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# Assessing trophic interactions in a guild of primary parasitoids and facultative hyperparasitoids: stable isotope analysis

**Gail A. Langellotto · Jay A. Rosenheim · Megan R. Williams**

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**Abstract** Facultative hyperparasitism is likely to be the most common form of intraguild predation among parasitoids. However, difficulties associated with studying facultative hyperparasitoids in the field have hampered a thorough understanding of their trophic ecology. In this study, we used a combination of stable isotope analysis and published natural history information to infer trophic interactions in a guild of field-collected primary parasitoids and facultative hyperparasitoids that attack a gall-making midge on *Baccharis pilularis*. Our three a priori hypotheses were: (1) stable isotope values should increase incrementally from the host plant to higher trophic levels; (2) the two species of ectoparasitoids should exhibit higher stable isotope signatures than the two endoparasitoids, and; (3) the two facultative hyperparasitoids should exhibit stable isotope signatures that fall between zero and one trophic level steps above that observed for the primary parasitoids. Food webs inferred from stable isotope data generally agreed with previously published accounts of community structure. As expected, both $\delta^{13}C$ and $\delta^{15}N$ were progressively enriched in the heavy isotope from the host plant to the herbivorous midge to the parasitic wasps. Multivariate analysis of stable isotope data revealed that the two primary ectoparasitoids occupied a similar trophic niche, but were significantly different from the primary endoparasitoids. We attribute this result to "coincidental intraguild predation" by ectoparasitoids that develop on already-parasitized midge larvae. One of the facultative hyperparasitoids, *Zatropis capitis*, exhibited a stable isotope signature approximately one trophic step above the primary parasitoids. Unexpectedly, the second facultative hyperparasitoid, *Mesopolobus* sp., appeared to be developing as a primary parasitoid at all sites. Coupled with independent assessments of community structure, stable isotope analysis validated trophic links constructed by previous researchers and identified potential taxon-specific differences in trophic interactions for two facultative hyperparasitoids in the *B. pilularis* gall community.

**Keywords** Stable isotopes · Food web · Facultative hyperparasitism · *Rhopalomyia californica* · Parasitoids

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G. A. Langellotto (✉) · J. A. Rosenheim · M. R. Williams
Department of Entomology, University of California, Davis, CA 95616, USA
e-mail: langellotto@fordham.edu

G. A. Langellotto
Louis Calder Center, Department of Biological Sciences, Fordham University, Bronx, NY 10458, USA

## Introduction

Facultative hyperparasitoids are omnivores that can successfully complete development on either herbivorous or primary parasitoid hosts. Facultative hyperparasitism is widespread among the Hymenoptera and is likely to be the most common form of intraguild predation among parasitoids (Brodeur 2000). Surprisingly little is known about the trophic ecology of facultative hyperparasitoids (Rosenheim et al. 1995), despite the fact that their plastic life history strategy confers the potential to

suppress *or* promote herbivore populations. A deeper understanding of the trophic ecology of facultative hyperparasitoids is important for both basic and applied insect community ecology.

Historically, the primary tools that have been employed to investigate the prevalence and importance of omnivory and intraguild predation within arthropod communities, in general, have been observation (i.e. descriptive studies of species' diets for the construction of food webs) and experimentation (i.e. manipulation of the community to quantify interaction strengths). These approaches provide rigorous analyses of trophic interactions and community structure, but are also logistically difficult and at times prohibitive due to the speciose nature of many terrestrial arthropod communities. Such approaches are especially difficult to employ with parasitoids that oviposit and feed on internally feeding herbivores, such as gall-formers.

Unlike more traditional ecological approaches, stable isotope analysis offers the potential to quickly and easily assess community structure in systems where direct observations of trophic interactions or manipulative studies are difficult. However, for many food webs involving arthropods, the prevalence of omnivory (Coll and Guershon 2002), including cannibalism (Wagner and Wise 1996) and intraguild predation (Rosenheim et al. 1995), complicate the interpretation of stable isotope data. Despite the challenges that arthropod communities present, stable isotope analysis has been used to infer trophic interactions in insect communities where omnivory was common (Tillberg and Breed 2004; Mooney and Tillberg 2005).

Here, we assess community structure and species interactions in a community of parasitoids that attack an herbivorous midge on coyote bush, *Baccharis pilularis* (Asteraceae), using a combination of stable isotope analysis and published natural history information. The confined and specific feeding habits of the herbivore and the primary parasitoids associated with *Baccharis* galls should reduce isotopic mixing associated with omnivory to some degree. Nonetheless, great variation still exists in the enrichment of both carbon and nitrogen stable isotopes for trophic transfers between (a) the plant and the herbivore and (b) the herbivore and primary parasitoids in this system (Langellotto et al. 2005). Although such variation complicates the use of stable isotope data to infer food web structure, specific hypotheses can be tested regarding the degree to which isotopes should enrich with trophic transfers. First, carbon and nitrogen isotope signals of individual taxa are expected to exhibit a progressive enrichment in the heavier isotope ($^{13}C$ or $^{15}N$) from the primary producer to the herbivorous midge and from midge to the primary parasitoids. Thus, stable isotope values should exhibit "trophic level steps" from the base of the food web to higher trophic levels. Second, because primary ectoparasitoids may consume midge hosts that have been previously parasitized by endoparasitoids, they may exhibit isotope signals that are enriched, relative to stable isotope values of primary endoparasitoids. Thus, these consumers should fall farther along the "diagonal" of increasing $\delta^{13}C$ and $\delta^{15}N$ on a standard isotope biplot, such that coincidental intraguild predation among parasitoids produces isotope signals that translate into different functional groups.

