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

Sean P. Kelly, Elvira Cuevas, Alonso Ramírez

Web-spinning spiders that inhabit stream channels are considered specialists of aquatic ecosystems and are major consumers of emerging aquatic insects~~,~~. O~~while o~~ther spider taxa are more commonly found in riparian forests and as a result consume more terrestrial insects. ~~In order~~ T~~t~~o determine if there was a difference in spider taxa abundance between riverine web-spinning spider assemblages within the stream channel and the assemblages 10m into the riparian forest, we compared both day and night 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 insect groups contributed more to the diet of the riverine spider assemblage and therefor stable isotope analyses of $\delta^{15}N$ and $\delta^{13}C$ for web-spinning spiders along with their possible prey were utilized. The results of the mixing model (IsoSource) however showed little difference in the diets of the riverine and riparian spider assemblages. This study highlights the strong connectivity between headwater streams and riparian forests. Despite the differences in taxa composition within the riverine and riparian areas both assemblages of spiders were shown to depend on emerging aquatic insects as a major food source.

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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 et al. 1993; Nakano & Murakami 2001). The importance of terrestrial subsidies 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 et al. 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 et al. 1997) and the abundance of aquatic insects can affect the distribution of generalist ~~predators~~ predators, such as insectivorous bats, reptiles, birds and spiders (Fukui et al. 2006; Iwata et al. 2003; Kato et al. 2003; Marczak & Richardson 2007; Sabo & Power 2002).

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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 (eg. *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 disproportionally 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~~most 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 in order 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, Lyniphiidae and Theridiidae) have also been shown to be more abundant along streams where there are greater densities of aquatic insects (Marczak & Richardson 2007).

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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 $\delta^{15}N$ and $\delta^{13}C$ 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 (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 as food for terrestrial predators (Akamatsu et al. 2004; Burdon & Harding 2008; Davis et al. 2011; Hicks 1997; Sanzone 2001; Sanzone et al. 2003; Walters et al. 2007).

This study had two main objectives ~~in order to better understand the complex relationship between web spinning spiders and stream ecosystems~~. The first ~~objective~~ was to determine if there were differences in the composition of taxa in the assemblages of web-spinning spiders that were found within the stream channel compared to those 10m into the riparian area. Due to some spiders being specialists of aquatic ecosystems we predicted that these taxa would be in far greater abundance within the stream channel.

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The second ~~objective of the study~~ was to determine if there were differences in the diets of these two assemblages using stable isotopic analyses. The majority of 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), ~~and~~ 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 600m above sea level and runs into the Quebrada Sonadora at around 310m above sea level with an average slope of 20% (Masteller 1993). The stream ranges from 2-4m in width and is mainly composed of large boulders and cobble with intermittent small pools with finer sediments of sand and silt. In the year of the study, total rainfall was 397.7cm 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 until around 600m 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 but ~~the majority of the~~most biomass is dominated by the freshwater shrimp, *Atya lanipes*, *Xiphocaris ~~elongata~~elongate,* and *Macrobrachium* spp. (Masteller 1993). Other members within the stream community are amphibious crabs, *Epilobocera sinuatifrons*, and one algivorous goby species, *Sicydium plumieri* (Masteller 1993).

Web-Spinning Spider Assemblages

A 100m reach of Quebrada Prieta was selected and then divided into four 25m 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 3m x 3m riverine quadrat was selected within the stream channel in the first 25m section of stream, measuring 3m from the stream's 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 2.5m in height were hand collected and preserved in 70% ethanol for later identification.

