

Patterns of trophic resource use and individual specialization in two species of darters (*Etheostoma*: Percidae)

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ABSTRACT

Background: Species are often grouped into distinct trophic guilds based on their average diets. But the diets of populations may be highly variable through space and time. Even populations that are described as foraging on a wide variety of dietary resources are often comprised of individuals with very narrow dietary preferences, and such individual specialization may vary among populations as a function of local conditions. River gradients provide subtle variation in environmental conditions, so that stream fishes that occur along such gradients may be studied to reveal how environmental conditions shape trophic resource use and individual specialization.

Questions: How does trophic resource use vary among species and populations? Does it correlate with local resource availability? What variability exists in individual dietary widths among populations and species? May we attribute variation in individual dietary widths among sites to any environmental variation?

Organisms: Two sympatric stream fishes (*Etheostoma flabellare* and *E. spectabile*, Percidae) from nine locations in Oklahoma, USA.

Methods: We analysed gut contents of the fish. We measured individual dietary width in each of the populations. We also measured resource diversity and densities as well as the number of competitor species.

Results: We found significant variation in the diets between species and among populations of the same species. Furthermore, most populations consisted of individuals with narrow dietary width (i.e. high individual dietary specialization). Variation in individual dietary widths in populations of *E. flabellare* was correlated with invertebrate density and the number of competitor species, and in *E. spectabile* with the number of invertebrate species and invertebrate density.

Keywords: diet variation, environmental variation, *Etheostoma flabellare*, *Etheostoma spectabile*, individual specialization, trophic ecology.

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INTRODUCTION

Ecologists commonly classify animals into distinct trophic guilds based on the average diet of their populations. However, trophic resource use may vary drastically through space and time, and populations described as foraging on a wide range of dietary resources are often comprised of individuals with highly reduced dietary widths (Bolnick *et al.*, 2003). Reduction of the individual trophic niche width occurs when individuals in a given population partition resources with conspecifics by specializing on specific diet items (Roughgarden, 1974), and such individual specialization may occur in response to local environmental conditions (Rosenblatt *et al.*, 2015). Studies across a broad range of taxa have investigated intraspecific variation in diet composition to understand variation in the magnitude of individual dietary widths (Bolnick *et al.*, 2002; Svanbäck and Persson, 2004; Araújo *et al.*, 2011). Individuals with narrow dietary widths (high individual diet specialization) may occur in particular in populations with high phenotypic variability (Snowberg *et al.*, 2015), allowing some individuals to efficiently exploit a particular subset of the population's total dietary niche (Van Valen, 1965). Frequency-dependent intraspecific competitive interactions can then favour uncommon strategies of individuals exploiting under-utilized subsets of the population's niche, leading to balancing selection and the maintenance of variation within populations (Bolnick, 2001). Alternatively, the width of individual diets can decrease following the expansion of a population's total trophic niche width in response to interspecific competitive release (Van Valen, 1965; Bolnick *et al.*, 2002; Costa *et al.*, 2008). Therefore, it is expected for populations with broader diets to be composed of individuals exhibiting narrower diet widths (Lister, 1976), but it remains unclear how ecological interactions influence individual foraging decisions (Araújo *et al.*, 2011; Evangelista *et al.*, 2014). Interspecific competition is expected to reduce ecological opportunity and reduce within-population diet variation (broad individual diets) (Costa *et al.*, 2008). Ecological opportunity may also vary in response to abiotic environmental factors, such as patch size and structure, resource availability, and habitat stability (Nosil and Reimchen, 2005). Because variation in individual diets can lead to among-individual differences in competition, predation, parasitism, and ultimately fitness, it is important to elucidate the ecological factors driving diet divergence among individuals (Araújo *et al.*, 2011). Widespread species of small-bodied stream fishes provide an excellent opportunity to understand the nature of intraspecific diet variation within and among populations in response to a gradient of ecological factors.

Ecologists and evolutionary biologists have long investigated the dietary habits of fishes to study mechanisms of species co-existence and trophic niche partitioning (Ross, 1986; Bouton *et al.*, 1997), food web dynamics (Winemiller, 1990; Pound *et al.*, 2011), as well as the function of trophic adaptations (López-Fernández *et al.*, 2014). Many fish species are opportunistic feeders consuming a broad array of locally abundant food items (Pratt and Lauer, 2013), but fish diversification has often coincided with adaptation for the exploitation of specific trophic resources (Streelman and Danley, 2003). Accordingly, fish are often classified into distinct trophic guilds based on the average diet of a species (Binning *et al.*, 2009), and such classifications have profoundly influenced our understanding of fish eco-morphology (Geerinckx *et al.*, 2007). Some fish species have extremely specialized feeding strategies with concomitant morphological and behavioural modifications (Westneat, 1991; Takahashi *et al.*, 2007; Berumen and Pratchett, 2008), and distantly related lineages belonging to the same trophic guild can exhibit a high degree of trait similarity that has evolved in convergence (Winemiller, 1991; Winemiller *et al.*, 1995). Nonetheless, the diets of fish species can be highly variable through space and time

depending on resource availability (Godinho *et al.*, 1997), and even species that typically exploit a narrow range of food items will use a broad range of trophic resources when competition is low and high-quality resources are abundant (Liem, 1980; Lowe-McConnell, 1987). For example, trophic niche widths within species and trophic niche overlap among species can be relatively high during the rainy season in many tropical systems, but intraspecific diet widths and interspecific diet overlap decrease in the dry season when resources are scarce (Jepsen *et al.*, 1997). Even though most work on fish dietary habits has focused on variation among species, diet variation within and among populations is also widespread and may profoundly affect a species' ecological function and evolutionary trajectories (Bolnick *et al.*, 2003).