Apart from the specific hypotheses outlined above, uncertainty remains regarding the use of isotope signals to infer trophic interactions in facultative hyperparasitoids. In theory, facultative hyperparasitoids should have stable isotope signals that fall on or between the signals of true secondary and tertiary consumers (e.g., an obligate hyperparasitoid). Furthermore, how the mean isotope value of a facultative hyperparasitoid falls in relation to the isotope values of secondary versus tertiary consumers can be used as a measure of the degree of hyperparasitism within the population. However, because we do not yet have an independent, controlled measure of what the facultative hyperparasitoids are consuming (as suggested in Gannes et al. 1997), we must be cautious in applying stable isotope values to infer trophic interactions. Nonetheless, in the absence of direct measures of consumption, we can still hypothesize that the stable isotope values of a facultative hyperparasitoid should fall somewhere between the isotope value of a strict primary parasitoid and the value that would be expected for an obligate hyperparasitoid (one trophic level step higher).

## Methods

### Natural history of system

Independent assessments of trophic interactions between the midge, its host plant and its natural enemies suggest that between three and four trophic levels are associated with *B. pilularis* galls (Doutt 1961; Force 1970, 1974; Ehler 1982; Hopper 1984; Ehler and Kinsey 1991; Latto and Briggs 1995; Briggs and Latto 1996, 2000, 2001) (Fig. 2). The lone herbivore in the system, the specialist midge *Rhopalomyia californica* Felt (Diptera: Cecidomyiidae), forms galls on *B. pilularis* shrubs, and is in turn subject to attack by a diverse assemblage of parasitoids. For example, the parasitoid *Platygaster californica* Ashmead (Platygasteridae) attacks the midge eggs and first-instar larvae while they are still on

the outside of the plant. Parasitized midge eggs and larvae continue to develop and produce galls along with the unparasitized midges. All of the other common parasitoid species oviposit into the chambers of already-developed galls. These include the primary larval endoparasitoid *Tetrastichus* sp. (Eulophidae) and the primary larval ectoparasitoids *Torymus koebelei* Huber (Torymidae) and *Torymus baccharidis* Huber (Torymidae). *Zatropis capitis* Burks (Pteromalidae) and *Mesopolobus* sp. (Pteromalidae) are both facultative hyperparasites that develop externally on either the midge larvae or on the larvae of the other wasp species (Doutt 1961; Force; 1970; Ehler and Kinsey 1991).

## Study sites/sampling

Galls were collected three times during 2002 from three sites in Northern California: Stebbins Cold Canyon Reserve (Solano County, CA, USA), Point Reyes National Seashore (Marin County, CA, USA), and Tomales Bay State Park (Marin County, CA, USA). For a more complete description of the study sites and sampling protocol, please refer to Langellotto et al. 2005.

## Midge and parasitoid emergence

Field-collected galls were brought back into the lab for rearing. Galls were individually placed into one dram shell vials topped with cotton stoppers and were reared on the lab bench at ambient temperature and photoperiod (approximately 23 °C and 14:10 L:D). Every 1–2 days, galls were checked for emerging adults. Adults that had emerged (both midges and parasitoids) were removed from the vials and frozen until they could be identified. The number of adult midge and wasp emergences from collected galls was used as a measure of midge and wasp abundance in the field. This method is known to underestimate actual abundance, especially for midges, which are particularly susceptible to desiccation and mortality in lab-maintained galls (Briggs and Latto 2001). However, gall dissections overestimate actual abundance, since not all insects will successfully emerge from the gall (Briggs and Latto 2001). Because several researchers have used adult midge and wasp emergences as a measure of field abundance (Force 1970 as reported by Hopper 1984; Ehler 1982), our use of emergence as a measure of abundance allowed for cross-study comparisons. If the relative abundance of the midge, primary parasitoids and facultative hyperparasitoids at our study sites is comparable to abundances reported by other researchers (e.g., Force 1970 as reported by Hopper 1984; Ehler 1982), it may be reasonable to assume that the general structure of the *Baccharis* food web at our study sites is also comparable to the trophic web constructed by other researchers (e.g., Force 1970, 1974; Hopper 1984; Ehler and Kinsey 1991) (Fig. 1).

