

ECOLOGICAL AND MORPHOLOGICAL VARIATION OF DARTERS AMONG
ASSEMBLAGES IN OKLAHOMA STREAMS

by

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Abstract

Environmental variation can shape phenotypic variation in organisms. Most evidence for trait differentiation along environmental gradients comes from analyses of dichotomous habitat types that differ in only one or few environmental factors. In reality, however, environmental variation is often more subtle, gradual, and multifarious. I investigated geographic variation in body shape, trophic resource use, and individual diet specialization in two species of darters (*Etheostoma spectabile* and *E. flabellare*; Percidae) that occur along river gradients. I explicitly tested how abiotic and biotic environmental factors shape trait variation within and between species. Results indicated significant among population variation in the body shape of both species. Population differences in body shape were correlated with variation in substrate composition. Although body shape analyses revealed a small but significant signal of convergent evolution of body shape when both species occur in sympatry, *E. spectabile* and *E. flabellare* mostly exhibited unique responses to shared sources of selection. The analyses of darter trophic resource use uncovered significant resource partitioning between the two species and geographic variation in diets that is likely driven by differences in resource availability. Furthermore, the majority of populations exhibited significant individual specialization. Variation in individual specialization in populations of *E. flabellare* was related to invertebrate density and competitor richness, and in *E. spectabile* to the combined effects of invertebrate density and invertebrate diversity. My results indicate substantial variation in trophic resource use among individuals, populations, and species of small-bodied fishes that are typically assumed to be generalist insectivores. Variation in diet specialization may be more widespread than previously considered, and ecological opportunity is an important factor in shaping trophic resource use of individuals and populations. Overall, the results indicate that even subtle and gradual

environmental variation can induce substantial variation in phenotypes on a relatively small spatial scale.

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Chapter 1 - Body shape variation of two species of darter and its relation to the environment

Introduction

Environmental variation affects biological processes at all levels of organization, scaling from subcellular biochemical processes to ecosystem dynamics. From an evolutionary perspective, spatial environmental variation is an important source of divergent selection driving phenotypic evolution, local adaptation, and even speciation (Kawecki and Ebert 2004; Rundle and Nosil 2005; Schluter 2000). Evolutionary responses to divergent selection are often studied in proximate habitats that differ in one or few key environmental parameters, whereby habitats are typically classified into dichotomous categories, such as cave *vs.* surface (Dowling et al. 2002; Tobler et al. 2008), low *vs.* high predation (Langerhans et al. 2007b; Reznick and Endler 1982), or benthic *vs.* limnetic habitats (Bernatchez et al. 1996; McKinnon and Rundle 2002). This approach has been extremely fruitful, leading to the documentation of a broad spectrum of abiotic and biotic factors shaping physiological, morphological, and life history traits in a wide variety of study systems (e.g., Langerhans and DeWitt 2004; Riesch et al. 2010; Schluter and Nagel 1995; Tobler et al. 2011; Vamosi 2005). For many systems, we are gaining an increasing understanding of both the functional significance of divergent traits (Ghalambor et al. 2004; Langerhans 2009) and their genomic underpinnings (Jones et al. 2012; Renaut et al. 2011). Moreover, evidence for the deterministic nature of natural selection comes from comparative studies indicating that independent evolutionary lineages show similar (i.e., convergent) patterns of trait differentiation when exposed to similar sources of divergent selection (Franssen 2011; Krabbenhoft et al. 2009; Landry and Bernatchez 2010; Tobler and Hastings 2011).

While the recognition of distinct habitat types has provided crucial insights about the role of abiotic and biotic environmental variation in shaping evolutionary trajectories of different populations, many organisms are exposed to more subtle and gradual, but often multifarious, sources of divergent selection. Here, we were interested in investigating whether two stream fish species exhibit geographic variation in body shape across Oklahoma's Ozark Highland ecoregion. In addition, we tested whether environmental variation across study sites predicts morphological variation and whether the two species show convergent patterns of differentiation across sites. Streams have long been recognized for exhibiting complex environmental gradients with abiotic and biotic factors co-varying from headwater streams to lowland rivers (Allan 1995; Vannote et al. 1980). The effects of environmental variation in streams on shaping the composition of biotic communities have been studied in great detail (e.g., Arrington et al. 2005; Godinho et al. 2000; Mykrä et al. 2007), but knowledge about the evolutionary effects on populations remains relatively scarce (but see Langerhans 2008; Schaefer et al. 2011).

We focused our study on two species of darters, the fantail darter and orange throat darter (family Percidae), that are common throughout the Ozark Highlands of Oklahoma. Darters are small-bodied stream fishes and among the most diverse freshwater fish groups in North America (Near et al. 2011). Most darters are sexually dimorphic, with males exhibiting bright nuptial coloration, and show a diversity of reproductive behaviors (Kelly et al. 2012). They mostly inhabit lotic environments in the United States east of the Rocky Mountains and generally have a benthic life style (Kuehne and Barbour 1983; Page 1983). Hence, darters exhibit a reduced swim bladder (Evans and Page 2003) and eco-morphological modifications mediating adaptation to variation in water depth, flow, and substrate composition (Carlson and Lauder 2011; Carlson and Wainwright 2010). Consequential to a benthic life style, many darter species have a low

propensity to disperse (Freeman 1995; Ingersoll et al. 1984; Reed 1968), which can also be reflected in strong population genetic structuring (Austin et al. 2011; Beneteau et al. 2009; Haponski et al. 2009). This high site fidelity renders darters as excellent study objects for examining phenotypic differentiation in response to environmental variation, as locally restricted divergent selection may outweigh the homogenizing effects of gene flow (Räsänen and Hendry 2008; Storfer et al. 1999).

We addressed the following questions: (1) Is there geographic (i.e., site-specific) variation in the body shape of *E. spectabile* and *E. flabellare*? We used geometric morphometric analysis of body shape to quantify geographic variation across 30 sites in the Ozark Highlands of Oklahoma. (2) Is intraspecific geographic variation correlated with environmental differences among sites? We tested whether morphological variation among populations is correlated with environmental variation using a dual approach. In addition, we tested for effects of geographic distance on body shape differences among populations, as spatially proximate populations may exhibit more similar body shapes due to potential homogenizing effects of gene flow. (3) Is there a signal of convergent evolution in morphological variation between species, and what environmental characteristics correlate with convergent aspects of body shape? Analyzing *E. spectabile* and *E. flabellare* from sites where both species occur sympatrically, we identified shared site-specific morphological variation between the species. We tested whether potential convergence in morphology was correlated with environmental conditions to identify factors potentially driving convergent body shape evolution.

Methods

Study species

Etheostoma spectabile (Orangethroat darter, Figure 1.1a) is a robust darter of the subgenus *Oligocephalus*, exhibits pronounced sexual dichromatism, and reaches up to 74 mm in standard length (Kuehne and Barbour 1983; Page 1983). The species lives in shallow gravel and cobble riffles of small to medium sized streams, but both young and adults can also occupy pools with little or no flow (Winn 1958; Ceas and Page 1997). *Etheostoma spectabile* inhabits much of the central United States, ranging 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 variation in phenotypic traits, including morphology, physiology, and life history (Feminella and Matthews 1984; Marsh 1984; Ceas and Page 1997). In Oklahoma, this species spawns between early February and late May (Miller and Robison 2004).

Etheostoma flabellare (Fantail darter, Figure 1.2b) is part of 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). During the spawning season (from March to May; Miller and Robison 2004), breeding males develop fleshy knobs mimicking eggs on the spiny portion of the first dorsal fin, which are preferred by females (Knapp and Sargent 1989; Strange 2001).

Etheostoma flabellare is typically restricted to shallow riffles and fast flowing runs with larger substrates (Winn 1958; Hlohowskyj and Wissing 1986). This species is widely distributed in North America, ranging from southern Quebec to Minnesota, south to South Carolina, Alabama, and northeastern Oklahoma (Braasch and Mayden 1985).

Sample collection

Specimens were collected during the months of May and June of 2012 and 2013 at thirty different sites in the Ozark Highlands of northeastern Oklahoma (Figure 1.1c, Table 1.1). Collections were made towards the end of the reproductive season of both species; hence, samples from each of the sites likely included both reproductive and non-reproductive adults. We sampled tributaries of three major drainages: the Neosho-Grand River, the Illinois River, and Spavinaw Creek. Fish were collected using 15-20 min intervals of backpack electrofishing (LR-24 Electrofisher, Smith-Root, Inc., Vancouver, WA, USA) within 100 m stream transects. Upon collection, specimens were immediately euthanized using MS-222 and fixed in a 10% formaldehyde solution. In the lab, specimens were rinsed in water and stored in 70% isopropyl alcohol. Overall, we collected 1063 individuals (542 *E. spectabile* and 521 *E. flabellare*; see Table 1.1 for details).

Assessing environmental variation

Physical environmental characteristics were evaluated at each sampling location based on a protocol for low gradient streams (Barbour et al. 1999). We focused predominantly on quantifying physical habitat characteristics related to habitat size, flow, and substrates (Carlson and Wainwright 2010). Stream size was characterized by measuring maximum width and depth at each sampling location. Substrate composition was classified into six categories based on particle size (bedrock: solid bedrock; boulder: >256 mm; cobble: 64-256 mm; gravel: 2-64 mm; sand: 0.06-2 mm; silt: 0.004-0.06 mm) and was estimated visually as percent coverage along each sampling transect. Finally, two hydrological variables (slope and flow accumulation) were obtained from the USGS HYDRO1k database (<https://lta.cr.usgs.gov/hydro1k>) based on the latitude and longitude of the collection locations. Stream slope quantifies the maximum change

in elevation between the grid squares including the sample site and the eight neighboring grids. Flow accumulation describes the number of cells that flow into each down-slope cell and translates into upstream drainage area in square kilometers, so that sites with higher values accumulate more flow and are typically more channelized.

Prior to analyses, habitat size and hydrological variables were log-transformed, and substrate composition variables were arcsine-square-root-transformed. We performed a principal components analyses (PCA) on each group of variables (i.e., hydrological, habitat size, and substrates), such that axes constructed based on environmental variables could be correlated with morphological variation. In summary, hydrological PC scores described an environmental gradient from streams with a high slope and a high flow accumulation to streams with a low slope and low flow accumulation, and stream size PC scores described a gradient from wide and deep streams to narrow and shallow streams (Table A.1). PCA on substrate composition yielded two axes of variation (Table A.1). The first axis invariably described a substrate gradient from smaller particle sizes (silt, sand, and gravel) dominating to larger particle sizes (cobble, boulder, and bedrock) dominating. The second axis described a gradient from extremely small particle sizes dominating (sand, silt, bedrock and boulder) to intermediate particle sizes (gravel and cobble) dominating.

Assessing phenotypic variation

For geometric morphometric analysis, lateral photographs of preserved specimens (stored in isopropyl alcohol) were taken using a Canon EOS 400D Digital camera mounted on a copy stand. We digitized 16 morphological landmarks (Figure 1.1a & b) using the software program tpsDig (Rohlf 2004). Landmarks included (1) the tip of the upper jaw; (2) the center of the eye; (3) the posterior head region (nape); (4) the anterior and (5) posterior insertions of the first, spiny

dorsal fin; (6) the anterior and (7) the posterior insertions of the second, soft dorsal fin; (8) the dorsal and (9) ventral insertions of the caudal fin; (10) the posterior and (11) anterior insertions of the anal fin; (12) the anterior insertion of the pelvic fin; (13) the bottom of the head where the operculum breaks away from the body outline; (14) the posterodorsal corner of the operculum; and (15) the ventral and (16) dorsal insertions of the pectoral fin. Since the distance of the camera varied between specimens (to allow for maximum resolution irrespective of specimen size), we size-corrected all pictures by digitizing a 10 mm distance on a size standard in each picture and resizing landmark coordinates based on the number of pixels per millimeter.

We performed a geometric morphometric analysis based on the coordinates of the digitized landmarks (Zelditch et al. 2004). Landmark coordinates were aligned using least-square superimposition as implemented in the program tpsRelw (Rohlf 2007) to remove effects of translation, rotation, and scale. Based on the aligned coordinates, we calculated centroid size and relative warp scores for each individual. To address the questions outlined in the introduction, we conducted a series of analyses first for the two species separately, and then for the two species together. Note that different sets of specimens were used for these analyses, because some sites were only inhabited by one of the two species (Table 1.1). All statistical analyses were performed using SPSS version 20 (IBM Inc., Armonk, NY, USA) unless otherwise stated.

Analytical approaches: Intraspecific geographic variation

For intraspecific comparisons of body shape variation, relative warp scores were used as dependent variables in a multivariate analysis of covariance (MANCOVA). Assumptions of multivariate normal error and homogeneity of variances and covariances were met for all analyses performed. F -values were approximated using Wilks' lambda and effect strengths by use of partial eta squared (η_p^2). We also calculated the relative variance as the partial variance for

a given term divided by the maximum partial variance value in a model (Langerhans and DeWitt 2004). We included “sex” and “site” as independent variables, and used “centroid size” as a covariate to control for multivariate allometry. If there were significant geographic variation in body shape, we would expect significant effects of the factor “site” in these analyses. For visualization of body shape variation among sites, we calculated divergence scores for each individual along two orthogonal site divergence vectors as defined by Langerhans (2009).

