Use of stable isotopes to assess the intraspecific foraging niche of males and female colour morphs of the damselfly *Enallagma hageni*

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Abstract. 1. For the first time, diet and isotopic niche overlap among males and two female-specific colour morphs of a damselfly were quantified to test whether sexual conflict could cause intra-sexual diet variation.

2. Relative to the green female morph, blue females, similar in coloration to the blue males, may be more likely overlooked by mate-searching males. If so, blue females should be more likely to forage unmolested at lake shores, where the density of males and prey is high. The blue morph's isotopic niche space should therefore differ from that of green females and be more similar to that of males.

3. Stable isotope analyses of two Michigan populations failed to support these predictions. Despite population differences in $\delta^{13}C$ and diet, within sites isotopic niches overlapped among males and females, with little difference between the two female colour morphs.

4. Males exhibited the broadest isotopic niche area, which increased across years, whereas that of blue females became more restricted, possibly due to greater sexual harassment in forest feeding sites.

5. There was an unexpectedly high shift in $\delta^{13}C$ from prey to predator. Future work is merited to determine whether such trophic shifts are characteristic of Odonata, a group of important aquatic and terrestrial predators.

Key words. Colour polymorphism, diet breadth, Odonata, sexual conflict, trophic shift.

Introduction

Intraspecific variation in foraging niches, known particularly from vertebrates, may result from intraspecific competition for food among adults (Johnson, 1986; Ehlinger & Wilson, 1988; Schluter, 1993; Alanärä *et al.*, 2001) or between juveniles and adults (Marrin, 1983; Chizinski *et al.*, 2007). Additionally, intraspecific niche partitioning can evolve as one consequence of sexual dimorphism in body size (Shine *et al.*, 2002; Lewis *et al.*, 2006) or other morphologies (Smith, 1990). The result of such niche partitioning is usually variation in dietary overlap within a species.

Although dietary overlap has historically been calculated by direct methods such as analysis of gut contents, increasingly, analysis of stable isotopes of carbon and nitrogen are used to determine diet preferences and/or habitat use (Fry *et al.*, 1978; Herrera *et al.*, 2008; Layman *et al.*, 2012). A major advantage of isotope analysis is that it provides a direct measure of food assimilated as opposed to food only recently ingested. An organism's niche space can be represented using stable isotope ratios as axes because energy flow is closely linked to carbon isotope ratios and trophic position to nitrogen isotope ratios (Bolnick *et al.*, 2003; Bearhop *et al.*, 2004). The $\delta^{13}C$ ratios reflect the basal resource in food webs and are thought to be fairly constant across trophic levels. On the other hand, $\delta^{15}N$ ratios indicate trophic position and are expected to be more enriched in the heavier isotope in predators, relative to their prey (Deniro & Epstein, 1981; McCutchan *et al.*, 2003). The resulting δ-space should be strongly correlated with the ecological niche space described by Hutchinson (1978) because both carbon and nitrogen ratios are conditional upon

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local environmental conditions (Newsome *et al.*, 2007). Using intra-population variation in isotopic signatures, ecologists have begun to re-examine individual specialisation, population dynamics and niche breadth (Bolnick *et al.*, 2003; Bearhop *et al.*, 2004; Kelly *et al.*, 2004; Matthews & Mazumder, 2004; Layman *et al.*, 2012).

Here, we use stable isotopes to estimate diet and trophic niche space to test, for the first time, the possibility of intraspecific partitioning of the foraging niches among male damselflies and their colour-polymorphic females. Like many insects, damselflies are typically sexually dimorphic in size; males are often slightly smaller than females (Westfall & May, 1996). Our primary interest here, however, lies not in the effects that sexual dimorphism may have on diet *per se*, but rather in determining possible dietary consequences of the female-specific colour polymorphism, which occurs in over 100 species of Holarctic odonates (reviewed by Fincke *et al.*, 2005). Females of our study species, *Enallagma hageni* Walsh, exhibit two distinct colour morphs: a blue morph that is similar to the blue male in coloration and a green morph whose colour reflectance differs from that of the male (Fincke *et al.*, 2007). Female body coloration and pattern are known to be a Mendelian trait in *Ischnura*, the sister genus to *Enallagma*, as well as in *Coenagrion* (reviewed by Van Gossum *et al.*, 2008). We thus assume that similar genetics control the polymorphism in *Enallagma*.