Darters belong to the family Percidae and are small, often brightly coloured stream fishes with a benthic lifestyle. Darters lack completely or exhibit a reduced swim bladder, which makes them inefficient swimmers with reduced dispersal capabilities, high site fidelity, and often strong population genetic structure (Mundahl and Ingersoll, 1983; Ingersoll *et al.*, 1984; Faber and White, 2000). Darters have been documented to exhibit considerable inter- and intraspecific variation in a suite of ecologically relevant traits (Near and Keck, 2005; Near *et al.*, 2011), including life-history strategies (Johnston and Johnson, 2000; Kelly *et al.*, 2012), body shape (Guill *et al.*, 2003; Hopper *et al.*, in press), and habitat use (Stauffer *et al.*, 1996). Consistent with their benthic lifestyle, darters typically feed on invertebrates found on or in the substrate of their habitat (Knight and Ross, 1994). Descriptions of darter feeding habits often classify them as opportunistic insectivores preying on items in proportion to their abundances (Stewart, 1988; Knight and Ross, 1994), and morphological differences among species coincide with feeding ecology (Carlson and Wainwright, 2010). Relatively little is known about resource partitioning among sympatric species and potential diet variation among and within populations of the same species (but see Van Snik Gray *et al.*, 1997; Gillette, 2012). Inter- and intraspecific variation of this kind could be prevalent in widely distributed darters, and discerning the influence of environmental selection on diet variation among and within populations is critical to predicting the ecological function and evolutionary trajectories of darters. Furthermore, descriptive studies of darter trophic resource use have suggested that diets consist of prey items found within distinct territories (Smart and Gee, 1979; Gillette, 2012), perhaps suggesting that individual diet specialization within species may be high and trophic niche differentiation among sympatric species may be low. However, explicit tests of such hypotheses remain mostly lacking.

In this study, we explored variation in trophic resource use and individual diet specialization of two sympatric species of darters, *Etheostoma spectabile* and *E. flabellare*, at multiple stream sites in eastern Oklahoma, USA. We used gut content analysis and surveys of biotic environmental factors to address the following questions: (1) How does trophic resource use vary among species and populations? Does it correlate with local resource availability? The competitive exclusion principle postulates that species partition resources along at least one niche axis (Hardin, 1960). Accordingly, we predicted that sympatric species of darters would differ in their diet. Furthermore, intraspecific geographic variation in diets should occur among populations in response to differences in local resource availability. (2) What variation exists in the degree of individual dietary specialization among populations and species? May we attribute variation in individual dietary specialization among sites to any environmental variation? Based on its ability to occupy various habitat types, we expected to find higher degrees of individual dietary specialization in *E. spectabile* than in *E. flabellare*. Furthermore, the degree of intraspecific variation should be expressed differently among sites, because local environmental conditions should influence resource availability and suitable foraging habitats. We selected a suite of biotic factors that could

influence ecological opportunity of individuals among populations and tested whether variability in these factors was correlated with the degree of individual dietary specialization in populations of *E. spectabile* and *E. flabellare*. Theory predicts that individuals will exploit small subsets of the population's total diet width when resources are abundant (Svanbäck and Persson, 2004; Bolnick *et al.*, 2010); thus, we predicted that individual diet specialization within populations would be positively correlated with number of aquatic invertebrate species (Araújo *et al.*, 2011) and the density of prey items (Pyke, 1984). Individual specialization may also increase following the expansion of a population's total diet width in response to ecological release (Van Valen, 1965). Hence, we also predicted that higher individual specialization (i.e. narrower individual diets) would occur in populations with lower numbers of competitor species and predator species present (Knudsen *et al.*, 2007).

MATERIALS AND METHODS

Study species

Etheostoma spectabile (orangethroat darter) is a robust species of the subgenus *Oligocephalus*, demonstrates pronounced sexual dichromatism, and reaches up to 74 mm in standard length (Kuehne and Barbour, 1983; Page, 1983). The species inhabits shallow gravel and cobble riffles of small to moderately sized streams, but both juveniles and adults can also occupy pools with minimal to no flow (Winn, 1958; Ceas and Page, 1997). *Etheostoma spectabile* can be found in much of the central United States, reaching from southeastern Michigan and Ohio to eastern Wyoming, south to Tennessee and northern Texas (Distler, 1968; Kuehne and Barbour, 1983; Page, 1983). Throughout its range, the species exhibits considerable phenotypic variation in morphological, physiological, and life-history traits (Feminella and Matthews, 1984; Marsh, 1984; Ceas and Page, 1997).

Etheostoma flabellare (fantail darter) is included in the subgenus *Catonotus* (Porterfield *et al.*, 1999) and is a slender darter reaching up to 78 mm in standard length (Kuehne and Barbour, 1983; Page, 1983). Breeding males develop egg mimicking knobs on the spiny portion of the first dorsal fin, which are preferred by females (Knapp and Sargent, 1989; Strange, 2001). *Etheostoma flabellare* is generally limited to shallow riffles and fast flowing runs with larger substrates (Winn, 1958; Hlohowskyj and Wissing, 1987). This species is widespread throughout North America, ranging from southern Quebec to Minnesota, south to South Carolina, Alabama, and northeastern Oklahoma (Braasch and Mayden, 1985).

Collection of specimens and gut content analysis

We collected fish at nine different stream sites in eastern Oklahoma during the summer of 2014. Fish were collected along 100-metre stream transects using 15–20 minute intervals of backpack electrofishing (LR-24 electrofisher, Smith-Root, Inc.). Specimens were immediately euthanized using MS-222 and fixed in a 10% formaldehyde solution. After fixation, specimens were rinsed in water and stored in 70% isopropyl alcohol. Overall, we collected 741 individuals (269 *E. flabellare* and 472 *E. spectabile*).

