Food source and trophic position analysis of spiders living in northern wetlands

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ABSTRACT

Wetland ecosystems are important ecological features that contain complex food webs due to their terrestrial and aquatic inputs. Feeding and diet habits of spiders in different types of wetlands is poorly understood, but is an essential subject to understand different interactions and community structure of organisms within these ecosystems. The $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures have been found to be effective tools to better understand and interpret species' diets and trophic levels. Spiders are generalist predators, and are often cannibalistic, causing their isotopic signatures to be further complicated. Given the low insect productivity in wetlands during the dry season, spiders are expected to occupy a higher trophic level. We analyzed the nitrogen and carbon isotopic signatures of spiders and vegetation during the dry season from three different wetland locations: two vernal pools and one bog. We didn't find any significant differences for isotopic signature between spider families or between sites. The average $\delta^{13}C$ value for spiders and vegetation were – 25.620 ± 1.095 and -28.758 ± 1.385 respectively. These values indicate that C3 vegetation serves as the basal carbon source and that spiders rely on terrestrial food source. The average $\delta^{15}N$ value for spiders and vegetation were 5.486 ± 0.940 and -1.790 ± 1.092, respectively. There was approximately a 7‰ ^{15}N enrichment in spiders. Using the literature value of approximately 3 to 4‰ ^{15}N increase per trophic level, the found 7‰ ^{15}N enrichment suggests spiders occupy two levels up in the trophic chain. Our study provides evidence for utilizing spider family isotopic signatures to better understand food webs in wetland ecosystems.

Keywords: Spiders; Isotopic composition; Generalist predators; Wetlands; Trophic interactions; Food source; Stable isotope analysis

INTRODUCTION

Spiders are dominant predators that consume a large variety of prey and engage in intraguild predation (Mestre et al. 2013). Their great abundance, biological functionality, and diversity have enabled them to inhabit a vast variety of ecosystems and living environments. They also have many important ecological roles, including serving as a source of nutrient input in ecosystems (Hodkinson et al. 2001). Spiders provide a mechanism for the natural deposition of dead insects, a ready source of nutrients that optimizes nutrient retention and distribution (Hodkinson et al. 2001). Despite this, information pertaining to spiders is sparse. We

still lack knowledge about the biology and feeding habits of spiders in their natural environment (Cardoso et al. 2011; Greenstone 1999; Mestre et al. 2013).

Diets of animals have been studied and used to better understand their relationship and interactions with their environment and other organisms, especially for trophic level studies (Mestre et al. 2013; Hyodo 2015; Iakovlev et al. 2017). Various studies (Akamatsu et al. 2004; Paetzold et al. 2005) have used the isotopic composition of riparian spiders to study their food source. Riparian spiders interact with both aquatic and terrestrial ecosystems, and as such their diets consists of a variety of food sources, which is reflected in their isotopic composition. Aquatic food sources have a different isotopic composition from terrestrial food sources (Hyodo 2015). However, these studies focus on spiders living in areas adjacent to large bodies of freshwater, and not in wetland ecosystems.

Wetlands have different ecological and biological properties from rivers or streams. They also sustain a different variety of terrestrial and aquatic organisms. The smallest and often the most ephemeral of the wetlands are temporary or vernal pools that fill from seasonal rains or snow melt (Batzer & Wissinger 1996). These pools can flood and dry repeatedly, fill for a few months, or remain flooded for much of the year. Insect productivity can be very high in temporary pools, possibly because decomposition during waterless stages enhances detrital food quality (Batzer & Wissinger 1996). Peat develops in wetlands where decomposition does not keep pace with plant production, such as bogs (Batzer & Wissinger 1996). Peat accumulation cause peatland surfaces to gradually rise above the water table and thus many of the insects found in these habitats are of terrestrial origin (Batzer & Wissinger 1996), although aquatic species can occur in the water around peatland vegetation itself (Batzer & Wissinger 1996). In these fishless habitats, predaceous insects can become very abundant, and they are often apex aquatic predators (Batzer & Wissinger

1996). Spiders are dominant generalist predators that consume a great variety of prey, engage in intraguild predation, and reduce herbivore numbers in terrestrial habitats (Hooks et al 2006). Therefore, they are an important component in these ecosystem interactions.

This study focuses on feeding habits, trophic position, and the diet of spiders living in northern wetlands, specifically bogs and vernal pools. The analysis of the natural variations in stable isotope ratios in animal tissue is a powerful tool to reveal feeding preferences of generalist predators such as spiders (Oelbermann & Scheu 2001). This approach is based on the fact that C and N isotopic signatures of body tissues of consumers reflect those of its diets (Hyodo 2015). Stable carbon isotope ratios are also used increasingly to examine the trophic flow across terrestrial and freshwater ecosystems (Kato et al. 2004). Stable isotope analysis enables characterization of trophic relationships between organisms because it tracks the energy flow in food webs (Mestre et al. 2013). The $\delta^{13}C$ isotopic signature has been proven to indicate the basal carbon source of a species (Hyodo 2015). The $\delta^{15}N$ of a species indicates the average number of trophic transfers between the species and the base of the food web with enrichment values ranging 3 to 5‰ per trophic level (Mestre et al. 2013).