Female-specific colour polymorphism is a common characteristic of damselflies in the family Coenagrionidae, whose non-territorial males must search for mates, often in multiple microhabitats, such as forests, fields and lake shores, where feeding also occurs. For damselfly species with mate-searching males, female colour variation has long been suspected to result in part from sexual conflict over mating rates. Specifically, the polymorphism has been considered an evolutionary response by females to reduce male harassment, although specific hypotheses differ in details (reviewed by Fincke, 2004; Van Gossum *et al.*, 2008). Of interest here is the fact that, because of their signal similarity to the blue males, blue females, typically viewed as sexual mimics, may frequent areas occupied by males without being as readily detected by males as potential mates, and hence enjoy lower harassment from males compared with the green females (Robertson, 1985; Sherratt, 2001). For example, Forbes *et al.* (1995) found that, at high-density sites, male-like female *Nehalennia* were more likely than green females to frequent lake shore areas. At the edges of these aquatic habitats, emerging insect prey is abundant, and males are often seen foraging there. Hence, if blue females indeed frequent a different feeding microhabitat from that of green females and, more specifically, forage in areas frequented by males, one would predict, respectively, that (i) the diets of blue and green females will differ, and (ii) relative to the trophic niche of green females, that of blue females should be more similar to that of males. We tested these hypotheses about adult diets in two different populations of *Enallagma hageni* in northern Michigan. Our results failed to support either hypothesis, but revealed a more complex picture of diet breadth than we expected.

Materials and methods

Study species and populations

Like all odonates (Corbet, 1999), our study species, *E. hageni* is a predator as both an aquatic larva and as an adult. Newly emerged 'teneral' adults are opaque in colour and typically take a day for the cuticle to harden, after which they begin feeding on small flying insects. Thus, the isotopic signature of newly emerged adults reflects their diet as aquatic larvae. During the week or so needed to reach sexual maturity, adults rapidly increase in body mass (Anholt *et al.*, 1991). *Enallagma* males are often observed feeding in the grassy areas along lake shores, where the density of males searching for mates and hence the operational sex ratio is high (Fincke, 1982, 1994). Between bouts of mating and oviposition at the water's edge, females and most males at our study sites returned to the forest where both sexes were observed feeding in forest light gaps (Fincke, 1986a; O. M. Fincke, unpublished).

Our study was conducted at two sites in northern Michigan during the summers of 2010, 2011 and 2012. Populations at both sites produced only one generation per year, with adults flying from mid-June to early August. The Sugar Island (SI) population in the eastern Upper Peninsula of Michigan was located in the Chase Osborn Preserve (46.4°N, 84.2°W) in Chippewa County. Here, adult *E. hageni* bred on Duck Lake and the contiguous Sweet Gale Lake, both of which are connected to the St Marys River. Based on water samples taken during our study, these two water bodies were only slightly alkaline (41–44 mg CaCO$_3$ l^{-1}) but had a relatively high concentration of dissolved organic carbon (DOC, 11–15 mg C l^{-1}). The macrophyte community consisted of *Najas flexilis*, *Vallisneria* sp. and *Elodea* sp. Emergent vegetation was dominated by *Carex stricta*, *Scripus acutus* and *Dulichium arundinaceum*. Close to shore was a shrubby zone of blueberry (*Vaccinium* sp.) and *Myica gale*. Most of the terrestrial habitat was conifer and mixed hardwood forest that was roughly 10–20 m from the water's edge.

The second study population bred on Larks Lake (LL, 45.6°N, 84.9°W) located in Emmet County, Michigan, roughly 143 km south of the SI population. Larks is a shallow alkaline lake (114–116 mg CaCO$_3$ l^{-1} with a DOC of 7–8 mg C l^{-1} at the time of our study). The substrate consisted of a sandy bottom, which supports a limited array of aquatic vegetation. Typical submerged vegetation included *Najas flexilis*, *Chara* sp. and the filamentous green alga *Spirogyra* sp. Emergent vegetation included *Scirpus americanus* and *S. acutus*. The shoreline consisted of a shrubby zone which graded into a mixed conifer-hardwood forest, about 5–10 m from the waterline.

Collection of damselflies

To standardise the reproductive condition of sexually mature adults, tandem pairs (i.e. females clasped by males prior to copula) were collected along the water's edge using an insect net. Two sample sets were taken from each site during each study year, one early in the reproductive season and another about 2 weeks later (total $N = 267$ sexually mature adults).

To calculate the approximate time needed for damselfly tissue to equilibrate with its diet as an adult, females from the LL population were sorted by age group. We used the presence of ectoparasitic water mites and the scars they leave as evidence of the relative age of females (i.e. younger and older damselflies, respectively). The mites attach to the venter of a damselfly's abdomen during its emergence from the final-instar larva (Smith, 1988), which occurs on emergent vegetation. The mites feed on their host and then detach from the damselfly when it returns to the water to breed and females submerge to lay eggs (Fincke, 1986b; Rolff & Martens, 1997). After detachment, a scar remains on the damselfly's abdomen, evidence that the adult has had one or more bouts of breeding. Additionally, because the wax that adults secrete on their bodies accumulates over time, we scored a female as relatively old if she had a very waxy (i.e. whitish) abdomen or exhibited extreme wing wear. We grouped individuals into four categories of relative age as follows: 0, tenerals; 1, young mature (i.e. with mites; > 5 and < 10 days old); 2, older mature adults (i.e. with scars of mites, 1.5–3 weeks old); and 3, old adults (with whitish abdomens, ≥ 3 weeks old).