## Stable isotope analysis

Once identified to species, midges and parasitoids were placed in a desiccating oven for at least 48 h before being processed for stable isotope analysis. Individual, whole insects (0.01–0.06 mg) or a sample of host plant gall tissue (2 mg) were separately loaded into tin capsules (8 × 5 mm, Elemental Microanalysis, Okehampton, UK). For all samples, dual isotope analysis (carbon and nitrogen) was performed on a Europa Hydra 20/20 isotope ratio mass spectrometer (PDZ Europa, Northwich, UK) at the University of California Stable Isotope Facility in Davis, CA, USA. Results for each element were expressed in delta notation (δ) as parts per thousand (‰). A total of 879 samples (plant and insect) were submitted for stable isotope analysis. However, after 51 outliers (e.g., those observations greater than 2 SD removed from the mean for each taxon at each site) were excluded, a total of 828 samples were retained for statistical analysis. We expected to observe outliers (all parasitoids), because many of our parasitoid samples were near the lower limit of total sample mass for proper isotope ratio determination by the spectrometer.

## Analysis of stable isotope data

To assess how stable isotope values ($\delta^{13}C$ and $\delta^{15}N$ together) vary according to species, study site and the

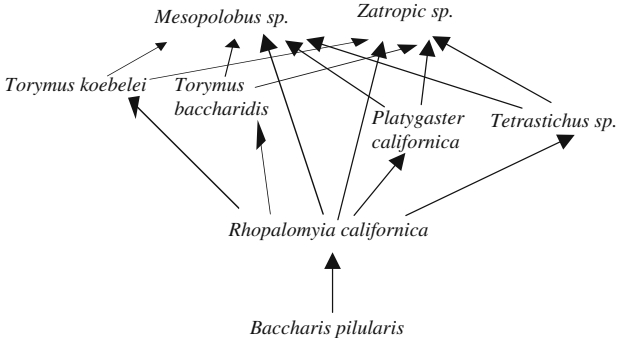


**Fig. 1** Trophic web, based on the results of previous studies (Force 1970, 1974; Hopper 1984; Ehler and Kinsey 1991). An *arrow* pointing from one species to another indicates that the former consumes or parasitizes the latter. Note that the two primary ectoparasitoids (*T. koebelei* and *T. baccharidis*) are placed somewhat higher in the food web than the two primary endoparasitoids (*P. californica* and *Tetrastichus* sp.), reflecting that these ectoparasitoids may incidentally consume the endoparasitoids if they are developing on a host that has been previously parasitized

interaction between species and study site, multivariate analysis of variance (MANOVA) was used. Assumptions of MANOVA were met for all analyses, without data transformation. Significance ($P$ value) is reported for Wilks' lambda (test of multivariate significance). Because there was a significant interactive effect of species and study site on the multivariate data (see results), we used MANOVA to further analyze stable isotope values as a function of species at each site. When there was a significant effect of species on stable isotope values, we used preplanned contrasts to compare multivariate mean vectors across all species within a single site. Because all comparisons were preplanned, we did not use a post hoc adjustment for significance in individual contrasts. Multivariate mean vectors of species' pairs were considered to be significantly different when $P \leq 0.05$. Where mean vectors differed significantly, species were assumed to be feeding on different trophic levels.

Stable isotope data are presented in biplot format ($\delta^{13}C$ versus $\delta^{15}N$). In order to make the results of pairwise multivariate mean comparisons easier to visualize, ellipses are superimposed upon each biplot. When two or more data points are enclosed by a single ellipse, the multivariate mean vectors of the enclosed group of taxa did not differ significantly from one another.

## Results

### Midge and parasitoid emergence

A general pattern in the relative abundance of midge and wasp species (measured as the percent of adult emergences) was found across our three study sites for the most common species (Table 1). At all three sites, *P. californica* was the most abundant species (range 35.1–41.5%). *T. koebelei* was the next most abundant (range 21.3–25.2%). However, the relative abundances of the less common species (e.g., *T. baccharidis*, *Testrastichus* sp., *Mesopolobus* sp., *Z. capitis* and *R. californica*) were not uniform across all three sites.

Overall, consistencies were apparent between the insect community at our sites and the communities examined by Hopper (1984), Ehler (1982) and Force (1970) (Table 1). For example, among wasp species, the dominant taxa across all sites and at all time periods were *P. californica* and the two *Torymus* species. In addition, although the rank abundance of the less common taxa differed between sites and studies, all taxa were present at all sites. The biggest difference between our study and those conducted by Ehler (1982) and Force (1970) was in the relative abundance of the herbivorous midge, which may reflect differences in habitat disturbance or habitat age among sites (Hopper 1984).

### Stable isotope analysis

Nitrogen stable isotope values, carbon stable isotope values and carbon to nitrogen ratios for each species at each site, as well as across all sites, can be found in Appendix 1. There was a significant effect of species ($F_{(14,1606)}=18.73$; $P<0.0001$), site ($F_{(4,1606)}=10.87$, $P<0.0001$) and their interaction ($F_{(28,1606)}=3.24$, $P<0.0001$) on stable isotope values of nitrogen and carbon (MANOVA). The significant site effect and site by species interaction were not due to differences in $\delta^{13}C$ and $\delta^{15}N$ values at the base of the food web; there was no significant effect of site on the stable isotope signals of *B. pilularis* galls ($F_{(4,176)}=1.67$; $P=0.16$; MANOVA). At each site, there was a significant effect of species on stable isotope values (Stebbins Cold Canyon

