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# Diet Affects Egg Laying, Biomass, and Stable Isotope Values in Tetragnathid Spiders

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**Abstract**
Riparian tetragnathid spiders are used as biosentinels of aquatic contamination because they are specialized feeders of aquatic emergent insects and are also prey items for terrestrial predators (e.g., birds). Analysis of both trophic position (e.g., stable nitrogen isotopes) and contaminant concentrations are needed to utilize tetragnathids as biosentinels, which can present challenges when collecting enough biomass to reach analytical detection limits, due to their relatively small size. The purpose of this study was to investigate the impacts of a controlled diet source on spider biomass, egg laying and stable isotope values ($\delta^{13}$C and $\delta^{15}$N). Diet significantly influenced the biomass and egg laying of tetragnathids, with some spiders losing up to 50% of their biomass in a single egg-laying event. $\delta^{13}$C had a faster turnover rate in the whole-body of spiders compared to legs, which is important, as spider legs are presently used as surrogates for whole-body $\delta^{13}$C values.

**Keywords** Growth · Stable Isotopes · Diet · Tetragnathids · Spider

## Introduction

Spiders from the genus Tetragnatha (Araneae, Tetragnathidae) are used as biosentinels of aquatic contamination (Chumchal et al. 2022). Most species from this genus (hereafter referred to as "tetragnathids") live near the land-water interface and spin horizontal webs to capture and consume aquatic emergent insects (Walters et al. 2008). This specialized predatory feeding has been the cornerstone of why tetragnathids have been used to advance the understanding of insect-mediated contaminant flux from aquatic to terrestrial ecosystems (Otter et al. 2020) and is why they are used as a line of evidence to assess remedy effectiveness of contaminated sediment (Walters et al. 2008, 2018; Otter et al. 2013; Kraus et al. 2017; Beaubien et al. 2023). However, much remains unknown about the impact of dietary shifts among this genus.

Stable isotopes of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) are often used to evaluate food web dynamics through the determination of food sources and trophic levels, respectively (Sanzone et al. 2003; Collier et al. 2002; Akamatsu et al. 2004; Speir et al. 2014). Previous research with stable isotopes and tetragnathids has shown that spider diet can be significantly affected by confounding factors such as seasonality (Akamatsu et al. 2004) and the presence of aquatic invasive predators (Gergs et al. 2014).

Individual tetragnathid spiders are relatively small, so it can be difficult to collect enough individuals to reach the needed biomass for analytical detection (Beaubien et al. 2019). To maximize biomass for analytical analysis, past researchers have focused on the collection of female tetragnathids and performed stable isotope analysis on a single spider leg, rather than a whole organism, under the assumption that the spider leg is an adequate surrogate for whole-body isotope values (Walters et al. 2008; Spier et al., 2014). Beaubien et al. (2019) directly addressed this issue when they compared the isotopic signatures of 31 spiders (from five different locations) and found significant correlations between tetragnathid legs to whole bodies for both $\delta^{13}$C ($R = 0.97$) and $\delta^{15}$N isotopes ($R = 0.87$). One goal of this

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study is to build upon the work of Beaubien et al. (2019) and investigate if these same relationships hold true after an abrupt change in diet.

To date, studies focused on the use of tetragnathids as biosentinels have mainly been limited to field collected samples without directly controlling spider diet. The purpose of this study was to use a controlled laboratory feeding experiment to investigate the impacts on spider biomass and stable isotope values. The specific objectives were to: (1) evaluate the impacts of diet on egg laying and individual biomass in female tetragnathid spiders; and (2) determine if the leg of an individual spider accurately reflected the $\delta^{13}C$ and $\delta^{15}N$ isotope values observed in the whole-body throughout the experiment.

## Methods and Materials

### Spider Collection

Adult female tetragnathid spiders ($n = 32$) were collected by hand from riparian vegetation during daylight hours within a 100 m reach from the East Fork Stones River (35.823485, -86.091313) on September 6, 2020. An additional eight spiders were collected on September 20, 2020, at the end of the experiment. The sex of each spider was confirmed by visually inspecting pedipalps at the time of collection. Each spider was placed into an individual polypropylene 50mL conical tube after collection for transport to the lab.

### Experimental Design

Spiders were divided into four feeding groups (0, 1, 3, and 5 flies offered daily) and placed in an environmental chamber set to 26.5 ± 0.5 °C and a 12:12 h light:dark cycle. Each spider was randomly assigned an individual 16oz transparent polycarbonate specimen container that contained an 18 cm mesh strip, 25 mL of tap water, and was covered with a 18 × 18 cm mesh top. Each container was then randomly assigned a location in the environmental chamber, which stayed constant for the 14-day experiment.