We used two complementary analytical approaches to uncover potential correlations between morphological variation and environmental characteristics. First, we used partial Mantel tests, as implemented in FSTAT (Goudet 2002), with 10,000 randomizations to correlate morphological similarity between sites with environmental similarity and geographic distance (Tobler and Carson 2010). Morphological similarity was quantified by calculating pairwise distances between sites in morphospace based on relative warp scores used in the MANCOVAs described above. Effects of “sex” and “centroid size” were first removed with a preparatory MANCOVA, and residuals were then used to calculate site-specific means. Pairwise Euclidean distances were then calculated between all site pairs, and the resulting phenotypic distance matrices were used as dependent variables in the partial Mantel tests. Predictor matrices were based on environmental and geographic distance matrices. To obtain the environmental distance matrix, environmental data (PC scores for hydrological, stream size, and substrate variables; see Table A.1) from each site were used to calculate pairwise environmental distances, as for the phenotypic data. Finally, the geographic distance matrix was obtained through ArcGIS (version 10.0) using the origin-destination cost matrix analysis implemented in the Network Analyst extension. We used a GIS-based streams layer obtained from the Oklahoma Water Resources Board (<http://www.owrb.ok.gov/maps/data/owrbdata.php>) to create a network dataset that

allowed us to measure stream distance (instead of straight line distance) between all pairwise sampling points. Geographic distances were log-transformed. The partial Mantel test approach allowed for disentangling the potential roles environmental similarity and geographic distance play in shaping morphological variation. Geographic distance might be a key explanatory variable, because more proximate sites could be more similar in environmental conditions and share more gene flow, which would counteract phenotypic differentiation.

While correlating distance matrices allows for the consideration of environmental similarity and geographic distances simultaneously, this approach has the disadvantage that specific environmental factors correlating with morphological variation cannot be identified. Hence, we also determined the relationship between environmental variables and body shape through a two-block partial-least-squares analysis (PLS) (Bookstein et al. 2003; Rohlf and Corti 2000). PLS reduces data dimensionality by creating new linear combinations of the dependent and independent variables, and singular axes are generated to maximize the co-variation between two sets of variables (i.e., morphological and environmental). We performed a PLS as implemented in the program tpsPLS (Rohlf and Corti 2000). Population-specific estimated means of aligned landmark coordinates (corrected for sex and centroid size with MANCOVA models as described above) were used as dependent variables. The PC scores summarizing environmental variation for each site were used as independent variables. A null distribution for establishing the significance of the correlation coefficients was determined by performing 10,000 random permutations of the morphological and environmental variables, and we compared the actual correlation between morphology and environment to this null distribution to obtain *P*-values.

Analytical approaches: Convergent geographic variation

To uncover potential convergent patterns of body shape variation between *E. spectabile* and *E. flabellare*, we realigned coordinates of individuals collected from sites with both species ($N=721$) and calculated relative warp scores. Relative warp scores were subjected to MANCOVA with “sex”, “species”, and “site” as independent variables. Centroid size was included as a covariate to control for allometry. We calculated individual divergence scores based on site divergence vectors for visualization of shape variation as for analyses described above. These scores represent shared (i.e., convergent) site-specific phenotypic variation between species across sites. We tested for correlations between shared morphological variation and environmental variables using the same approaches outlined above.

Results

Geographic variation: Etheostoma spectabile

Analyzing body shape variation across *E. spectabile* populations using MANCOVA indicated significant effects of size, sex, site, as well as the interaction between sex and site (Table 1.2a). Sex, centroid size, and site explained the bulk of variation in our dataset. The nature of sexual dimorphism in body shape is visualized in Supplementary Figure A.1. The nature of shape variation among sites is illustrated along the first two site-specific divergence axes of shape variation using thin plate spline transformation grids (Figure 1.2). Along the first axis of shape variation, *E. spectabile* populations varied particularly in body height and the size of the caudal peduncle. Along the second axis, they varied in terms of head position and the degree of dorsal arching.

A Mantel test correlating phenotypic similarity among all pairwise population comparisons with environmental similarity and geographic distance explained 11% of variation in body shape. Phenotypic similarity was significantly and positively correlated with environmental similarity across sites ($r = 0.302$, $P < 0.001$). In contrast, geographic proximity of populations did not significantly predict similarity in body shape ($r = 0.053$, $P = 0.324$). The PLS analysis produced four dimensions of co-variation between body shape and environmental variables, of which only the second pair of singular axes explained significantly more co-variation than would be expected by chance (Table 1.3a). The second dimension accounted for 42% of the co-variation between the two blocks, and the correlation between the two variable blocks was 0.76 ($P = 0.02$). Correlation between body shape and environmental variable vectors indicated that populations varied particularly along a gradient of substrate composition and stream size (Figure 1.3). The body shapes associated with positive values (larger streams with smaller substrate particles) were predominantly characterized by higher bodies, and smaller, more down-turned heads (Figure 1.3). Note that the significant correlation between variable blocks was partially driven by an outlier site (see Figure 1.3; Sager Creek). Excluding this outlier did not yield qualitatively different results (i.e., variation explained by PLS analysis still primarily pertained to the substrate gradient described by PC axis 1 and changes in body height and head shape), however, correlation between variable blocks was marginally non-significant ($P = 0.07$).

Geographic variation: *Etheostoma flabellare*

MANCOVA of shape variation in *E. flabellare* indicated significant effects of size, sex, site, as well as the interaction between sex and site (Table 1.2b). Variation in body shape was largely explained by sex and size, and to a lesser extent explained by site (Table 1.2b). The

nature of sexual dimorphism is visualized in Supplementary Figure A.1, and shape variation across sites is visualized in Figure 1.4. Along the first axis of shape variation, *E. flabellare* populations particularly varied in body height and the shape of the caudal peduncle. Along the second axis, they varied in terms of head position and the degree of body arching.

A partial Mantel test correlating phenotypic similarity among *E. flabellare* populations with environmental similarity and geographic distance explained 10 % of variation. Both environmental similarity ($r = 0.273$, $P < 0.001$) and geographic distance ($r = 0.148$, $P = 0.034$) explained similarity in body shape. PLS analysis of *E. flabellare* yielded four dimensions of co-variation between the environmental and body shape variables, of which only the first pair of singular axes exhibited significantly more co-variation than expected by chance ($P = 0.02$). This dimension accounted for 53% of the co-variation between the two blocks (Table 1.3b), and the correlation between the two variable blocks was 0.85 ($P = 0.02$). As for *E. spectabile*, body shape variation in *E. flabellare* particularly varied along a gradient of substrate composition (Table 1.3b). Body shapes associated with positive values (larger streams with larger and more homogenous substrates) are primarily characterized by a posterior shift in the dorsal fin insertions and the dimensions of the caudal peduncle (Figure 1.5).

Patterns of convergence

Analyzing body shape variation with both species combined revealed a significant and strong effect of species, explaining the bulk of shape variation (Supplementary Figure A.1). In addition, size and sex, as well as all interaction terms had significant effects (Table 1.2c). Most importantly though, we detected a small but significant effect of the factor “site”, representing convergent body shape variation between the two species across different collection locations. Assessment of thin plate spline transformation grids indicated that the convergent aspect of body

shape particularly pertained to body height, the proportions of the caudal peduncle, and the degree of arching of the body (Figure 1.6). The PLS revealed no significant correlations between convergent aspects of body shape and environmental variables (Table 1.3c).

Discussion

Our study uncovered significant geographic variation in body shape of two species of darters across stream sites in Oklahoma's Ozark Highlands. In both species, body shape variation across sites was correlated with environmental variables. We documented both convergent and unique patterns of shape differences in the two species wherever they occurred sympatrically. Overall, our study highlights geographic variation in phenotypes in response to subtle and gradual, but complex environmental gradients within a relatively small spatial scale. The mechanisms underlying geographic variation in the *Etheostoma* species investigated here remain unclear. Phenotypic differences could be entirely due to phenotypic plasticity (Pigliucci 2001), as the presence of predators and variation in flow regimes affect developmental trajectories and the expression of alternate body shapes within populations (Pakkasmaa and Piironen 2001; Eklöv and Jonsson 2007; Burns et al. 2009). Alternatively, local adaptation and the evolution of heritable differences in body shape in response to divergent selection from environmental habitat differences even on small spatial scales is not unprecedented (Woods 2007; Janhunen et al. 2009; Langerhans 2009; Palacios et al. 2013). Additional studies exploring the mechanistic underpinnings of darter shape variation are warranted.

Body shape variation among populations of both *E. spectabile* and *E. flabellare* was significantly correlated with local environmental conditions. In contrast, geographic distance among populations explained similarity in body shape only in *E. flabellare*. This could be indicative of higher population connectivity in *E. flabellare*, even though movement studies

indicate high site fidelity (Ingersoll et al. 1984). Estimates of gene flow and potential population genetic structuring would facilitate uncovering potential relationships between population connectivity and phenotypic divergence in the future. In general, the relatively strong correlations between phenotypic and environmental similarity (compared to geographic distance) indicate that shared features among populations are likely due to parallel evolution in response to similar environmental factors rather than gene flow among proximate populations. This result is congruent with the known low dispersal potential and high site fidelity of darter species (e.g., Ingersoll et al. 1984), and the low propensity of darters to move could in fact be crucial in facilitating local adaptation on small spatial scales.

Overall, populations of *E. spectabile* and *E. flabellare* particularly varied in the proportions of the caudal peduncle, body height, the shape and position of the head, as well as the degree of arching of the body (see Figures 1.2 and 1.4). However, only a subset of that variation was actually correlated with environmental variables. Partial least squares analyses indicated that body shape in both species was primarily related to substrate composition and – to a lesser degree – with stream size, which is not unexpected considering that darters generally are benthic fishes inhabiting lotic environments (Carlson and Wainwright 2010; Carlson and Lauder 2011). Along a substrate gradient from smaller (silt, sand, and gravel) to larger particle sizes (cobble, boulder, and bedrock), *E. spectabile* populations changed in body height, head size and position, as well as the shape of the caudal peduncle (Figure 1.3). In contrast, populations of *E. flabellare* mostly varied in the position and length of the dorsal fins, as well as the depth of the caudal peduncle (Figure 1.5). Many of these morphological differences have clearly established functional consequences based on theoretical and empirical studies of fish body shapes (see Domenici and Kapoor 2009 for an overview). Both variation in caudal peduncle proportions and

body height have been associated with tradeoffs between steady and unsteady swimming (Domenici 2003; Langerhans 2009). Specifically, narrow, tapered caudal peduncles and increased anterior portions of the body act to minimize drag and maximize thrust during steady swimming, while high bodies and wide caudal regions increase stability during rapid turns and maximize velocity and acceleration during fast-starts (Langerhans 2008). In contrast, the functional consequences of variation in head position and the degree of body arching are less well studied, but population differences in these traits were strikingly similar to behavioral responses of *E. flabellare* and *E. tetrazonum* exposed to varying flow conditions (see Carlson and Lauder 2011). In these flow plume experiments, individuals exposed to low flows held their head slightly upward or parallel with the substrate and the dorsal line remained relatively straight. As flow increased, they lowered their head downward and their bodies becomes arched dorsally (Carlson and Lauder 2011). Overall, the morphological responses detected in both species are congruent with studies documenting body shape variation in response to differential flow regimes, and the nature of body shape variation documented here insinuates that flow regimes likely play an essential role in influencing body shape across darter populations. Substrate composition likely serves as an indicator of local flow conditions, and the presence of different substrates sizes may also directly modulate darter body shape, since particle size likely determines the availability of low flow microhabitats where darters can effectively hold their position even in swift waters (Carlson and Lauder 2011).

Even though we uncovered trait-environment correlations in both species examined, our analyses also indicate that a majority of variation in body shape remains unexplained, such that other factors likely influence geographic variation documented here. Unexplained shape variation could be attributed to differences in the reproductive state or body condition among

sites (Wesner et al. 2011). Darters also diversified in trophic resource use (Carlson and Wainwright 2010), and character displacement in response to the presence of congeners has been documented in various clades (Knouft 2003; Carlson 2008; Carlson et al. 2009). Hence, variation in resource availability and darter communities across sites may affect eco-morphological traits in *E. spectabile* and *E. flabellare*, and future studies need to address how resource competition and niche space may influence phenotypic evolution. Furthermore, shape variation could also be affected by sexual selection. Male darters are known to exhibit strong territorial behavior during breeding season, and there is evidence of intra- and interspecific competition for breeding sites (Ingersoll et al. 1984). In addition, there is intersexual selection in many darter species (e.g., Williams and Mendelson 2011), and female *E. flabellare* show mating preferences for males exhibiting egg-mimicking knobs on the dorsal fin (Knapp and Sargent 1989). Variation in environmental characteristics among sites could modulate the intensity of intrasexual selection and female preferences, ultimately precipitating in population differences in body shape.

