Patterns of Trophic Shift in $\delta^{15}N$ and $\delta^{13}C$ Through a Cynipid Gall Wasp Community (*Neuroterus* sp.) in *Quercus turbinella*

C. T. YARNES,[1] J. N. ROCKWELL, AND W. J. BOECKLEN

Department of Biology, New Mexico State University and Laboratory for Ecological Chemistry, Institute for Natural Resource Analysis and Management, Las Cruces, NM 88003

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ABSTRACT The degree of shift in stable isotope ratios between trophic levels, known as trophic shift, can help elucidate trophic interactions in systems not amenable to conventional analyses. Gall wasp communities have long been a model system for community ecologists, yet much remains to be explored concerning trophic interactions between hosts, herbivores, and natural enemies. Before stable isotopes can be successfully applied to trophic interactions within gall communities, quality estimates of trophic shift between community members are required. In this study, we document the degree of trophic shift in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes within a simple cynipid gall wasp community, *Neuroterus* sp. (Cynipidae), in *Quercus turbinella* Greene (Fagaceae). The trophic shift in $\delta^{15}N$ between *Neuroterus* sp. and *Q. turbinella* was much lower than values reported for Cecidomyiid gallers, whereas the shift in $\delta^{15}N$ between *Neuroterus* sp. and its parasitoid, *Omyrus* sp., was similar to that reported for parasitoids. The trophic shift in $\delta^{13}C$ was considerably greater in *Neuroterus* sp. than previous estimates from other types of herbivores, whereas *Omyrus* sp. exhibited a trophic shift in $\delta^{13}C$ similar to other biological systems. The unusual trophic shift in $\delta^{13}C$ in *Neuroterus* sp. is likely a result of metabolic differences between host and gall tissues. We discuss commonalities in the observed trophic shift of $\delta^{13}C$ and $\delta^{15}N$ in the *Neuroterus* sp. community to other biological systems and postulate physiological mechanisms for deviations from reported estimates of trophic shift.

KEY WORDS Cynipidae, stable isotope, parasitoid, *Quercus*, trophic shift

CYNIPID GALL WASPS (Hymenoptera: Cynipidae, Cynipini) on oaks often harbor diverse, complex communities of parasitoids, hyperparasitoids, inquilines, and predators. These closed communities have long served as empirical models for community ecology, yet much remains to be explored surrounding the role of direct and indirect trophic interactions in structuring communities (Price et al. 1980, Washburn and Cornell 1981, Stone et al. 1995, Plantard et al. 1996, Roininen et al. 1996, Schönrogge et al. 2000). The complex trophic interactions within galls may be further elucidated through the use of stable isotope techniques. Stable isotopes have proven useful in determining the structure of complex communities and the trophic position of species in other biological systems (France 1995, Post 2002), as well as revealing important population-level aspects of organism nutrition (O'Brien et al. 2002). This is particularly true for trophic systems that are not amenable to more traditional dietary or behavioral analysis (Callaham et al. 2000, Bluthgen et al. 2003).

Patterns of consumer-diet fractionation (Δ; e.g., $\Delta = \delta^{13}C_{consumer} - \delta^{13}C_{diet}$) in carbon ($\Delta\delta^{13}C$) and nitrogen ($\Delta\delta^{15}N$) isotopes (DeNiro and Epstein 1978, 1981) between trophic levels is known as trophic shift. Nitrogen generally becomes more enriched (greater relative amount of the heavier isotope, e.g., $^{15}N/^{14}N$) across trophic levels where the realized level of enrichment is balanced by exogenous and endogenous variation in nitrogen assimilation and excretion (Steele and Daniel 1978). Carbon becomes only slightly enriched across trophic levels, and less reliably so than nitrogen; carbon isotope enrichment is primarily determined by the ratio of respiration to growth (McCutchan et al. 2003).

The degree of trophic shift in nitrogen and carbon through food webs is known to be variable between systems because of the underlying physiological variation between organisms (Post 2002, McCutchan et al. 2003, Vanderclift and Ponsard 2003). However, this does not reduce the current use of stable isotopes in the examination of trophic interactions in insect communities (Ostrom et al. 1997, Callaham et al. 2000, Markow et al. 2000, McNabb et al. 2001). Moreover, progress depends on the documentation of patterns in the field and the integration with mechanistic physiological studies across a wide range of organisms (Gannes et al. 1997, Martínez del Rio and Wolf 2004), including endoparasites and parasitoids.