To evaluate trophic resource use, we isolated the stomach of each darter under a dissection microscope to access its contents and identify and quantify prey items (Schlosser and Toth, 1984). We only examined the anterior portion of the gut where prey items had not been fully digested. The keys provided by Merritt *et al.* (2008) were used to identify prey items

to the lowest feasible taxonomic level. Note that partial prey items were only counted if head capsules were present. We recognized 18 prey categories overall, 13 of which were insects. Eleven diet categories represented genus-level identifications, including *Baetis*, *Heptagenia*, and *Isonychia* (Ephemeroptera), *Limonia*, *Tipula*, *Tabanus*, and *Simulium* (Diptera), *Chimarra* and *Hydropsyche* (Tricoptera), as well as *Agnatina* and *Neoperla* (Plecoptera). Two categories represented family-level groupings (Chironomidae and Crambidae) due to complexity of further identification. The remaining diet categories were Amphipoda, Isopoda, Ostracoda, water mites (Arachnida), and fish eggs. Count data of all 18 categories were used for calculating indices of individual diet specialization, but *Limonia*, *Tabanus*, *Tipula*, and *Simulium* were grouped together as ‘Other Diptera’ to reduce the number of zero-distances when performing ordinations (see below). For analyses, we calculated the relative proportion of each item in the diet for each of the specimens, and proportions were arc-sin-square-root-transformed prior to multivariate analysis. We also recorded the sex of each fish and measured its standard length to the nearest millimetre.

Collection of biotic environmental variables

To test how biotic environmental conditions affect trophic resource use and the degree of individual diet specialization, we characterized resource availability and the fish community at each collection site. To quantify resource availability, a Surber sampler was used to collect three samples of the benthic invertebrate community (900 cm² each) at each site from the same riffle areas where the darters were collected (Barbour *et al.*, 1999). Benthic invertebrates were preserved in 50% isopropyl alcohol. Specimens were later identified to the lowest feasible taxonomic level (typically genus) using dichotomous keys (Merritt *et al.*, 2008) and counted to estimate prey availability at each site. Overall, we collected 36 categories of prey items spanning seven insect orders and six other invertebrate taxa (see www.evolutionary-ecology.com/data/2963Appendix.pdf, Table S1). The three samples from each site were pooled for analysis. To determine the effects of interactions with other members of the fish community on individual dietary specialization, we identified all fish species collected during the electrofishing surveys based on keys provided by Miller and Robison (2004). Overall, we identified 26 species of fish belonging to eight different families (2963Appendix.pdf, Table S2).

Analysing patterns of diet use and its relation to the environment

We first used non-metric multidimensional scaling (NMDS) to perform an ordination analysis using the relative proportions of diet items found in each individual using the VEGAN package (Oksanen *et al.*, 2015). All analyses were performed in the R statistical platform (R Development Core Team, 2013) unless otherwise noted. Data attribute plots were produced for each NMDS, which summarized the compositional data by constructing a low dimensional space in which darters with similar diets exhibit small pairwise distances in NMDS space, while those with dissimilar diets are further apart. Stress plots and a goodness-of-fit test were used to assess the distances assigned by the NMDS related to the original distances of the data (Borcard *et al.*, 2011). The goodness-of-fit test ensured that diet ordination distances displayed in the NMDS were representative of actual diet distances in the data set ($R^2 = 0.986$). We extracted the individual NMDS scores and used them as dependent variables in a multivariate analysis of covariance (MANCOVA) in SPSS v.20 to explicitly test

for variation in diets between species and among populations. Assumptions of multivariate normal error and homogeneity of variances and covariances were met for this analysis. We used Wilks' lambda to approximate F -values, and partial eta-squared values (η_p^2) were used to estimate effect strengths. We also calculated the relative variance as the partial variance for a given term divided by the maximum partial variance value in a model. We included 'site', 'species', and 'sex' as independent variables, and used 'standard length' as a covariate to control for potential effects of body size.

Darters were expected to feed on prey taxa in proportion to their relative abundance (Pratt and Lauer, 2013). Hence, we used a Mantel test to examine the correlation between the composition of local invertebrate communities and the composition of gut contents using the VEGAN package (Borcard and Legendre, 2012). Pairwise Bray-Curtis similarities were calculated between all populations based on matrices describing the composition of gut contents (dependent variable) and the composition of local invertebrate communities (independent variable) based on the arc-sin-square-root-transformed proportional occurrence of each invertebrate taxon. Matrix correlations were performed for each species separately since trophic resource use varied between species (see below).

Individual diet specialization and its relation to the environment

We analysed the degree of individual diet specialization across populations of *E. spectabile* and *E. flabellare*. To test the hypothesis that there is variation in individual dietary widths among populations, we first calculated the total niche width (TNW), which corresponds to the Shannon-Wiener index (Roughgarden, 1974), for each species at a site separately using the count data of stomach contents. Total niche width is calculated by summing the within-individual component (WIC) of variation and the between-individual component (BIC) (Roughgarden, 1974). The ratio of WIC/TNW provides information on the contribution of the within-individual variation to the total diet width (Roughgarden, 1974). When WIC/TNW nears 0, the individual dietary niche width is narrow and exhibits low overlap among individuals (high individual diet specialization); when the ratio is 1, there is complete overlap in trophic resource use among individuals (low individual diet specialization) (Roughgarden, 1974). We also calculated Schoener's proportional similarity index (PS_i) adapted to the individual level to evaluate the overlap of each individual's diet with the other darters at a site (Bolnick *et al.*, 2002). The PS_i was calculated based on the following formula, where p_{ij} is the frequency of food category j in the diet of individual i , and q_j is the frequency of food category j in the entire population:

$$PS_i = 1 - 0.5 \sum_j |p_{ij} - q_j|$$

The PS_i ranges from 1 (the individual's diet overlaps entirely with all darters at a site) to 0 (the individual utilizes only one specific diet category j). The mean value of all individual PS_i expresses the mean individual specialization (IS) of a particular group of interest. Statistical significance for individual specialization metrics (both for WIC/TNW and IS) was tested using 999 Monte Carlo permutations of the data sets, yielding a null model distribution against which the observed IS values were tested (Bolnick *et al.*, 2002; Araújo and Gonzaga, 2007). All trophic indices and Monte Carlo null models were calculated using the RINSP package (Zaccarelli *et al.*, 2013). We tested for variation in the degree of individual specialization among species and sites by performing an ANCOVA (performed in SPSS)

using extracted PS_i values as dependent variables with ‘site’, ‘species’, and ‘sex’ as independent variables and ‘standard length’ as a covariate.

