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PHYLOGENETIC AND PROXIMATE MECHANISMS AFFECT DARTER (PERCIDAE: ETHEOSTOMATINAE) COMMUNITY STRUCTURE

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A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

BY

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For my mother and father.

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Abstract

Understanding the relative influences of biotic and abiotic mechanisms responsible for generating patterns of community diversity remains a fundamental theme in ecology. Although studies have recovered patterns of community structure, the mechanisms responsible for such patterns are often unclear. To better understand assembly mechanisms, I implemented a framework that incorporated phylogeny, morphology, and habitat use data among co-occurring darters (Percidae: Etheostomatinae). Darters are small bodied stream fishes endemic to North America, and due to the high degree of co-occurrence of species in fine scale communities, these systems are ideal for testing assembly mechanisms where inter-specific interactions are plausible. My goals were to identify patterns of habitat use structure, describe mechanisms influential on assembly, and demonstrate the effects of spatial scale on assembly mechanisms within darter communities.

To test for mechanisms influential on community assembly, I incorporated metrics of phylogenetic relatedness, habitat use similarity, and morphologic similarity, among co-occurring species within communities at several spatial scales. At the stream site scale, communities showed consistent phylogenetic clustering and habitat use clustering among co-occurring taxa, indicating that habitat filtering (i.e., co-occurrence of species with similar ecological requirements) was the main driver of community assembly; although some degree of evolutionary convergence in habitat use among co-occurring species was found. There were separations in habitat use but these occurred between groups of species rather than among all individual species. Additionally, phylogenetic/habitat use relationships indicated displacement in habitat use among recently diverged taxa. Across multiple spatial scales (from within to across four watersheds) darter communities showed an increase in the prevalence of habitat filtering from fine to intermediate spatial scales, and in two of the four systems there was a signal for habitat filtering at the most broad scale. There was a strong signal for competitive exclusion in only one of the river systems. In conclusion, darter communities showed group separation in habitat use, where similar habitat use is found within groups of species suggesting that competition may not act strongly among individual species within these communities, but rather, competition could act between species groups. Habitat filtering appeared to be the dominate mechanism influencing the assembly of communities, and the relative influence of habitat filtering increased with larger spatial scales. However, the prevalence of simultaneous signal for habitat filtering and competitive exclusion based on trait distributions and phylogenetic patterns further illustrates the complexities of community assembly processes.

Chapter 1: **Separation in habitat use, and phylogenetic influence on habitat use among fishes in diverse temperate stream communities**

Abstract

The investigation of habitat use and its relationship to phylogeny among co-occurring species provides knowledge of ecological and evolutionary parameters important for the structuring and maintenance of communities. Among communities of darters (Percidae: Etheostomatinae) in the Duck River, TN, U.S.A., I tested for inter-specific separations in habitat use (i.e., based on flow velocity, substrate composition, and depth) to identify patterns and potential processes important for maintaining community structure. Furthermore, I incorporated phylogeny to test for influence of evolutionary history on patterns of contemporary habitat use. Multivariate analyses recovered two significant species clusters (based on habitat use), suggesting that separations in habitat use occurred between groups of species rather than among all individual species. Phylogenetic relationships among species did not significantly correlate with species habitat use; however I recovered patterns of displacement in habitat use among the most closely related taxa, which indicated limiting similarity between close phylogenetic pairs. My results suggest that group separations in habitat use, and displacement in habitat use between phylogenetic close relatives, can help explain how communities are structured and maintained. These findings are important for understanding ecological community organization, and I demonstrate the value of examining contemporary ecological traits in the light of evolutionary relationships.

Introduction

Comparing habitat use among species provides valuable knowledge about environmental parameters important in structuring and maintaining ecological communities. Moreover, examining separation among species' habitat use traits, and phylogenetic signal for such traits, may have important implications for understanding community assembly mechanisms (i.e., competitive interactions and habitat filters) (Robertson 1996, Poff 1997, Losos et al. 2003, Vamosi et al. 2009). Especially interesting are habitat use studies in communities with taxonomically closely related species occurring sympatrically, because these studies provide insight into how diversity can persist at local spatial scales (e.g., Schoener 1974). Studies in systems such as these allow us to better determine the influences of both environmental parameters and phylogeny on community structure, due to relatively recent shared evolutionary histories among community members (Vamosi et al. 2009). Stream fish communities often harbor high species diversity at small spatial scales, and provide an excellent opportunity to examine ecological and evolutionary relationships among potentially interacting species. In this study, I examined habitat use and separation in habitat use among stream communities of darters (Percidae: Etheostomatinae), and assessed the relationships between species habitat use and phylogeny to test the relative influence of evolutionary history on environmental preferences.

Assessing phylogenetic relationships among community members is important for understanding community structure in relation to species ecological functions (Webb 2000, Webb et al. 2002, Kozak et al. 2005, Webb et al. 2006, Vamosi et al. 2009). Because phylogenetically closely related species are hypothesized to be more ecologically similar than phylogenetically distanced species, under a Brownian motion model of evolution, species habitat use within communities may directly correlate with phylogenetic structure (Blomberg et al. 2003, Kraft et al. 2007). Because of this predicted relationship, the inclusion of phylogeny with habitat use data can aid in the understanding of whether ecological functions are influenced by evolutionary histories, and or contemporary competitive interactions among species.