[1] Corresponding author: PO Box 30001, MSC 3AF, Las Cruces, NM 88003 (e-mail: chris.yarnes@gmail.com).

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The successful application of stable isotopes to trophic interactions requires a valid a priori expectation of trophic shift (Vander Zanden and Rasmussen 2001, Post 2002, McCutchan et al. 2003). Trophic positioning of complex communities with high levels of omnivory, cannibalism, or parasitism are especially susceptible to error in assumptions concerning $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$. Unfortunately, trophic positioning within complex communities using stable isotopes is often performed before adequate estimates of trophic shift are known (e.g., Tooker and Hanks 2004). It is important to determine the isotopic baseline of the community (Vander Zanden and Rasmussen 2001), as determined by the isotopic composition of the primary consumers, and obtain estimates of trophic shift for secondary consumers. Moreover, stable isotope ecology research has historically been limited to certain types of organisms and ecosystems making the extrapolation of most existing estimates of trophic shift to different systems problematic.

Patterns in the trophic shift of stable isotopes have only recently been reported for any taxa of gall-former or their associated parasitoids (Tooker and Hanks 2004, Langellotto et al. 2005). The establishment of an isotopic baseline of primary consumers (gall-formers) and quality estimates of trophic shift in gall-forming communities is critical because global estimates of trophic shift based on metadata compiled across different taxa may be invalid for gall communities because of physiological differences between organisms (Martínez del Rio and Wolf 2004). In this study, we established the baseline isotopic composition of the oak gall cynipid, *Neuroterus* sp., in the oak, *Quercus turbinella* Greene and document patterns of trophic shift in carbon and nitrogen ($\Delta\delta^{13}C$, $\Delta\delta^{15}N$) for *Neuroterus* sp. and its parasitoid, *Omyrus* sp. (Chalcidoidea, Omyridae). We compare the degree of $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ in the *Neuroterus* sp. community relative to global estimates constructed from a wide variety of ecosystems ($\Delta\delta^{15}N \approx 3‰$; $\Delta\delta^{13}C \approx 1‰$) and discuss physiological parameters important to the analysis of trophic shift in gall communities.

Materials and Methods

The southwest United States contains a high diversity of oaks (Nixon 1993) and consequently oak cynipid gall formers (Kinsey 1937). In this region, the leaf-galling cynipid, *Neuroterus* sp., is known to use *Quercus turbinella* and possibly other white oaks (Weld 1960). *Neuroterus* sp. forms a number of galls (8–20) along the abaxial mibrib of a single leaf. We observed only a single parasitoid species during gall rearing, an *Omyrus* sp. (Chalcidoidea, Omyridae). *Neuroterus* sp. was identified from existing collections of cynipids at New Mexico State University (W. J. Boecklen and K. C. Larsen), while *Omyrus* sp. was keyed using Grisell and Schauff (1997). Voucher specimens of adult insects and galls were deposited in the Arthropod Museum at NMSU (*Neuroterus* sp. acc. no. 26265; *Omyrus* sp. acc. no. 26266).

On 15 October 2004, galls were collected from *Q. turbinella* near Aguirre Springs Campground in the Organ Mountains, Dona Ana County, NM. Here *Q. turbinella* forms dunes of shrubs, 1–2 m. Six trees were haphazardly chosen from a small stand of *Q. turbinella* and censused for *Neuroterus* sp. galls. All galled leaves were collected from each tree (range: 10–32 galls/tree). We also collected five ungalled, undamaged leaves from each tree to examine the effect of gall-formation on leaf isotopic composition.