Along with significant geographic variation in both species, our data indicate that there is a small – but significant – signal of convergent evolution in *E. spectabile* and *E. flabellare* from the same locality. Shared aspects of body shape include body height, proportions of the caudal peduncle, as well as the shape and angle of the head (Figure 1.6). However, convergent aspects of body shape variation were not correlated with any of the environmental variables included in this study. These results support the idea that shared environmental conditions can cause two species to converge toward similar phenotypic characteristics, but considering that *E. spectabile* and *E. flabellare* body shapes varied differently along the gradient of substrate composition (see results from PLS analyses above), non-convergent responses to shared sources of selection seem to be prevailing in this system. The balance between convergent and non-convergent

evolutionary responses to shared sources of selection can be shaped by a variety of factors (Kaeuffer et al. 2012). For example, non-convergent responses may be caused by the effects of genetic drift (De Brito et al. 2005), or by differences in genetic variation and genomic architecture between species that favor alternative evolutionary trajectories (Schluter 1996). In addition, non-convergent responses may be caused by hidden ecological variation, and even though we analyzed both species from the same sites, the two species may actually be exposed to slightly different sources of selection. Although we found significant correlations between darter body shape and environmental variables that were assessed at the stream reach scale, natural selection likely operates at the microhabitat scale to shape body shape evolution. For example, different microhabitat use and adaptation to those microhabitats may in part explain the non-convergent changes in body shape along the gradient of substrate composition as well as the large proportion of unexplained body shape variation within each of the species. Indeed, *E. spectabile* utilizes multiple microhabitat types, ranging from riffles and riffle margins to slow moving pools and runs (Pratt and Lauer 2013), and it typically forages on the surface of the substrate (Dewey 1988). In contrast, *E. flabellare* is restricted to riffles and uses interstitial crevices to avoid predation and exposure to direct flow (Dewey 1988). Hence, future studies linking environmental variation and body shape variation in benthic stream fishes should consider quantifying environmental factors at the microhabitat scale to better understand the selective forces shaping trait variation in different species.

Overall, our results are consistent with the growing body of literature documenting variation in fish body shape in response to abiotic and biotic environmental factors (Langerhans et al. 2004; Hendry et al. 2006; Langerhans et al. 2007a; Tobler et al. 2008; Tobler and Carson 2010; Fluker et al. 2011). To date, most studies on evolutionary responses to selection have

focused on dichotomous environmental parameters, when in reality selective forces are often multifarious. This study highlights that even subtle and continuous environmental variation can elicit organismal responses in the expression of phenotypes (also see Langerhans 2008; Schaefer et al. 2011; Haas et al. 2015). Future studies should combine field assays of morphological and environmental variation with population genetic analyses and laboratory experiments in a broad collection of species to uncover generalities about organismal responses to stream and river gradients.

Figures and Tables

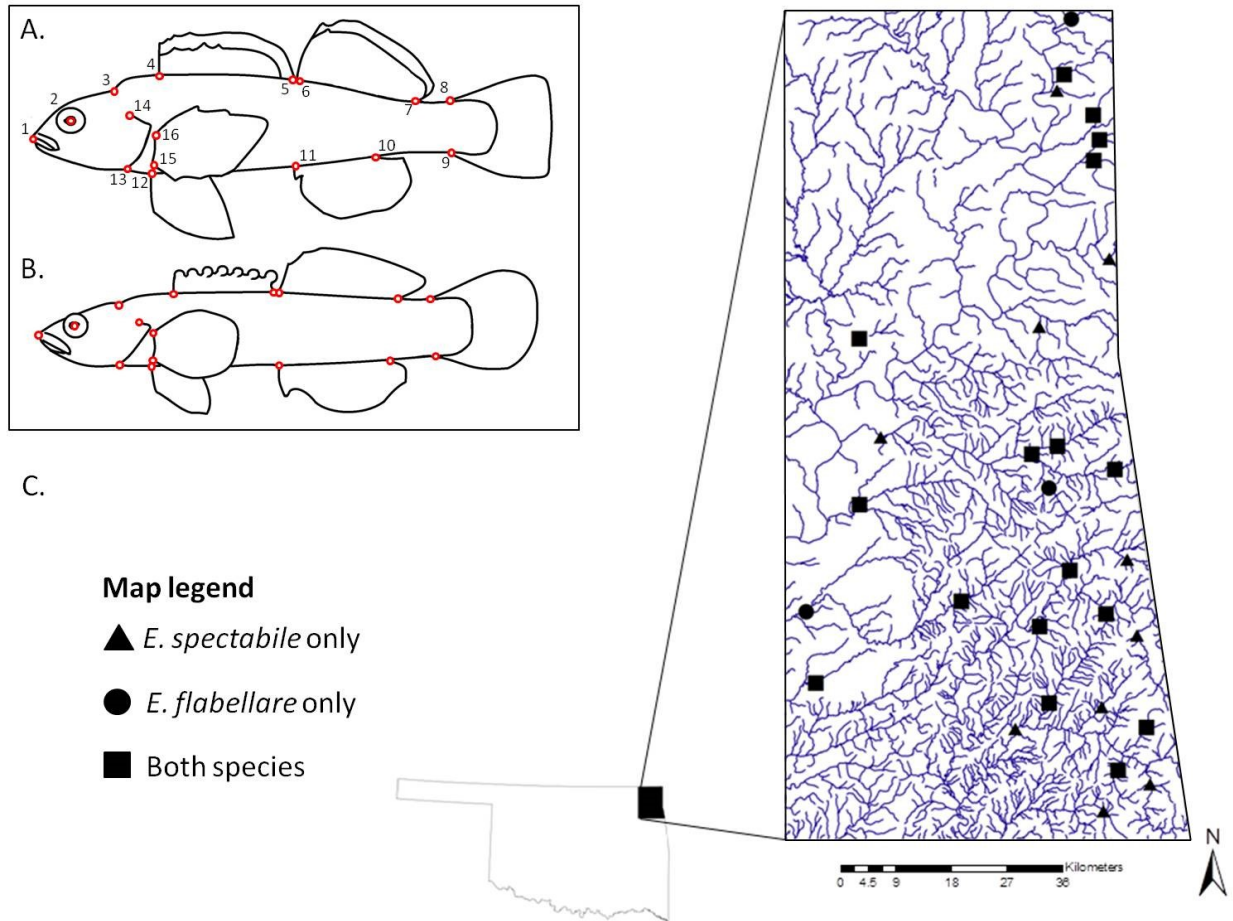


Figure 1.1 Body outlines of male specimens of (a) *Etheostoma spectabile* (including landmark locations used for geometric morphometric analyses; see main text for landmark descriptions) and (b) *E. flabellare*. (c) Map of the collection sites in the Ozark Mountains in northeastern Oklahoma

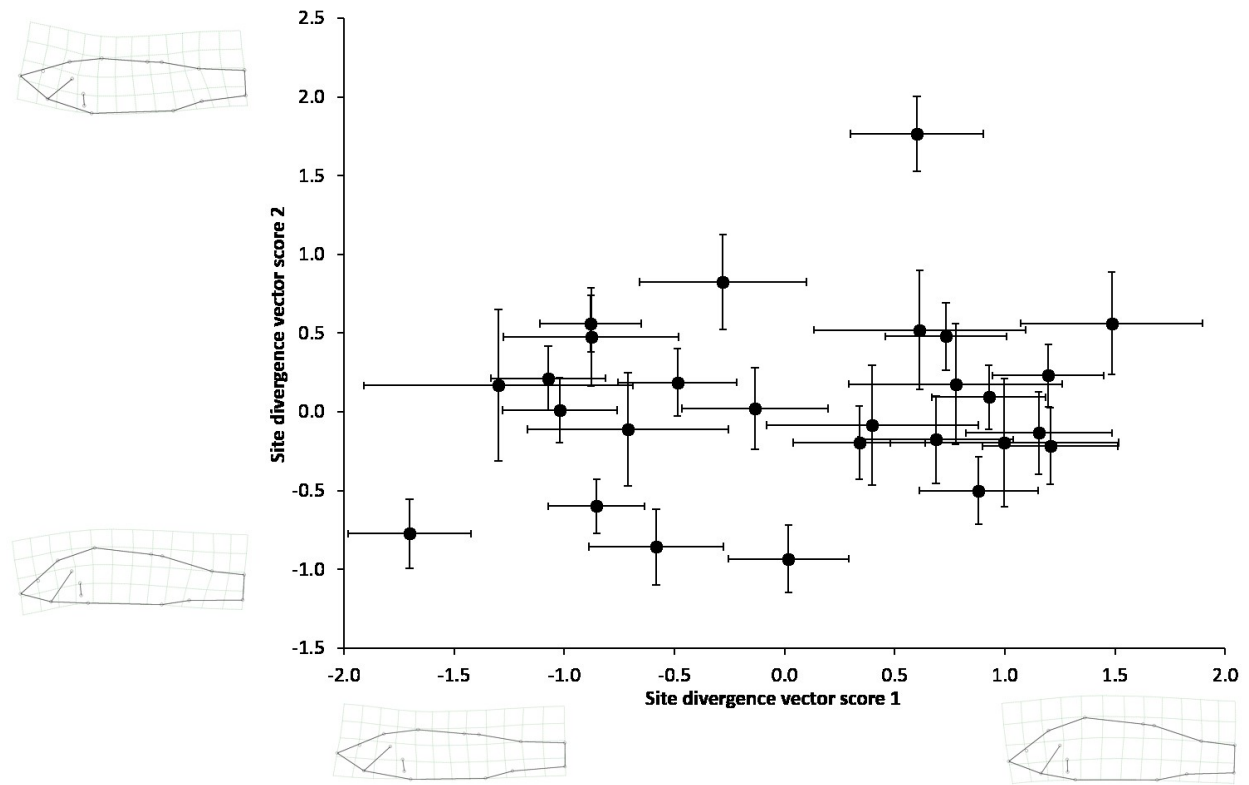


Figure 1.2 Phenotypic variation in *E. spectabile* from different sites investigated. Depicted are site-specific estimated marginal means (\pm SEM) of individual divergence vector scores (IDS1 on x-axis, IDS2 on y-axis). The thin plate transformation grids illustrate shape variation along each divergence axis.

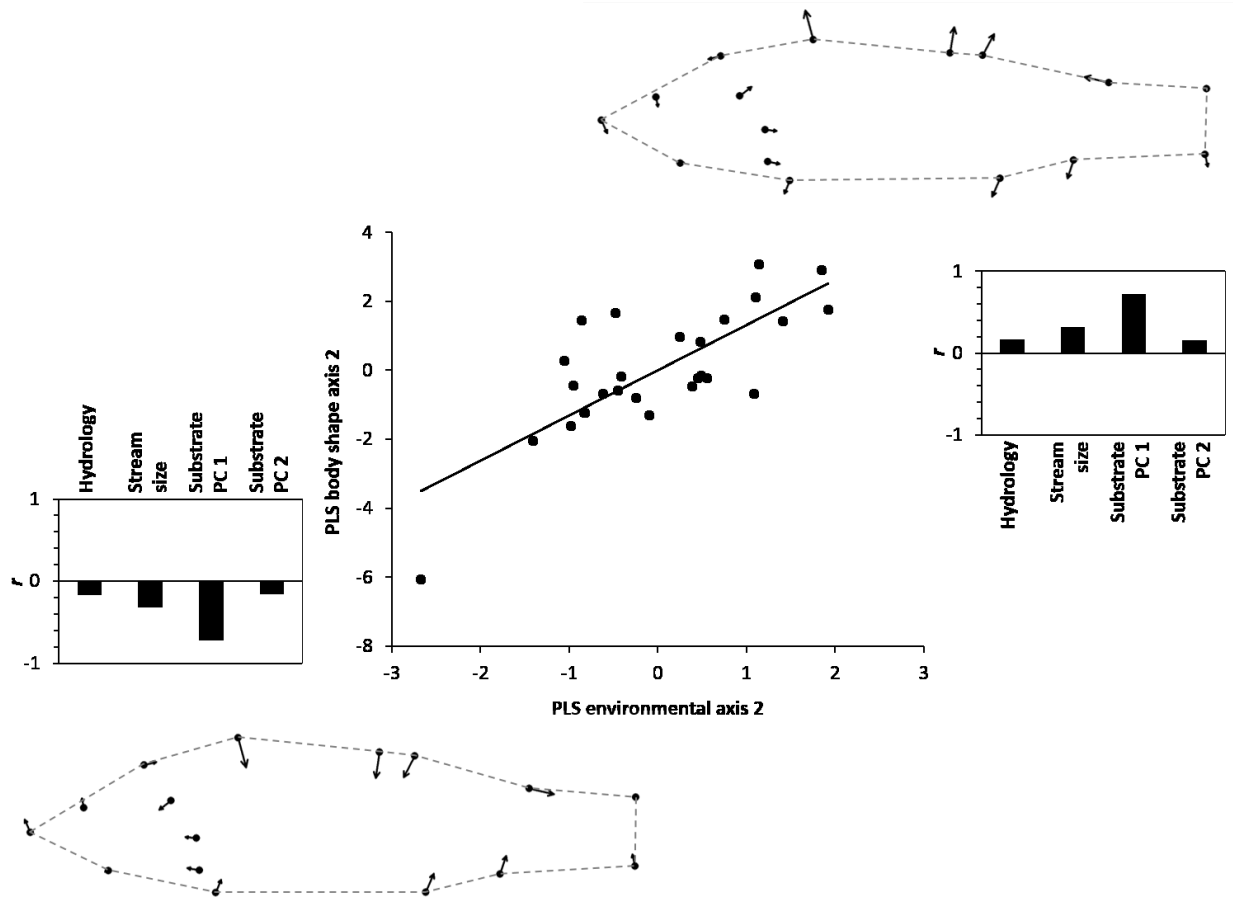


Figure 1.3 Correlation of coupled partial least square latent vectors of body shape and environment for different populations of *E. spectabile* ($r = 0.76$, $P = 0.02$). Vector diagrams illustrate the body shapes at either extreme of the latent body shape vectors. Histograms depict the correlation of environmental variables with the corresponding body shape.