To determine the influence of biotic environmental factors on individual diet specialization, we used the fish community data and invertebrate community data to calculate biotic indices used to address the questions outlined in the Introduction. First, we calculated the relative density of invertebrate prey items in the guts, and the percentage composition for major taxonomic groups were tabulated (arc-sin-square-root-transformed). Relative density of invertebrate communities (\log_{10} -transformed) was then calculated by dividing the total number of invertebrates collected in each Surber sample by the total area sampled, and ranged from 811 to 9422 individuals per m^2 . Since there is often a positive relationship between number of species and sample size, we created rarefaction curves as implemented in the package VEGAN to estimate the number of invertebrate taxa at every locality. To do so, Shannon-Wiener diversity indices for invertebrate communities were first calculated. We then used a subsample of $n = 70$ to estimate rarefied number of species using rarefaction curves. Values of the rarefied number of species used in the analysis ranged from 8.99 to 14.41. Because we were interested in the influence of competition on individual specialization, we estimated the number of competitor species present at each site. We considered all benthic insectivores as competitors (Todd and Stewart, 1985) for the analysis (number of competitor species = 5–8; [2963Appendix.pdf](#), Table S2). As we were simultaneously concerned with the effect of predation on individual specialization (Araújo *et al.*, 2011), we estimated the number of predator species at each site as any piscivorous fish species capable of consuming darters (number of predator species = 0–2; [2963Appendix.pdf](#), Table S2).

We used a model selection approach to evaluate the effects of number of invertebrate species and invertebrate density (ecological opportunity), as well as the number of competitor and predator species on individual specialization using the GMULTI package (Calcagno and Mazancourt, 2010). The influence of biotic variables on individual specialization was analysed separately for each species using estimated marginal means from the ‘site-by-species’ interaction term of the ANCOVA model described above. These were used as the dependent variables in model selection based on general linear models (GLM) with ‘number of predator species’, ‘number of competitor species’, ‘invertebrate density’, and ‘number of invertebrate taxa’ as the independent variables. All possible models were considered, ranging from the full model and all pairwise interactions to the null model including the intercept only. Collinearity of independent variables was explored via calculation of variance inflation factors (VIF), which measure the proportion of variance that a particular regression coefficient is inflated by the presence of other variables (Borcard *et al.*, 2011). Following the examination of VIFs, the number of predator species (VIF > 6) was excluded to avoid over-inflating models. We then performed model selection using the three remaining variables. Models were evaluated using the Akaike Information Criterion with finite sample correction [AIC_C (Johnson and Omland, 2004)]. In addition, we report the difference between the AIC_C score of a given model and the lowest AIC_C score (i.e. ΔAIC_C). Models that differ within 2 AIC_C units from the model with the lowest AIC_C ($\Delta AIC_C < 2$) are considered equally supported (Burnham and Anderson, 2002). Ultimately, we evaluated the relative importance of main effects and interaction terms individually by using the sum of the relative evidence weights for each model in which a given term appears (model averaging). Terms that exceed an importance value of 0.8 were considered critical predictors of the dependent variable (Buckland *et al.*, 1997; Calcagno and Mazancourt, 2010). Significant interaction terms were visualized using non-parametric thin-plate spline regression to create a surface

illustrating individual diet specialization (Arnold, 2003; Lee *et al.*, 2008). Estimation of individual specialization surfaces was performed using R statistical software (R Development Core Team, 2013) using the FIELDS package (Furrer *et al.*, 2012) (smoothing parameter $\lambda = 0.005$).

RESULTS

Variation in darter diets

After removing darters with empty stomachs (75 *E. flabellare* and 223 *E. spectabile*), the final data set was comprised of 443 darters (194 *E. flabellare* and 249 *E. spectabile*; Table 1). Overall, *E. flabellare* and *E. spectabile* incorporated a similar number of prey categories into their diet, foraging on 14 and 13 categories, respectively (2963Appendix.pdf). Chironomid larvae dominated the diet of both species, constituting over half of each species' diet. When comparing the relative frequencies of diet items of each species, *E. flabellare* foraged on prey items other than chironomid larvae more often than *E. spectabile*. Although both species foraged on a similar number of prey types, each species incorporated unique prey items into their diets. We identified four prey items that were unique to *E. spectabile* (*Isonychia*, *Chimarra*, mites, and Ostracoda), all of which occurred at very low frequencies. In *E. flabellare*, we recognized five items that occurred exclusively in its diet (*Neoperla*, *Simulium*, *Tabanus*, *Tipula*, and *Limonia*) at relatively low frequencies (2963Appendix.pdf).

The MANCOVA used to analyse NMDS scores describing diet composition of *E. spectabile* and *E. flabellare* yielded significant effects of species and site, indicating geographic variation among populations and interspecific differences in diets. In addition, there was a significant interaction between the site and species terms, suggesting that the nature of trophic niche partitioning was variable among sites (Table 2). Species, site, and their interaction term explained the majority of variation in the data set (Table 2). Visualization of dietary resource use in an NMDS plot indicated that both the magnitude and direction of species differences varied among sites (Fig. 1a). Although not consistent across all of the examined sites, two general patterns emerged from the analysis. First, *E. spectabile* and *E. flabellare* tended to partition trophic resources at the majority of sites. Second, the diets of *E. flabellare* tended to be more biased towards larger Dipteran prey items, classified as

Table 1. List of collection sites with latitude and longitude based on GPS coordinates (for each site, the number of *E. spectabile* and *E. flabellare* are also listed)

Site name	Latitude	Longitude	<i>E. spectabile</i>		<i>E. flabellare</i>	
			Males	Females	Males	Females
Peavine Creek	35.897	-94.627	16	14	5	11
Tributary to Flint Creek	36.187	-94.709	22	17	17	13
Spring Creek at Rocky Ford State Park	36.144	-94.907	9	12	10	15
Sycamore Creek	36.808	-94.645	19	15	14	19
Whitewater Creek	36.539	-94.759	27	18	5	1
Beaty Creek at 456 Bridge	36.355	-94.776	19	19	24	21
Sallisaw Creek	35.464	-94.862	7	1	7	2
Little Lee Creek	35.566	-94.532	7	3	4	13
Shawnee Creek	34.768	-94.628	12	11	1	10

Table 2. Results of multivariate analyses of covariance examining the NMDS scores from the combined diets of both species

Effect	<i>F</i>	Hypothesis d.f.	Error d.f.	<i>P</i>	η_p^2	Relative variance
Length	1.898	2	413	0.151	0.009	0.116
Site	4.387	16	826	<0.001	0.078	1.000
Species	13.058	2	413	<0.001	0.059	0.759
Sex	2.067	2	413	0.128	0.010	0.127
Site × Species	3.245	16	826	<0.001	0.059	0.755
Site × Sex	1.370	16	826	0.149	0.026	0.330
Species × Sex	3.000	2	413	0.074	0.013	0.160

Note: Significant effects with a relative variance > 0.5 are highlighted in **bold**.