Cage rearing is common for the examination of oak cynipid gall communities; it allows for the reliable collection of all emerging (surviving) insects from the gall. As such, this study focused on adult insect isotopic composition. Leaves bearing galls were placed in petri dishes, stored at room temperature and monitored weekly for emergence. For each tree, ungalled leaves were processed for stable isotope analysis at the time of first emergence from that tree; all leaf and gall tissues were frozen, dried to completeness using a freeze-dryer, and ground using a ball mill before analysis. Adult *Neuroterus* sp. and *Omyrus* sp. were collected after emergence, frozen overnight at −20°C, and freeze-dried; the corresponding galled leaf and gall tissues were processed at that time as well. Weekly collection was completed at 3 mo, with 82% of emergence occurring during weeks 3 and 4. Adults were not ground and were analyzed whole to maximize sample size for isotope-ratio mass spectrometry (IRMS) analysis. When possible, analysis of whole body isotopic composition is considered more appropriate than tissue-specific analysis (DeNiro and Epstein 1978). IRMS requires a minimum of 60 μg of nitrogen within a single sample for successful analysis. Because of the small weights of adult *Omyrus* sp. (range: 55–100 μg), individuals were pooled for IRMS analysis across leaves within trees. All other samples were pooled and analyzed in triplicate. Galls harboring inquilines and other community members were not used in this study to minimize their potential influence on estimates of trophic shift between *Q. turbinella*, *Neuroterus* sp., and *Omyrus* sp.

Stable isotope analysis was performed at the Laboratory for Ecological Chemistry (LEC) in the Institute for Natural Resource Analysis and Management, Las Cruces, NM. Samples were combusted using a Costech Analytical Elemental Analyzer (Valencia, CA) and introduced to a ThermoFinnigan DeltaPlus XP IRMS in continuous-flow through a ConFlo III Interface. As dictated by the International Atomic Energy Agency (IAEA), results are reported as the difference, as a small delta value (δ) in measures of "per mil" (‰) or parts per thousand, between the isotopic ratio of the sample and that of the recognized international standard, "VPDB" (Vienna Pee Dee Belemnite, a carbonate rock) for ^{13}C and "Air" (atmospheric nitrogen) for ^{15}N, each standard representing 0‰. Standardized results are then obtained by the following formula:

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

Table 1. $\delta^{15}N \pm SE$, $\delta^{13}C \pm SE$, and $\%N \pm SE$ for the host tree, *Q. turbinella* (including all plant tissues), the gall-forming Cynipid, *Neuroterus* sp., and its parasitoid, *Omyrus* sp.

	Galled leaf	Ungalled leaf	Galls	*Neuroterus* sp.	*Omyrus* sp.
$\delta^{13}C$	−27.562 ± 0.72‰	−27.126 ± 0.53‰	−25.975 ± 0.83‰	−21.521 ± 0.74‰	−21.756 ± 0.90‰
$\delta^{15}N$	1.747 ± 0.50‰	1.584 ± 0.71‰	2.018 ± 0.82‰	2.346 ± 0.73‰	4.417 ± 0.91‰
%N	1.3 ± 0.2%	1.1 ± 0.2%	0.6 ± 0.2%	13.1 ± 0.3%	15.9 ± 1.3%

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Trophic shift is a measure of consumer-diet fractionation (α) and is expressed as capital delta (Δ), the difference in small delta (δ) between two phases:

$$\Delta\delta^{13}C = \delta^{13}C_{consumer} - \delta^{13}C_{diet} \text{ or } \Delta\delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{diet}$$

Laboratory quality control and analysis at LEC follows that of Werner and Brand (2001); all analyses are traceable to the NIST (National Institute of Standards and Technology, Washinton, DC) isotopic standard reference materials IAEA-N3, USGS-32, USGS-24, and NBS-19. Internal laboratory standards are both accurate and precise and readily yield measurements ±0.2‰ for both $\delta^{15}N$ and $\delta^{13}C$. Results of the batches processed for this experiment yielded a ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$ in the internal laboratory standard. Estimates of %N for two samples of each trophic level and leaf material were measured using a ThermoFinnigan Flash EA 1112 Elemental Analyzer (CE Elantech, Lakewood, NJ). Dumas combustion provides measurements of ±0.3% for nitrogen and was standardized using the elemental standard reference material NIST 1547 Peach Leaves.

We analyzed the significance and pattern of isotopic fractionation between the leaves of *Q. turbinella*, *Neuroterus* sp. galls, *Neuroterus* sp., and *Omyrus* sp. Isotopic differences between the different plant tissues (galled leaves, ungalled leaves, and galls) were determined through analysis of variance (ANOVA). All assumptions of ANOVA were verified and met. Post hoc comparisons were carried out using Fisher least significant difference (LSD). Significance in the pairwise trophic shift between *Q. turbinella*, *Neuroterus* sp., and *Omyrus* sp. within trees was analyzed using Student's paired *t*-test. All analyses were carried out using SYSTAT Version 10.2 (SSI, Richmond, CA). All reported estimates of trophic shift are accompanied by their respective SD of the difference (±1 SDD).