**Table 1** Percent of adult emergences from *B. pilularis* galls collected from different sites in Northern California. Percent of adult emergences is used as a measure of relative species abundance because it allows comparison across studies (modified from Hopper 1984)

| Source | This study | | | Hopper (1984) | Ehler (1982) | | Force (1970)[a] | |
|---|---|---|---|---|---|---|---|---|
| Location | Point Reyes | Cold Canyon | Tomales Bay | Point Reyes | Davis | Davis | Tomales Bay | Bodega Bay |
| Date | 2002 | | | 1978–1980 | 1978 | 1979 | 1967–1972 | 1967–1972 |
| Wasp species | | | | | | | | |
| *Torymus koebelei* | 22.8 | 21.3 | 25.2 | 37.4 | 35 | 33.5 | 12.8 | 6.6 |
| *Platygaster californica* | 41.5 | 38.7 | 35.1 | 18.8 | 17.8 | 23.1 | 32.6 | 31.3 |
| *Torymus baccharidis* | 11.2 | 14.7 | 11.9 | 11.4 | 1.3 | 0.5 | 16.3 | 20.8 |
| *Tetrastichus* sp. | 3.6 | 13.3 | 3.3 | 7.9 | 0.5 | 1.8 | 0.5 | 1 |
| *Mesopolobus* sp. | 8.7 | 4.4 | 21.2 | 6.7 | 0.07 | 0.06 | 5.8 | 7.9 |
| *Zatropis capitis* | 1.7 | 5.8 | 2.0 | 1.4 | 9.5 | 9.8 | 1.6 | 1.9 |
| Midge | | | | | | | | |
| *Rhopalymyia californica* | 10.4 | 1.8 | 1.3 | 12.8 | 35.8 | 31.2 | 30.3 | 30.9 |
| Number of emergences | 412 | 225 | 151 | 4528 | 7655 | 6351 | 12957 | 12064 |

[a] Emergence data from Force 1970 is as reported in Hopper 1984

$F_{(14,494)}$=18.23, $P$<0.0001; Point Reyes $F_{(14,828)}$=22.41, $P$<0.0001; Tomales Bay $F_{(14,280)}$=3.78, $P$<0.0001).

Trophic interactions across all sites combined and at each individual site were inferred via pairwise species contrasts of multivariate mean vectors (see Appendix 2 for the results of all pairwise multivariate mean vector contrasts). Across all sites (Fig. 2a), the stable isotope signals for the *B. pilularis* host plant and the herbivorous *R. californica* midge were distinct from all other taxa. Among the parasitic wasps, stable isotope signals formed four slightly overlapping groups. The first group comprised the primary endoparasitoid *P. californica* and the facultative hyperparsitoid *Mesopolobus* sp., which were not significantly different from one another ($F_{(2,803)}$=2.45, $P$=0.09). The second group included the primary endoparasitoid *Tetrastichus* sp., which was marginally different from the facultative hyperparasitoid *Mesopolobus sp*. ($F_{(2,803)}$=2.87, $P$=0.06) and quite distinctly different from the other primary endoparasitoid, *P. californica* ($F_{(2,803)}$=9.86, $P$<0.0001). That *Tetrastichus* sp. and *P. californica* are significantly different from each other is fairly striking, since we expect both to be "pure" primary parasitoids that are not predisposed to coincidental intraguild predation. The third group comprised the two primary ectoparasitoids *T. baccharidis* and *T. koebelei*, which were not significantly different from each other ($F_{(2,803)}$=1.01, $P$=0.37) but were distinct from all other taxa, with one exception. *T. baccharidis* did not significantly differ from the facultative hyperparasitoid *Mesopolobus* sp. ($F_{(2,803)}$=0.98, $P$=0.38). These two species thus comprised the fourth group. The fifth and final group included the facultative hyperparasitoid *Z. capitis*, which had a multivariate mean vector that was significantly distinct from all other taxa. Overall, our results were fairly concordant with our three a priori hypotheses, with the exception of the position of *Tetrastichus* sp. (relative to the other primary parasitoids) on the isotope biplot.

When sites were examined individually (e.g., Stebbins Cold Canyon, Point Reyes, or Tomales Bay alone), patterns in isotope enrichment were generally quite similar to the analysis of samples across sites. However, the smaller sample sizes of many of the individual taxa reduced the overall power of multivariate analyses at individual sites. Thus, some of the trophic groups identified in the analysis for all sites combined were not statistically distinguishable in the analysis of samples at individual sites. Multivariate analyses of stable isotope data from individual sites, however, did not suggest any major differences in trophic groups from the analysis of all sites combined. Specifically, results at each site were fairly concordant for both $\delta^{13}C$ and $\delta^{15}N$ values.