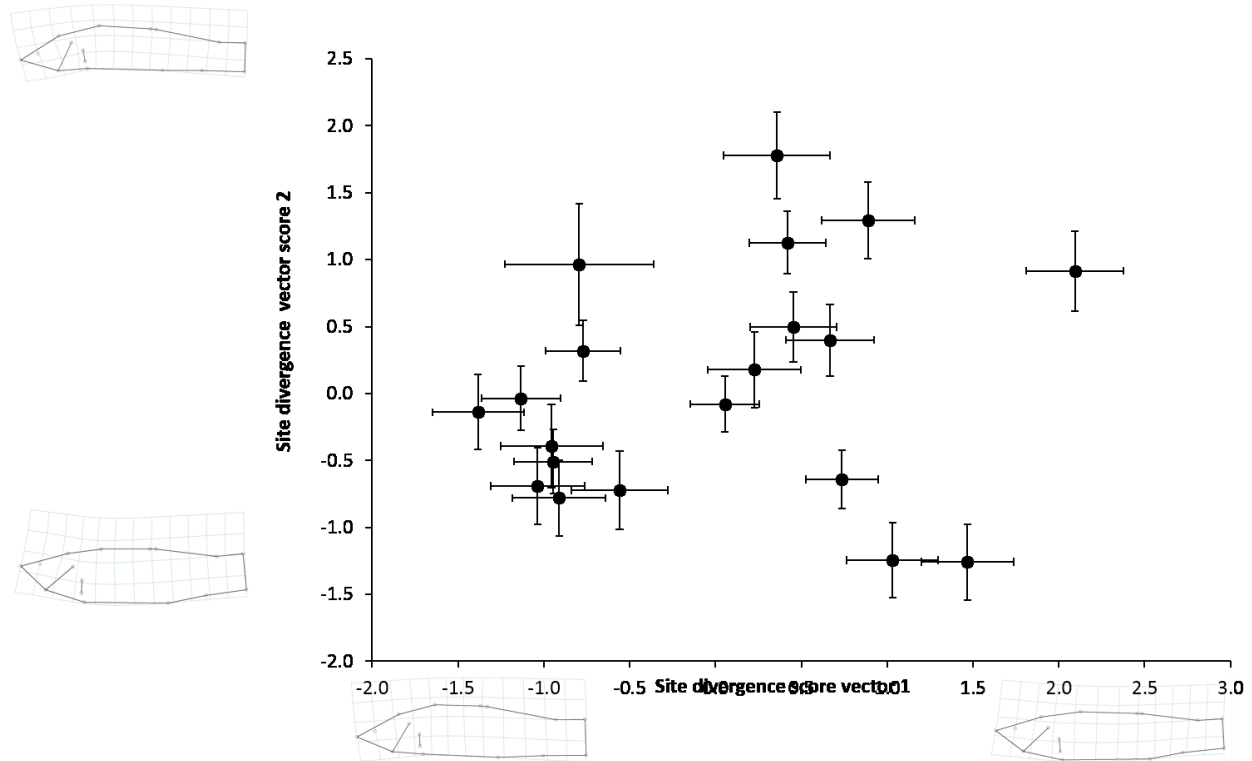


Figure 1.4 Phenotypic variation in *E. flabellare* from different sites investigated. Depicted are site-specific estimated marginal means (\pm SEM) of individual divergence vector scores (IDS1 on x -axis, IDS2 on y -axis). The thin plate transformation grids illustrate shape variation along each divergence axis.

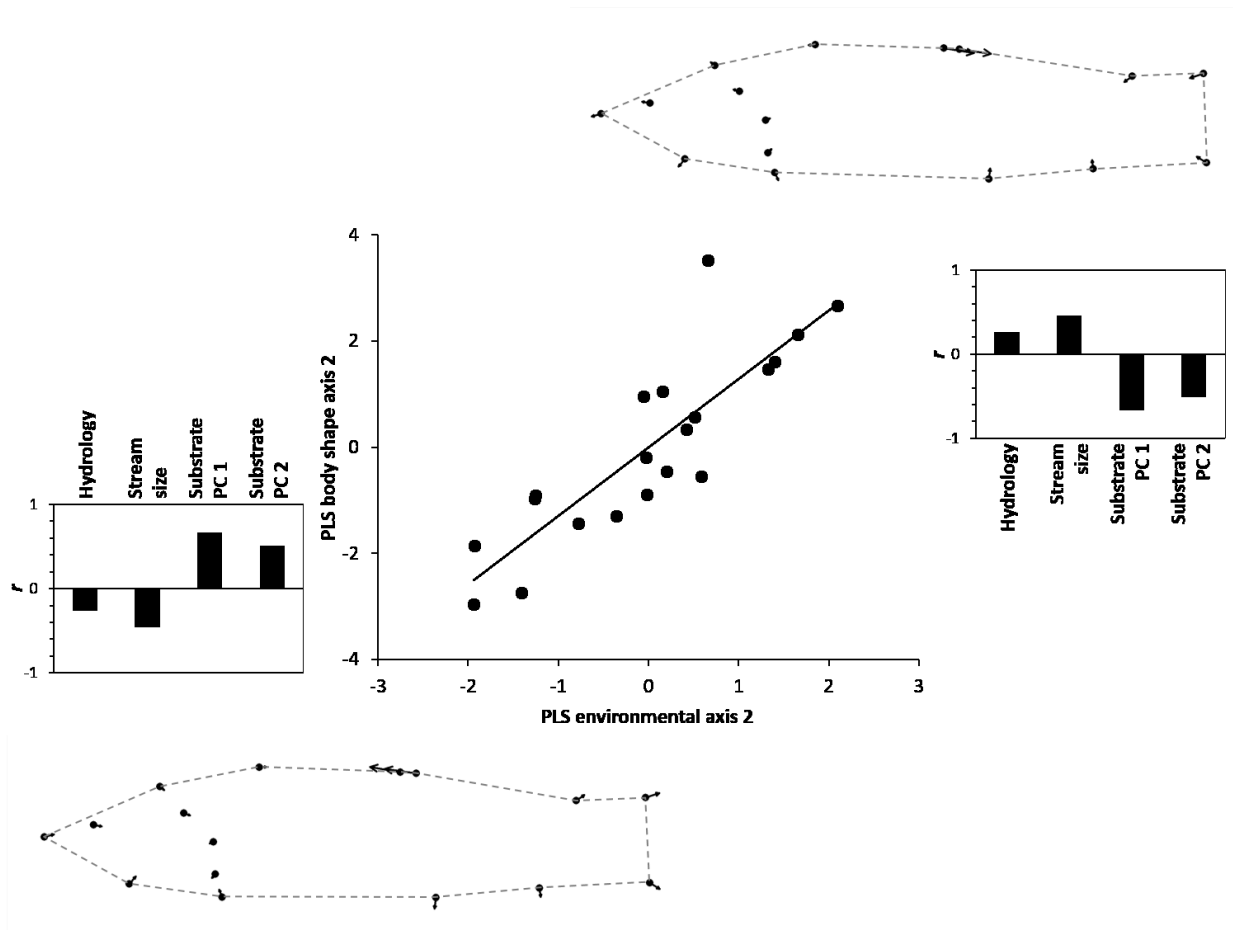


Figure 1.5 Correlation of coupled partial least square latent vectors of body shape and environment for different populations of *E. flabellare* ($r = 0.85$, $P = 0.02$). Vector diagrams illustrate the body shapes at either extreme of the latent body shape vectors. Histograms depict the correlation of environmental variables with the corresponding body shape.

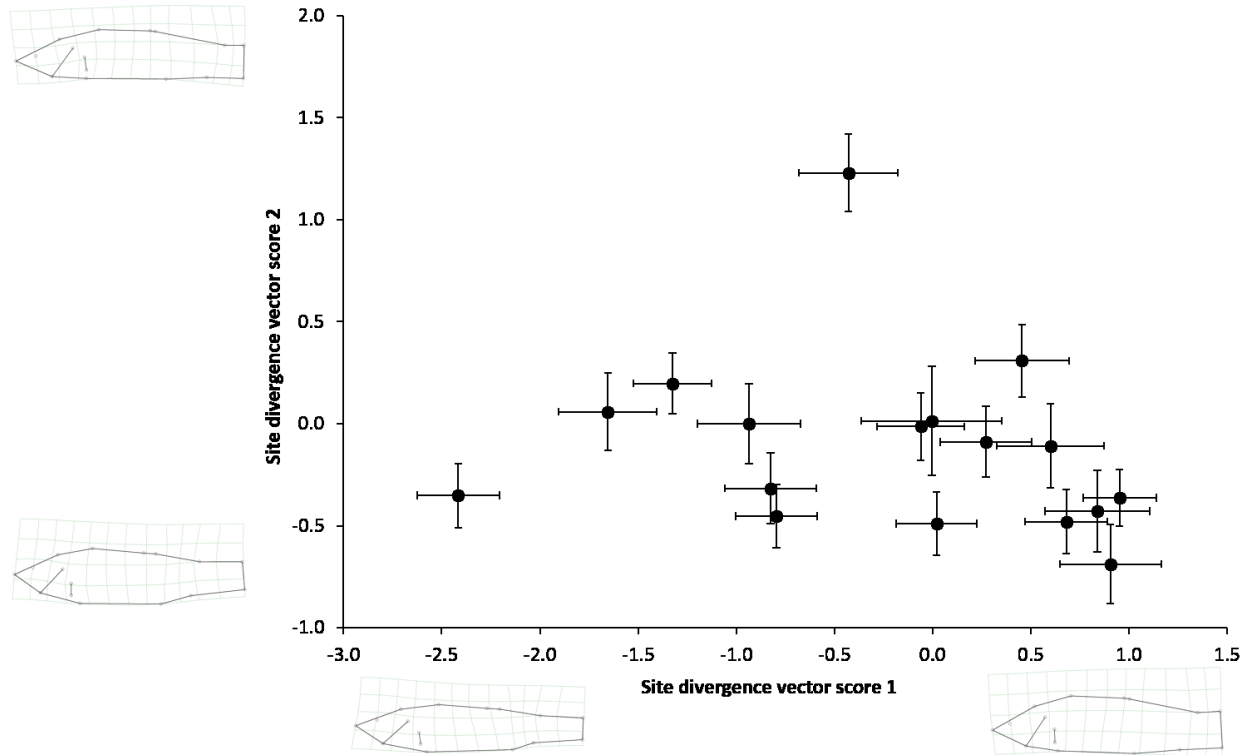


Figure 1.6 Shared (i.e., convergent) phenotypic variation between *E. spectabile* and *E. flabellare* from different sites investigated. Depicted are site-specific estimated marginal means (\pm SEM) of individual divergence vector scores (IDS1 on x -axis, IDS2 on y -axis). The thin plate transformation grids illustrate convergent aspects shape variation along the divergence axis

Table 1.1 List of collection sites with latitude and longitude based on GPS coordinates. Sites are grouped according to river drainages. For each site, the number of *E. spectabile* and *E. flabellare* are also listed

Name/Location	Latitude	Longitude	<i>E. spectabile</i>		<i>E. flabellare</i>	
			Males	Females	Males	Females
Illinois River drainage						
Ballard Creek	36.09138	-94.58956	15	10		
Baron Fork at Camp Egan	35.95843	-94.81205	26	14		
Caney Creek	35.83789	-94.65479	15	14		
Evansville Creek	35.87596	-94.57000	35	11		
Peacheater Creek @ 4700	35.98862	-94.65512	15	6		
Peavine Creek	35.89667	-94.62730	10	10	11	13
Sager Creek	36.20124	-94.60518	14	12		
Shellbranch Creek	35.95813	-94.57475	10	3	9	10
Tributary of Illinois near chance road	36.12270	-94.64512	7	5	20	23
Tributary to Flint Creek	36.18657	-94.70946	19	9	12	16
Tributary of Illinois North of Chewey	36.10606	-94.76579	4	1	20	14
Tyner Creek	35.99514	-94.75010	4	5	9	12
Neosho-Grand River drainage						
Brush Creek	36.77839	-94.65563	8		12	12
Buffalo Creek	36.63608	-94.62994	14	12		
Clear Creek	36.02794	-95.17210	4	4	11	16
Five Mile Creek	36.98299	-94.69307			15	8