This process was then repeated for a riparian site 10m laterally from the stream edge into the riparian forest from the riverine sampling site. The same sampling procedure was then repeated for the next 25m section of the stream. On the following sampling date we would sample the remaining two 25m sections of the stream not sampled during the previous visit. Each sampling date consisted of two riverine and two riparian quadrats, with each sampling lasting about four hours for both nocturnal and diurnal sampling. We conducted both diurnal and nocturnal sampling because some taxa of web-spinning spiders (e.g. *Tetragnatha*) are more active at night and rarely build webs during the day. Nocturnal sampling on average was conducted from 1900-2300, while diurnal sampling on average was from 1000-1400. Sampling was only conducted during favorable weather conditions, ~~due to the fact that~~ because spider webs are many times easily destroyed by wind and rain (Foelix 2011). A total of four riverine quadrats and four riparian quadrats were sampled both diurnally and nocturnally for a total of 16 quadrats sampled. 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 et al. 2001).

Stable Isotopes

~~In order to~~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 100m 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 haphazardly from C3 plants within the riparian forest.

Possible insect prey of the spider assemblages were collected for isotope analysis using several methods. Flying insects were collected using a passive sampling method with three Malaise traps that were placed between 0-25m, 25-75m, and 75-100m within the stream channel for approximately four hours during the diurnal and nocturnal spider sampling. Aquatic insect larvae were collected using hand nets throughout the 100m stream reach. Sampling was conducted in pools, riffles, and cascades to ensure that all major microhabitats were accounted for. 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 due to their feeding habits as aquatic nymphs. Adult stages were used for the ~~orders~~ Odonata and Tipulidae.

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~~In order to~~ To compare the δ^{15}N and δ^{13}C stable isotope signals for the two different spider assemblages, individuals were collected from a riverine transect within the stream channel and again from a riparian transect 10m parallel from the edge of the stream. 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, ~~in order~~ to allow for the digestion of

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prey that may have been recently consumed to reduce the influence of the prey's isotopic signal.

Specimens were frozen (-20˚C) for a minimum of 24 hours, then placed in a drying oven for a minimum of 48hrs (70˚C) and finally ground to a fine powder for isotopic analysis. Insects were identified to the family level (except for Lepidoptera identified to order) and spiders were identified to the genus level (except for *Wendilgarda clara* Keyserling 1886, identified to species). Composite taxa samples of a minimum of four individuals for spiders 1±0.05mg of animal tissue and 5±0.05mg 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 $\delta^{13}C$ and $\delta^{15}N$ were calculated as:

$\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) – 1] \times 1000$

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

The stable isotopes ^{15}N and ^{13}C of insects were analyzed as composite samples with aquatic insect taxa compiled 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=2) or predacious (n=2). For simplicity all seven groups of insects will be referred to as functional feeding groups, while understanding that the term is more generally used only for aquatic insects. Spider taxa were identified to genus and were grouped as either having been collected in riverine (n=7) or riparian (n=5) transects. Mean averages of $\delta^{13}C$ and $\delta^{15}N$ for each group were used in subsequent analyses with the program IsoSource (Phillips & Gregg 2003). The source increment was set at 1% and the mass balance tolerance level was 0.1%. This mixing model was used to determine the contribution of each insect group to the diet of the riverine and riparian spiders.

RESULTS

Web-Spinning Spider Assemblages

~~There were a total of f~~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 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 with a Shannon-Weiner index of H'=1.30 while in the riparian habitat 142 spiders were collected with a Shannon-Weiner index of H'=1.56.

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

Stable isotope analyses of the basal C sources showed a difference of $\delta^{13}C$ in terrestrial vegetation, periphyton and stream leaf litter. Terrestrial vegetation (-34.90‰) was more depleted in $\delta^{13}C$ than aquatic periphyton (-32.40‰) and stream leaf litter (-25.50‰) (Table 2). $\delta^{15}N$ values were very similar for C3 vegetation (-1.30‰) and periphyton (-0.80‰) while stream leaf litter had the highest $\delta^{15}N$ value (0.80‰). Despite these differences in $\delta^{13}C$ there was no clear separation between aquatic and terrestrial C signatures. $\delta^{13}C$ and $\delta^{15}N$ values for the composite samples of individual insects varied among taxa. The family Helicopsychidae (Trichoptera) was the most depleted in $\delta^{13}C$ (-34.88‰), while the family Lampyridae (Coleoptera) was the most enriched in $\delta^{13}C$ (-25.30‰) (Table 2). The family Cicadoidea (Hemiptera) had the lowest $\delta^{15}N$ value (-0.55‰), while Lampyridae, a terrestrial predator, was not only the most enriched in $\delta^{13}C$ but was also the most enriched in $\delta^{15}N$ (6.31‰) (Table 2). When taxa were analyzed as either terrestrial (n=4) or aquatic (n=10) insects no significant difference was found between $\delta^{13}C$ nor $\delta^{15}N$ in the two groups.