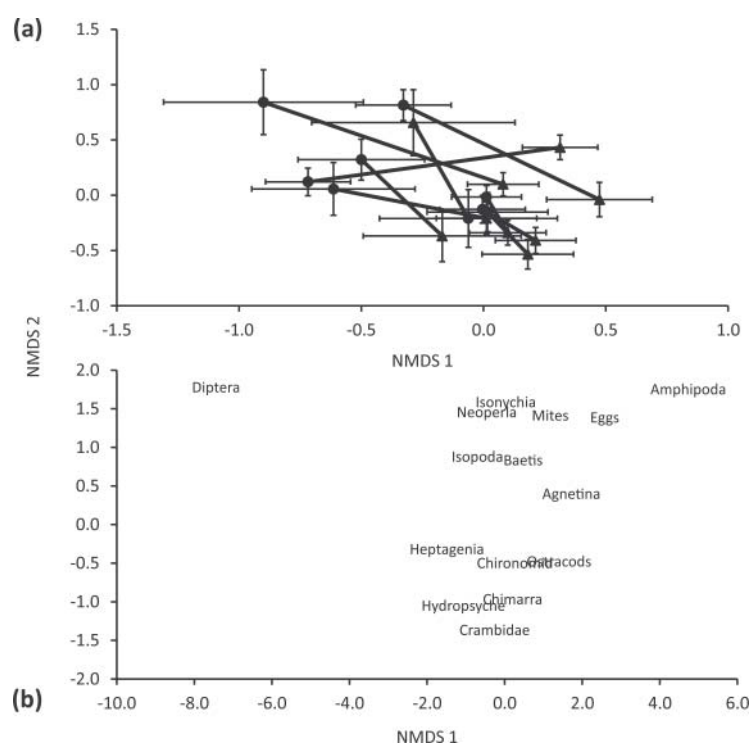


Fig. 1. Trophic resource use variation from the combined analysis at different sites investigated. (a) Estimated marginal means calculated from the site-by-species interaction term from the MANCOVA of the NMDS scores (\pm S.E.M.). Circles are *E. flabellare* and triangles are *E. spectabile*. Lines connect populations of *E. flabellare* and *E. spectabile* from the same site. (b) Illustration of diet item vectors used in the NMDS.

‘Other Dipterans’, as indicated by the often more negative scores along NMDS axis 1 and/or more positive scores along NMDS axis 2 (Fig. 1b). Results of the Mantel test indicated that there was no significant association between the diets of *E. flabellare* populations and their local invertebrate communities ($r = -0.012$, $P = 0.47$). However, there was a significant

correlation between the composition of *E. spectabile* diets and the composition of invertebrate communities ($r = 0.378$, $P = 0.038$).

Variation in individual diet specialization and its relation to the environment

When calculating indices of individual diet specialization of darters across the nine sites, IS values ranged from 0.374 to 0.871 for *E. spectabile* (Table 3a) and from 0.461 to 0.694 for *E. flabellare* (Table 3b), indicating intermediate values of individual diet specialization for both species. Despite the narrow range of categories consumed at some sites, individual diet widths were significantly narrower than predicted based on the null model produced by the Monte-Carlo procedure for all populations of *E. spectabile*, except for Sallisaw Creek (Table 3a). Likewise, all populations of *E. flabellare*, except for Whitewater Creek, had individuals with significantly narrower diet widths than predicted by the null model (Table 3b). ANCOVA of the combined PS_i scores revealed significant effects of site and species, as well as the interaction between site and species (Table 4). With the exception of Sallisaw Creek, *E. flabellare* tended to exhibit narrower individual diet widths (lower values

Table 3. Indices of individual diet width calculated for the diets of *E. spectabile* and *E. flabellare* at each site investigated using the proportion of similarity index adapted to the individual level

Site	WIC	BIC	TNW	WIC/TNW	IS	<i>N</i>	<i>P</i>
(a) <i>E. spectabile</i>							
Sycamore Creek	0.190	0.098	0.289	0.660	0.871	34	0.001
Whitewater Creek	0.375	0.672	1.047	0.358	0.374	45	0.001
Tributary to Flint Creek	0.414	0.861	1.275	0.325	0.423	39	0.001
Spring Creek	0.221	0.195	0.416	0.531	0.814	18	0.001
Peavine Creek	0.092	0.123	0.215	0.430	0.827	30	0.001
Beaty Creek	0.215	0.180	0.395	0.544	0.771	38	0.001
Sallisaw Creek	0.542	0.288	0.829	0.653	0.651	8	0.298
Little Lee Creek	0.398	0.530	0.929	0.429	0.556	11	0.160
Shawnee Creek	0.494	0.457	0.951	0.520	0.594	23	0.003
(b) <i>E. flabellare</i>							
Sycamore Creek	0.46	0.334	0.794	0.579	0.604	34	0.001
Whitewater Creek	0.375	0.361	0.736	0.510	0.694	6	0.297
Tributary to Flint Creek	0.604	1.018	1.622	0.372	0.434	31	0.001
Spring Creek	0.624	0.702	1.327	0.471	0.525	25	0.001
Peavine Creek	0.248	0.327	0.575	0.432	0.651	16	0.001
Beaty Creek	0.379	0.589	0.968	0.391	0.558	45	0.001
Sallisaw Creek	0.429	0.35	0.779	0.551	0.677	9	0.001
Little Lee Creek	0.304	0.721	1.025	0.297	0.461	17	0.015
Shawnee Creek	0.379	0.589	0.968	0.391	0.578	11	0.001

Note: *P*-values for individual specialization metrics (both for WIC/TNW and IS) were attained using 999 Monte Carlo permutations of the data sets, yielding a null model distribution against which the observed IS values were tested. Values of IS near 0 represent narrow individual diet widths, whereas values nearing 1 represent broad individual diet widths within populations. Cases with significant *P*-values are highlighted in **bold**. WIC is the within-individual component of variation, BIC is the between-individual component of variation, TNW is the total niche width of the population, while IS represents the average diet width of individuals within a population.