Flint Branch	36.88017	-94.72110	15	6		
Little Saline Creek	36.28513	-95.08994	26	3	17	21
Lost Creek	36.84374	-94.65531	9	8	16	8
Mustang Creek	36.52480	-95.08723	15	15	10	11
Spring Creek #2	36.13083	-95.18826			10	8
Spring Creek at Rocky Ford State Park	36.14394	-94.90664	7	10	11	12
Sycamore Creek	36.80793	-94.64468	5	10	15	24
Warren Branch	36.90317	-94.70763	7	2	13	13
Whitewater Creek	36.53923	-94.75930	12	12		
Spavinaw Creek drainage						
Beaty Creek #2	36.36610	-94.72894	13	7	15	18
Beaty Creek at 456 Bridge	36.35522	-94.77576	2	9	14	10
Cloud Creek	36.30595	-94.74545			13	10
Spavinaw Creek	36.33155	-94.62527	4	4	4	5
Tail waters of Spavinaw Lake	36.38228	-95.05000	16	9		

Table 1.2 Results of multivariate analyses of covariance examining body shape variation in *E. spectabile* (a), *E. flabellare* (b), and both species combined (c). Effects with a relative variance >0.3 are highlighted in bold

Effect	<i>F</i>	Hypothesis <i>df</i>	Error <i>df</i>	<i>P</i>	η_p^2	Relative variance
<u>a. <i>E. spectabile</i></u>						
Centroid size	15.872	28.0	450.0	<0.001	0.497	1.00
Sex	8.349	28.0	450.0	<0.001	0.342	0.69
Site	2.590	728.0	8712.0	<0.001	0.134	0.27
Sex × Site	1.223	700.0	8524.7	<0.001	0.069	0.14
<u>b. <i>E. flabellare</i></u>						
Centroid size	11.285	28.0	459.0	<0.001	0.408	0.82
Sex	16.074	28.0	459.0	<0.001	0.495	1.00
Site	3.675	532.0	7313.8	<0.001	0.178	0.36
Sex × Site	1.382	532.0	7313.8	<0.001	0.076	0.15
<u>c. Combined analysis</u>						
Centroid size	13.773	28.0	626.0	<0.001	0.381	0.41
Sex	11.094	28.0	626.0	<0.001	0.332	0.36
Species	303.503	28.0	626.0	<0.001	0.931	1.00
Site	2.960	448.0	8779.7	<0.001	0.115	0.12
Sex × Species	8.498	28.0	626.0	<0.001	0.275	0.30
Sex × Site	1.231	448.0	8779.7	0.001	0.052	0.06

Species × Site	1.914	448.0	8779.7	<0.001	0.078	0.08
Sex × Species × Site	1.323	420.0	8353.7	<0.001	0.055	0.06

Table 1.3 Results of partial least squares analyses examining the co-variation between environmental variables and body shape for *E. spectabile* (a), *E. flabellare* (b), and both species combined (c). Dimensions with significant co-variation are highlighted in bold.

	PLS dimension			
	1	2	3	4
<u>(a) <i>E. spectabile</i></u>				
Hydrology	-0.210	0.167	-0.351	0.358
Stream size	-0.403	0.320	0.460	0.108
Substrate PC1	0.091	0.720	-0.169	-0.180
Substrate PC2	0.612	0.160	0.207	0.221
Singular value	0.429	0.359	0.169	0.042
Correlation	0.698	0.766	0.629	0.598
<i>P</i>	0.364	0.021	0.242	0.160
<u>(b) <i>E. flabellare</i></u>				
Hydrology	-0.259	-0.211	0.114	0.500
Stream size	-0.459	0.353	0.362	-0.120
Substrate PC1	0.670	-0.342	0.295	-0.043
Substrate PC2	0.511	0.658	-0.004	0.203
Singular value	0.533	0.285	0.100	0.082
Correlation	0.857	0.829	0.533	0.620
<i>P</i>	0.029	0.074	0.965	0.568
<u>(c) Combined</u>				

Hydrology	-0.150	-0.245	0.693	-0.218
Stream size	-0.471	-0.074	0.324	0.324
Substrate PC1	0.726	-0.347	0.219	0.153
Substrate PC2	0.255	0.706	0.382	0.034
Singular value	0.440	0.287	0.224	0.049
Correlation	0.832	0.808	0.813	0.562
<i>P</i>	0.187	0.321	0.094	0.853

Chapter 2 - Patterns of diet variation and individual specialization in two darter species

Introduction

Ecologist and evolutionary biologists have long investigated the dietary habits of fishes to study mechanisms of species coexistence 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 generalists feeding on 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 behavioral 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, diet use of fish species can be highly variable through space and time depending on resource availability (Godinho et al. 1997), and even dietary specialists can maintain a generalist feeding strategy 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 dietary specialization and a reduction in interspecific niche

overlap increase 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). Within population variation in diet use occurs when individuals in a given population specialize on specific diet items and partition resources with other individuals of the same species (Roughgarden 1974), and analyses of such intraspecific variation in diet composition have recently increased to understand diet specialization across a broad range of taxa (Bolnick et al. 2002; Svanbäck and Persson 2004; Araújo et al. 2011). Individual specialization may particularly occur in populations that demonstrate high phenotypic variability, allowing some individuals to be more efficient at exploiting a particular subset of the total species niche (Van Valen 1965). Frequency-dependent intraspecific competitive interactions can then favor uncommon strategies of individuals exploiting underutilized subsets of the population's overall niche, leading to balancing selection and the maintenance of variation within populations (Bolnick 2001). Alternatively, individuals may become more specialized following the expansion of a population's total 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 wider niches to display higher degrees of individual specialization (Lister 1976), but it remains unclear how biotic interactions and other environmental factors influence individual diet specialization (Araújo et al. 2011; Evangelista et al. 2014).

Darters belong to the family Percidae and are small, often brightly colored stream fishes with a benthic lifestyle. Most darters completely lack 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), 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). Diet 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 specialization within species may be high and trophic niche specialization among sympatric species may be low. Explicit tests of such hypotheses remain mostly lacking though.

In this study we explore diet variation and individual diet specialization of two sympatric species of darters, *Etheostoma spectabile* and *E. flabellare*, occurring at multiple stream sites in eastern Oklahoma. 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 and does trophic resource use 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) Is there variation in the degree of individual specialization among populations and species of darters? Based on its more generalistic ecology, we expected to find higher degrees of individual specialization in *E. spectabile* than in *E. flabellare*. Furthermore, the degree of individual specialization should vary among sites, because local environmental conditions should influence resource availability and suitable foraging habitats. (3) Is variation in the degree of individual specialization among sites attributed to any environmental variation? 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 specialization in populations of *E. spectabile* and *E. flabellare*. We predicted that individual diet specialization of populations would be positively correlated with resource richness (Araújo et al. 2011) and the density of prey items (Pyke 1984). We also predicted that populations with lower competitor richness and predator richness would exhibit higher levels of individual specialization (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 (Ceas and Page 1997; Winn 1958). *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 (Ceas and Page 1997; Feminella and Matthews 1984; Marsh 1984).

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 (Hlohowskyj and Wissing 1986; Winn 1958). 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 meter stream transects using 15-20 minute intervals of backpack electrofishing (Smith-Root, Inc. LR-24 electrofisher). 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 over all, thirteen 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 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-sine-square root transformed prior to multivariate analysis. We also recorded the sex of each fish and measured the standard length to nearest millimeter.

Collection of biotic environmental variables

To test how biotic environmental conditions affect trophic resource use and the degree of individual specialization, we characterized resource availability and competitive regimes 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 (Table B.1). 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 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 (Table B.2).

Analyzing 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. 2013) in the R statistical platform (R Development Core Team 2013). 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 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). We extracted the individual NMDS scores and used them as dependent variables in a multivariate analysis of covariance (MANCOVA) 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's 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 analyzed the degree of diet specialization in individuals across populations of *E. spectabile* and *E. flabellare*. To test the hypothesis of a site-specific variation in individual diet specialization, we first calculated the total niche width (TNW), which corresponds to the Shannon–Weaver index (Roughgarden 1974), for each species at a site separately using the count data of stomach contents. TNW 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, there is complete niche specialization; when the ratio is 1, there is complete niche overlap (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).

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:

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

PS_i ranges from 1 (the individual's diet overlaps entirely with all darters at a site) to q (the individual is specialized on a 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 by using 999 Monte Carlo permutations of the data sets, yielding 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 in R (Zaccarelli et al. 2013). We tested for variation in the degree of individual specialization among species and sites by performing an ANCOVA using extracted PS_i values as dependent variables with "site", "species", and "sex" as independent variables and using "standard length" as a covariate.

To determine the influence of biotic environmental factors on individual 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 percent composition for major taxonomic groups were tabulated (arcsin-square-root-transformed). Relative density of invertebrate communities (log-transformed) was then calculated by dividing the total number of invertebrates collected in each Surber sample by the total area sampled, and ranged from 811-9422 individuals/m². Since there is often a positive relationship between species richness and sample size, we created rarefaction curves as implemented in the package VEGAN to estimate invertebrate richness at

every locality. To do so, Shannon-Weaver diversity indices for invertebrate communities were first calculated. We then used a subsample of $N=70$ to estimate rarified richness using rarefaction curves. Values used in the analysis ranged from 8.99-14.41. Because we were interested in the influence of competition on individual specialization we estimated fish competitor richness at each site. We considered all benthic insectivores as competitors (Todd and Stewart 1985) for the analysis (competitor richness 5-8; Table B.2). We were simultaneously concerned with the effect of predation on individual specialization (Araújo et al. 2011), therefore we estimated predator richness at each site as any piscivorous fish species capable of consuming darters (predator richness 0-2; Table B.2).

We used a model selection approach to evaluate the effects of invertebrate density and diversity (rarified richness), as well as competitor and predator species richness on individual specialization using the `GMULTI` package (Calcagno and Mazancourt 2010) in the R statistical software platform (R Development Core Team 2013). The influence of biotic variables on diet specialization was analyzed 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 “predator richness”, “competitor richness”, “invertebrate density”, and “rarified richness” 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 VIF’s, predator richness was excluded to avoid overinflating our models ($VIF > 6$). We then performed model selection using

the three remaining variables. Models were evaluated using Akaike Information Criteria 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 ($\Delta\text{AIC}_c, 2$) 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 specialization (Arnold 2003; Lee et al. 2008). Estimation of individual specializations 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 dataset was comprised of 443 darters (194 *E. flabellare* and 249 *E. spectabile*; Table 2.1). Overall, *E. flabellare* and *E. spectabile* incorporated a similar number of prey categories into their diet, foraging on 14 and 13 categories respectively (Table 2.2). 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 exclusively occurred in its diet (*Neoperla*, *Simulium*, *Tabanus*, *Tipula*, and *Limonia*) at relatively low frequencies (Table 2.2).

The goodness of fit test ensured that ordination distances displayed in the NMDS were representative of actual distances in the data set ($R^2=0.986$). Using MANCOVA to analyze NMDS scores of *E. spectabile* and *E. flabellare* indicated significant effects of species and site on the composition of diet. In addition, there were significant interaction effects between site and species as well as between site and sex (Table 2.3). Species, site, and their interaction term explained the majority of variation in the data set (Table 2.3). Visualization of dietary resource use in an NMDS plot indicated that both the magnitude and direction of species differences varied among sites (Figure 2.1a). Although not consistent across all of the examined sites, two general patterns emerged from the analysis. Firstly, *E. spectabile* and *E. flabellare* tended to partition trophic resources at the majority of sites. Secondly, the diets of *E. spectabile* tended to be more biased towards chironomids, as indicated by the often more negative scores along NMDS axis 1 and/or more positive scores along NMDS axis 2 (Figure 2.1b). Results of the Mantel test indicated that there was no significant association between the diets of *E. flabellare* populations and their local invertebrate communities ($r^2 = - 0.012$, $P = 0.47$). However, there was a significant correlation between the composition of *E. spectabile* diets and the composition of invertebrate communities ($r^2 = 0.378$, $P=0.038$).

Variation in individual diet specialization and its relation to the environment

When calculating indices of individual specialization of darters across the nine sites, IS values ranged from 0.374-0.871 for *E. spectabile* (Table 2.4a) and 0.461-0.694 for *E. flabellare* (Table 2.4b), indicating intermediate levels of individual specialization for both species. Despite the narrow range of categories consumed at some sites, the level of individual specialization was significantly higher than predicted based on the null model produced by the Monte-Carlo procedure for all populations of *E. spectabile*, except for Sallisaw Creek (Table 2.4a). Likewise, all populations of *E. flabellare*, except for Whitewater Creek, had significantly higher specialization than the null model used to determine population level individual specialization (Table 2.4b). ANCOVA of the combined PS_i scores revealed significant effects of site and species, as well as the interaction between site and species (Table 2.5). Overall, *E. flabellare* tended to exhibit a higher level of individual specialization (lower values of PS_i) at any given site, although the magnitude of difference was highly variable, and *E. spectabile* was more specialized at Sallisaw Creek (Figure 2.2).