Aquatic and Terrestrial Insects

There was a large amount of variation seen in the $\delta^{13}C$ and $\delta^{15}N$ values for the seven different functional feeding groups used in the dietary analysis of the spider assemblages. The terrestrial predator group of insects was the most enriched in $\delta^{13}C$ (-26.06 ±1.75‰), followed by collector-gatherers (-26.63‰), aquatic predators (-27.26±0.68‰), shredders (-27.82 ±1.03‰), herbivores (-28.05 ±0.91‰), filterers (-28.59 ±1.17‰) and scrapers (-31.52 ±4.75‰) (Figure 2). Terrestrial predators (5.07±1.75‰) were the most enriched in $\delta^{15}N$, followed by aquatic predators (4.35±1.20‰), filterers (3.16±0.76‰), collector-gatherers (2.63‰), scrapers (2.27±0.45‰), shredders (1.81±1.47‰) and herbivores (0.69±1.76‰) (Figure 2).

Web-Spinning Spiders

Stable isotope analyses of the individual spider taxa showed less variation in $\delta^{13}C$ and $\delta^{15}N$ values than was seen in the insect taxa. The genus *Modisimus* (Pholcidae) collected along the riparian transect was the most depleted in $\delta^{13}C$ (-28.49‰), while *Modisimus* collected from the riverine transect was the most enriched in $\delta^{13}C$ (-26.65‰) (Table 2). The genus *Chrysometa* (Tetragnathidae) from the riverine transect had the highest $\delta^{15}N$ value (5.19), while riparian *Miagrammopes* (Uloboridae) had the lowest

$\delta^{15}N$ value (2.54‰) (Table 2). There was only a slight difference in $\delta^{13}C$ between the combined riverine (-27.07±0.38‰) and riparian taxa (-27.62±0.52‰) (Figure 2). $\delta^{15}N$ values only showed a slight difference between riverine (3.99±0.75‰) and riparian (3.76±0.90‰) assemblages (Figure 2).

Dietary Analyses

Results from the mixing model, IsoSource (Phillips & Gregg 2003), showed that shredders (0-93%) and herbivores (0-65%) were the two insect groups that contributed the most to the riverine spider diet while scrapers (0-24%) and terrestrial predators (0-25%) contributed the least (Figure 2). Shredders (0-79%) and filterers (0-61%) contributed the most to the riparian spider diet, while terrestrial predators (0-26%) and scrapers (0-34%) once again contributed the least to the spiders' diet (Figure 2).

DISCUSSION

The influence of emerging aquatic insects has been shown to affect web-spinning spider distributions in riparian areas, especially within the first 10m from the stream edge (Collier et al. 2002; Kato et al. 2003; Kato et al. 2004; Sanzone et al. 2003). ~~The majority of~~Most emerging aquatic insects follow a negative power function abundance curve and over 50% of their "signature" has been found to be within only 1.5m from the stream (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 10m 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 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.

In contrast, t~~T~~he results did not entirely support our second hypothesis. The analyses of stable isotopes showed no clear separation between the δ^{13}C signature for aquatic and terrestrial prey ~~due to the fact that~~because the aquatic food web was driven by leaf litter inputs from the terrestrial vegetation that resulted in similar δ^{13}C ranges for both terrestrial and aquatic primary consumers, which are the typical prey for web-spinning spiders. The dietary analysis showed that the group of insects that contributed the most to both spider assemblages was aquatic shredders.