Availability of potential prey

We assessed the relative abundance and microhabitat distribution of suitably small, flying prey at both study sites by placing sticky fly paper strips in the field in mid-season. Sticky traps were placed along the shore within 10 m of the water's edge, and at different small light gaps in the forest where damselflies were observed to feed. Traps were left in the field for 24 h of clear weather and then returned to the laboratory where trapped insects were identified to family and their relative distribution determined.

Isotope analyses of damselfly predators and their potential prey

Live adult *E. hageni* were held in small glassine envelopes under cool conditions for transport to the laboratory where they were then frozen at −80 °C until analysed. Samples were dried in a lyophiliser until reaching a constant weight. After drying they were kept in a tightly closed container and stored in a freezer until further processing. Specimens did not appear to discolour during storage. Only the thorax, cut into quarters, was used for isotopic measurement, except in 2010 when the abdomen was also used. Lipids were not removed prior to isotopic analysis because the molar C : N ratio for all adults was consistently low, ranging from 4.36 to 5.71, suggesting a minimal effect of fat on our results. To test whether inorganic carbonates might be affecting the organic carbon signal, a subset of damselfly adults was treated with dilute HCl (1 M) overnight and then redried. The carbon signal before and after acid treatment showed no statistical difference (data available upon request).

For carbon and nitrogen analyses, approximately 0.4 mg of sample was weighed into a tin capsule (5 × 9 mm) and placed in a Costech 4010 Elemental Analyzer (Costech Analytical

Technologies Inc., Valencia, California), which was coupled to a Thermo Finnigan Delta Plus XP mass spectrometer (Thermo Electron Corp., San Jose, California). During each run, standards were placed after every 10 samples to correct for instrumental drift. All results are expressed using the δ notation, with units expressed in per mil fashion. The reference materials were secondary standards previously calibrated against international standards. Carbon results are reported relative to Vienna PeeDee Belemnite (VPDB) and nitrogen relative to atmospheric N_2. In addition to standards, a control sample (caffeine) was analysed with every run throughout the study period and used to estimate instrumental precision. Nitrogen control values had a precision of ± 0.2‰, while carbon was ± 0.1‰. Because our measures were at odds with some in the literature (see the Discussion section), we had another laboratory measure 13 of our individuals. Those results were no different from ours, and thus were included in the analyses.

Suitable damselfly prey were collected with insect nets from each site, identified to family, then dried, ground and analysed for $\delta^{15}N$ and $\delta^{13}C$ as described earlier. To estimate variability of isotopic signatures of prey, individual prey items were analysed whenever possible; otherwise they were pooled to achieve sufficient mass for analysis. Although several types of chironomid species were analysed, we failed to sample *Chironomus anthracinus* and *Chironomus plumosus*, both of which are known from the study sites. These species of chironomid typically inhabit deeper waters and consume methanotrophic bacteria, resulting in very depleted ^{13}C isotope signals. We included them as possible prey items, using the average carbon and nitrogen values obtained from the literature (Hecky & Hesslein, 1995; Grey *et al.*, 2004; Kelly *et al.*, 2004). The prey data were used to construct bi-plots of isotopic signatures of damselfly predators and their potential prey.

Analysis of trophic enrichment and foraging niche space

Before one can assess the diet of a predator, it is necessary to determine the trophic shift (i.e enrichment) that occurs as carbon and nitrogen move up the food chain. Trophic enrichment Δ is defined as $\delta X_{consumer} - \delta X_{diet}$, where X represents either the carbon or nitrogen isotopic signal. The variance $v(\Delta)$ can be calculated from the equation: $v(\Delta) = (n^f - 1) \ (s^f)^2 + (n^c - 1)(s^c)^2/(n^f + n^c - 2)$, where n^f and n^c are sample sizes of the food and consumer, and $(s^f)^2$ and $(s^c)^2$ are the variances for the food and consumer, respectively (Hedges & Olkin, 1985). Trophic enrichment was determined using 25 SI females that had been collected as tenerals and held in a flight cage until sexual maturation (5–8 days). These were fed *ad libitum* on a diet of mosquitoes (*Culex* sp.) collected on-site. The females were subsequently harvested and their thoraxes as well as a sample of 24 mosquitoes were analysed for $\delta^{13}C$ and $\delta^{15}N$ as described earlier.

In order to reconstruct the diet of adult damselflies, we corrected the isotopic signal of the adults for trophic enrichment by subtracting the trophic enrichment factor calculated earlier. The resource proportions in the diet of *E.*

Table 1. Mean ± SD of isotope results for *Enallagma hageni* adults on Sugar Island (SI) and Larks Lake (LL), 2010–2012 (ranges in parentheses).