Table 4. Results of ANCOVA analysing individual specialization among sites with *E. spectabile* and *E. flabellare* combined analysis

Effect	d.f.	<i>F</i>	<i>P</i>	η_p^2	Relative variance
Length	1	0.015	0.904	0.000	0.000
Site	8	24.215	<0.001	0.319	0.885
Species	1	90.990	<0.001	0.180	0.500
Sex	1	0.002	0.965	0.000	0.000
Species × Site	8	29.132	<0.001	0.360	1.000
Site × Sex	8	0.913	0.505	0.017	0.048
Species × Sex	1	0.223	0.637	0.001	0.001

Note: Effects with a relative variance > 0.5 are highlighted in **bold**.

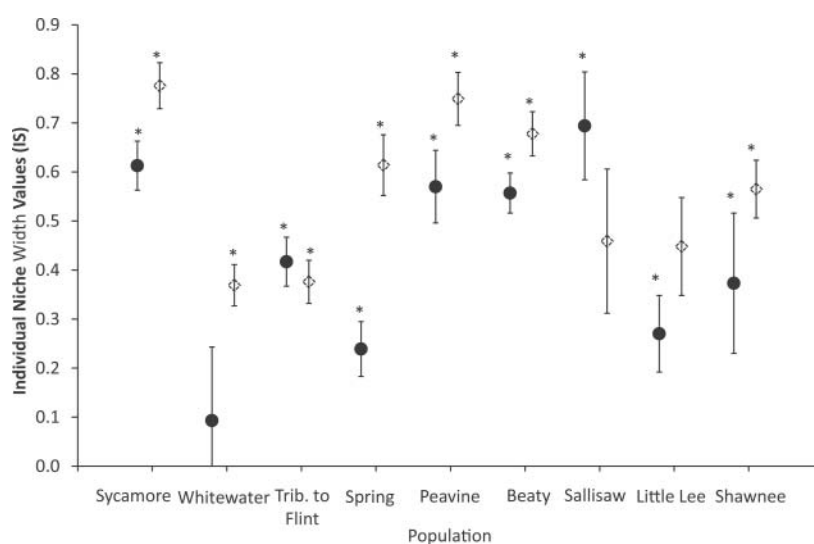


Fig. 2. Visualization of population differences in individual niche width using estimated marginal means for the site-by-species interaction term. *Etheostoma flabellare* (solid symbols) and *E. spectabile* (open symbols) calculated using proportions of diet items collected from stomach contents of individuals from nine sites. Asterisks indicate individual dietary widths that are significantly narrower than the null model created using the Monte Carlo resampling procedure.

of PS_i , higher individual specialization) at any given site, although the magnitude of difference was highly variable (Fig. 2).

In *E. spectabile*, individual diet specialization was strongly influenced by the number of invertebrate taxa present and invertebrate density. We found support for only two models ($\Delta AIC_C < 2$; Table 5), which included invertebrate density as well as the interaction between invertebrate density and the number of invertebrate taxa. Model averaging indicated that all factors and interactions had importance values < 0.8 (Fig. 3a). Visualizing the effects of invertebrate density and the number of invertebrates indicated that the highest levels of individual diet specialization in *E. spectabile* were associated with a combination of low to moderate numbers of invertebrate taxa and low to moderate invertebrate densities (Fig. 3b).

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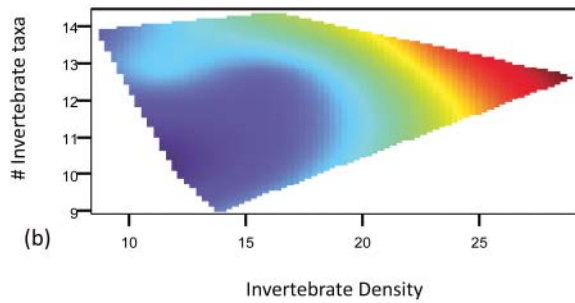
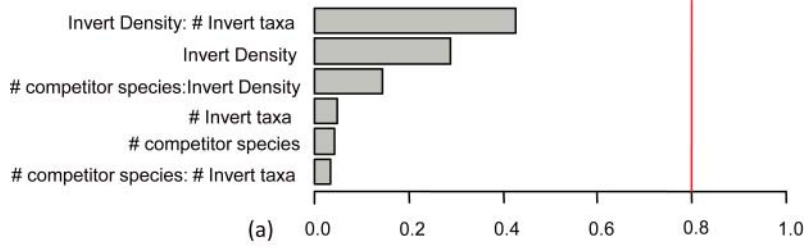


Fig. 3

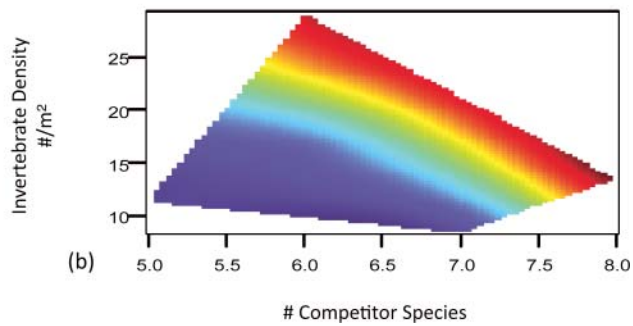
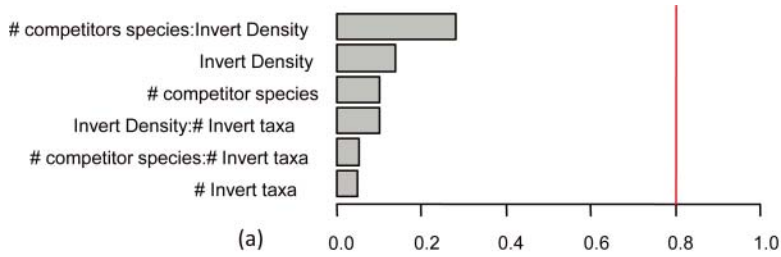