Each day, during the light cycle, all containers were removed, and the number of flies consumed by each spider was recorded. Each spider was removed from its container, placed in a pre-weighed 50-mL polypropylene conical tube, and weighed to the nearest 0.1 mg (Mettler Toledo, New Classic ML104). If an egg sac was laid, it was removed with forceps and weighed. Containers were cleaned each day (removal of egg sacs & adding of freshwater) and new flies were added, according to feeding group assignment. At the end of the experiment, all spiders were frozen at -20 °C, prior to stable isotope analysis.

### Spider Feeding

Adult house flies (*Musca domestica*) were collected daily from an in-house culture and placed briefly (1–2 min) into a -20 °C freezer to temporarily suppress their activity (no longer able to fly). The appropriate number of flies were then added to each container, according to feeding group assignment using forceps. Flies returned to full activity within one minute of being removed from the freezer. All spiders consumed flies after active capture of their prey. The house fly culturing techniques followed the guidelines outlined by Carolina Biological Supply Company (Burlington NC, USA). Note: sex difference among adult house flies was not considered in this design.

### Experimental Controls

On the first day and last day of the experiment eight spiders (from the field) and seven flies (from feeding culture) were collected and frozen (-20 °C) identically to all other samples to examine if significant changes (in the field or fly culture) occurred during the experiment.

### Stable Isotope Analysis

Spiders were prepared for analysis by removing a single leg from each spider so it could be independently analyzed from its respective whole-body value (Beaubien et al. 2019). All samples (spider-legs, spider-whole body, and flies-whole body) were dried and homogenized and filtered using a 40-um mesh screen. Stable isotope analysis was performed on an EA-Isolink Elemental analyzer interfaced via ConFlo-IV to a Delta V Advantage Plus isotope ratio mass spectrometer (IRMS) (all Thermo Electron, Bremen Germany). In short, samples were combusted and reduced to pure $CO_2/N_2$. The resulting gas is separated on a gas chromatography (GC) column before being admitted to the mass spectrometer. Samples were dried and weighed into tin capsules on a micro balance (0.25–0.3 mg). Three different standards were used in the data normalization. White River Trout (UASIL house standard) were used for carbon and nitrogen content, through the construction of a calibration curve, five samples covering the sample peak sizes. All isotope values are reported in δ-notation in part per thousand, or per mil (‰) and represent the heavy to light isotopic ratio, $\delta^{13}C$ ($^{13}C/^{12}C$) and $\delta^{15}N$ ($^{15}N/^{14}N$) relative to reference standards. Precision was greater than 0.1‰ (1 SD) for both elements.

### Statistical Analysis

To test if differences existed in average egg sac mass, we excluded feeding group 0 then used a 2-way ANOVA with

Tukey's post host test (variables of feeding group and egg sac number). To test differences in carbon and nitrogen isotope values we used separate 2-way ANOVAs with Tukey's post-hoc test (variables of feeding group and tissue type (whole-body and legs)). Linear regression analysis was used to determine whether the total number of flies consumed by an individual spider was correlated with carbon isotope values. We used an ANCOVA (tissue type as covariate) to determine if this relationship differed by tissue type.

For analysis of quality control samples, the following carbon and nitrogen isotope comparisons were made using separate 1-way ANOVAs for: (1) Day 0 field spiders were compared to day 14 field spiders to ensure no changes occurred at the sampling location; (2) Day 14 field spiders were compared to feeding group 0 on day 14 to ensure they still reflected the isotope values of spiders in the field; and (3) flies from day 0 and 14 were compared to ensure diet consistency.

Significance level was defined as $\alpha = 0.05$. JMP was used for all analyses.

## Results

### Isotope Controls

No significant differences in $\delta^{13}C$ or in $\delta^{15}N$ values were observed for any of the three quality control comparisons.

### The Impact of Prey Availability on Egg Laying and Individual Female Biomass

Spider mass fluctuations and egg laying events were more prevalent in treatment groups that were offered more flies (Fig. 1). Spiders in feeding group 0 did not produce egg sacs subsequent to the first egg laying event. Average spider mass lost with each egg laying event ranged from 0.006 to 0.099 g and increased as treatment groups were offered more flies (excluding first egg sacs) (group 1, $n = 7$, 0.021 g; group 3, $n = 9$, 0.042 g; group 5, $n = 10$, 0.0491 g).