Site	Year	Group	N	δ^{13}C (‰)	δ^{15}N (‰)
SI	2010	M	24	−25.38 ± 1.70 (−29.18 to −21.36)	2.3 ± 0.4 (1.7–3.0)
SI	2010	BF	12	−24.68 ± 1.29 (−26.72 to −22.77)	2.1 ± 0.4 (1.2–3.0)
SI	2010	GF	11	−24.93 ± 0.64 (−25.84 to −23.70)	2.9 ± 0.7 (2.0–4.2)
SI	2011	Teneral	17	−27.63 ± 0.62 (−29.05 to −26.32)	3.0 ± 0.3 (2.6–3.8)
SI	2011	M	37	−27.01 ± 2.06 (−30.23 to −23.20)	3.7 ± 0.6 (2.3–5.1)
SI	2011	BF	27	−27.59 ± 1.30 (−29.46 to −24.32)	3.8 ± 0.5 (2.9–4.9)
SI	2011	GF	25	−26.97 ± 1.90 (−29.39 to −21.92)	3.6 ± 0.7 (2.3–4.8)
SI	2012	M	11	−26.77 ± 2.18 (−29.05 to −22.24)	3.7 ± 0.8 (2.3–5.7)
SI	2012	BF	10	−24.89 ± 0.87 (−26.75 to −24.14)	3.7 ± 0.3 (3.3–4.2)
SI	2012	GF	11	−24.34 ± 1.57 (−26.45 to −20.50)	4.0 ± 0.5 (3.1–5.2)
LL	2011	Teneral	4	−21.57 ± 1.1 (−22.80 to −20.57)	3.2 ± 0.5 (2.7–3.8)
LL	2011	M	33	−23.45 ± 0.80 (−25.22 to −22.21)	4.3 ± 0.7 (3.3–5.8)
LL	2011	BF	26	−24.38 ± 0.95 (−26.17 to −22.67)	4.2 ± 0.5 (3.1–5.5)
LL	2011	GF	40	−24.02 ± 0.96 (−26.47 to −21.84)	4.0 ± 0.7 (2.4–5.4)

M, male; BF, blue female; GF, green female.

hageni adults were calculated using ISOSOURCE (Philips & Gregg, 2003), a computer program available online which calculates likely mixing proportions satisfying the isotopic mass balance equation of the predator, as follows:

$$\delta X_{predator} = (f_a)(\delta X_a) + (f_b)(\delta X_b) + (f_n)(\delta X_n)$$

where X is the carbon or nitrogen isotopic signal of the predator, f is the proportion of resource contributing to mix, and X_n is the isotopic signal of resource.

Because the number of possible prey contributing to the mix exceeded $n + 1$ isotopes measured, an exact solution to the mixing problem was not possible; instead ISOSOURCE returned a range of solutions satisfying the mass balance equation. In order to better constrain the model (reduce the number of possible prey classes), we averaged the isotopic signals of trichoptera and emphemeroptera (aquatic resource) and homoptera (plant hoppers), lepidoptera and coleoptera (terrestrial resource). We accepted solutions to the mixing problem that were ≤ 0.02‰ different from the average predator signal.

Additionally, we calculated the trophic niche area for males and each female colour morph for the SI population for three consecutive breeding seasons (2010–2012), using Bayesian inference techniques (after Jackson *et al.*, 2011). The total area of the resulting ellipses, an indirect measure of trophic niche space, is based on δ^{15}N of the individuals in each of the three groups (i.e. males plus the two female colour morphs), corrected for sample size. The areas were bootstrapped ($n = 10\,000$) to account for natural variations in the data set. The metrics were calculated using the download package SIBER (Stable Isotope Bayesian Ellipses in R) which is part of the Stable Isotope Analysis for the R statistical environment. The corrected ellipse area was thus less sensitive to sample size, and provides confidence intervals around the means, in lieu of comparator statistics, which remain a challenge. Bayesian inference has the added advantage of generating a range of probable values for the calculated areas, whose confidence intervals can be calculated, an improvement on a metric describing niche width developed by Layman *et al.* (2007).

Statistical analyses

Because diets differed between the two populations, analyses were done by population. A few of the isotopic data sets were not normally distributed in some years, even after log transformation. Hence non-parametric Kruskl–Wallis tests (NPAR1WAY in SAS, version 9.2) were used to analyse the isotopic data. Analysing the normally distributed isotopic data with parametric tests did not change any of our conclusions. General linear models (GLM, SAS) were used for all other data (i.e. determined to be normally distributed using Shapiro–Wilk tests). Throughout, means are given ± SD.

Results

Isotopic signatures of E. hageni *predators*

Table 1 summarizes isotope data from the two study populations of *E. hageni*. There was no difference between male and female tenerals in δ^{13}C ($H = 1.03$, 1 d.f., $P = 0.31$) or δ^{15}N ($H = 0.00$, 1 d.f., $P = 1.0$). Tenerals from SI were more depleted in δ^{13}C relative to those at LL ($H = 9.27$, 1 d.f., $P < 0.002$), although there was no difference in δ^{15}N ($H = 0.08$, 1 d.f., $P = 0.93$). There was no difference between the δ^{13}C signal of tenerals and adults at the SI site ($H = 0.48$, 1 d.f., $P = 0.83$), but at LL, tenerals were more enriched than the adults ($H = 10.08$, 1 d.f., $P < 0.001$). At both sites, tenerals were depleted in δ^{15}N relative to adults ($H = 20.82$, 1 d.f., $P < 0.001$ and $H = 6.75$, 1 d.f., $P = 0.01$ for SI and LL, respectively). As shown in Fig. 1, newly emerged teneral *E. hageni* reached equilibrium with their diet as terrestrial adults within a week or less of emergence.