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 10m into the riparian zone (Sanzone 2001; Sanzone et al. 2003). However, most ~~the majority of~~ these studies have only been conducted in temperate regions, and so far~~there are still~~ few studies that have investigated whether this distribution of spider taxa also occurs along tropical streams ~~as well~~. Some of the proposed biotic and abiotic factors that could explain the shift in spider distributions range from differences in vegetative complexity and structure to changes in humidity and temperature, but the most common factor associated with the distribution of web spinning spiders has been the abundance of aquatic insects(Kato et al. 2003; Kato et al. 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 and riparian) (Table 2) were all found to have isotopic signals within the range of reported values from other

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2006; Trudeau 2003). Terrestrial vegetation was the most depleted in $\delta^{13}C$ and $\delta^{15}N$, stream leaf litter was the most enriched in $\delta^{13}C$ and $\delta^{15}N$ and periphyton was the intermediate of the two (Table 2). Allochthonous and autochthonous C sources in riparian food webs can vary considerably in their $\delta^{13}C$ signature (±10‰) depending on several factors such as plant taxa, water velocity, and canopy cover(Lau et al. 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, but not in determining a difference in aquatic or terrestrial resources for web spinning spiders.

Isotopic values of insect taxa were all found to be within the range of basal C sources and mean values for each functional feeding group reflected their particular food source~~,~~; however, there was no clear separation in the isotope signals between terrestrial and aquatic insects. Terrestrial predators and herbivores showed little variation in their $\delta^{13}C$ signal, -28.05±0.91‰ and -26.06±1.08‰ respectively. The enriched $\delta^{13}C$ 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 $\delta^{15}N$ values respectively, similar to what was reported in a study done by Kato et al. (2004) in Japan where they also found a difference of around 4‰ between terrestrial herbivores and predators (Kato et al. 2004).

The $\delta^{13}C$ signature for the aquatic insect groups, as mentioned earlier, was not statistically different from the terrestrial insects, and ~~the majority~~most of the functional feeding groups had overlapping values with terrestrial herbivores, emphasizing the importance of leaf litter inputs in the aquatic food web. Scrapers were found to be the most depleted in $\delta^{13}C$, and this group also showed the greatest range in their $\delta^{13}C$ signature (-1.52±4.75). ~~The~~ This variation is most likely the result of the two taxa that were collected for this functional group. Helicopsychidae were severely depleted in $\delta^{13}C$ due to them being obligate scrapers, feeding on C sources depleted in $\delta^{13}C$ 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). In a study by Sanzone et al. (2002), Helicopsychidae were also found to be one of the most depleted aquatic insect taxa (Sanzone et al. 2003). The aquatic insect groups had $\delta^{15}N$ signatures that fell within the two terrestrial extremes with aquatic predators (4.35±1.20‰) and shredders (1.81±1.47‰) having respectively the highest and lowest $\delta^{15}N$ signatures. Shredders along with being the most depleted in $\delta^{15}N$ also showed the greatest variation, most likely due to the influence of Tipulidae in this group, which are generally considered as shredders although some taxa have been found to be predacious (Ramirez & Gutierrez-Fonseca 2014). Collector-gatherers, scrapers and filterers were found to be intermediary with relatively little variation in their $\delta^{15}N$ values (1.96-3.69‰).

The two spider assemblages showed very little difference in both their $\delta^{13}C$ and $\delta^{15}N$ signatures with riverine spiders being only slightly more enriched in both instances. Both spider assemblages had intermediary $\delta^{15}N$ values between predacious and non-predacious insects similarly to other riparian studies conducted along both temperate forest and desert streams (Kato et al. 2004; Sanzone et al. 2003). In our study we included only web-spinning spiders and this may explain the similarity between the two assemblages because 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 et al. 2002; Kato et al. 2004; Sanzone et al. 2003).