In this study, we seek to understand how multiple spider families use the available food sources from terrestrial and aquatic ecosystems. More specifically, we seek to study food source preference, aquatic or terrestrial, of spiders using their isotopic signatures to better understand trophic interactions in wetland ecosystems during the dry season. The questions being addressed are 1) are terrestrially or aquatically derived food sources the larger contributor to spiders' diet and 2) how can we describe their trophic position in northern wetland ecosystems, specifically vernal pools (sites 1 & 3) and a bog (site 2) during the dry season? To determine this, spiders and vegetation from each site were collected and run on an elemental analyzer coupled to an Isotope

ratio mass spectrometer (EA-IRMS) for bulk analyses of stable C and N isotopes. Carbon stable isotopes are useful for tracing basal carbon sources, whereas nitrogen stable isotopes are greater indicators of trophic level (Newton 2016). We hypothesized terrestrial food sources will be a greater contributor to spiders' diet in northern wetland ecosystems than aquatic food sources and that spiders are predominantly two to three trophic transfers away (7-12‰ ^{15}N enrichment) from the basal carbon source, since they serve as generalist dominant predators in the ecosystem (Lang et al. 1999) and are also cannibalistic.

MATERIALS AND METHODS

Field sampling

Sweep nets and pitfall traps were used to collect spiders at three different locations (Figure 1) at the University of Notre Dame Environmental Research Center (UNDERC) in Wisconsin, USA. Sampling locations included three wetland areas, including two vernal ponds and one bog. The pitfall traps consisted of five buckets that were dug into the ground at approximately 2 meters away from each other and netting placed between them in a cross arrangement (Figure 2). Sampling periods took place in the morning and evening for three weeks throughout the summer (May 25th - June 3rd, June 11th - 17th and July 9th -12th). Spiders were collected using labeled ethanol rinsed glass jars and vials, and were carried back to the lab. Dominant vegetation samples were also collected for basal carbon source analysis on 17/06/13. Plants were selected based on their commonality between all three sites, as well as visible evidence of insect interactions, such as bite marks, larvae on leaves, etc. Site assessment for the three locations was done during the last research week. Notes were taken on site vegetation composition. Soil moisture and pH were measured using a Kelway Soil Tester sensor.

Sample processing

Samples were allowed to stay in the jars overnight or for 12 hours to give enough time for complete food digestion. Samples were then frozen in a -20˚C freezer for at least 8 hours. A Leica EZ4 HD photographic dissecting microscope was used to identify spiders down to family level. Photos were taken using Leica Application Suite software. After identification, samples were placed in a drying oven at 50˚C for 12 hours, or until dry. Samples were ground to a fine powder using an ethanol rinsed pestle and mortar. Each powdered sample was transferred into a labeled penny-envelope and sealed for transport to Notre Dame's stable isotope facility located in Center for Environmental Science and Technology (CEST).

Each sample was weighed to around 0.8 mg on a microbalance and placed into 4 x 6 mm pressed tin capsules. This process was repeated for each sample. The stable carbon and nitrogen isotope ratios were found using an elemental analyzer coupled to a Delta V Isotope ratio mass spectrometer (EA-IRMS). Stable C and N isotope ratios are expressed as:

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

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ or $\delta^{15}N$, respectively. The standard for C is the Peedee Belemnite (PDB), and for N the standard is atmospheric N2.

Data analysis

Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ using an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS). In-house standards of sorghum flour, protein powder, and peach leaves standards were used to correct isotopic values. An acetanilide standard was used to calculate the %C and %N using the following equations:

a) $\%C = \frac{71.09 \text{ x std. amount (mg)}}{\text{std. peak area 44}} \text{ X } \frac{\text{sample peak area 44}}{\text{sample amount (mg)}}$

b) $\%N = \frac{10.36 \text{ x std. amount (mg)}}{\text{std. peak area 28}} \text{ X } \frac{\text{sample peak area 28}}{\text{sample amount (mg)}}$

All four standards were run at the beginning and the end, with three run in the middle to ensure no drifting occurred during the run. Only samples from runs with an R^2 value greater than 0.99 were utilized for analysis. All the samples were run in duplicate to ensure reproducibility.

Statistical analysis

Measured and expected isotope signatures for the standards were analyzed using regressions to obtain linear equation to be used for correcting data. Three spider samples were dropped when running statistical tests and descriptive statistics since they fell outside the 2 standard deviation and 95% of the data was still represented. Since data was not normally distributed, Kruskal-Wallis test was used to test for any C:N differences between families and sites. All statistical tests were run using the program Systat, version 10.0.

RESULTS

Data was not normally distributed and could not be transformed, so non-parametric Kruskal-Wallis testing was employed. The first Kruskal-Wallis tested for any difference in C:N ratio between families. No significant difference in C:N ratios was detected between spider families using a Kruskal-Wallis test (test statistic= 7.621, df=4, p-value=0.1062).