Among sexually mature adults on SI, there were year-to-year differences in δ^{13}C ($H = 42.30$, 2 d.f., $P < 0.0001$) and δ^{15}N ($H = 80.80$, 2 d.f., $P < 0.0001$). Thus, only 2011 data
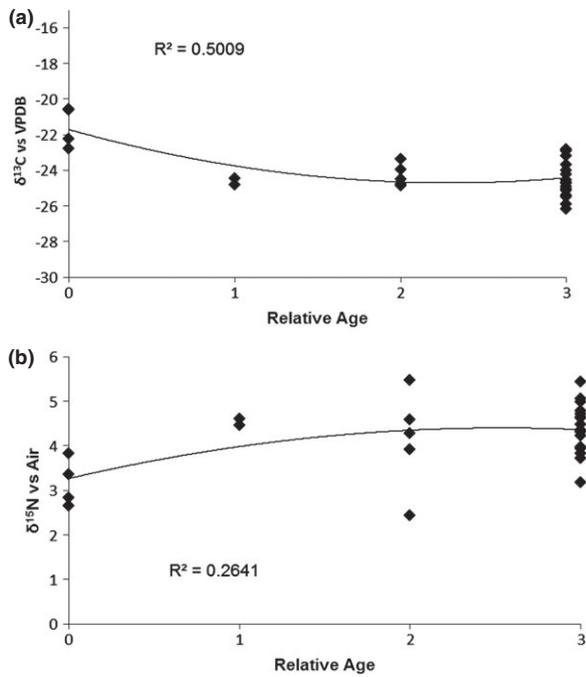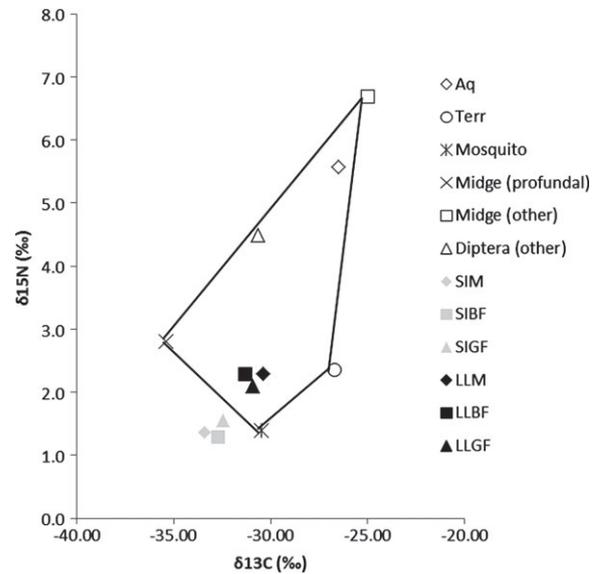
Fig. 1. Isotopic shift with relative age of Larks Lake females: (a) δ^{13}C; (b) δ^{15}N. Age class $= 0$ refers to newly emerged tenerals, which should reflect the larval diet. See text for description of relative ages.

Fig. 2. Bi-plot of predator types from Larks Lake (LL) and Sugar Island (SI) and the prey mixing model. M, male; BF, blue female; GF, green female. Sample sizes for damselflies are in Table 1; for prey, see Table 3.

were used for comparison of the two study sites. Compared with LL, adults on SI were more depleted in δ^{13}C ($H = 93.86$, 1 d.f., $P < 0.0001$) and δ^{15}N ($H = 23.01$, 1 df, $P < 0.0001$). In the SI population pooled across years, there was no difference in the isotopic signatures between the two female colour morphs in δ^{13}C ($H = 2.15$, 1 d.f., $P = 0.34$) or δ^{15}N ($H = 2.95$, 1 d.f., $P = 0.23$), or between males and females (for δ^{13}C: $H = 1.24$, 1 d.f., $P = 0.26$; for δ^{15}N: $H = 2.54$, 1 d.f., $P = 0.11$). Similarly, in the LL population, neither δ^{13}C ($H = 2.03$, 1 d.f., $P = 0.15$) nor δ^{15}N ($H = 3.63$, 1 d.f., $P = 0.06$) differed between the female morphs. However, compared with males, females were more depleted in δ^{13}C ($H = 12.54$, 1 d.f., $P = 0.0004$), whereas the sexes did not differ in δ^{15}N ($H = 0.86$, 1 d.f., $P = 0.35$).

