Iwata & Wada (2004)* in Japan where they also found a difference of around 4‰ between terrestrial herbivores and predators (*Kato, Iwata & Wada, 2004*).

The $\delta^{13}\text{C}$ signature for the aquatic insect groups, as mentioned earlier, was not statistically different from the terrestrial insects and most of the functional feeding groups had overlapping values with terrestrial herbivores emphasizing the importance of leaf litter inputs in the aquatic food web. Overall, aquatic insects were more depleted in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than terrestrial insects, similarly to what has been found in other studies (*Kato, Iwata & Wada, 2004*). Scrapers were found to be the most depleted in $\delta^{13}\text{C}$ and this group also showed the greatest range in their $\delta^{13}\text{C}$ signature (-1.52 ± 4.75). This variation is most likely the result of the two taxa that were collected for this functional group. Helicopsychidae were severely depleted in $\delta^{13}\text{C}$ due to them being obligate scrapers, feeding on C sources depleted in $\delta^{13}\text{C}$ such as periphyton and possibly other more depleted C sources that were not sampled in this study (e.g., aquatic moss). Leptophlebiidae are considered to be more generalists and at times may feed as collector-gatherers,

despite the families overall classification as scrapers (*Ramirez & Gutierrez-Fonseca, 2014*). Similar isotopic values were found for Leptophlebiidae in a nearby stream ($\delta^{13}\text{C}$: $-24.25 \pm 0.72\text{‰}$ and $\delta^{15}\text{N}$: $2.51 \pm 0.20\text{‰}$) (*March & Pringle, 2003*). The small change in $\delta^{13}\text{C}$ could have been a result of the stream in the study by *March & Pringle (2003)* having a more open canopy and therefore a possible greater presence of algae. The aquatic insect groups had $\delta^{15}\text{N}$ signatures that fell within the two terrestrial extremes with aquatic predators ($4.35 \pm 1.20\text{‰}$) and shredders (0.78‰) having respectively the highest and lowest $\delta^{15}\text{N}$ signatures. Collector-gatherers, scrapers and filterers were found to be intermediary with relatively little variation in their $\delta^{15}\text{N}$ values ($1.96\text{--}3.69\text{‰}$).

The three spider groups showed only slight differences in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures with upland spiders being the most depleted in both instances. A study conducted in Japan similarly found only minimal changes in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between riparian and upland web spinning spiders of the same taxa (*Kato, Iwata & Wada, 2004*). However, in our study we did not analyze individual taxa and included only web-spinning spiders. This may explain some of the similarity between riverine, riparian and upland groups. Other studies have found that differences in stable isotopes values can be associated with different hunting strategies (i.e., sit and wait, wandering or web building) (*Collier, Bury & Gibbs, 2002; Kato, Iwata & Wada, 2004; Sanzone et al., 2003; Yuen & Dudgeon, in press*).

The Bayesian analysis in SIAR found that upland spiders relied the most upon aquatic insects although there were only slight differences among the three spider groups. Although the vast majority of aquatic insect biomass is concentrated within the first few meters or so from the stream edge, some taxa are known to disperse laterally up to hundreds of meters from the stream (*Muehlbauer et al., 2014*). For an example, even at around 13.3 m from the stream edge an estimated 50% of the abundance of chironomids would still be present (*Muehlbauer et al., 2014*). Around 10% of the abundance for ephemeropterans and trichopterans was estimated to be found still even 160 m and 650 m respectively from the stream edge (*Muehlbauer et al., 2014*). We found that aquatic insects made up around 50% of the spiders' diet which was slightly less than what has been reported in some other studies which have found that aquatic insects can make up $\sim 70\text{--}90\%$ of the diet of riparian spiders (*Akamatsu, Toda & Okino, 2004; Sanzone et al., 2003*). The dominant riparian taxa in those studies however were species of *Tetragnatha*, which have been found to be specialists in trapping emerging aquatic insects. Along our site there were extremely few *Tetragnatha* and were therefore not included in our isotopic analyses. Our results were more similar to those reported for other tropical (*Yuen & Dudgeon, in press*) and sub-tropical (*Collier, Bury & Gibbs, 2002*) sites. In Hong Kong, *Yuen & Dudgeon (in press)* found that riparian web-building spiders had a mean dependence of $\sim 36\text{--}55\%$ on aquatic insects. In New Zealand, *Collier, Bury & Gibbs (2002)* found that the mean contribution of aquatic insects to all riparian spider taxa was $\sim 58\%$. Emergence patterns of aquatic insects can vary greatly among and even within tropical, sub-tropical and temperate streams and this could have a large influence on the importance of stream subsidies to surrounding terrestrial predators. Some of the variability found among studies may be related to the type of isotopic mixing model that was applied (linear, algorithmic,

or Bayesian), differences in which spider taxa were present, or differences between stream localities (tropical, subtropical and/or temperate).