Results and Discussion

The $\delta^{13}C$ composition of galls was significantly enriched relative to galled and ungalled leaves, whereas galled leaves were somewhat depleted relative to ungalled leaves in *Q. turbinella* ($F_{2,51} = 24.378$, df = 51, $P < 0.001$; Fisher LSD: $P < 0.001$ and $P < 0.069$, respectively; Table 1). The $\delta^{13}C$ composition of *Neuroterus* sp. was heavier than its galled leaf ($t = -24.791$, df = 5, $P < 0.001$); the mean $\delta^{13}C_{consumer\text{-}diet}$ between *Neuroterus* sp. and *Q. turbinella* was 5.964 ± 1.13‰ (Fig. 1A). The $\delta^{13}C_{consumer\text{-}diet}$ between *Omyrus* sp. and *Neuroterus* sp. was near zero ($t = -0.362$, df = 5, $P < 0.732$; Fig. 1A), and accordingly, their respective $\delta^{13}C$ values were nearly identical (*Omyrus* sp. $\delta^{13}C = -21.756$‰; *Neuroterus* sp. $\delta^{13}C = -21.521$‰; $\delta^{13}C_{consumer\text{-}diet} = -0.314 \pm 1.064$‰).

We found no difference in the $\delta^{15}N$ composition of galled leaves, ungalled leaves, or galls ($F_{2,51} = 1.816$, df = 51, $P < 0.173$). The $\delta^{15}N_{consumer\text{-}diet}$ between *Neuroterus* sp. and *Q. turbinella* was also near zero per

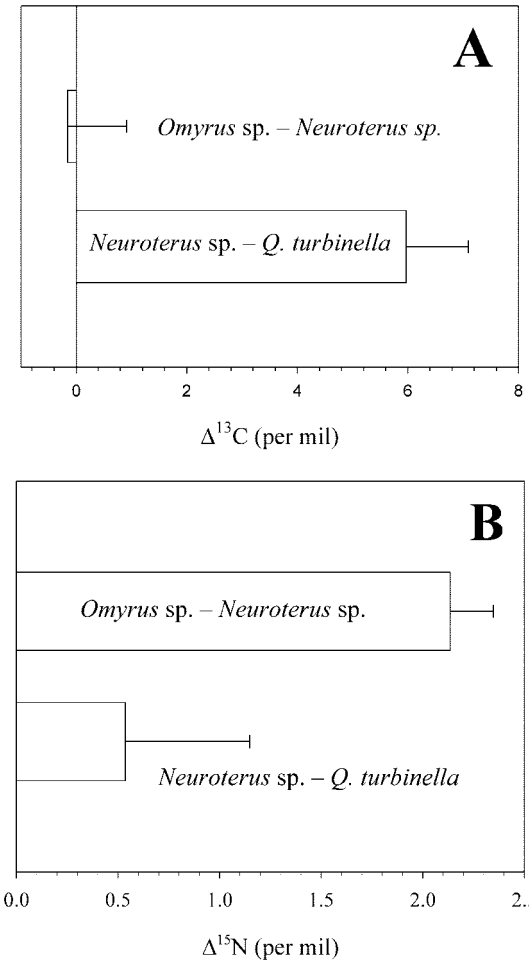

Fig. 1. (A) Trophic shift in the stable isotope ratios of carbon ($\Delta\delta^{13}C$; per mil) between primary producer and primary consumer (*Q. turbinella*–*Neuroterus* sp.) and primary to secondary consumers (*Neuroterus* sp.–*Omyrus* sp.). (B) Trophic shift in the stable isotope ratios of nitrogen ($\Delta\delta^{15}N$; per mil) between primary producer and primary consumer (*Q. turbinella*–*Neuroterus* sp.) and primary to secondary consumers (*Neuroterus* sp.–*Omyrus* sp.).

mil ($t = -2.140$, df = 5, $P < 0.085$; Fig. 1B). *Omyrus* sp. were significantly enriched in $\delta^{15}N$ relative to their *Neuroterus* sp. hosts ($t = -12.944$, df = 5, $P < 0.001$), with a mean within-tree $\delta^{15}N_{consumer\text{-}diet}$ of 2.135 ± 0.211‰ (Fig. 1B). Percent nitrogen for *Q. turbinella* tissue was similar to other studies for nonagricultural woody plants (Mattson 1980), while also showing increase into consumers at higher trophic levels (Table 1), consistent with ecological stoichiometry theory (Sterner and Elser 2002).