At Stebbins Cold Canyon (Fig. 2b), the stable isotope signal of the host plant was distinct from all gall-associated arthropods. However, using stable isotope data alone, it was difficult to distinguish the herbivorous midge from the facultative hyperparasitoid *Mesopolobus* sp. ($F_{(2,247)}$=1.60, $P$=0.20) and the primary parasitoid *P. californica* ($F_{(2,247)}$=2.01, $P$=0.14). This lack of distinction between the herbivore and two parasitic wasps was most likely due to the small sample of *R. californica* midges ($n$=4) that successfully emerged from collected galls. In addition, the stable isotope signals of individual primary parasitoids and facultative hyperparasitoids at Stebbins Cold Canyon were difficult to distinguish from one another. No parasitoid had a significantly distinct multivariate mean isotope vector at this site. Instead, there was a great degree of overlap in the stable isotope signals of parasitic wasps at this site. Nonetheless, there was a progressive increase in $\delta^{13}C$ and $\delta^{15}N$ from the base of the food web to the parasitic wasps. Furthermore, the two *Torymus* spp. fell further along the "diagonal" of increasing $\delta^{13}C$ and $\delta^{15}N$ on the isotope biplot (Fig. 2b) than the other primary parasitoids, consistent with an expectation of the coincidental intraguild predation that should be associated with their ectoparasitic habit.

A similar picture emerged from stable isotope analysis of samples from Tomales Bay (Fig. 2c). Here, the sample size of several taxa was small (e.g., *B. pilularis*, *R. californica*, *Tetrastichus* sp. and *Z. capitis*, all with $n$<10), and this likely contributed to the lack of statistical distinction for taxa at this site. Despite this amalgamation of ellipses on the stable isotope biplot for Tomales Bay, there was an apparent increase in $\delta^{13}C$ and $\delta^{15}N$ values from the primary producer, to the herbivore and to the parasitic wasps (Fig. 2c).

At Point Reyes (Fig. 2d), the stable isotope signals of the *B. pilularis* host plant and the herbivorous *R. californica* midge were distinct from all other taxa. In addition, the stable isotope signals of the primary endoparasitoids (*P. californica* and *Tetrastichus* sp.) as well as the isotope signal of the facultative hyperparasitoid *Z. capitis* were distinct from all other taxa. However, the stable isotope signals of the primary ectoparasitoids *T. baccharidis* and *T. koebelei* ($F_{(2,414)}$=2.69, $P$=0.07) and *T. baccharidis* and the facultative hyperparasitoid *Mespolobus* sp. ($F_{(2,414)}$=0.71, $P$=0.49) did not significantly differ from one another. Overall, patterns of isotope enrichment from Point Reyes were reassuringly similar to both Stebbins Cold Canyon and Tomales Bay (i.e., progressive increase in isotope signals from the primary producer to the parasitic wasps; position of the ectoparasitoids along the diagonal of the isotope biplot, relative to other wasps).