Fig. 4

Table 5. Results of model selection using the estimated marginal means (IS) calculated from the analysis of covariance for each species separately with the biotic factors number of competitors, invertebrate densities and invertebrate diversity indices

Species	Model	AIC _C	ΔAIC _C	Effect	Estimate	S.E.	<i>t</i>	<i>P</i>
<i>E. spectabile</i>	1	-5.309	0.000	Number of Invertebrate Taxa × Invertebrate Density	0.321	0.091	3.530	0.010
	2	-4.467	0.842	Invertebrate Density	0.001	0.000	2.881	0.024
<i>E. flabellare</i>	1	1.447	0.000	Intercept	0.139	0.157	0.883	0.406
	2	2.365	0.918	Number of Competitor Species × Invertebrate Density	0.002	0.001	1.943	0.093

Note: See [2963Appendix.pdf](#), Tables S2 and S3 for biotic indices used in the models.

Wider individual diets occurred with higher numbers of invertebrate taxa or when invertebrate density was high.

Individual diet specialization in *E. flabellare* was associated with number of competitors and invertebrate density (Table 5). Model averaging indicated the interaction between invertebrate density and the number of competitor species was the best predictor of individual diet specialization in *E. flabellare*, even though it had an importance value considerably lower than 0.8 (Fig. 4a). Visualization of the interaction effect illustrated that narrow individual diet widths in *E. flabellare* coincided with low to moderate numbers of competitor species when invertebrate densities were low (Fig. 4b).

DISCUSSION

Our study of trophic resource use in sympatric populations of *E. spectabile* and *E. flabellare* indicated significant geographic variation in diets and frequent trophic niche partitioning between species. *Etheostoma spectabile* appeared to consume prey items in proportion to their relative abundances among sites. In contrast, gut contents of *E. flabellare* were not correlated with the composition of local invertebrate communities, indicating that this species may be a more selective forager. In addition, the individual dietary width (i.e.

Fig. 3. (a) Importance of factors used to predict variation in individual niche widths among populations of *E. spectabile*. The red line indicates the importance value used to assess model importance. (b) Surface visualization of the interaction between the number of invertebrate taxa and number of competitor species and its effect on individual niche widths among populations of *E. spectabile*. Values used in the analysis are the IS values derived from the individual PS_i values. Narrow niche widths are represented in dark blue (low IS values), broad niche widths are represented in red (high IS values).

Fig. 4. (a) Importance of factors used to predict variation in individual niche widths among populations of *E. flabellare*. The red line indicates the importance value used to assess model importance. (b) Surface visualization of the interaction between the number of invertebrate taxa and number of competitor species and its effect on individual niche widths among populations of *E. flabellare*. Values used in the analysis are the IS values derived from the individual PS_i values. Narrow niche widths are represented in dark blue (low IS values), broad niche widths are represented in red (high IS values).

individual specialization) varied between species and among sites. Variation in individual dietary width among sites was associated with the number of invertebrate taxa and invertebrate density in *E. spectabile*, and with invertebrate density and the number of competitor species in *E. flabellare*. Overall, our results show substantial variation in trophic resource use among individuals, populations, and species of small-bodied fishes that are typically assumed to be insectivores utilizing a wide range of trophic resources.

Previous studies of diets have relied on qualitative comparisons of the mean diets of different species, which can be problematic given the patterns of within- and among-population variation uncovered here. An early study investigating the feeding habits of co-occurring species of darters found that the diets of sympatric species were more similar to each other than to those of conspecifics from other stream sites (Martin, 1984), which aligns well with the geographic variation in diets documented here. These results support our predictions and suggest that resource availability is likely a key driver in determining dietary resource use. Indeed, darters exhibit high site fidelity (Ingersoll *et al.*, 1984) and experience variable environmental conditions that are likely to influence the availability of invertebrate prey species (Atilla *et al.*, 2005). Our results also correspond to previous studies documenting interspecific differences in the diets of *E. spectabile* and *E. flabellare* (Martin, 1984). *Etheostoma flabellare* populations in our study tended to consume Dipteran prey more often, and we speculate that differences perhaps coincided with microhabitat use of the two species (Dewey, 1988). *Etheostoma spectabile* is known to utilize a variety of stream habitats, including riffles, riffle margins, and pools (Vogt and Coon, 1990), where it gleans prey from the substrate's surface. In contrast, *E. flabellare* is typically confined to riffle-type habitats (Paine *et al.*, 1982; Matthews, 1985) and feeds in the interstices of the substrate (Paine *et al.*, 1982). Thus, *E. flabellare* is more apt to forage on rheophilic invertebrate taxa like *Hydropsyche* (Merrit *et al.*, 2008). Furthermore, trophic resource partitioning between darters has previously been attributed to the range in the size of prey selected by the two species (Martin, 1984; Todd and Stewart, 1985). Our results are consistent in that *E. spectabile* typically focused its feeding efforts on very small taxa (chironomids) compared with *E. flabellare*, which more frequently selected relatively larger prey items (*Tabanus*, *Tipula*, *Hydropsyche*) (Hlohowskyj and White, 1983; Fisher and Pearson, 1987).