In *E. spectabile*, individual specialization was strongly influenced by invertebrate richness and invertebrate density. Only two models were supported ($\Delta AIC_C < 2$, Table 2.6), which included invertebrate density as well as the interaction between invertebrate density and invertebrate richness. Model averaging indicated that all factors and interactions had importance values < 0.8 (Figure 2.3a). Visualizing the effects of invertebrate density and invertebrate richness indicated that the highest levels individual specialization in *E. spectabile* was associated with a combination of low to moderate levels of invertebrate richness and low to moderate invertebrate densities (Figure 2.3b). Low individual specialization occurred whenever invertebrate richness was very high or invertebrate density was high.

Individual specialization in *E. flabellare* was primarily associated with invertebrate density and competitor richness. Only a single model was supported (Table 2.6), including the interaction term between invertebrate density and competitor richness. Model averaging also indicated the interaction between invertebrate density and competitor richness was the best predictor of individual specialization in *E. flabellare*, even though it had an importance value considerably smaller than 0.8 (Figure 2.4a). Visualization of the interaction effects illustrated that the highest levels of individual specialization in *E. flabellare* coincided with low to moderate levels of competitor richness when invertebrate densities were low. (Figure 2.4b).

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* appears 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 degree of individual diet specialization varied between species and among sites. Individual specialization among sites was associated with invertebrate richness and invertebrate density in *E. spectabile*, and with invertebrate density and competitor richness in *E. flabellare*. Overall, our results indicated substantial variation in trophic resource use among individuals, populations, and species of small-bodied fishes that are typically assumed to be generalist insectivores.

Previous studies of darter 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 already 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, as the factor site described most variation in our analysis of diet composition. These results suggest that resource availability is likely a key driver in darter diet use. Indeed, darters exhibit high site fidelity (Ingersoll et al. 1984) and experience variable environmental conditions likely influencing the availability of invertebrate prey species (Atilla et al. 2005). Our results also match previous studies documenting interspecific differences in the diets of *E. spectabile* and *E. flabellare* (Martin 1984). *Etheostoma spectabile* populations in our study tended to have a more chironomid biased diet, 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 Jr. and Coon 1990), where it gleans prey from the surfaces of substrate. In contrast, *E. flabellare* is typically confined to riffle type habitats (Paine et al. 1982b; Matthews 1985) and feeds in the interstices of the substrate (Paine et al. 1982a). Thus, *E. flabellare* is more apt to forage on rheophilic invertebrate taxa like *Hydropsyche* (Merritt et al. 2008). Trophic resource partitioning between darters has also been attributed to the range in the size of prey selected by the two species (Martin 1984). In sympatric populations of *E. spectabile* and *E. mihilize* (Sunburst darter) collected from Oklahoma's Ozark Plateau, prey items were partitioned between species on the basis of size, with *E. spectabile* selecting smaller prey items on average (Todd and Stewart 1985). Our results are consistent in that *E. spectabile* typically focused its feeding efforts on very small taxa (chironomids) compared to *E. flabellare*, which more frequently selected relatively larger prey items (*Baetis*, *Heptagenia*, *Hydropsyche*) (Hlohowskyj and White 1983; Fisher and Pearson 1987).

Our results also indicated that populations of *E. flabellare* and *E. spectabile* exhibit geographic variation in individual diet specialization. Darters have previously been described as dietary generalists, consuming invertebrate prey items in proportion to their relative abundances (Pratt and Lauer. 2013). Our analysis indicated that the degree to which individuals become specialized is shaped by differences in ecological opportunity among populations. Individual specialization in populations of *E. flabellare* was low where invertebrate density is high and competitor richness is high. In *E. spectabile*, high individual specialization was associated with low invertebrate richness and low invertebrate densities. Interestingly, these findings are opposite of theoretical predictions and the findings of previous empirical studies. (Darimont et al. 2009; Semmens et al. 2009). For example, Layman et al. (2007) found that habitat fragmentation in estuarine tidal wetlands lead to lower resource diversity and simplified food webs, which in turn decreased individual specialization in gray snapper (*Lutjanus griseus*). Optimal foraging theory predicts that individuals will expand their niche to include previously unutilized resources (i.e., become less specialized) when resources are limited. Individual niche width thus relies both on the diversity of available resources and resource abundance. However, a recent study investigating the diet of *E. spectabile* from spatially separated microhabitats in streams experiencing drought-like conditions found diets did not reflect the invertebrate community, providing evidence that darters were preferentially selecting prey items under resource limiting conditions (Christian and Adams 2014).

Within population diet variation in *E. spectabile* has previously been established in conjunction with individuals that consume readily available prey taxa from their respective habitat patches (Gillette 2012), which could mean that individuals are adopting a generalist foraging strategy by consuming the most abundant prey within a patch causing populations to

appear more specialized (Araújo et al. 2011). Ultimately, stream systems are heterogeneous and invertebrate resources and darters could be patchily distributed. If individuals are in reality generalists and feeding on the most abundant resource within a patch, high degrees of individual specialization may represent patchiness in resource distribution rather than variation in feeding strategies within populations. Evidence derived from empirical studies of resource patchiness (Effenberger et al. 2011) and the high site fidelity exhibited by darters (Ingersoll et al. 1984) supports this hypothesis, and suggest that individual diet specialization may not be as prevalent within generalists 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 specialization is to be thoroughly understood in this system.

Populations of *E. flabellare* were more specialized when competitor richness was lower and invertebrate densities were low. Interspecific competition is expected to alter diet specialization, and the direction of its effect depends on variation in resource preference of the focal species and the nature of diet overlap with competing species (Svanbäck and Bolnick 2008). Since populations of *E. flabellare* maintain high levels of individual specialization under lower competitive regimes with lower resource densities, we speculate that interspecific competition with species having high diet overlap may be an important factor in determining specialization. For example, *E. flabellare* may encounter higher competition within its narrow range of habitat types reducing opportunity for population niche expansion, ultimately decreasing individual specialization. For example, *Noturus exilis* (slender madtom), *Cottus carolinae* (banded sculpin), and *E. mihilize* were all captured consistently in riffles inhabited by *E. flabellare* and could impart considerable competition for space and trophic resources where these species co-occur (Miller and Robison 2004). The distribution of darters within stream sites

might be affected by their tendency to partition microhabitats with other benthic stream fishes (Welsh and Perry 1998), which could result in specialization on invertebrate taxa confined to specific patches and cause individuals to appear as specialists by feeding opportunistically, further contributing to geographic variation in specialization documented here (Konrad et al. 2008; Evangelista et al. 2014).

Our analyses could not rule out the potential effects predation on individual specialization (Peacor and Pfister 2006). In this system, fish predation is likely not very important, considering the habitats darters are generally found in are less than a meter deep, and large piscivorous fish species are generally absent. However, the threat to fish by avian or mammalian predators remains poorly understood and could have a profound influence on foraging habits of stream fishes populating shallow waters. Empirical studies have shown 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 diet specialization among populations, because foraging individuals can be susceptible to predation associated with a particular diet (Svanbäck and Bolnick 2008). 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 underlying variation in the degree of individual specialization among natural populations.

The diets of species are often described as the mean of a single population, 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 specialization among populations. Our study contributes additional evidence to the notion that generalist populations are collections of specialized individuals (Bolnick et al. 2003) and lends insight to answering key questions about the ecological causes of individual 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 degree of individual specialization is variable among populations and conclude that ecological opportunity determines the strength of specialization among natural populations. Variation in diet specialization among naturally occurring populations and the underlying causes of specialization in those populations is rarely documented (Rosenblatt et al. 2015) and suggest that these patterns may be more widespread than previously considered. Future studies should work to elucidate the generalities associated with our findings and determine the importance of each in maintaining individual specialization in natural populations.

Figures and Tables

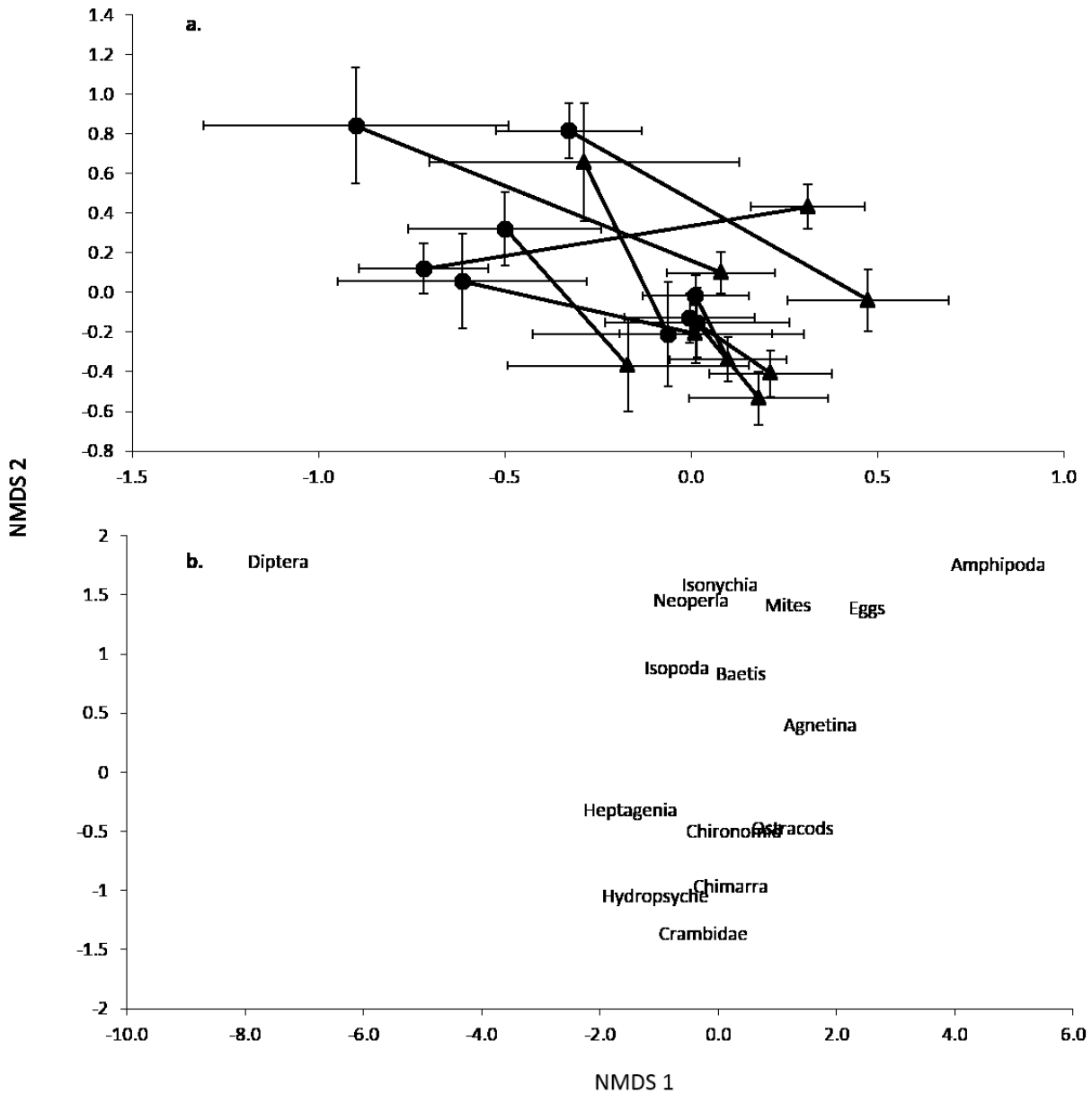
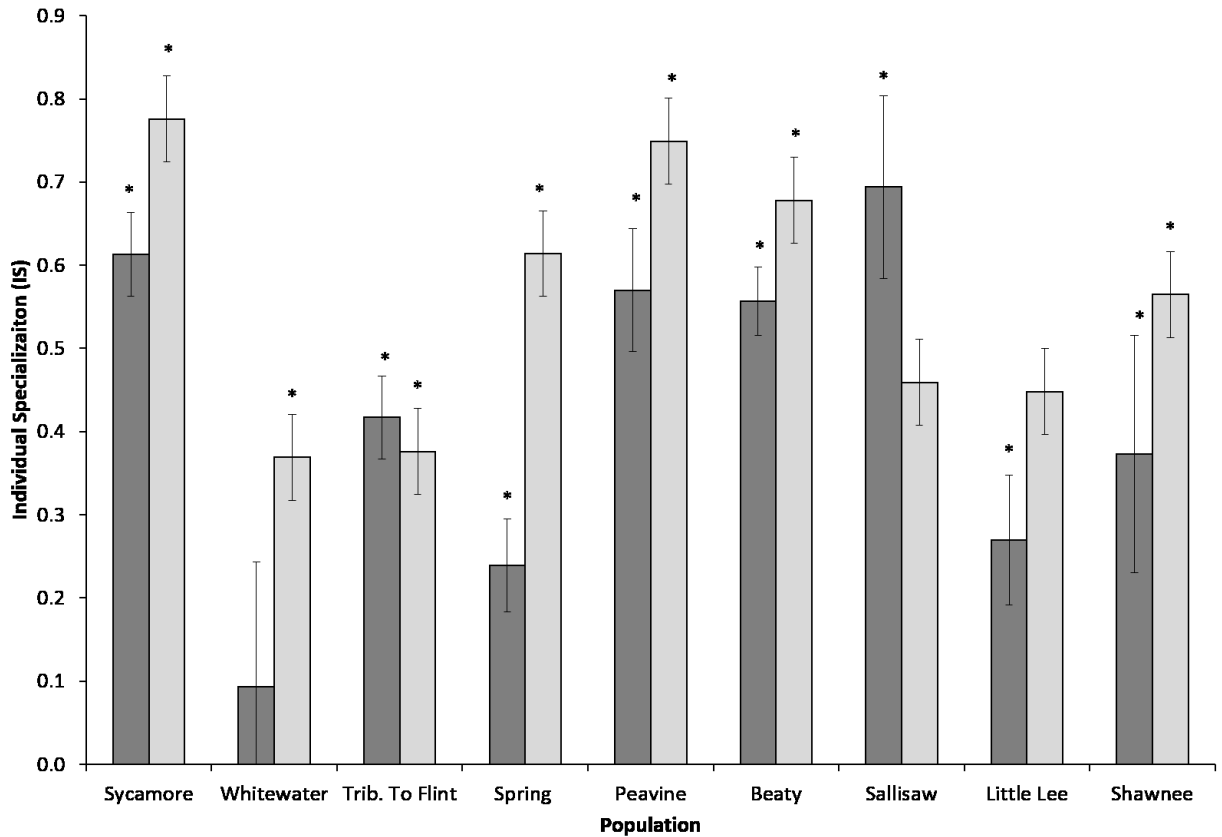
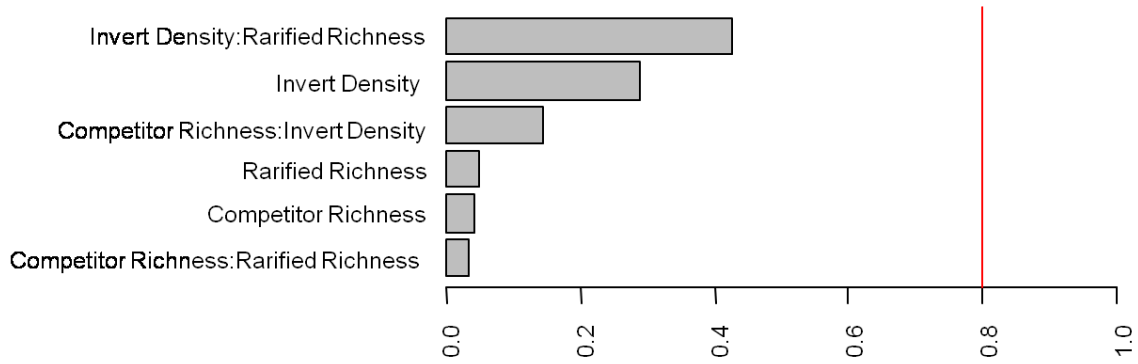