No significant differences between treatment groups were observed for the mass of the first egg sacs laid, but significant differences did exist for all subsequent egg sacs ($F_{(5,35)} = 7.91$; $p < 0.0001$; interaction ($p = 0.0226$); feeding group ($p = 0.0003$); egg sac number ($p = 0.0690$)) (Fig. 2). Subsequent egg sac mass averages were not significantly different from the first egg sacs laid for feeding groups 3 ($p = 0.992$) or 5 ($p = 0.993$), however for feeding group 1 the average egg mass of the first egg sac was significantly greater than subsequent egg sacs laid. ($p = 0.025$)

### Carbon and Nitrogen Stable Isotopes

Significant differences were observed in $\delta^{13}C$ values ($F_{(7,47)} = 7.72$; $p < 0.0001$;) (Fig. 3), but not in $\delta^{15}N$ values ($F_{(7,47)} = 1.96$; $p = 0.0848$) when investigating differences related to feeding group and tissue type. No interaction effect was observed for carbon values ($p = 0.096$) so we focused on each main effect separately. Leg $\delta^{13}C$ values were not significantly different across feeding groups, however significant whole-body $\delta^{13}C$ differences were observed between treatment group 0 and treatment groups 3 and 5. Leg $\delta^{13}C$ values were also significantly different from whole-body $\delta^{13}C$ values in treatment groups 3 and 5, but not in groups 0 or 1.

Linear regression analysis showed a significant relationship between the total number of flies consumed by an individual spider and carbon isotope values in legs and whole-body (leg: $y = 0.0416x - 26.42$; $R^2 = 0.519$; $p < 0.001$; whole-body: $y = 0.1047x - 25.8$; $R^2 = 0.4082$;

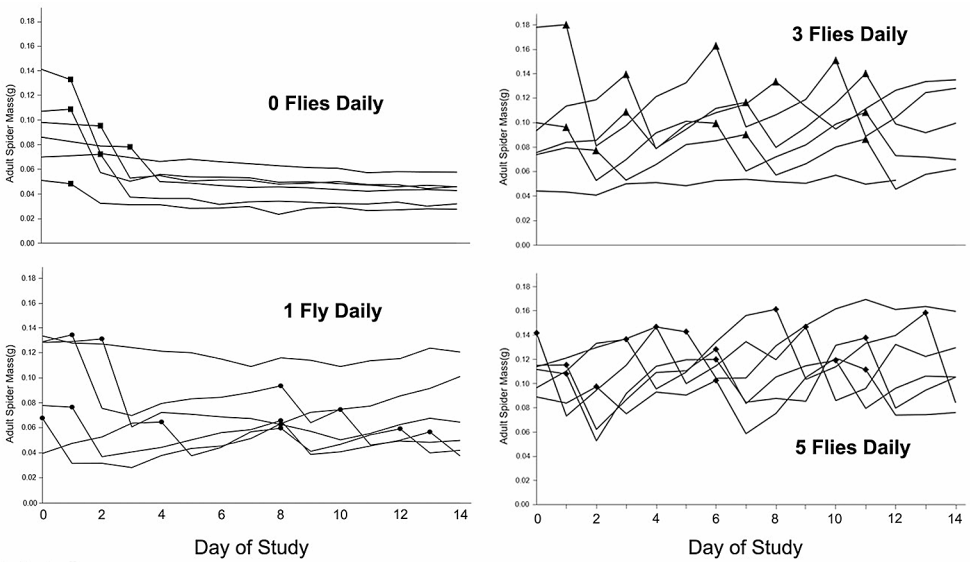


**Fig. 1** Daily mass (g) of individual adult female spiders over 14-day experiment. Points (square = feeding group 0; circle = feeding group 1; triangle = feeding group 3; and diamond = feeding group 5) on each line represent an egg laying event for each individual female