Although darters have previously been described as consuming invertebrate prey items in proportion to their relative abundances (Pratt and Lauer, 2013), our results show that populations of *E. flabellare* and *E. spectabile* exhibit geographic variation in the diet widths of individuals, and that variation in such individual diet specialization is shaped by differences in ecological opportunity among populations. Individual diet widths in populations of *E. flabellare* are narrower where invertebrate density is low and the number of competitor species is high. In *E. spectabile*, narrow individual diet widths are associated with low numbers of invertebrate taxa and low invertebrate densities. Interestingly, these findings are opposite to theoretical predictions and the outcomes of previous empirical studies of intra-population diet variation (Semmens *et al.*, 2009). For example, in the fruit bat, *Rousettus aegyptiacus*, individual diet widths were narrower when the number of fruit-bearing plant species were higher (Herrera *et al.*, 2008). Among-individual diet variation was also higher in populations of grey wolves (*Canis lupus*) with access to terrestrial and marine resources compared with populations with access to only terrestrial resources (Darimont *et al.*, 2009). Furthermore, Layman *et al.* (2007) found that habitat fragmentation in tidal wetlands reduced resource diversity and simplified food webs, which in turn increased individual diet widths in grey snapper (*Lutjanus griseus*). Optimal foraging theory predicts that individuals will expand their diets to include previously unutilized resources when resources are limited

(Pyke, 1984). Individual diet widths are thus affected both by the diversity of available resources and resource abundance (Araújo *et al.*, 2011).

Individuals of *E. flabellare* had narrow diet widths when the number of competitor species was higher and invertebrate densities were low. Interspecific competition is expected to alter dietary widths, and the direction of its effect depends on variation in resource preference of the focal species and the nature of diet overlap among competitors (Svanbäck and Bolnick, 2008). Support for the idea that competitive release leads to expansion of the population-level niche width and a concomitant reduction of individual niche widths is evident from a variety of systems (Bolnick *et al.*, 2003); however, our data suggest that this pattern may not occur ubiquitously. Since populations of *E. flabellare* maintain narrow individual diet widths under higher competitive regimes with lower resource densities, we speculate that interspecific competition with species having high diet overlap (exploitative competition) may be an important factor in determining the diet width of individuals (Bolnick *et al.*, 2010). In other systems, diet divergence within populations is hypothesized to occur as a mechanism to avoid competition with co-occurring species. For example, in Galapagos sea lions, *Zalophus wollebaeki*, high intra-population diet variability is a strategy that reduces interspecific competition with Galapagos fur seals (*Arctocephalus galapagoensis*) (Paez-Rosas *et al.*, 2014). Alternatively, partitioning microhabitats with other species (Welsh and Perry, 1998) could restrict the diet of individuals to trophic resources confined to specific patches (interference competition) (Svanbäck *et al.*, 2011), and cause the diet widths of individuals to appear narrow (Konrad *et al.*, 2008; Evangelista *et al.*, 2014).

Ultimately, ecosystems are heterogeneous and resources and individuals are patchily distributed. If individuals are actually capable of including a range of food items in their diets, but feeding on the most abundant resource within a patch, narrow individual diets may represent patchiness in resource distribution rather than variation in feeding strategies within populations (Araújo *et al.*, 2011). For example, a study investigating diet widths of nesting pairs of great tits (*Parus major*) found that habitat partitioning for nest sites among conspecifics produces high among-site variation in the diet widths of nesting pairs (Pagani-Núñez *et al.*, 2011), which could mean that nesting pairs are adopting a generalist foraging strategy by consuming the most abundant prey items within a patch and causing the population to appear as though individuals are dividing the total trophic niche into small subsets (Araújo *et al.*, 2011). Evidence derived from empirical studies of resource patchiness of streams (Effenberger *et al.*, 2011) and the high site fidelity exhibited by darters used for this study (Ingersoll *et al.*, 1984) also support this hypothesis, and suggest that individuals specializing on a narrow subset of the diet may not be as prevalent within populations as previously considered. Hence, longitudinal studies of individual diets using isotopic analysis coupled with gut content analysis are warranted if the true nature of individual diet widths are to be thoroughly understood.

Our analyses could not rule out the potential effects of predation on individual diet widths (Peacor and Pfister, 2006). In our system, fish predation is likely not very strong, considering that the habitats darters are commonly found in are less than a metre deep, and large piscivorous fish species are generally rare. However, the threat to small-bodied fishes by avian or mammalian predation remains poorly understood and could have a profound influence on foraging habits of stream fishes populating shallow waters. Empirical studies from other systems have generated evidence demonstrating that non-consumptive predator effects can influence foraging efforts and the distribution of individuals within populations by imposing a larger risk to individuals during foraging (Peckarsky *et al.*, 2008). For example,

some individuals select resources that minimize risk to predation, while bolder individuals act to maximize energy gain (Nannini *et al.*, 2012). The presence of predator species could thus play a role in determining individual dietary width among populations, because foraging individuals can be susceptible to predation associated with a particular diet (Svanbäck and Bolnick, 2008). In the Atlantic mud crab, *Panopeus herbstii*, the introduction of a predator cue reduced consumption of mussel prey and activity levels in individuals, demonstrating the effects of higher-order predators on the foraging behaviour of intermediate predators (Toscano and Griffen, 2014). Further exploration of interactions between populations of organisms occupying intermediate positions in food webs and their predators or competitors will be required to understand the mechanisms that underlie variation in individual diet widths among natural populations.

The diets of species are often described as the mean of their populations, even though there is considerable variation among populations and even among individuals (Bolnick *et al.*, 2003). Relatively few studies have investigated diet variation at the individual level and attempted to disentangle the ecological causes that shape variation in individual diet widths among populations (Araújo *et al.*, 2011). Our study contributes additional evidence to the notion that populations exhibiting wider trophic niches are actually collections of individuals with narrow diets (Bolnick *et al.*, 2003) and lends insight to answering key questions about the ecological causes of individual diet specialization (Araújo *et al.*, 2011). We have provided evidence of geographic variation in the diets of two sympatric species of stream fish, along with further support of trophic niche partitioning between these species. Furthermore, we present new evidence that the variation in individual diet specialization is variable among populations and conclude that ecological opportunity affects the width of individual diets among natural populations. Variation in individual dietary widths among naturally occurring populations and the underlying mechanisms in those populations are rarely documented (Rosenblatt *et al.*, 2015), and we suggest that these patterns may be more widespread than previously considered.

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