Figure 2.1 Trophic resource use variation from the combined analysis at different sites investigated. (a) Depicted are the estimated marginal means calculated from the site by species interaction term from the MANCOVA of the NMDS scores (\pm SEM). Circles are *E. flabellare* and triangles are *E. spectabile*. Lines connect populations of *E. flabellare* and *E. spectabile* from the same site. (b) Illustration of diet item vectors used in the NMDS.

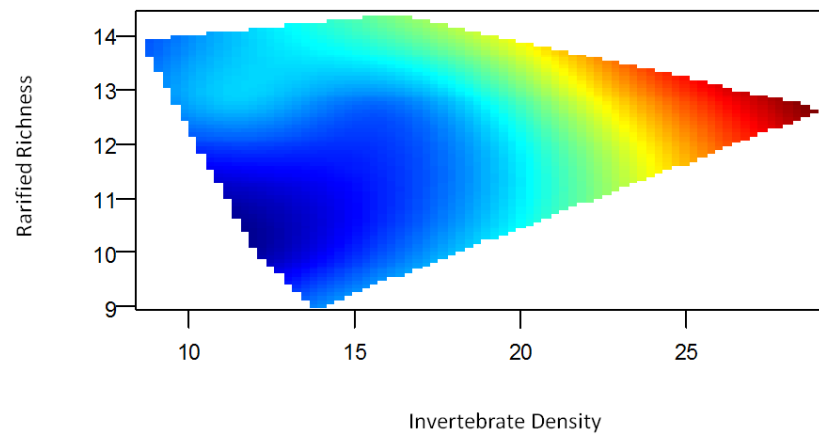


1
 2 **Figure 2.2 Visualization of population differences in individual specialization using**
 3 **estimated marginal means for the site by species interaction term *E. flabellare* (darker**
 4 **grey) and *E. spectabile* (lighter grey) calculated using proportions of diet items collected**
 5 **from stomach contents of individuals from 9 sites. Asterisk indicate individual**
 6 **specialization values that are significantly higher than the null model created using the**
 7 **Monte Carlo resampling procedure.**
 8

a.



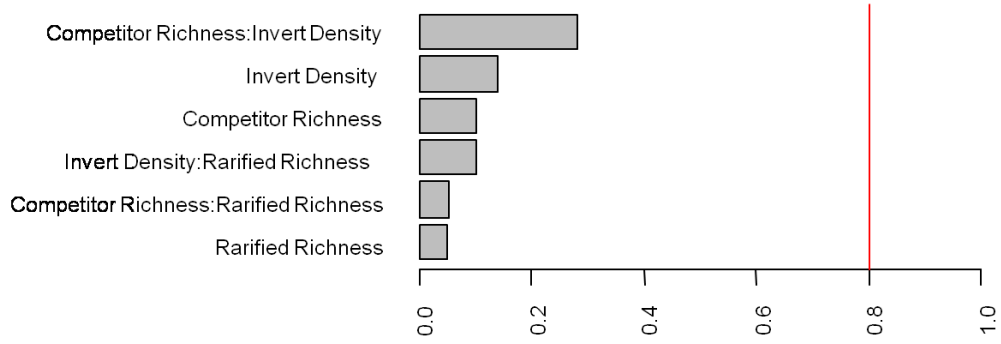
b.



10

11 **Figure 2.3 (a) Importance of factors used to predict variation in the strength of**
 12 **specialization among populations of *E. spectabile*. (b) Surface visualization of the**
 13 **interaction between invertebrate density and fish community richness and its effect on the**
 14 **strength of individual specialization among populations of *E. spectabile*. The highest levels**
 15 **of specialization are represented in dark blue, low specialization is represented in red.**

a.



b.

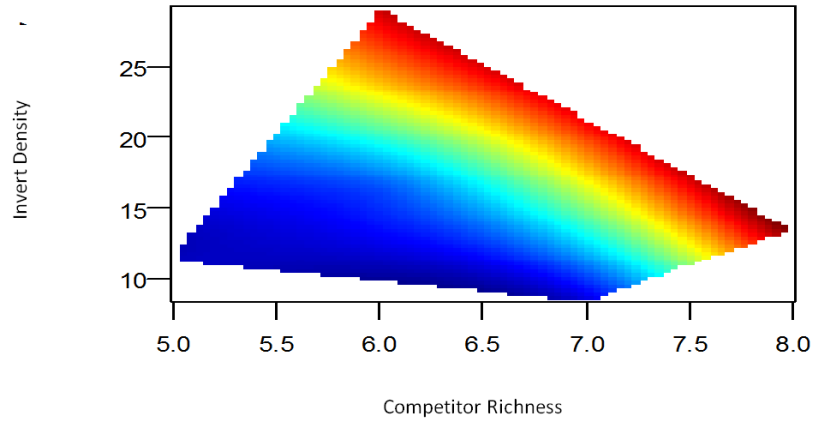


Figure 2.4 (a) Importance of factors used to predict variation in the strength of specialization among populations of *E. flabellare*. (b) Illustration of the effect of invertebrate diversity on the strength of individual specialization among populations of *E. flabellare*.

Table 2.1 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 2.2 List of diet categories obtained from stomachs of (a) *E. spectabile* and (b) *E. flabellare*. The percentage of each prey category in the diets of each population is also indicated.

	Chironomid	<i>Limonia</i>	<i>Simulium</i>	<i>Tabanus</i>	<i>Tipula</i>	<i>Baetis</i>	<i>Heptagenia</i>	<i>Isonychia</i>	<i>Chimarra</i>
<i>a. E. spectabile</i>									
Sycamore Creek	32.64%	0.00%	0.00%	0.00%	0.00%	19.32%	5.08%	0.00%	0.00%
Whitewater Creek	8.06%	0.00%	0.00%	0.00%	0.00%	10.63%	52.54%	0.00%	100.00%
Trib. to Flint Creek	2.98%	0.00%	0.00%	0.00%	0.00%	23.67%	0.00%	0.00%	0.00%
Spring Creek	10.37%	0.00%	0.00%	0.00%	0.00%	8.70%	8.47%	0.00%	0.00%
Peavine Creek	24.98%	0.00%	0.00%	0.00%	0.00%	3.38%	23.73%	100.00%	0.00%
Beaty Creek	17.32%	0.00%	0.00%	0.00%	0.00%	11.11%	8.47%	0.00%	0.00%
Sallisaw Creek	0.45%	0.00%	0.00%	0.00%	0.00%	6.28%	0.00%	0.00%	0.00%
Little Lee creek	0.67%	0.00%	0.00%	0.00%	0.00%	3.86%	0.00%	0.00%	0.00%
Shawnee Creek	2.54%	0.00%	0.00%	0.00%	0.00%	13.04%	1.69%	0.00%	0.00%
	<i>Hydropsyche</i>	<i>Agnatina</i>	<i>Neoperla</i>	Crambidae	Amphipoda	Eggs	Isopoda	Ostracods	Mites
Sycamore Creek	40.00%	7.69%	0.00%	0.00%	0.00%	0.00%	37.50%	23.81%	0.00%
Whitewater Creek	6.67%	7.69%	0.00%	0.00%	0.00%	77.50%	12.50%	23.81%	9.52%
Trib. to Flint Creek	0.00%	65.38%	0.00%	0.00%	100.00%	17.50%	0.00%	0.00%	0.00%
Spring Creek	0.00%	3.85%	0.00%	20.00%	0.00%	0.00%	37.50%	19.05%	66.67%
Peavine Creek	6.67%	0.00%	0.00%	0.00%	0.00%	2.50%	12.50%	9.52%	9.52%
Beaty Creek	26.67%	15.38%	0.00%	0.00%	0.00%	2.50%	0.00%	19.05%	9.52%

Sallisaw Creek	6.67%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Little Lee creek	6.67%	0.00%	0.00%	10.00%	0.00%	0.00%	0.00%	4.76%	0.00%
Shawnee Creek	6.67%	0.00%	0.00%	70.00%	0.00%	0.00%	0.00%	0.00%	4.76%
	Chironomid	<i>Limonia</i>	<i>Simulium</i>	<i>Tabanus</i>	<i>Tipula</i>	<i>Baetis</i>	<i>Heptagenia</i>	<i>Isonychia</i>	<i>Chimarra</i>
<u>b. E. flabellare</u>									
Sycamore Creek	43.37%	0.00%	0.00%	0.00%	0.00%	27.21%	9.76%	0.00%	0.00%
Whitewater Creek	0.00%	0.00%	0.00%	0.00%	0.00%	4.41%	4.88%	0.00%	0.00%
Trib. to Flint Creek	6.63%	100.00%	100.00%	100.00%	100.00%	16.91%	9.76%	0.00%	0.00%
Spring Creek	2.68%	0.00%	0.00%	0.00%	0.00%	20.59%	17.07%	0.00%	0.00%
Peavine Creek	19.01%	0.00%	0.00%	0.00%	0.00%	2.57%	9.76%	0.00%	0.00%
Beaty Creek	17.35%	0.00%	0.00%	0.00%	0.00%	11.40%	26.83%	0.00%	0.00%
Sallisaw Creek	8.42%	0.00%	0.00%	0.00%	0.00%	2.94%	0.00%	0.00%	0.00%
Little Lee creek	1.15%	0.00%	0.00%	0.00%	0.00%	6.25%	17.07%	0.00%	0.00%
Shawnee Creek	1.40%	0.00%	0.00%	0.00%	0.00%	7.72%	4.88%	0.00%	0.00%
	<i>Hydropsyche</i>	<i>Agnatina</i>	<i>Neoperla</i>	Crambidae	Amphipoda	Eggs	Isopoda	Ostracods	Mites
Sycamore Creek	31.25%	0.00%	0.00%	6.67%	50.00%	0.00%	69.23%	0.00%	0.00%
Whitewater Creek	0.00%	0.00%	5.41%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Trib. to Flint Creek	14.06%	20.00%	0.00%	20.00%	0.00%	0.00%	30.77%	0.00%	0.00%

Spring Creek	3.13%	20.00%	86.49%	6.67%	0.00%	0.00%	0.00%	0.00%	0.00%
Peavine									
Creek	7.81%	0.00%	0.00%	0.00%	50.00%	45.45%	0.00%	0.00%	0.00%
Beaty Creek	1.56%	60.00%	8.11%	0.00%	0.00%	54.55%	0.00%	0.00%	0.00%
Sallisaw									
Creek	29.69%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Little Lee									
creek	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Shawnee									
Creek	12.50%	0.00%	0.00%	66.67%	0.00%	0.00%	0.00%	0.00%	0.00%

1 **Table 2.3 Results of multivariate analyses of covariance examining the NMDS scores from**
 2 **the combined diets of both species. Significant effects with a relative variance > 0.3 are**
 3 **highlighted in bold.**

Effect	<i>F</i>	Hypothesis <i>df</i>	Error <i>df</i>	<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 x Species	3.245	16	826	<0.001	0.059	0.755
Site x Sex	1.370	16	826	0.149	0.026	0.330
Species x Sex	3.000	2	413	0.074	0.013	0.160

4

5 Table 2.4 Indices of individual diet specialization calculated for the diets of *E. spectabile*
6 and *E. flabellare* at each site investigated using the proportion of similarity index adapted
7 to the individual level. *P*-values for individual specialization metrics (both for WIC/TNW
8 and IS) was attained by using 999 Monte Carlo permutations of the data sets, yielding a
9 null model distribution against which the observed IS values were tested. IS values near 0
10 represent high diet specialization, whereas values nearing 1 represent high diet overlap
11 within the populations. Significant *P*-values are highlighted in bold.