The second Kruskal-Wallis tested for C:N ratio differences between type of site; site 1 being vernal pools and site 2 being one bog. No significant difference in C:N ratios was detected between types of sites using a Kruskal-Wallis test (test statistic= 2.256, df=2, p-value=0.324). Thus, there was not a significant difference in C:N ratio depending on type of site.

The averages for $\delta^{13}C$ and $\delta^{15}N$ for spiders were -25.620 ± 1.095 and 5.486 ± 0.940, and for plants they were -28.758 ± 1.385 and -1.790 ± 1.092, respectively. The $\delta^{13}C$ values for the plants and spiders were similar. For $\delta^{15}N$, there was approximately an increase of 7‰. The C:N ratio average for plants was 3.310 ± 0.852. For spiders, it was 7.076 ± 0.225 (Figure 5). The average C:N ratio by type of site for both spiders and plants was also calculated. For site 1 (vernal pool), spiders had an average C:N ratio of 7.091 ± 0.078 , and plants had an average of 2.915 ± 0.059. For site 2 (bog), spiders had an average C:N ratio of 7.032 ± 0.410, and plants had an average of 3.569 ± 0.634. Generally, spiders have a larger C:N ratio than plants in both types of wetlands studied (Figure 6). In addition, values for C and N isotope signature for spider families showed no significant variation (Table 1).

DISCUSSION

There was not a significant difference for C isotope signature between different spider families (Figure 7). This suggests that basal carbon source does not significantly vary between spider families. However, different abiotic and biotic factors could have influenced these results. Since the study was carried during summertime, drought and water evaporation rates dried the sites. In addition, aquatic insect composition would have been reduced drastically, as conditions were not favorable anymore and water was scarce. A previous study (Kato et al. 2004) carried in Japan found that $\delta^{13}C$ for linyphiid spiders showed a preference for aquatic prey, except in June-July; that is, the isotopic composition started to resemble that of terrestrial prey during summertime. In contrast, araneid spiders relied on terrestrial prey throughout the whole study period. This suggests different groups of spiders can have different diet preference, but prey can also change seasonally. In addition, it is possible that aquatic insect productivity was low for that particular vernal pool, and so there would have been less aquatic food resources. If wetlands are

flooded infrequently or for short durations, their invertebrate productivities may be low (Batzer & Wissinger 1996). Petit (2017) explained that the capacity of some wetlands in Australia to provide refugia for aquatic biota during the dry season is likely to be highly dependent on either localized runoff or ground water inputs. It is possible that the same criteria can also be applied for some northern wetlands.

Additionally, there also wasn't a significant difference in $\delta^{15}N$ values between families. Spiders are mostly known as predators, and so are expected to prey on similar functional organisms. However, this is not always the case. A previous study showed that jumping spiders have $\delta^{13}C$ values similar to acacia plant species, and $\delta^{15}N$ values higher by 2.9‰ than the Beltian food bodies of acacia species (Hyodo 2015). However, this study was carried in grasslands, and nutritious plants were abundant.

The values for $\delta^{13}C$ shows us the basal carbon source, whereas $\delta^{15}N$ indicates trophic level (Hyodo 2015; Perkins et al. 2014; Michener & Kaufman 2008). The similarity of N isotopic signatures values between spiders indicates that they tend to occupy similar trophic levels. This suggests that spiders tend to prey on similar functional organisms, at least during the period this study was held. On the other hand, the similarity of C isotopic signatures suggests that they belong to similar food chains, since their C isotope signature reflects that of the same C3 vegetation and thus their primary source of carbon. The C isotopic signature helps us differentiate between carbon sources that greatly differ in their $\delta^{13}C$ values, such as C3 or C4 photosynthetic pathways or aquatic and terrestrial sources (Michener & Kaufman 2008). For the collected plants, the average C isotopic signature was -28.758 ± 1.385. C_3 plants have $\delta^{13}C$ values averaging about -26.5‰, while values for C_4 species average about -10 to -12.5‰ (Hyodo 2015; Michener & Kaufman 2008). The C isotopic signature of the plants resemble that of the plants that undergo C3 photosynthetic

pathways. This makes sense since most northern deciduous trees and conifers use C3 photosynthetic pathway.

The $\delta^{13}C$ average for spiders was -25.620 ± 1.095, which indicates that the primary carbon source is the C3 plants. Overall, there is a small (0.5-1.5‰) enrichment in $\delta^{13}C$ in the organism relative to its diet (Michener & Kaufman 2008). Variations in $\delta^{13}C$ could be due to the variation of isotopic composition of individual tissues within organisms, which can reflect differences in amino acid and lipid composition and concentration, variation among turnover time for different tissues and different rates of tissue turnover (Michener & Kaufman 2008). Moreover, cases where $\delta^{13}C$ depletion occurs are not unknown. In a previous study, larvae of some aquatic insects such as Helode ssp. in detritus of stream backwater pools, and the adults collected on the stream bank, were both highly depleted in $\delta^{13}C$ (−67‰) (Kohzuet al. 2004). This methane-derived C is suggested to support terrestrial predators such as spiders (Jones & Grey 2011).