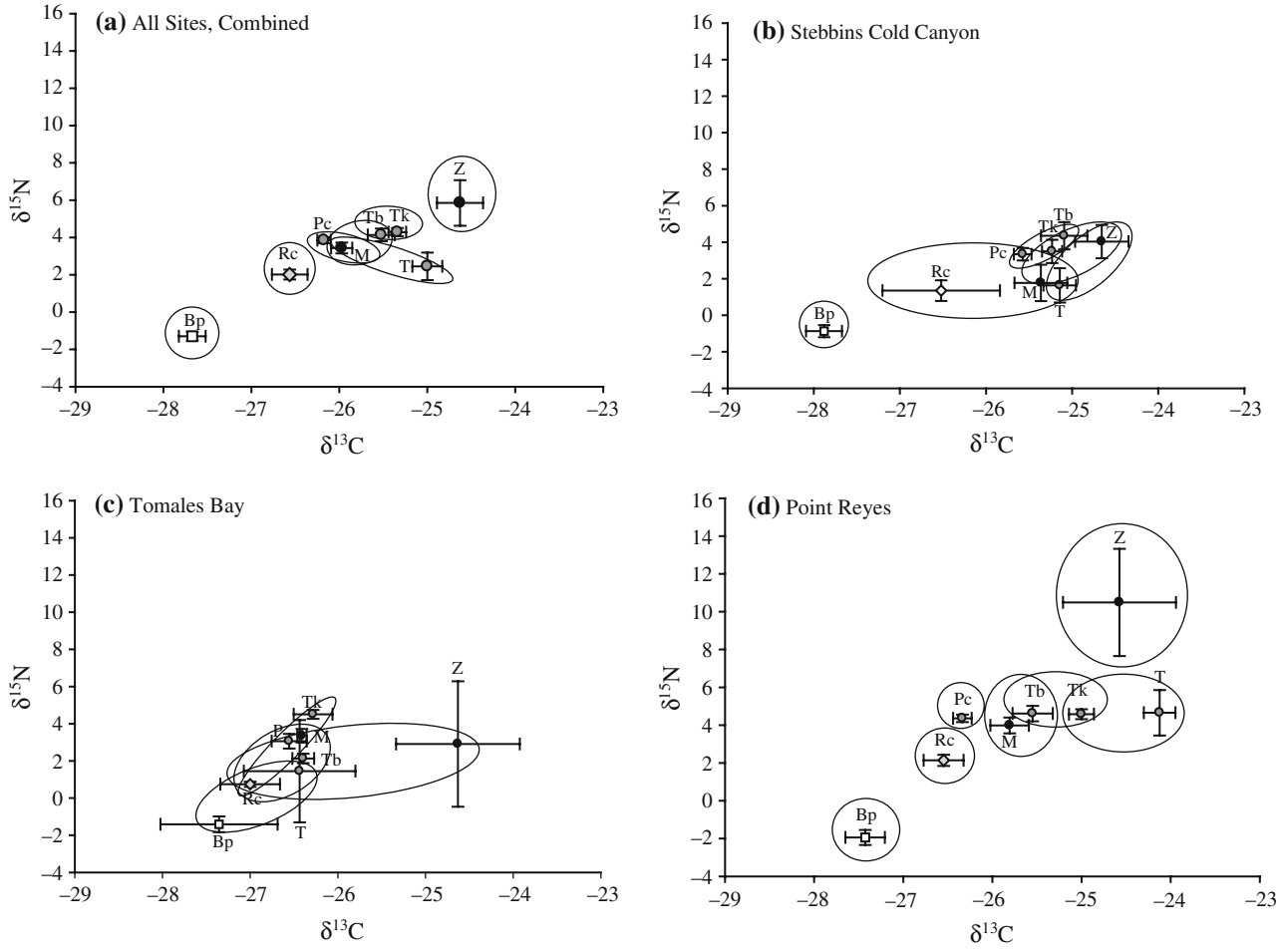


**Fig. 2a–d** $\delta^{13}C$ and $\delta^{15}N$ stable isotope biplots (mean ± 1 SE) for eight species associated with *B. pilularis* galls across all study sites combined (**a**), at Stebbins Cold Canyon (**b**), at Tomales Bay (**c**) and at Point Reyes (**d**). The producer, *B. pilularis*,is represented by the *open square* (Bp). The herbivore, *R. californica*, is represented by the diamond (Rc). Those wasps known to be primary parasitoids are represented by the *gray circles*, and include *P. californica* (Pc), *Tetrastichus sp.* (T), *T. baccharidis* (Tb) and *T. koebelei* (Tk). Those wasps known to be facultative hyperparasitoids are represented by the *black circles*, and include *Mesopolobus sp.* (M) and *Z. capitis* (Z). Where two or more data points are enclosed by a *single ellipse*, the multivariate mean vectors of the enclosed group of taxa did not differ significantly from one another ($P \leq 0.05$)

## Discussion

The goal of this study was to evaluate trophic interactions in a community of primary and facultative hyperparasitoids, across three sites in Northern California, using a combination of stable isotope analysis and published natural history information. Because consistencies were apparent between the insect community at our sites and the communities examined by Hopper (1984), Ehler (1982) and Force (1970) (Table 1), we felt that it was reasonable to compare trophic interactions inferred from our stable isotope data to the trophic interactions inferred from the observations and experiments of other researchers who have worked with the *B. pilularis* gall community (Force 1970, 1974; Ehler 1982; Hopper 1984). Although the relative strength of specific trophic linkages may differ between studies, the general structure of the food webs should be comparable.

Specifically, we tested three a priori hypotheses. First, carbon and nitrogen isotope signals of individual taxa were expected to exhibit a progressive enrichment in the heavier isotope ($^{13}C$ or $^{15}N$) up successive trophic levels. Second, the two *Torymus* spp. ectoparasitoids were expected to exhibit significantly enriched isotope signals relative to stable isotope values of the primary endoparasitoids. Third, the stable isotope values of the facultative hyperparasitoids were expected to fall somewhere between the isotope value of a strict primary parasitoid and the value that would be expected for an obligate hyperparasitoid.

The food webs we inferred from our stable isotope data generally agreed with previously published accounts of community structure for the *Baccharis* gall

system (Force 1970, 1974; Ehler 1982; Hopper 1984; Ehler and Kinsey 1991). At each individual site, there was a progressive increase in both $\delta^{13}C$ and $\delta^{15}N$ from the primary producer *B. pilularis* to the herbivore *R. californica* to the parasitic wasps (Fig. 2b–d). This result was verified statistically when data were pooled and analyzed across sites (Fig. 2a), and is consistent with the expectation that nitrogen and carbon stable isotopes should become enriched in the heavier isotope up successive trophic levels (Epstein and DeNiro 1978, 1981; Minagawa and Wada 1984).

Stable isotope data for the primary parasitoids yielded mixed support for our hypotheses. In the analysis of stable isotope data across all sites, as predicted, the two primary ectoparasitoids (*T. baccharidis and T. kobelei*) had $\delta^{13}C$ and $\delta^{15}N$ signals that were distinct from the signals of the two primary endoparasitoids (*P. californica* and *Tetrastichus* sp.) (Fig. 2a). This difference is likely due to instances of "coincidental intraguild predation" that could occur when endoparasitic larvae develop on a midge host that has previously been parasitized. Unexpectedly, the stable isotope signals of the two primary endoparasitoids were distinct from one another. Although the $\delta^{15}N$ signals of the two species were comparable, the $\delta^{13}C$ signal of *Tetrastichus* sp. was consistently enriched relative to *P. californica*. In fact, not only were the stable isotope signals of the two endoparasitoids statistically different from one another; to some extent, these two species "bracketed" the stable isotope signals of the ectoparasitic *Torymus* spp. These results could thus reflect either (a) general support for our hypotheses, with *Tetrastichus* being an outlier species for an as-yet unknown reason, or (b) no support for our hypothesis regarding expected isotope enrichment of ecto- versus endoparasitoids, with idiosyncratic and unexplainable variation among the primaries, regardless of their feeding habits.