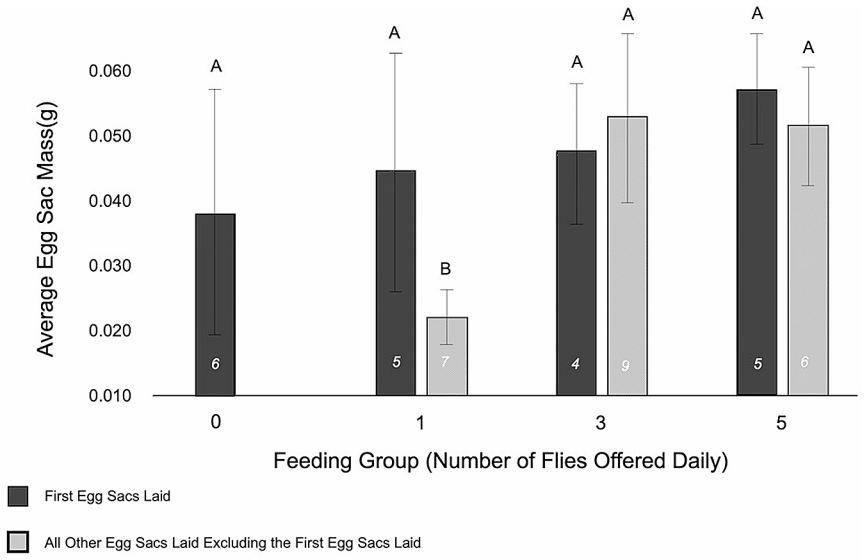


**Fig. 2** Average egg sac masses(g) across feeding groups. Dark bars represent the average masses(g) of first egg sacs laid by each spider within that feeding group. Light bars represent the average mass(g) of subsequent egg sacs laid by each spider within that feeding group. White numbers represent sample size and significant differences between masses(g) are indicated by different upper-case letters. Data presented as mean ± SE.

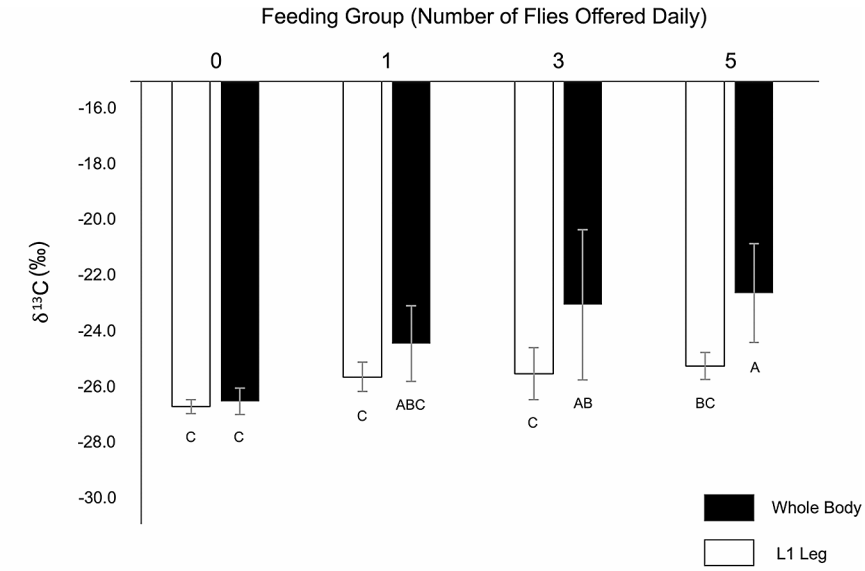


**Fig. 3** Average spider leg and whole body $\delta^{13}C$ values across feeding groups. Filled bars represent whole body $\delta^{13}C$ averages and open bars represent leg L1 $\delta^{13}C$ averages. Significant differences between values are indicated by different upper-case letters. Each bar represents a sample size of $n = 6$. Data is presented as mean ± SE.

$p < 0.001$) (Fig. 4). The ANCOVA analysis revealed that there was a difference between the slopes of the two regressions ($p = 0.03$).

## Discussion

Spiders are biosentinels of aquatic contamination because they are globally distributed, easy to collect and are linked directly to the aquatic environment because of their diet (Chumchal et al. 2022).

In the present study, the impact of food availability had a drastic effect on adult female biomass and egg sac laying over a 14-day period (Figs. 1 and 2). Spiders that were offered more food laid more egg sacs and showed greater fluctuation in mass surrounding egg sac laying events, with some females losing up to 50% of their biomass in a single egg-laying event. Spiders that acquired more mass between egg laying events on average produced heavier egg sacs. However, egg sac mass was also strongly correlated with adult mass lost, meaning that spiders with higher biomass due to higher consumption also lost more mass in egg laying events. These results indicate that higher prey consumption may not always lead to higher overall biomass, due to the loss of biomass that occurs with each egg laying event. Though excess prey consumption may not lead to higher individual spider biomass, prey availability should still be considered when collecting spiders for contaminant analysis, as these results also indicate that sufficient prey consumption is necessary to spiders having multiple egg laying events and egg laying events do influence biomass. The results expand upon previous research conducted by Wise