Availability of potential prey

The data on the small prey caught in the sticky traps are summarized in Table 2. There was no difference in total number of suitable small prey items between the two study sites ($F_{1,5} = 1.26$, $P = 0.36$), or between the shore and the forest locations ($F_{1,5} = 0.04$, $P = 0.84$), and no interaction effects. At both sites small diptera, particularly midges and phorid flies, were the most abundant prey. LL had more leafhoppers and other hompterans than did the SI site ($F_{1,5} = 8.67$, $P = 0.03$). Finally, at both sites, more Tricoptera ($F_{1,5} = 6.98$, $P = 0.045$) and Lepidoptera ($F_{1,5} = 6.64$, $P = 0.05$) occurred near the shore than in the forest.

Isotopic niche space of predators and their potential prey

The mean enrichment of δ^{13}C from prey to predator was 6.9 ± 1.3‰, the difference between the mean δ^{13}C of 25 damselflies (-23.58 ± 1.9) and that of the mosquito prey on which they were raised (-30.50 ± 1.6, $n = 24$). The mean δ^{15}N enrichment value was 1.9 ± 1.1‰, the difference between the mean δ^{15}N of the 25 damselflies (3.3 ± 0.72) and that of the 24 mosquito prey (1.4 ± 1.1). Figure 2 shows the bi-plot of predator types from both populations, corrected for the above trophic shift, and the prey mixing model. We were unable to model the diet from the SI site because the predator signal fell outside the mixing polygon, indicating that a possible prey item was missing from our data set (Table 3). It should be noted however, that the predator signal fell very close to the edge of the mosquito-profundal midge contour, suggesting that these items probably played a significant role in the diet of the SI population. The LL population fed heavily on mosquitoes (50–60%) followed by midges (profundal) (15–30%) and terrestrial insects (8–12%) along with lesser amounts of other items (Table 4).

A summary of the average ellipse area for each colour morph is shown in Fig. 3. Whereas the ellipse areas of the male and female colour morphs in 2010 did not vary greatly in either population, by 2012 blue females in the SI population appeared to utilise a smaller niche area, while green females and males, in particular, enlarged their niche space. Figure 4 shows the density plots of the confidence intervals of the standard ellipse areas; the niche space of males expanded, whereas that of the blue females contracted over the 3-year study of the SI population.

Table 2. Mean ± SD of small prey caught in sticky traps on shore and in forest light gaps at each study site (*n* = 2 traps per location per site; eight in total).

Location/site	Total	Diptera	Tricoptera	Lepidoptera	Homoptera
Larks Lake					
Shore	97.0 ± 31.11	81.50 ± 29.00	5.5 ± 3.54	4.0 ± 1.41	1.0 ± 0
Forest	83.5 ± 13.43	70.00 ± 14.12	2.00 ± 1.41	1.50 ± 0.70	1.0 ± 0
Sugar Island					
Shore	50.0 ± 8.49	36.00 ± 8.49	7.00 ± 2.82	1.50 ± 0.70	1.50 ± 0.71
Forest	74.0 ± 66.47	70.00 ± 68.59	1.50 ± 2.12	1.50 ± 2.12	0.50 ± 0.71
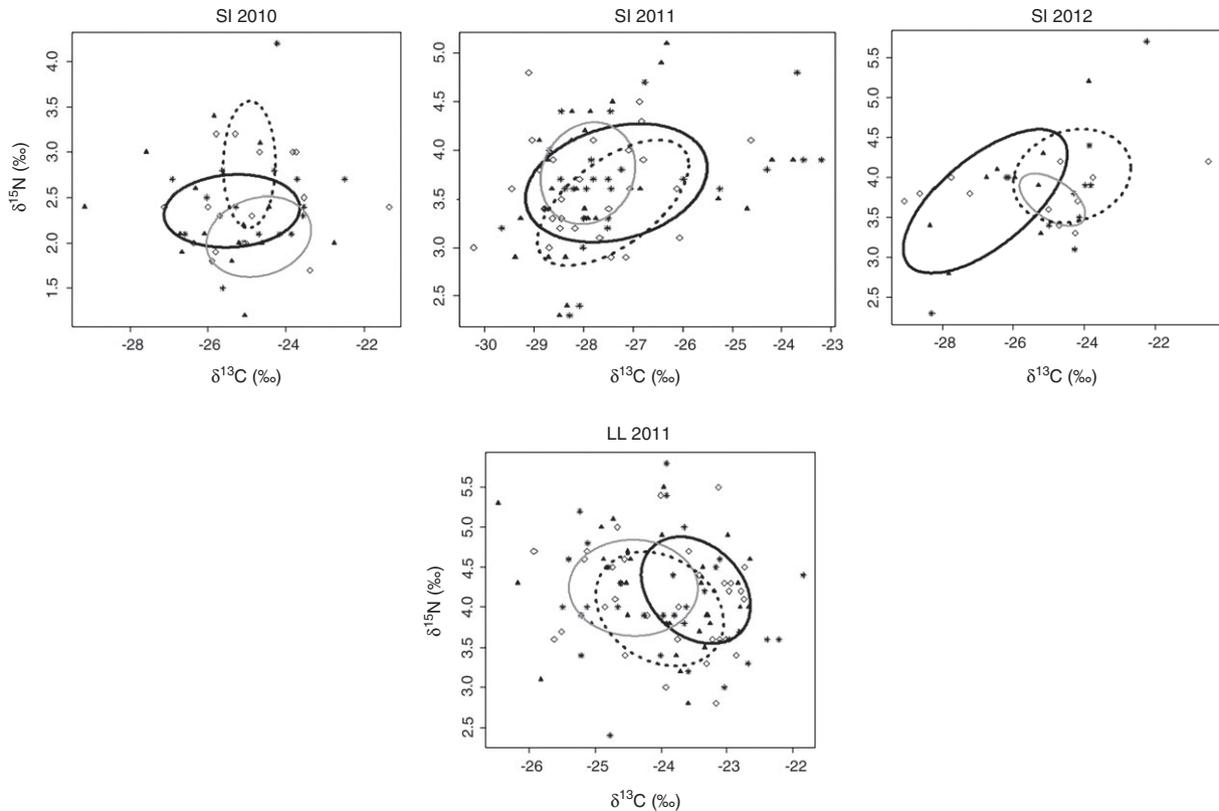
Fig. 3. Standard ellipse areas for *Enallagma hageni* male (black), blue female (gray) and green female (dotted) for Sugar Island (SI), 2010–2012, and Larks Lake (LL, 2011 only). The standard ellipse area represents the central 45% of the data in each set.