Deciduous plant tissues are subject to considerable nitrogen recycling resulting in the homogenization of $^{15}N/^{14}N$ (Kolb and Evans 2002). For *Q. turbinella*, intraplant $\delta^{15}N$ composition of plant tissues (leaves and structural gall tissue) was relatively homogeneous as expected (Table 1). The $\delta^{13}C$ values for *Q. turbinella* leaves (both galled and ungalled) and galls were typical of C_3 trees (terrestrial C_3 range: $\delta^{13}C = -35$ to -21‰). The $\delta^{13}C$ enrichment of gall tissue relative to leaf tissue is likely caused by differences in metabolic activities between the tissues (Gleixner et al. 1998). The more negative $\delta^{13}C$ value indicates greater physiological stress in galled leaves than ungalled leaves (Farquhar and Sharkey 1982), consistent with the findings for *Acer opalus* leaves bearing aphid galls as reported by Verdu et al. (2004).

While $\Delta\delta^{13}C$ is much less variable than is $\Delta\delta^{15}N$, herbivores and omnivores typically exhibit higher $\Delta\delta^{13}C$ than do secondary consumers depending on diet composition (DeNiro and Epstein 1978, Teeri and Schoeller 1979). In rare instances, $\Delta\delta^{13}C$ may range to near 4‰ (McCutchan et al. 2003), yet in most systems, $\Delta\delta^{13}C$ is generally observed between 0 and 1‰. As expected, we observed a $\Delta\delta^{13}C$ between *Omyrus* sp. and *Neuroterus* sp. of ≈0‰. However, the same was not true for *Neuroterus* sp. on *Q. turbinella.* The $\delta^{13}C$ composition of pupated *Neuroterus* sp. is determined by the balance of carbon assimilated minus that which was excreted and respired over its lifetime. It is unlikely that *Neuroterus* sp. would require an abnormally high respiration rate for growth leading to such an elevated $\Delta\delta^{13}C$ (≈5.9‰ over whole-leaf tissue). However, it is quite probable that the nutritive parenchyma cells or inner nutritive cells used by gall-forming insect larvae (Rohfritsch and Arnold-Rinehart 1991, Bronner 1992, Brooks and Shorthouse 1998) have a much different $\delta^{13}C$ composition than leaf tissue or whole-gall tissue. If so, whole-gall or leaf $\delta^{13}C$ would poorly represent the actual carbon sources used by gall-former larvae. Gleixner et al. (1998) found primary plant carbon storage compounds (sucrose, starch) in sink tissues to be enriched in $\delta^{13}C$ composition over their counterparts in source tissues. Sink tissue $\delta^{13}C$ enrichment was attributed to post-transport metabolic conversions of primary compounds. Galls are known to be metabolically intense, and similar source-sink dynamics and patterns of post-transport metabolic activities within galls (Larson and Whitham 1991, Harper et al. 2004) would contribute to variation in $\delta^{13}C$ between different gall tissues. The dissection and extraction of nutritive cells from active gall tissue may clarify the relatively large $\Delta\delta^{13}C$ between *Neuroterus* sp. and *Q. turbinella* leaf tissue. Information on sources of variation in the $\delta^{13}C$ composition between gall tissues in would be extremely advantageous for future studies into trophic interactions in gall communities, particularly for the trophic positioning of suspected inquilines.