Our study highlights the importance of riparian ecotones as areas that contain a unique biodiversity of web-spinning spider taxa that are specialists in aquatic habitats and are rarely found even after only a few meters from the water's edge. Dietary analyses revealed that aquatic insects comprised ~50% of the diet in riverine, riparian and upland spiders with only a slightly greater dependence on aquatic insects in the upland spider group. We found that isotopic signals between terrestrial and aquatic insects were not exclusively distinct and this can impact the effectiveness of isotopic mixing models, which has been shown to be a problem for other studies along forested headwater streams. Despite the overlapping of isotopic signals, the results of the dietary analysis were similar to other studies conducted along tropical streams. Our study provides further evidence for the importance of aquatic subsidies for terrestrial consumers even within upland areas from the stream.

CONCLUSION

The environment provided by the stream channel and that of the riparian forest clearly created two unique web-spinning spider assemblages, in which specialized taxa of aquatic ecosystems were shown to be the major difference between the two study areas. However, differences between these two habitats were potentially the result of structure and microenvironment, rather than prey resources.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Sean P. Kelly conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Elvira Cuevas and Alonso Ramírez analyzed the data, contributed reagents/materials/analysis tools, reviewed drafts of the paper.

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REFERENCES

- Aiken M, Coyle FA. 2000.** Habitat distribution, life history and behavior of tetragnatha spider species in the great smoky mountains national park. *Journal of Arachnology* **28**:97–106 DOI [10.1636/0161-8202\(2000\)028\[0097:HDLHAB\]2.0.CO;2](https://doi.org/10.1636/0161-8202(2000)028[0097:HDLHAB]2.0.CO;2).
- Akamatsu F, Toda H, Okino T. 2004.** Food source of riparian spiders analyzed by using stable isotope ratios. *Ecological Research* **19**:655–662 DOI [10.1111/j.1440-1703.2004.00680.x](https://doi.org/10.1111/j.1440-1703.2004.00680.x).
- Alvarez-padilla F, Hormiga G. 2011.** Morphological and phylogenetic atlas of the orb-weaving spider family Tetragnathidae (Araneae: Araneoidea). *Zoological Journal of the Linnean Society* **162**:713–879 DOI [10.1111/j.1096-3642.2011.00692.x](https://doi.org/10.1111/j.1096-3642.2011.00692.x).
- Burdon FJ, Harding JS. 2008.** The linkage between riparian predators and aquatic insects across a stream-resource spectrum. *Freshwater Biology* **53**:330–346 DOI [10.1111/j.1365-2427.2007.01897.x](https://doi.org/10.1111/j.1365-2427.2007.01897.x).
- Caut S, Angulo E, Courchamp F. 2009.** Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology* **46**:443–453 DOI [10.1111/j.1365-2664.2009.01620.x](https://doi.org/10.1111/j.1365-2664.2009.01620.x).
- Chan EKW, Yu Y-T, Zhang Y, Dudgeon D. 2008.** Distribution patterns of birds and insect prey in a tropical riparian forest. *Biotropica* **40**:623–629 DOI [10.1111/j.1744-7429.2008.00420.x](https://doi.org/10.1111/j.1744-7429.2008.00420.x).
- Chan EKW, Zhang Y, Dudgeon D. 2009.** Substrate availability may be more important than aquatic insect abundance in the distribution of riparian orb-web spiders in the tropics. *Biotropica* **41**:196–201 DOI [10.1111/j.1744-7429.2008.00463.x](https://doi.org/10.1111/j.1744-7429.2008.00463.x).
- Coddington JA. 1986.** The genera of the spider family Theridiosomatidae. *Smithsonian Contributions to Zoology* **422**:1–96 DOI [10.5479/si.00810282.422](https://doi.org/10.5479/si.00810282.422).
- Collier KJ, Bury S, Gibbs M. 2002.** A stable isotope study of linkages between stream and terrestrial food webs through spider predation. *Freshwater Biology* **47**:1651–1659 DOI [10.1046/j.1365-2427.2002.00903.x](https://doi.org/10.1046/j.1365-2427.2002.00903.x).
- Davis JM, Rosemond AD, Small GE. 2011.** Increasing donor ecosystem productivity decreases terrestrial consumer reliance on a stream resource subsidy. *Oecologia* **167**:821–834 DOI [10.1007/s00442-011-2026-9](https://doi.org/10.1007/s00442-011-2026-9).
- Eberhard WG. 2001.** Trolling for water striders: active searching for prey and the evolution of reduced webs in the spider *Wendilgarda* sp. (Araneae, Theridiosomatidae). *Journal of Natural History* **35**:229–251 DOI [10.1080/00222930150215350](https://doi.org/10.1080/00222930150215350).
- Eberhard-Crabtree WG. 1989.** Niche expansion in the spider *Wendilgarda galapagensis* (Araneae, Theridiosomatidae) on Cocos Island. *Revista de Biología Tropical* **37**(2):163–168.