For the facultative hyperparasitoids, multivariate analysis of stable isotope signals yielded novel results that suggest unique trophic interactions. Isotope data for *Mesopolobus* sp., a facultative hyperparasitoid that exhibits a preference for primary parasitoids over the herbivore *R. californica* in the lab (Hopper 1981), suggest that this species largely operates as a primary parasitoid in the field. Across all sites and at each individual site, the stable isotope signals for *Mesopolobus* sp. were more similar to the isotope signals of the four primary parasitoids than they are to the facultative hyperparasitoid *Z. capitis* (Fig. 2). However, this result must be interpreted with caution; our suggestion that *Mesopolobus* sp. largely behaves as a primary parasitoid in the field is best viewed as a testable hypothesis rather than a definitive conclusion. Ultimately, the isotope signal of *Mesopolobus* sp. may reflect an unexpected trophic role (operative primary parasitoid rather than facultative hyperparasitoid), or may simply reflect a less-than-expected isotope enrichment pattern.

Stable isotope data for *Z. capitis* suggest (1) that, averaged across the three sites, this species occupies a trophic level distinctly higher than the primary parasitoids, and (2) that this species may vary in the extent to which it acts as a hyperparasitoid among sites. At Stebbins Cold Canyon, the isotope signal of *Z. capitis* is similar to (yet still slightly higher than) those of the other parasitic wasps. It is thus possible that *Z. capitis* may forage largely as a primary parasitoid at this site. However, at Point Reyes, the isotope signal of *Z. capitis* is distinct from the other wasps. Could *Z. capitis* be acting predominantly as a hyperparasitoid at this site? Once again, the possibility that *Z. capitis* exhibits different life history strategies across sites (as inferred from stable isotope data) should be viewed as a testable hypothesis rather than a definitive conclusion.

To date, no study has quantified the dynamics of host use by facultative hyperparasitoids (Brodeur 2000), and there is only limited knowledge of hyperparasitoid life history strategies and foraging behavior (Sullivan and Völkl 1999). This is in part because hyperparasitism is notoriously difficult to study in the field. However, stable isotope analysis allowed us to identify possible alternative life history strategies and resource use by two species of facultative hyperparasitoids in the field. Our hypotheses regarding the functional roles of *Mesopolobus* sp. and *Z. capitis* in the *Baccharis* food web can be tested via the dissection of field-collected galls and the direct observation of larval feeding habits (as in Ehler 1982).

Why might *Mesopolobus* sp. behave as a primary parasitoid across all sites? Variation in host abundance and host quality could modulate host selection in the field (Brodeur 2000). In terms of host abundance, the number of midges that emerged from galls across all study sites was low ($n$=49) relative to the cumulative number of emerged primary parasitoids ($n$=739). Thus, it is not likely that *Mesopolobus* sp. parasitizes *R. californica* over other species due to the relative abundance of the herbivore in the field. Alternatively, the extremely low percentage of midges that emerged from galls may be a consequence of high parasitism rates by *Mesopolobus* sp. However, this possibility is not likely. *R. californica* eggs are subject to parasitism by *P. californica* earlier than midge larvae are subject to parasitism by larval parasitoids such as *Mesopolobus* sp. (Briggs and Latto1996). The egg parasite *P. californica* was the most abundant insect that emerged from galls

collected at our study sites ($n$=311). Thus, it is likely that the abundance of midge larvae available for oviposition by *Mesopolobus* sp. was extremely low and that the primary parasitoid life history habit of *Mesopolobus* sp. occurred despite the low abundance of midge hosts in the field.

The benefit of parasitizing an herbivore could outweigh the benefit of developing in a parasitoid host (Brodeur 2000) if the herbivore represents a higher quality diet. Indeed, herbivorous midges may represent a higher quality diet than parasitic wasps for the facultative hyperparasitoid *Mesopolobus* sp., as *R. californica* is generally larger and thus may provide more food for developing parasitoid larvae. However, *Mesopolobus* sp. does not appear to discriminate among species based on diet quality in the lab; Hopper (1981) demonstrated that when *Mesopolobus* sp. is given a choice, the midge is among the least preferred of five possible hosts. A third possibility is that *Mesopolobus* sp. is behaving as a hyperparasitoid in the field, yet the stable isotope signal of this species does not reflect that fact. However, that stable isotope data agreed with independent assessments of food web structure for all taxa but *Mesopolobus* sp. suggests that this third possibility is not the most parsimonious explanation for our results.