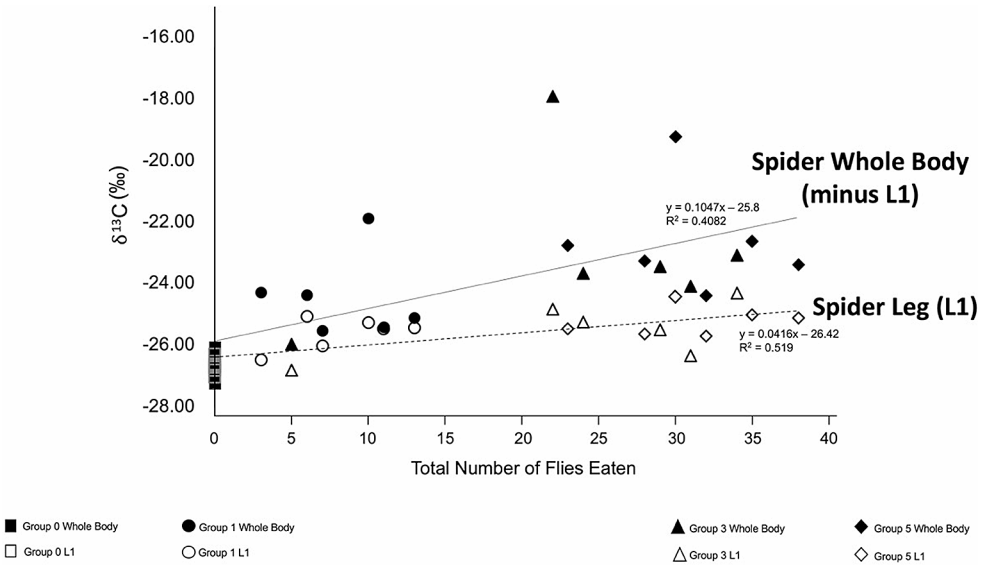


**Fig. 4** Individual spider $\delta^{13}C$ values relative to the total number of flies eaten by each individual spider. Shape is indicative of feeding group. Filled shapes represent whole body samples and open shapes represent leg samples

(1979), which demonstrated that an increase in prey led to a corresponding increase in the number of eggs produced by female individuals in two orb-weaving spider species. Additionally, Danielson-Francois et al. (2002) established a correlation between egg-cluster volume and adult female mass in *Tetragnatha elongata*.

In the present study all spiders were successful in laying a first egg sac, regardless of feeding group (Fig. 1), and the mass of those egg sacs were all similar (Fig. 2). However, food availability did significantly impact both the number and mass of subsequent egg sacs laid. Spiders offered no food (feeding group 0) laid no subsequent egg sacs and those offered one fly daily laid egg sacs with significantly less mass. These results were similar to those of Wise (1979) and Gillespie and Caraco (1987) that showed diet to be a limiting factor when examining fecundity and number of eggs laid in spiders.

To conserve biomass for contaminant analysis, previous researchers have utilized (Walters et al. 2008; Spier et al. 2014) and investigated (Beaubien et al. 2019) the use of a single spider leg as a representative of whole-body $\delta^{13}C$ and $\delta^{15}N$ values. The present study addressed the impact of food availability by investigating differences between whole body (minus one leg) and leg stable isotope values across feeding groups (Fig. 3) and the cumulative number of flies consumed throughout the entire experiment (Fig. 4). When viewed collectively, our findings indicate that $\delta^{13}C$ turns over more quickly in the whole-body than in the leg of tetragnathids. These results show that the use of a spider leg as a surrogate for the whole body of the spider may be confounded if a recent change in diet has occurred. Given the strong correlations observed by Beaubien et al. (2019) between legs and whole-bodies in field collected spiders, our results highlight the need for more detailed investigations into the rate at which this stable isotope enrichment occurs within tetragnathid spiders for both $\delta^{13}C$ and $\delta^{15}N$.

## Conclusion

Diet significantly influenced the biomass and egg laying of female tetragnathids, with some spiders losing up to 50% of their biomass in a single egg-laying event. Stable isotopes of carbon had a faster turnover rate in the whole-body of spiders compared to legs, which is important since presently spider legs are used as surrogates for whole-body values.

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