- Ferrington Jr LC, Buzby KM, Masteller EC. 1993.** Composition and temporal abundance of chironomidae emergence from a tropical rainforest stream at El Verde, Puerto Rico. *Journal of the Kansas Entomological Society* **66**:167–180.
- Flint Jr OS, Masteller EC. 1993.** Emergence composition and phenology of trichoptera from a tropical rainforest stream at El Verde, Puerto Rico. *Journal of the Kansas Entomological Society* **66**:140–150.
- Foelix RF. 2011.** *Biology of spiders*. New York: Oxford University Press.
- Fry B. 1991.** Stable isotope diagrams of freshwater food webs. *Ecology* **72**:2293–2297
[DOI 10.2307/1941580](https://doi.org/10.2307/1941580).
- Fukui DAI, Murakami M, Nakano S, Aoi T. 2006.** Effect of emergent aquatic insects on bat foraging in a riparian forest. *Journal of Animal Ecology* **75**:1252–1258
[DOI 10.1111/j.1365-2656.2006.01146.x](https://doi.org/10.1111/j.1365-2656.2006.01146.x).
- Gillespie RG. 1987.** The mechanism of habitat selection in the long-jawed orb-weaving spider *Tetragnatha elongata* (Araneae, Tetragnathidae). *Journal of Arachnology* **15**:81–90.
- Hammer O, Harper DAT, Ryan PD. 2001.** Past: paleontological statistics software package for education and data analysis. *Palaentologia Electronica* **4**:1–9.
- Hicks BJ. 1997.** Food webs in forest and pasture streams in the Waikato region, New Zealand: a study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. *New Zealand Journal of Marine and Freshwater Research* **31**:651–664
[DOI 10.1080/00288330.1997.9516796](https://doi.org/10.1080/00288330.1997.9516796).
- Iwata T, Nakano S, Murakami M. 2003.** Stream meanders increase insectivorous bird abundance in riparian deciduous forests. *Ecography* **26**:325–337 [DOI 10.1034/j.1600-0587.2003.03355.x](https://doi.org/10.1034/j.1600-0587.2003.03355.x).
- Kato C, Iwata T, Nakano S, Kishi D. 2003.** Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. *Oikos* **103**:113–120 [DOI 10.1034/j.1600-0706.2003.12477.x](https://doi.org/10.1034/j.1600-0706.2003.12477.x).
- Kato C, Iwata T, Wada E. 2004.** Prey use by web-building spiders: stable isotope analyses of trophic flow at a forest-stream ecotone. *Ecological Research* **19**:633–643
[DOI 10.1111/j.1440-1703.2004.00678.x](https://doi.org/10.1111/j.1440-1703.2004.00678.x).
- Lau DCP, Leung KMY, Dudgeon D. 2009.** What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong. *Freshwater Biology* **54**:127–141
[DOI 10.1111/j.1365-2427.2008.02099.x](https://doi.org/10.1111/j.1365-2427.2008.02099.x).
- March JG, Pringle CM. 2003.** Food web structure and basal resource utilization along a tropical Island stream continuum, Puerto Rico. *Biotropica* **35**:84–93.
- Marczak LB, Richardson JS. 2007.** Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. *Journal of Animal Ecology* **76**:687–694
[DOI 10.1111/j.1365-2656.2007.01240.x](https://doi.org/10.1111/j.1365-2656.2007.01240.x).
- Masteller EC. 1993.** Comparison of tropical and temperate emergence phenology of aquatic insects from Puerto Rico and Pennsylvania. *Journal of the Kansas Entomological Society* **66**:192–199.
- Masteller EC, Buzby KM. 1993a.** Composition and temporal abundance of aquatic insect emergence from a tropical rainforest stream, Quebrada Prieta, at El Verde, Puerto Rico. introduction. *Journal of the Kansas Entomological Society* **66**:133–139.
- Masteller EC, Buzby KM. 1993b.** Emergence phenology of empidiidae, ceratopogonidae, and Simuliidae (Diptera) from a tropical rainforest stream at El Verde, Puerto Rico. *Journal of the Kansas Entomological Society* **66**:187–191.