The $\delta^{13}C$ values of aquatic plants usually lie in an intermediate position between the values of terrestrial C3 and C4 plants, depending on their carbon sources. Aquatic fauna will usually have carbon isotopic values that are less negative than those of terrestrial animals feeding on C3-based foods and more negative than those feeding on C4-based foods (Michener & Kaufman 2008). However, freshwater aquatic food webs can appear to have C3-like carbon isotopic compositions.

The stable N isotopic signatures of plants are known to be influenced by various factors such as climate and the successional stage of the ecosystem (Hyodo 2015). However, the three locations had characteristics and vegetation of an early successional stage ecosystem, so this wouldn't be a determining factor. Nitrogen enrichment increases 3-4 ‰ with trophic level (Hyodo 2015; Perkins et al. 2014). The $\delta^{15}N$ values of most modern plants are between 0 and 5‰. Therefore, the nitrogen isotopic values of populations that rely on terrestrial animal protein in their

diet (two trophic transfers), would average from around 6-9‰. The average $\delta^{15}N$ value for plants collected was -1.980 ‰. This may have been due to different climatic and physiological factors. In contrast, the average for spiders was 5.486‰. If we compare the values for plants and spiders, we see that there was approximately an increase of 7‰. This indicates that spiders occupy two positions up in the trophic chain. In addition, this also suggests that spiders in these wetland areas rely more heavily on terrestrial animal protein. The nitrogen levels of a consumer will increase more than 6-9‰ when relying more heavily on aquatic resources (Schoeninger & DeNiro, 1984).

As expected, spiders have a larger C:N ratio than plants (Figure 6). This could possibly reflect the spiders' usage of carbon for more structural features. This also suggests that plants are, as expected, a better quality food source than spiders, indicated by the lower C:N ratio (Petit 2017). Overall, stable isotopes were found to be a useful tool for better understanding the food web of three wetlands in northern Wisconsin. The stable isotopic ratios for C and N were found to be useful indicators of basal plant sources and trophic positions. Improvements, such as having more replicates and identifying spiders' species could reflect the existence of marked trophic differences between spider species belonging to the same family (Mestre et al 2013). This could allow for a complete construction of food webs and would help to better understand interactions between organisms in wetland ecosystems.

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REFERENCES

Achim Paetzold, Carsten J. Schubert, and Klement Tockner; Aquatic Terrestrial Linkages Along a Braided-River: Riparian Arthropods Feeding on Aquatic Insects. Ecosystems 2005 8: 748–759

Akamatsu, F., Toda, H. and Okino, T. Food source of riparian spiders analyzed by using stable isotope ratios. Ecological Research 20014; 19 (6): 655-662

Batzer, D. and Wissinger, S. Ecology of Insect Communities in Nontidal Wetlands. Annu Rev. Entomol. 1996; 41:75-100

Hyodo, F. Use of stable carbon and nitrogen isotopes in insecttrophic ecology. Entomological Science 2015;18,295–312

Iakovlev, I. et al. Trophic position and seasonal changes in the diet of thered wood ant Formica aquilonia as indicated by stable isotope analysis. Ecological Entomology 2017; 42: 263–272

Ian D. Hodkinson, Stephen J. Coulson, Joanna Harrison, Nigel R. Webb. What a wonderful web they weave: spiders, nutrient capture and early ecosystem development in the high Arctic – some counter-intuitive ideas on community assembly. Oikos 2001: 95(2): 185–360

Kato, C., Iwata, T. and Wada, E. Prey use by web-building spiders: stable isotope analyses of trophic flow at a forest-stream ecotone. Ecological Research 2004; 19(6): 633-643

Keith A. Hobson, Harold E. Welch; Determination of trophic relationships within a high Arctic marine food web using carbon-13 and nitrogen-15 analysis. Marine Ecology Progress Series 1992; 84(1): 9-18

Lang, A., Filser, J. and Henschel J. Predation by ground beetles and wolf spiders on herbivorous insects in a maize crop. Agriculture, Ecosystems and Environment 1999; 72:189-199

Margaret J Schoeninge, Michael J. DeNiro; Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Elsevier 1984; 48 (4): 625-639

Meier-Augenstein, W. and Kemp, H. F. 2012. Stable Isotope Analysis: General Principles and Limitations. Wiley Encyclopedia of Forensic Science.

Mestre, L et al. Trophic structure of the spider community of a Mediterranean citrus grove: A stable isotope analysis. Basic and Applied Ecology 2013; 14 (413-422)

Michener, L. K. and Kaufman. R. 2007. Stable isotope ratios as tracers in marine food webs: and update. Pages 238-282 Stable Isotopes and Ecology in Environmental Science. Blackwell Publishing, Oxford, UK.