That trophic interactions inferred from stable isotope data generally agreed with interactions inferred from more traditional methods (e.g., Force 1970, Hopper 1984, Ehler and Kinsey 1991) is encouraging, particularly for ecologists studying arthropod communities. However, for most terrestrial arthropod communities, such detailed a priori analyses of food web interactions are lacking. Thus, in order to maximize the contribution of stable isotope data to studies of arthropod community structure, it is important that independent assessments of key trophic interactions be conducted (Gannes et al. 1997).

In particular, independent assessments of trophic interactions can counteract the possibility that idiosyncratic variation in stable isotope values among species obfuscates underlying differences in trophic position. For example, the degree to which relatively sessile, specialist herbivores are enriched in $\delta^{13}C$ and/or $\delta^{15}N$ relative to their host plants is extremely variable (Spence and Rosenheim 2005), despite the fact that these organisms are often considered as a single functional group. In fact, variation in enrichment within this single trophic level was similar to enrichment values that might be expected to occur across two or three trophic levels (Spence and Rosenheim 2005). Thus, although our study system had the distinct advantage of being dominated by relatively sessile specialists (most of whom had feeding habits that were well-characterized), our results revealed a fair degree of variation in the isotope signals of parasitoids feeding at the same trophic level. Nonetheless, when data was pooled across sites, multivariate analysis of stable isotope data validated many trophic links constructed via observation or experimentation and provided unexpected leads, in the form of testable hypotheses, regarding trophic interactions involving the same species complex.

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## Appendix 1

Sample sizes ($n$), nitrogen stable isotope ratios ($\delta^{15}N$), carbon stable isotope ratios ($\delta^{13}C$) and carbon to nitrogen ratios (C:N) of whole insects and the *B pilularis* galls from which they emerged. Galls were collected from Stebbins Cold Canyon, Tomales Bay, and Point Reyes, CA, USA.

## Appendix 2

$F$ statistics and $P$ values for pairwise multivariate mean vector contrasts for all possible species pairs associated with the *B. pilularlis* gall community analyzed (1) across all sites combined, (2) at Stebbins Cold Canyon, (3) at Point Reyes and (4) at Tomales Bay, CA, USA.

## References

Briggs CJ, Latto J (1996) The window of vulnerability and its effect on relative parasitoid abundance. Ecol Entomol 21:128–140

Briggs CJ, Latto J (2000) The effect of dispersal on the population dynamics of a gall-forming midge and its parasitoids. J Anim Ecol 69:96–105

Briggs CJ, Latto J (2001) Interactions between the egg and larval parasitoids of a gall-forming midge and their impact on the host. Ecol Entomol 26:109–116

Brodeur J (2000) Host specificity and trophic relationships of hyperparasitoids. In: Hochberg ME, Ives AR (eds) Parasitoid population biology. Princeton University Press, Princeton, NJ, pp 163–183

Coll M, Guershon M (2002) Omnivory in terrestrial arthropods: mixing plant and prey diets. Annu Rev Entomol 47:267–297

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Doutt RL (1961) The dimensions of endemism. Ann Entomol Soc Am 54:46–53

Ehler LE (1982) Ecology of *Rhopalomyia californica* Felt and its parasites in an urban environment. Hilgardia 50:1–32

Ehler LE, Kinsey MG (1991) Ecological recovery of a gall midge and its parasite guild following disturbance. Environ Entomol 20:1295–1300

Force DC (1970) Competition among four hymenopterous parasites of an endemic host. Ann Entomol Soc Am 63:1675–1688

Force DC (1974) Ecology of insect host-parasitoid communities. Science 184:624–632

Gannes LZ, O'Brien DM, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271–1276

Hopper KR (1981) Host-finding and hyperparasitism in a guild of parasitic wasps. Dissertation. University of California, Santa Barbara, CA

Hopper KR (1984) The effects of host-finding and colonization rates on abundances of parasitoids of a gall midge. Ecology 65:20–27

Langellotto GA, Rosenheim JA, Williams MR (2005) Enhanced carbon enrichment in parasitoids (Hymenoptera): a stable isotope study. Ann Entomol Soc Am 98:205–213

Latto J, Briggs CJ (1995) Factors affecting distribution of the gall forming midge *Rhopalomyia californica* (Diptera: Cecidomyiidae). Environ Entomol 24:679–686

Minagawa M, Wada E (1984) Stepwise enrichment of $^{15}N$ along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48:1135–1140

Mooney KA, Tillberg CV (2005) Temporal and spatial variation to ant omnivory in pine forests. Ecology 86:1225–1235

Rosenheim JA, Kaya HK, Ehler LE, Marois JJ, Jaffee BA (1995) Intraguild predation among biological control agents: theory and evidence. Biol Cont 5:303–335

Spence KO, Rosenheim JA (2005) Isotopic enrichment in herbivorous insects: a comparative field-based study of variation. Oecologia 146:89–97

Sullivan DJ, Völkl W (1999) Hyperparasitism: multitrophic ecology and behavior. Annu Rev Entomol 44:291–315

Tillberg CV, Breed MD (2004) Placing an omnivore in a complex food web: dietary contributions to adult biomass of an ant. Biotropica 36:266–271

Wagner JD, Wise DH (1996) Cannibalism regulates densities of young wolf spiders: evidence from field and laboratory experiments. Ecology 77:639–652