The IsoSource analyses showed that aquatic shredders was the insect group that contributed the most to the diets in both riverine and riparian spider assemblages, with the group representing 0-79% of the diet in riparian spiders and 0-93% in riverine spiders. Allocthonous inputs from riparian forests has been shown to be extremely important for the food webs in headwater streams (Vannote et al. 1980; Wallace et al. 1997) and it has been calculated that terrestrial runoff can account for 75 to 90 percent of the C budget of a stream (Polis et al. 1997). This energy is then transferred back to riparian web-spinning spiders in the form of emerging aquatic insects that feed primarily on stream leaf litter, such as shredders (Kato et al. 2004). The importance of allocthonous energy that indirectly effect riparian spider diets was also seen in headwater streams of Japan, where 52.6% of the total dry biomass of prey caught in Tetragnathid webs were insect taxa that feed primarily on terrestrial inputs(Kato et al. 2004).

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 shredders made up the majority of the prey in the two spider assemblages. These results emphasize the importance of allocthonous inputs to aquatic food webs in headwater streams and consequently the reciprocal importance of this energy being converted by aquatic primary consumers that then emerge as adults and become an important food source for riparian predators.

CONCLUSION

The environment provided by the stream channel and that of the riparian vegetation 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 resources. Our diet analysis using stable isotopes identified aquatic insects belonging to the shredder functional feeding group as the main food source for both spider assemblages. Our findings highlight the importance of leaf litter as a major energy source not only for aquatic consumers, but also for riparian food webs as well. We also found strong evidence for the importance of subsidies across ecotones, in this case from aquatic to terrestrial food webs, supporting current ecological literature that highlights the importance of ecological subsidies.

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LITERATURE CITED

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Table 1. Total number of individuals for each spider taxa collected from the eight riverine and eight riparian quadrats

Family	Genus	Riverine (n)	Riparian (n)
Araneidae		1	1
Pholcidae			
	Modisimus	20	8
Tetragnathidae			
	Chrysometa	21	7
	Leucauge	50	62
	Tetragnatha	6	0
Theridiosomatidae			
	Theridiosoma	5	22
	*Wendilgarda**	155	17
Uloboridae			
	Miagrammopes	7	25
Total		265	142

* Identified to species, *Wendilgarda clara* (Keyserling, 1886)

Table 2. Stable isotope values of all samples used in subsequent IsoSource(Phillips & Gregg 2003) analyses. Insects were grouped by functional feeding groups: CG = Collector-Gatherer, Fi = Filterer, Pr = Predator, Sc = Scraper, Sh = Shredder, He = Herbivore.

	Order	**Family**	**Genus**	**$\delta^{13}C$**	**$\delta^{15}N$**
Stream leaf litter				-25.5	0.8
Terrestrial vegetation				-34.9	-1.3
Periphyton				-32.4	-0.8
Aquatic CG	Diptera	Chironomidae		-26.63	2.63
Aquatic Fi	Diptera	Simuliidae		-27.76	2.62
	Trichoptera	Hydropsychidae		-29.41	3.69
Aquatic Pr	Hemiptera	Veliidae		-27.83	2.96
	Odonata	Coenagrionidae		-27.42	4.98
	Trichoptera	Hydrobiosidae		-26.51	5.09
Aquatic Sc	Ephemeroptera	Leptophlebidae		-28.15	2.59
	Trichoptera	Helicopsychidae		-34.88	1.96
Aquatic Sh	Diptera	Tipulidae		-27.08	2.85
	Trichoptera	Calamoceratidae		-28.55	0.78
Terrestrial He	Hemiptera	Cicadoidea		-28.69	-0.55
	Lepidoptera			-27.40	1.94
Terrestrial Pr	Coleoptera	Lampyridae		-25.30	6.31
	Hymenoptera	Evaniidae		-26.82	3.83
Riparian	Aranea	Tetragnathidae	*Chrysometa*	-27.40	4.51
	Aranea	Tetragnathidae	*Leucauge*	-27.25	4.76
	Aranea	Theridiosomatidae	*Theridiosoma*	-27.74	3.55
	Aranea	Uloboridae	*Miagrammopes*	-27.24	2.54
	Aranea	Pholcidae	*Modisimus*	-28.49	3.44
Riverine	Aranea	Tetragnathidae	*Chrysometa*	-26.72	5.19
	Aranea	Tetragnathidae	*Leucauge*	-26.66	4.53
	Aranea	Tetragnathidae	*Tetragnatha*	-27.54	3.87
	Aranea	Theridiosomatidae	*Theridiosoma*	-27.34	3.84
	Aranea	Theridiosomatidae	*Wendilgarda**	-27.24	4.24
	Aranea	Uloboridae	*Miagrammopes*	-27.35	2.90
	Aranea	Pholcidae	*Modisimus*	-26.65	3.40