N. E. Pettit, D. M. Warfe, P.G. Close, B. J. Pusey, R. Dobbs, C. Davies, D. Valdez, C and P. M. Davies. Carbon sources for aquatic food webs of riverine and lacustrine tropical waterholes with variable groundwater influence Marine and Freshwater Research, 2017,68, 442–451

Newton, J. (2016). Stable Isotopes as Tools in Ecological Research. eLS.

Perkins, M et al. (2014) Application of Nitrogen and Carbon Stable Isotopes ($\delta^{15}N$ and $\delta^{13}C$) to Quantify Food Chain Length and Trophic Structure.

Oelbermann K, Scheu S (2002) Stable isotope enrichment ($\delta^{15}N$ and $\delta^{13}C$) in a generalist predator (Pardosa lugubris, Araneae: Lycosidae): effects of prey quality. Oecologia 130: 337–344.

Schoeninger, M. J. and M. J. DeNiro. "Nitrogen and Carbon Isotopic Composition of Bone Collagen from Marine and Terrestrial Animals", Geochimica et Cosmochimica Acta 1984; 48:625-639.

GRAPHS AND FIGURES

Family	Average C isotope signature	Average N isotope signature
Lycosidae	-24.928±0.736	5.144±0.947
Thomisidae	-23.816±0.842	5.309±0.337
Pisuridae	-24.476±0.057	6.106±0.041
Araneidae	-26.923±0.225	6.951±0.0710
Agelenidae	-26.695±0.034	6.318±0.008

Table 1. Average C and N isotope signature for each isotope family. There is not significant variation among families.