Discussion

Before reaching sexual maturity, adult *E. hageni* had equilibrated to their terrestrial diet (Fig. 1). This is similar to the time of 5–8 days required for laboratory-reared locusts to equilibrate (Webb *et al.*, 1998). Newly emerged *E. hageni* at LL (but not on SI) had an enriched ^{13}C signal, reflecting their aquatic prey, which would derive most of their energy indirectly from carbonates dissolved in the water (i.e. a resource much higher in LL relative to SI). Adult diets differed slightly but significantly between the two Michigan populations, but at both sites *E. hageni* fed at the same trophic level as indicated by similar ^{15}N values. Although the two populations differed significantly in their prey base, the isotopic signatures across

the sexes and colour morphs at each site were remarkably consistent (Table 1). Mosquitoes seemed to dominate the diet of the LL population, while the SI population was probably influenced by a prey item highly depleted in ^{13}C, as indicated by its downfield shift from the LL population (Fig. 2). Small diptera dominated our samples of available prey at both sites, accounting for 72–95% of available prey caught in the sticky traps (Table 2). Surprisingly, prey abundance wasn't greater near the shore than in the forest, probably because in the latter, sampling was done only in light gaps where damselflies foraged. Nevertheless, it suggests that females may not be paying an increased cost in foraging efficiency by remaining away from the water while unreceptive to mating.

For each study population, our results did not reveal significant differences between the diets of blue and green

Table 3. Mean ± SD of nitrogen and carbon isotope ratios for representative prey items.

Sample	N	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$
Mosquito	14	−30.50 ± 1.6	1.7 ± 1.6
Lepidoptera	4	−25.21 ± 2.1	2.6 ± 1.8
Trichoptera	7	−25.93 ± 2.5	5.5 ± 0.9
Emphemeroptera	5	−28.56 ± 4.0	4.5 ± 0.5
Midge (profundal)	–	−35.38	2.8
Midge (other)	6	−25.02 ± 1.0	6.7 ± 1.3
Homoptera (plant hopper)	6	−28.23 ± 0.9	0.4 ± 2.3
Diptera (other)	3	−30.65 ± 0.14	4.5 ± 2.3
Coleoptera	4	−26.77 ± 2.3	4.1 ± 0.6

*Mean literature values (see text).
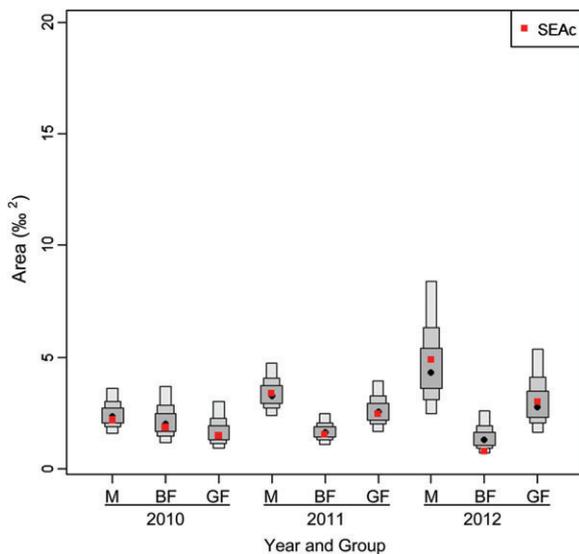
Fig. 4. Density plots showing the confidence intervals of the standard ellipse areas for *Enallagma hageni* populations on Sugar Island (3 years) and Larks Lake (1 year). SEAc (corrected for small sample size) = SEA × (n − 1)/(n − 2). The box areas reflect the 95%, 75% and 50% confidence areas. M, male; BF, blue female; GF, green female.

females, suggesting that the two morphs foraged on similar prey (Fig. 2). This was contrary to expectations if blue females were more likely than green ones to forage in the same areas as males, which foraged at the water as well as in forests. The latter assumes that the isotopic signatures of actual damselfly prey differs between shoreline and forest. It's possible that regardless of where they forage, damselflies select similar prey even though foraging efficiency may differ between locations.