* Identified to species, *Wendilgarda clara* (Keyserling)

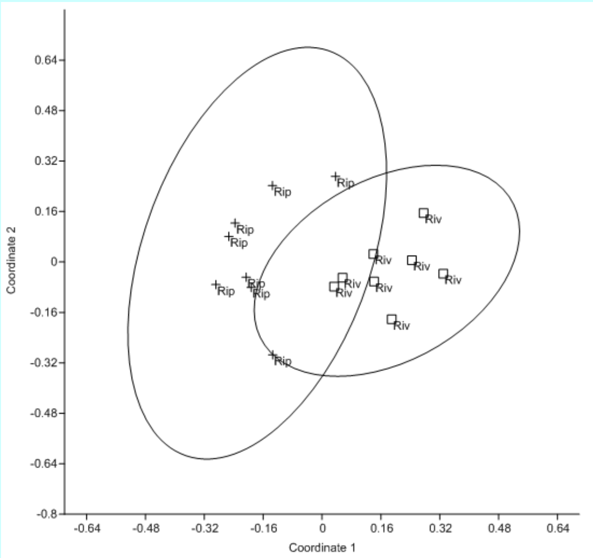

Figure 1. NMDS of web-spinning spider abundances for each sampling date. Riparian (Rip) quadrats (+) and riverine (Riv) quadrats (□). Bray-Curtis 95% ellipses. ANOSIM Bonferroni-corrected p=0.002, R=0.7224

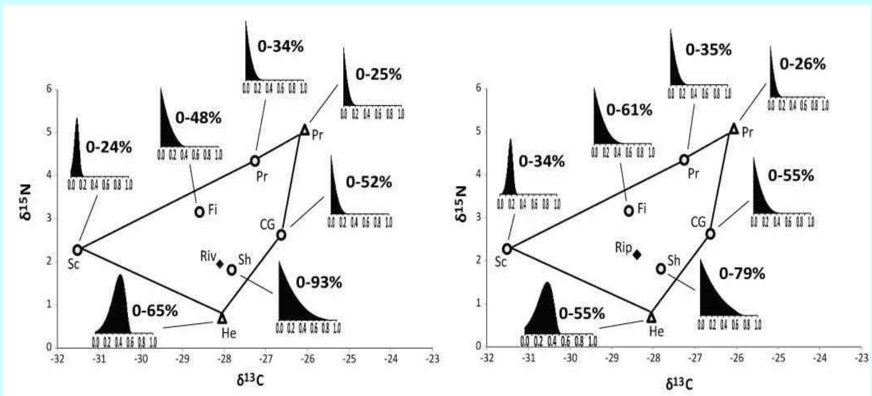

Figure 2. Mixing polygon for Riparian (Rip) and Riverine (Riv) spider assemblages (◆) (adjusted for trophic fractionation) along with terrestrial and aquatic insects (○) that are possible prey. Insects are grouped by their functional feeding groups: CG = Collector-Gatherer, Fi = Filterer, He = Herbivore, Pr = Predator, Sc = Scraper and Sh = Shredder. Histograms show the distribution of the possible percentages that the insect groups represent in the diet of each spider assemblage calculated using IsoSource (Source Increment: 1% , Tolerance Level: 0.1‰)(Phillips & Gregg 2003).