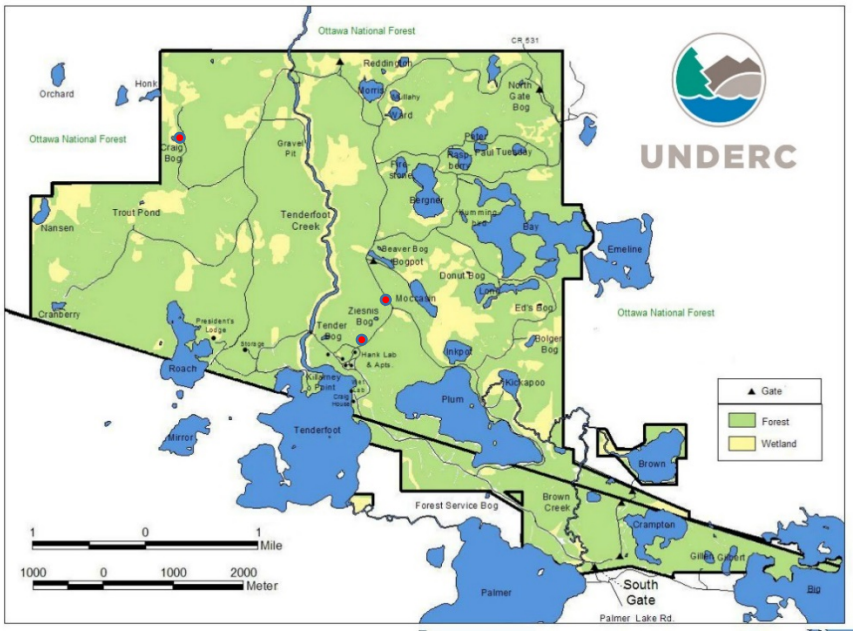

Figure 1: Red markings represent sampling locations

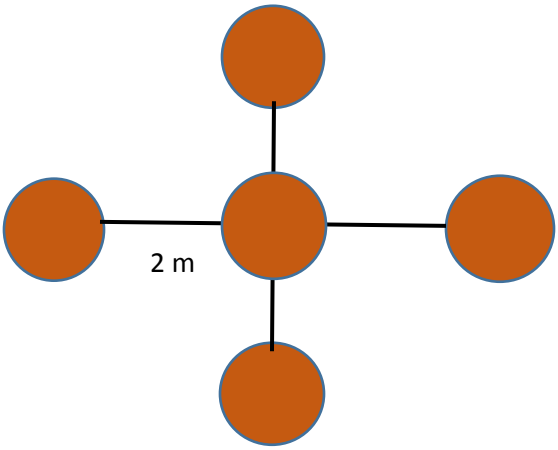

Figure 2: Pitfall trap arrangement

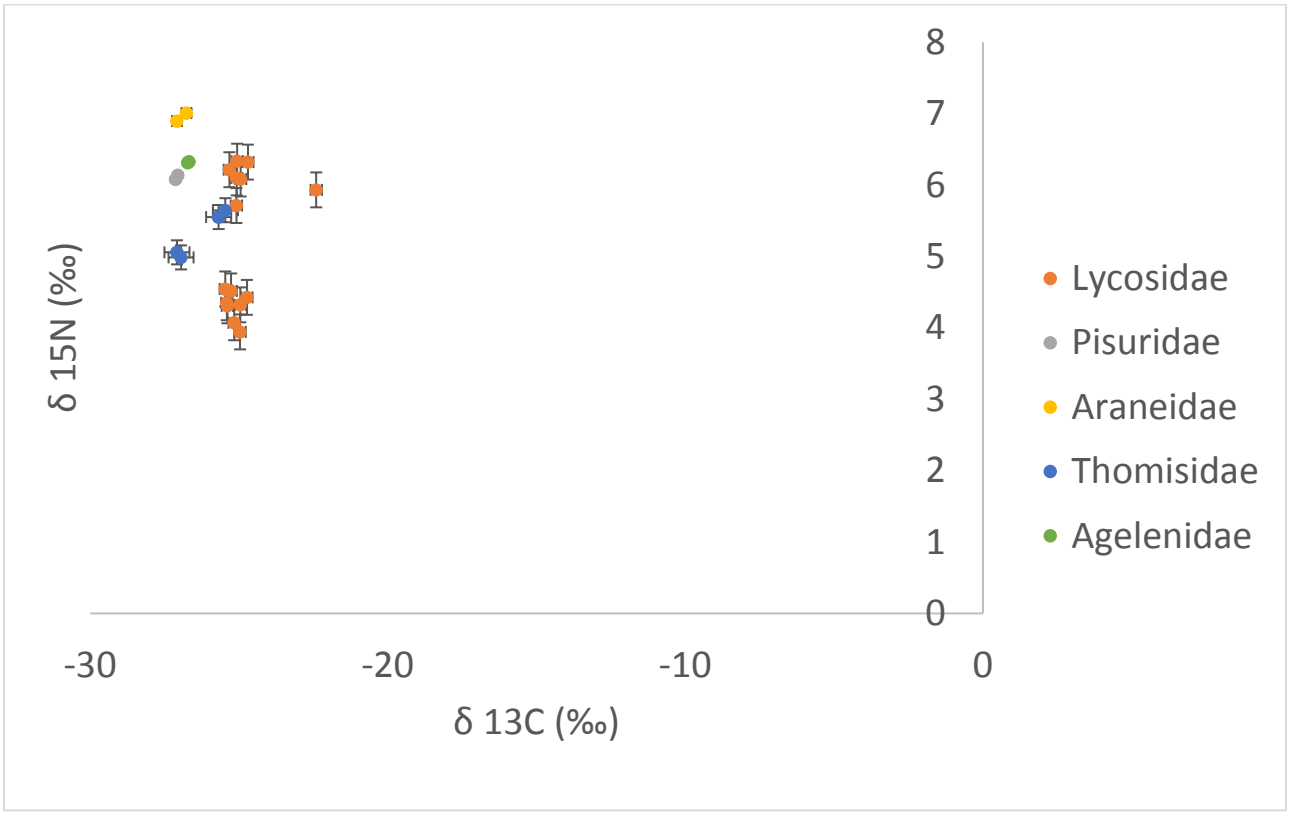

Figure 3: N and C isotopic signatures for spider families

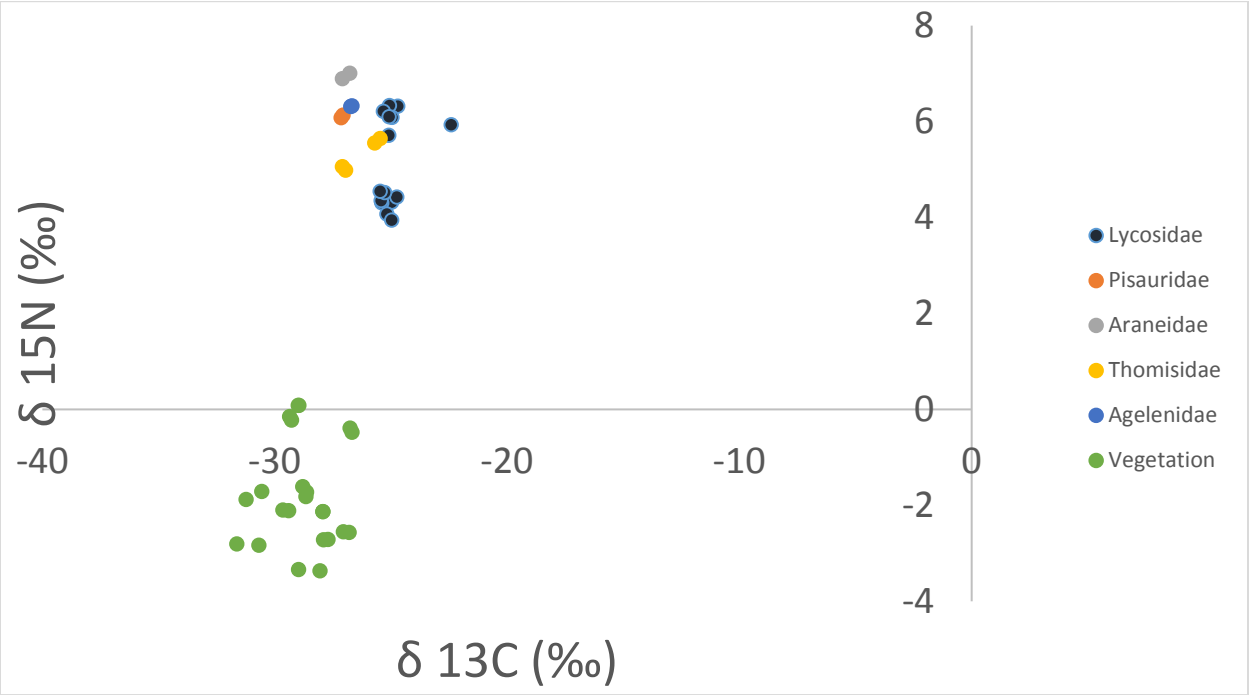

Figure 4: N and C isotopic signatures for spider families