- Muehlbauer JD, Collins SF, Doyle MW, Tockner K. 2014. How wide is a stream? Spatial extent of the potential stream signature in terrestrial food webs using meta-analysis. *Ecology* **95**:44–55 DOI [10.1890/12-1628.1](https://doi.org/10.1890/12-1628.1).
- Naiman RJ, Decamps H. 1997. The ecology of interfaces: riparian zones. *Annual Review of Ecology and Systematics* **28**:621–658 DOI [10.1146/annurev.ecolsys.28.1.621](https://doi.org/10.1146/annurev.ecolsys.28.1.621).
- Naiman RJ, Decamps H, Pollock M. 1993. The role of riparian corridors in maintaining regional biodiversity. *Ecological Applications* **3**:209–212 DOI [10.2307/1941822](https://doi.org/10.2307/1941822).
- Nakano S, Murakami M. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences* **98**(1):166–170 DOI [10.1073/pnas.98.1.166](https://doi.org/10.1073/pnas.98.1.166).
- Ometto JHB, Ehleringer J, Domingues T, Berry J, Ishida F, Mazzi E, Higuchi N, Flanagan L, Nardoto G, Martinelli L. 2006. The stable carbon and nitrogen isotopic composition of vegetation in tropical forests of the Amazon Basin, Brazil. *Biogeochemistry* **79**:251–274 DOI [10.1007/s10533-006-9008-8](https://doi.org/10.1007/s10533-006-9008-8).
- Parnell A, Jackson A. 2013. *Siar: stable isotope analysis in R*. R Package version 42.
- Pescador ML, Masteller EC, Buzby KM. 1993. Composition and phenology of ephemeroptera from a tropical rainforest stream at El Verde, Puerto Rico. *Journal of the Kansas Entomological Society* **66**:151–159.
- Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**:293–320 DOI [10.1146/annurev.es.18.110187.001453](https://doi.org/10.1146/annurev.es.18.110187.001453).
- Phillips DL, Gregg JW. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* **136**:261–269 DOI [10.1007/s00442-003-1218-3](https://doi.org/10.1007/s00442-003-1218-3).
- Polis GA, Anderson WB, Holt RD. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* **28**:289–316 DOI [10.1146/annurev.ecolsys.28.1.289](https://doi.org/10.1146/annurev.ecolsys.28.1.289).
- Ramirez A, Gutierrez-Fonseca PE. 2014. Functional feeding groups of aquatic insect families in latin America: a critical analysis and review of existing literature. *Journal of Tropical Biology* **62**:155–167.
- R Core Team. 2012. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Sabo JL, Power ME. 2002. River–watershed exchange: effects of riverine subsidies on riparian lizards and their terrestrial prey. *Ecology* **83**:1860–1869.
- Sanzone DM. 2001. Linking communities across ecosystem boundaries: the influence of aquatic subsidies on terrestrial predators. PhD, University of Georgia.
- Sanzone DM, Meyer JL, Marti E, Gardiner EP, Tank JL, Grimm NB. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. *Oecologia* **134**:238–250 DOI [10.1007/s00442-002-1113-3](https://doi.org/10.1007/s00442-002-1113-3).
- Trudeau V. 2003. The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton. *Limnology and Oceanography* **48**:2194 DOI [10.4319/lo.2003.48.6.2194](https://doi.org/10.4319/lo.2003.48.6.2194).
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**:130–137 DOI [10.1139/f80-017](https://doi.org/10.1139/f80-017).
- Wagner RH, Masteller EC. 1993. Composition and temporal abundance of mothflies (Diptera, Psychodidae) from a tropical rainforest stream at El Verde, Puerto Rico. *Journal of the Kansas Entomological Society* **66**:181–186.

Walters DM, Fritz KM, Phillips DL. 2007. Reach-scale geomorphology affects organic matter and consumer $\delta^{13}\text{C}$ in a forested piedmont stream. *Freshwater Biology* 52:1105–1119
[DOI 10.1111/j.1365-2427.2007.01735.x](https://doi.org/10.1111/j.1365-2427.2007.01735.x).

Yuen EYL, Dudgeon D. 2015. Dietary dependence of predatory arthropods on volant aquatic insects in tropical stream riparia. *Biotropica* In Press.