The trophic shift in nitrogen isotopes ($\Delta\delta^{15}N$) has been proposed as an index for diet protein quality for consumers (Martínez del Rio and Wolf 2004) because it represents the balance between nitrogen assimilation and excretion. As the quality and assimilation efficiency of diet protein increases, trophic shift decreases. The $\Delta\delta^{15}N$ between *Neuroterus* sp. and galled leaves was relatively small (≈0.5‰), indicating that *Neuroterus* sp. is highly efficient at using the nitrogen provided by *Q. turbinella.* This is much lower than the 3.31‰ trophic shift reported for *Rhopalomyia californica* (Cecidomyiidae) on *Baccharis pilularis* (Compositae) by Langellotto et al. (2005). This may be caused by differences in the physiological mechanisms of nitrogen supply between the Cecidomyiidae and Cynipidae. Moreover, some members of the Cynipidae may regulate the nitrogen supply from their hosts (Hartley and Lawton 1992, Koyama et al. 2003), helping to maintain nitrogen balance and thereby reducing $\delta^{15}N$ enrichment. Additional examples from the Cecidomyiidae and the Cynipidae, as well as other galling taxa, are clearly needed before estimates of host-galler trophic shift in $\delta^{15}N$ are established.

The potential also exists that laboratory cage rearing may have an effect on the isotopic composition of *Q. turbinella* tissues and gall inhabitants relative to that in the field, potentially affecting our estimates of $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ caused by stress. All *Q. turbinella* tissues received equal treatment—processed at the time of first emergence for ungalled tissue and at emergence for gall tissue; presumably, any experimental effect would be the same between galled leaves, ungalled leaves, and gall tissues. Variation in the processing of insects was inevitable because of differences in development between gallers and differences in the timing of emergence between gallers and parasitoids. Insects with longer time in the cages may have been subjected to nutritional stress because of postcollection changes in quality of the leaf/gall tissues. Nutritional stress in animals typically results in $\delta^{15}N$ enrichment of tissues (Young and Marcini 1990, Hobson et al. 1993) and has even been proposed as an indicator of nutritional stress. Our estimates of $\Delta\delta^{15}N$ from *Q. turbinella* to *Neuroterus* sp. as near zero, whereas *Neuroterus* sp. and *Omyrus* sp. was not enriched relative to literature estimates of 3‰. Therefore, while we cannot exclude any effect of nutritional stress on our estimate of $\Delta\delta^{15}N$, it does not affect the overall result that, in *Q. turbinella*, $\Delta\delta^{15}N$ is much lower for gallers and parasitoids relative to published estimates. Recent estimates of isotopic fractionation caused by nutritional stress in Collembola indicate a slight depletion of body $\delta^{13}C$ composition while undergoing starvation (Haubert et al. 2005). None of our reported estimates of $\Delta\delta^{13}C$ would indicate depletion of $\delta^{13}C$ in consumers relative to published estimates. However, a com-

parison of our estimates with that of careful field collections of adult *Neuroterus* sp. and *Omyrus* sp. from their hosts at the time of emergence would be required to completely resolve any potential experimental effects of cage rearing on $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$.

Literature surveys of predator-based studies point to a generalized 3‰ shift in $\delta^{15}N$ across trophic levels for secondary consumers (Post 2002). The lower observed trophic shift for *Omyrus* sp. of ≈2‰ is consistent with the high nitrogen assimilation efficiency often observed in parasitoids (Greenblatt et al. 1982), and is similar to values reported for other parasitoids (Langellotto et al. 2005). Tooker and Hanks (2004) reported no trophic shift in $\delta^{15}N$ between a braconid parasitoid and its host, a mordellid predator present in the cynipid galls of two *Silphium* aster species. However, that result may be confounded by unknown levels of conspecific cannibalism in the mordellid. More importantly, direct comparisons of the trophic position among secondary consumers cannot be made without the use of established estimates of trophic shift in linear mixing models (Phillips 2001).

We observed differences in the trophic shift in carbon and nitrogen isotopes between *Neuroterus* sp. and *Omyrus* sp. that indicate stable isotopes have the potential to provide considerable insight into the trophic interactions within gall communities. Additional information is still needed to set a baseline for $\delta^{15}N$ and $\delta^{13}C$ within gall communities for the examination of higher-level trophic interactions. This may be obtained through the examination of nonstructural gall tissues and the physiological ecology of gall-forming insect larvae. Importantly, the most difficult questions in food web ecology require a comparative approach. The ultimate use of stable isotopes to the study of food web ecology within galls will depend on the repeatability of patterns in trophic shift and the applicability to more complex communities that include inquilines, predators, and hyperparasitoids

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