and sampled vegetation. There is approximately a 7 permil increase in nitrogen isotope signature. C isotope signature values are consistent and similar, indicating C3 vegetation as basal carbon source.

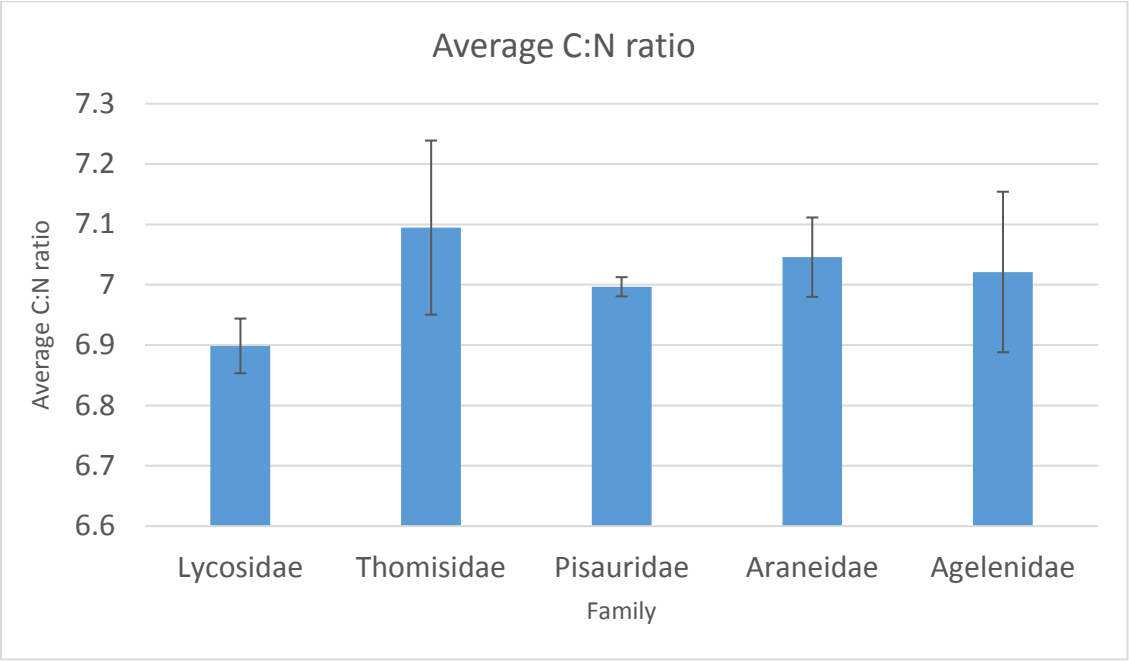

Figure 5: Average C:N ratio for different spider families. There is not a significance difference for C:N ratio among families.