Site	WIC	BIC	TNW	WIC/TNW	IS	N	<i>P</i>
<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
Trib. 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	0.53	0.929	0.429	0.556	11	0.160
Shawnee Creek	0.494	0.457	0.951	0.520	0.594	23	0.003
<i>E. flabellare</i>							
Sycamore Creek	0.460	0.334	0.794	0.579	0.604	34	0.001
Whitewater Creek	0.375	0.361	0.736	0.51	0.694	6	0.297
Trib. 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	0.350	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

12

13 **Table 2.5 Results of the analysis of covariance analyzing individual specialization among**
 14 **site with *E. spectabile* and *E. flabellare* combined analysis. Effects with a relative variance >**
 15 **0.30 are highlighted in bold.**

Effect	df	F	P	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 x Site	8	29.132	<0.001	0.360	1.000
Site x Sex	8	0.913	0.505	0.017	0.048
Species x Sex	1	0.223	0.637	0.001	0.001

16

17 **Table 2.6 Results of model selection using the estimated marginal means (IS) calculated from the analysis of covariance for**
 18 **each species separately with the biotic factors fish community richness, invertebrate densities and invertebrate diversity**
 19 **indices (See Table S2 and S3 for biotic indices used in the models).**

Species	Model	AIC _C	ΔAIC _C	Effect	Estimate	SE	<i>t</i>	<i>P</i>
<i>E. spectabile</i>	1	-5.309	0.000	Rarified Richness × 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	Competitor Richness × Invertebrate Density	0.002	0.001	1.943	0.093

20

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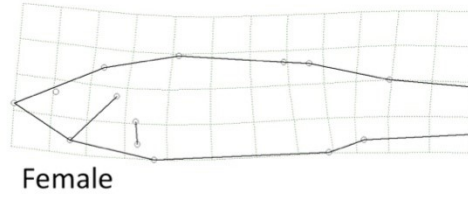
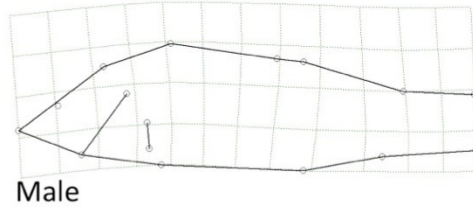
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Appendix A - Supplementary Material for Chapter 1

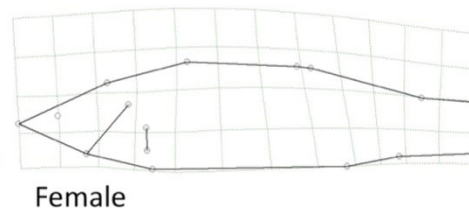
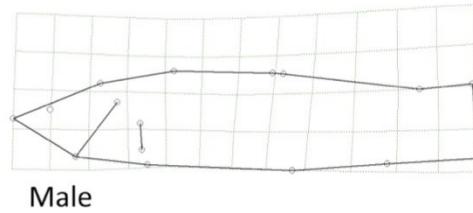
Table A.1 Results of the principal component analyses on environmental data from sites with *E. spectabile*, *E. flabellare*, and both species. Provided are component loadings, eigenvalues, and percent variance explained for each of the axes.

	<i>E. spectabile</i>		<i>E. flabellare</i>		Combined	
<u>Hydrological PC</u>	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
Slope	0.747		0.709		0.715	
Flow Accumulation	0.747		0.709		0.715	
Eigenvalue	1.117		1.007		1.022	
% variance	55.8		50.3		51.1	
 <u>Stream Size PC</u>						
Stream width	0.829		0.788		0.810	
Stream depth	0.829		0.788		0.810	
Eigenvalue	1.375		1.234		1.312	
% Variance	68.8		62.1		65.6	
 <u>Substrate PC1 & PC2</u>						
Bedrock & Boulder	-0.545	0.741	-0.392	0.847	0.527	0.604
Cobble	-0.649	-0.668	-0.858	-0.430	0.871	0.354
Gravel	0.833	-0.408	0.858	-0.360	-0.773	-0.511
Sand & Silt	0.518	0.600	0.615	0.443	-0.600	0.672
Eigenvalue	1.680	1.523	2.004	1.227	1.994	1.205
% Variance	42.0	38.1	50.1	30.7	49.9	30.1

Sex difference:
E. spectabile



Sex difference:
E. flabellare



Species difference

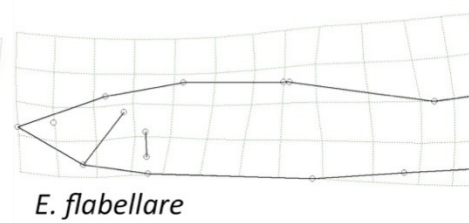
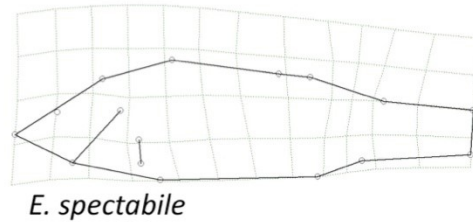


Figure A.1 Visualization of shape variation between sexes for *E. spectabile* and *E. flabellare* (based on the separate analyses for each species), and between the two species (based on the combined analysis)

Appendix B - Supplementary Material for Chapter 2

Table B.1 Percent of occurrence for each invertebrate taxa collected from three combined Surber samples at each site (Beaty Creek only 2). Shannon diversity indices for the invertebrate community was calculated for each site and used as an independent variable in the model selection analyses.

	Peavine Creek	Trib. To Flint Creek	Spring Creek @ Rocky Ford St. Park	Sycamore Creek	Whitewater Creek	Beaty Creek at 456 Bridge	Sallisaw Creek	Little Lee Creek	Shawnee Creek
Amphipoda	3.3%		0.4%	0.1%	3.5%	2.3%			
Dytiscidae			0.4%						
<i>Optioservus</i>	1.7%	0.8%	0.8%	10.8%	0.7%		5.4%	9.6%	0.5%
Stenelmis					0.7%			4.1%	
<i>Ectopria</i>		2.8%	0.8%	0.4%					
<i>Psephenus</i>		1.2%	10.5%	7.4%	1.4%	2.3%	4.9%	2.7%	1.1%
Chironomidae	19.0%	15.8%	4.9%	24.6%	2.8%	29.1%		6.8%	12.6%
Culcidae	0.2%	0.4%				1.2%			
<i>Hexatoma</i>						15.1%			1.1%
<i>Antocha</i>			1.1%	0.1%					
Tabanidae	0.2%								
<i>Tabanus</i>		0.4%	0.4%	0.6%					
<i>Tipula</i>		0.4%	0.8%	0.4%	0.7%				
<i>Limonia</i>				0.1%					
<i>Limnophora</i>									
<i>Simulium</i>							0.5%		
<i>Tipula</i>									
Baetidae	9.5%								
<i>Baetis</i>	0.3%	2.4%	0.8%	7.9%	9.8%		13.0%	9.6%	18.9%

<i>Caenis</i>				0.2%	0.7%	1.2%	57.8%	8.2%	3.7%
<i>Ephemerella</i>	4.3%	18.2%	8.6%	2.1%	0.7%	19.8%			
<i>Heptagenia</i>	4.0%	14.2%	11.3%	2.2%	49.0%	8.1%	7.0%	21.9%	30.5%
<i>Isonychia</i>	0.3%	1.6%	3.0%	1.1%		1.2%			
<i>Tricoryctodes</i>	1.9%					4.7%		1.4%	
Paraleptaphlebia							1.1%	9.6%	1.6%
<i>Amphiagrion</i>							0.5%	2.7%	0.5%
<i>Ampylla</i>			0.8%				0.5%		
Cordulidae									
Corydalus				0.5%					
Ostrococha									
<i>Alloperla</i>	6.1%	10.5%			0.7%				
<i>Agnetina</i>	0.9%		0.4%	0.5%		1.2%			
<i>Isoperla</i>	9.3%	24.7%	17.7%	0.4%					
<i>Neoperla</i>		1.6%	0.4%	1.1%	23.1%	11.6%	0.5%	5.5%	1.1%
Isopoda	27.9%		20.3%	7.4%	5.6%				
Crambidae									
<i>Glossossoma</i>			0.4%	0.1%					
<i>Chimarra</i>	0.7%	1.6%	1.1%	4.4%					0.5%
<i>Helicopsyche</i>	1.2%	0.4%	4.9%	8.5%		1.2%			
<i>Hydropsyche</i>	4.2%	1.2%	9.8%	18.2%			2.2%	1.4%	27.4%
<i>Polycentropus</i>	0.3%								
<i>Agarodes</i>			0.8%				0.5%		
Hirudinea				0.1%			0.5%	5.5%	
Oligochaeta	0.9%	1.6%			0.7%	1.2%	1.6%	9.6%	
Planaria	2.1%								
Mites									
Gastropoda	1.7%			0.2%			3.8%		0.5%
Fish eggs									

SW Diversity	2.331	2.155	2.400	2.331	1.597		1.565	2.444	1.73478
Relative									
Density(inv./m ²)	24.0416	15.716234	16.30950643	29.12044	11.9582607	11.35781669	13.60147	8.544004	13.78405

Table B.2 Presence and absences data for the fish species present at each site. Fish species richness was determined and used for the multiple regression analyses. (1 is present, 0 is absent, *competitor, °predator).

	Peavine Creek	Trib. To Flint Creek	Spring Creek	Sycamore Creek	Whitewater Creek	Beaty Creek	Sallisaw Creek	Little Lee Creek	Shawnee Creek
<i>C. anomalum</i>	1	1	1	1	1	1	0	1	0
<i>C. carolinae</i> *	1	1	1	1	1	1	0	0	1
<i>C. whipplii</i>	0	0	0	0	0	0	1	0	0
<i>E. blenniodes</i> *	0	0	0	0	0	0	1	1	0
<i>E. flabellare</i> *	1	1	1	1	1	1	1	1	1
<i>E. mihilize</i> *	1	1	1	1	1	1	1	1	1
<i>E. spectabile</i> *	1	1	1	1	1	1	1	1	1
<i>E. whipplii</i> *	0	0	0	0	0	0	1	1	1
<i>E. zonale</i> *	0	0	0	0	0	0	1	1	0
<i>F. catenatus</i>	1	0	1	0	0	0	0	0	0
<i>F. notatus</i>	0	0	0	1	0	0	0	0	0
<i>G. affinis</i>	0	1	0	1	0	0	0	0	0
<i>H. nigricans</i>	0	0	1	0	0	0	0	0	0
<i>L. cyanellus</i> °	1	0	1	0	1	0	0	0	0
<i>L. cyanellus</i> x <i>L. megalotis</i>	0	0	1	0	0	0	0	0	0
<i>L. macrochirus</i>	0	0	0	0	0	0	0	1	0
<i>L. megalotus</i>	0	0	0	0	0	0	1	1	0
<i>L. osseus</i>	0	0	0	0	0	0	1	0	0
<i>L. cardinalis</i>	1	1	1	0	1	1	1	1	1
<i>M. dolomieu</i> °	1	0	1	0	0	0	0	1	0
<i>M. salmoides</i> °	0	0	0	1	0	0	1	0	0
<i>N. exilis</i> *	1	0	1	1	1	1	1	0	1
<i>P. caprodes</i> *	0	1	0	0	0	0	1	1	0
<i>C. erythrogaster</i>	1	0	1	0	1	0	0	0	0
<i>S. atromaculatus</i> *	1	1	1	1	0	0	0	0	0

Richness	12	9	14	10	9	7	13	12	7
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