Nevertheless, compared with the blue morph, green females were consistently more similar to males in having a relatively broad isotopic niche space compared with blue females (Figs 3 and 4). The ellipse area reflects the isotopic variance among individuals and hence incorporates the breadth of prey taken weighted by their abundance. Males had the largest ellipse areas overall, suggesting that they had the most variable diet, whereas females exhibited less variation in their isotopic signal and generally smaller ellipse areas. This is consistent with

other results based on direct observations of feeding adults. Males fed as mature adults on the shore as well as in the forest, whereas females of both colour morphs fed primarily in forest light gaps (O. M. Fincke, unpublished). Similarly, in an earlier study of *Enallagma civile*, although there was a tendency for blue females to frequent areas closer to shore than green females, the difference was not significant (Miller & Fincke, 1999). By contrast, Forbes *et al.* (1995) found that, relative to green females, in five of seven samples at high-density sites, more male-like blue females of *Nehalennia irene* were close to shore. Such habitat partitioning has also been seen in *Ischnura verticalis*, a sister genus to *Enallagma*, but in developmental colour morphs. The orange and black, sexually immature females feed in areas away from the sexually mature, gray-blue females (Fincke, 1987).

Surprisingly, across the three summers in the SI population, there was a general trend of increasing ellipse area among males that was roughly paralleled by that of the green females, whereas the ellipse area of the blue morph seemed to decrease over the same time (Figs 3 and 4). Because all females were collected in tandem at the breeding site, there is no reason to suspect that such differences reflected age differences among the morphs. Although diets were similar for both sexes and morphs (Fig. 2), the changing ellipse area most likely reflects a change in the variety of prey eaten. Although a lower mosquito density in 2012, a particularly dry year, may account for the unusually variable isotope area, particularly of males, it is unlikely to account for the contraction of the trophic niche of blue females. A second possible cause of a reduced foraging niche of the blue female morph might be greater sexual harassment by males, which increases with blue morph frequency (Xu & Fincke, 2011). However, the frequency of blue females was lower in 2012 than in 2011 (O. M. Fincke, unpublished). Interestingly, because blue females were found to be harassed more in the forest relative to the shore (O. M. Fincke, unpublished), if population size and thus male harassment were relatively higher there in 2012, blue females might have responded by narrowing their foraging niche space, by avoiding large light gaps frequented by males. Regardless of the cause of the trends, confidence intervals around the ellipses (Fig. 4) strongly suggest that males and their polymorphic females varied in their trophic niches across years on SI (the only population for which we collected data in multiple years).

Although trophic enrichment from prey to predator is known to be quite variable, in a review of the literature, McCutchan *et al.* (2003) suggested that consumers are enriched by about 2.3 ± 0.18‰ in ^{15}N and 0.5 ± 0.13‰ in ^{13}C. In our study, the ^{15}N enrichment factor was similar, whereas our mean carbon enrichment (6.9‰) was much higher than those in the literature to date. The primary pathway for carbon loss is through respiration and therefore we speculate that odonates may exhibit an unusual respiration pattern, resulting in a larger-than-expected isotopic enrichment. Chapman (1998) points out that the metabolic rate of insects in flight is often 50–100 times larger than when at rest. In actively flying insects, the O_2 demand of flight muscles greatly exceeds all other tissues, which may explain the unexpectedly large carbon trophic shift that we found in *E. hageni*. Respiratory CO_2 is depleted in

Table 4. Source contribution (%) to *Enallagma hageni* diet at Larks Lake (LL). The Sugar Island population fell outside of mixing polygon so no solutions could be calculated.

ID	Aquatic (pooled)		Terrestrial (pooled)		Mosquito		Midge (profundal)		Midge (other)		Diptera (other)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
LLM	4	0–17	12	0–45	59	23–74	14	0–32	3	0–13	8	0–28
LLBF	3	0–12	9	0–38	52	25–63	28	16–42	2	0–9	6	0–22
LLGF	3	0–11	8	0–28	64	42–74	19	8–30	2	0–8	5	0–19

M, male; BF, blue female; GF, green female.

^{13}C, resulting in consumers being enriched relative to their diets. This difference should be greatest for animals with high respiration rates (McCutchan *et al.*, 2003). Future work that focuses on isotopic niche space of dragonfly predators is needed to test the generality of the trophic shift in ^{13}C that we found, work that would also more accurately quantify the potential impact these important predators have on their aquatic and terrestrial prey.

Acknowledgements

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