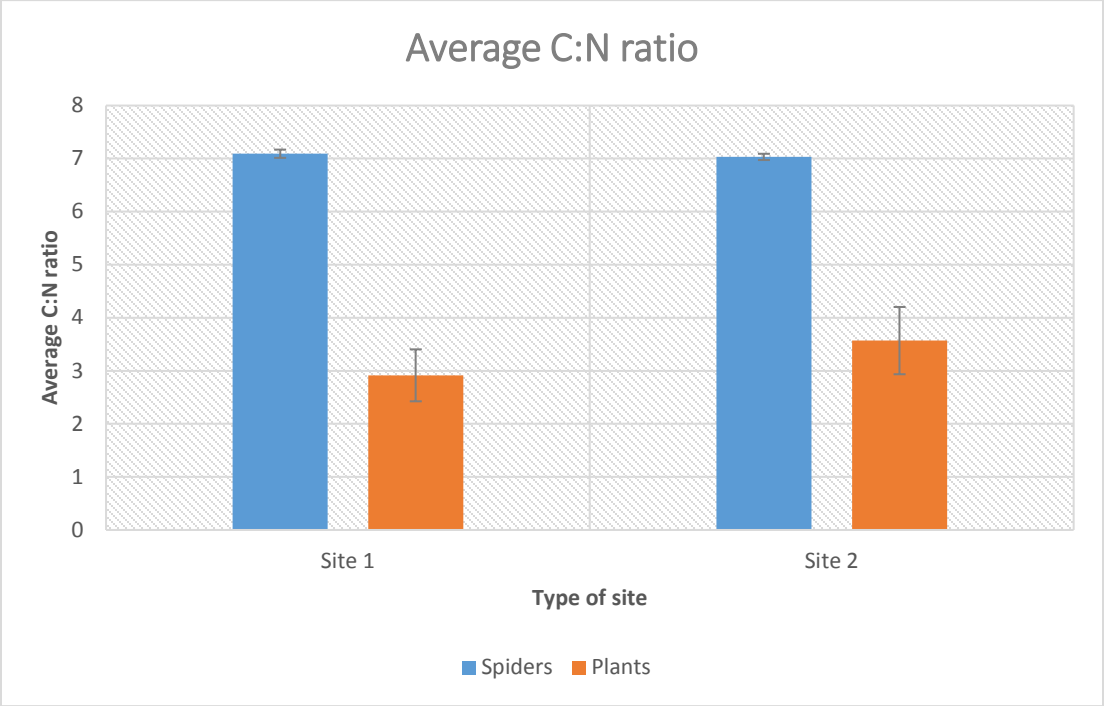

Figure 6: Average C:N ratio for different spider families by type of site. There is not a significant difference for C:N ratio among families depending on type of site. This suggests type Site 1 refers to vernal pool and site 2 to a bog.

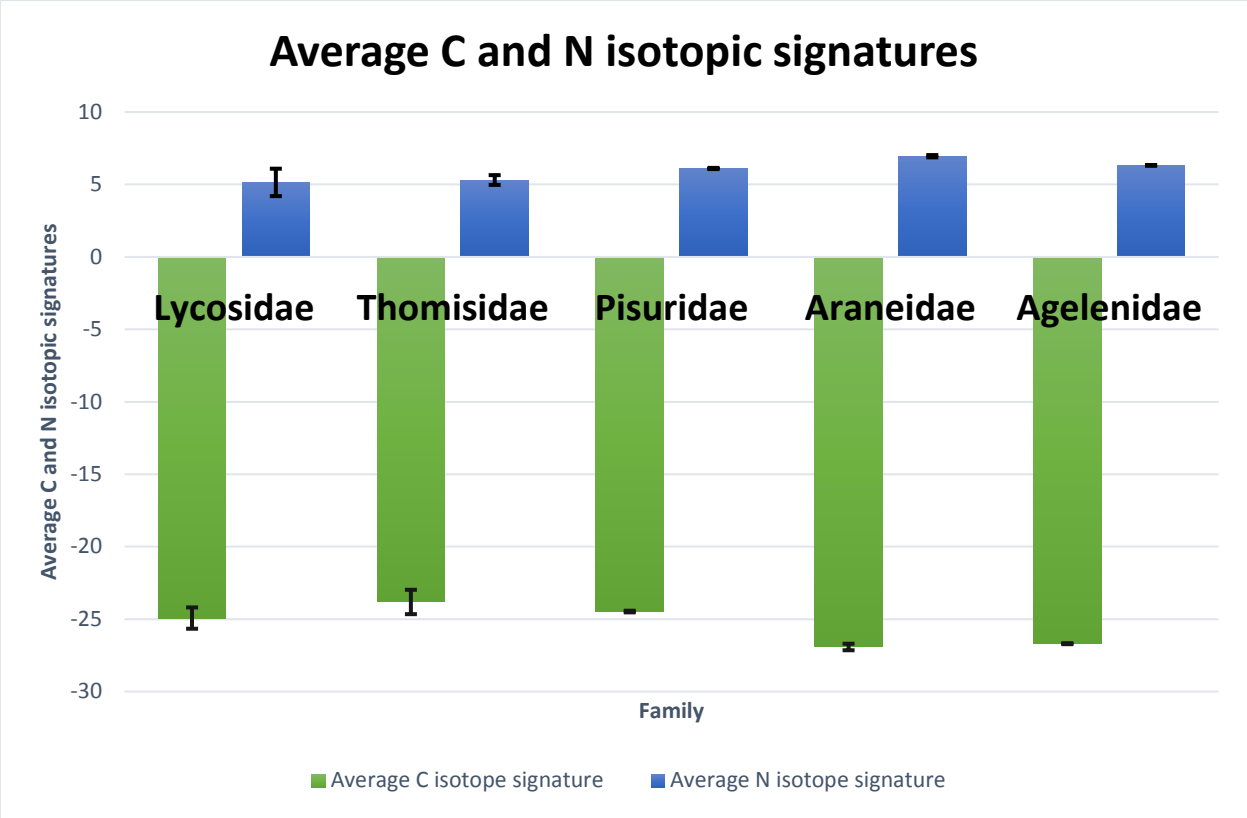

Figure 7: Average C and N isotopic signatures for different spider families. There is not a significant difference in C and N isotopic signatures among families.

Figure 8: *Lycosidae*

Figure 9: *Pisauridae*