Inter-specific habitat use differences contribute to the structure of ecological communities, and identifying such differences allows us to better understand past interactions among species which may drive current habitat selection (Resetarits & Wilbur 1989, Resetarits 2001). Furthermore, quantifying habitat use separation among congeneric species, where general environmental requirements are expected to be relatively similar, can aid in our understanding of competitive interactions that influence such separations.

Studies examining species-specific habitat use within stream fish communities have detected some degree of separation in habitat use across multiple habitat variables, or a combination of variables, including flow velocity, stream depth, and substrate size/class (e.g., Matthews 1985, Greenberg 1991, Kessler & Thorp 1993, Chipps et al. 1994, Stauffer et al. 1996, Welsh & Perry 1998, Pratt & Lauer 2013). However, these studies have rarely examined differential habitat use among species in highly diverse assemblages, and most work has focused on comparisons among smaller groups of species (≤ five species). Moreover, studies characterizing stream fish habitat use often fail to examine how these ecological traits relate to evolutionary histories among community members.

Darters are small bodied, benthic stream fishes endemic to North America, and the group comprises a substantial part of the freshwater fish diversity (≥ 20%) (Lundberg et al. 2000, Near et al. 2011). Furthermore, many darter species are habitat specialists and often occur sympatrically in local communities (Gorman & Karr 1978, Etnier & Starnes 1993, Pratt & Lauer 2013). Accordingly, many species rely on specific habitat parameters, such as substrate composition, flow, and depth, for ecological functioning such as feeding and reproduction (Greenberg 1991, Ross et al. 1992).

The goal of this study was to quantify habitat use and its relationship to phylogeny in communities of darters in the Duck River, TN. I examined habitat use and separation in habitat use among 14 darter species to determine relative importance of habitat use and phylogeny in structuring darter communities. Due to the high diversity of closely related species in the system, I hypothesized that there would be separation in habitat use among species, assuming that separations may aid in the avoidance of competitive interactions. Additionally, I used a phylogenetic hypothesis to assess the influences of evolutionary history on patterns of contemporary habitat use.

Study system – The southeastern United States harbors the most diverse freshwater fish fauna of any temperate region in the world (Warren et al. 1997, Burr & Mayden 1992, Lundberg et al. 2000). At the center of this diversity are systems of the Tennessee River drainage (Warren et al. 1997), which includes the Duck River system in the Lower Tennessee River drainage. Approximately 30 species of darters occur in this system, and many co-exist in local communities (Etnier & Starnes 1993). In this study, I measured habitat use among darters at 15 stream sites spread across approximately 300km of the Duck River.

Material & Methods

***Fish collections* –** Fishes were collected from 15 sites within the main channel of the Duck River, TN during June 2012 (Figure 1). Sites were chosen for the presence of riffle habitat, which was defined as stream areas containing rocky or sandy shoals producing turbulent flows, and were spaced across ~300km of the drainage. Each site was divided into 3 equal sections (lower, intermediate, and upper) based on individual riffle length. Transects perpendicular to stream flow were delineated in each of these sections, and fishes were collected from six quadrats (1.5 x 1.5m) along each transect. Quadrats were evenly spaced, spanning the width of the stream along each transect (Figure 1). At each quadrat, fishes were collected via kick seining using a weighted 1.8m seine net (3mm mesh) set at the downstream edge of each quadrat. Substrate was agitated at the upstream quadrat edge by manually churning the substrate with our feet, so that fishes were dislodged and driven downstream into the net. Specimens were preserved in 10% commercial grade formalin for later sorting. All fishes collected were cataloged in the Sam Noble Oklahoma Museum of Natural History (SNOMNH) at the University of Oklahoma.

***Habitat variables* –** Habitat parameters were collected from each of the above described quadrats following fish collections. Parameters measured included: stream depth, flow velocity (m/s) and substrate size. Within each quadrat 3 flow velocity measures were taken using a FLO-MATE flowmeter (model 2000, Marsh-McBirney, Inc.). Each measurement was taken at 2/3 stream depth at a random point within the quadrat. Mean flow velocity was measured across a one minute period at each point (n=810).

Additionally, stream depth was measured at the same points as flow velocity in each quadrat. Substrate size was quantified by taking 5 random substrate grabs per quadrat, where each grab consisted of an individual particle, and each particle was measured using a substrate sizer (AL-SCI Field Sieve). All particles were measured across their intermediate axes, of the three mutually perpendicular particle axes, to account for potential obscure shaped particles. Substrate was later categorized as gravel (2-16mm), pebble (16-64mm), cobble (64-256mm), boulder (> 256mm), and bedrock (Compton & Taylor 2013), and each category was represented as percent available within each quadrat.

***Data analysis of species habitat use* –** Darter species abundances are reported for each of the 15 sites. Additionally, the mean and standard deviation for each habitat variable are reported for each species across the seven habitat variables.

Principal components analysis (PCA) was used to illustrate the relationships among all 270 quadrats based on the measured habitat variables. This analysis reduced the dimensionality of the habitat data resulting in a more reasonable depiction of relationships among quadrats based on all measured variables. Prior to PCA, data were normalized by subtracting the mean and dividing by the standard deviation for each variable separately (*see* Clarke & Gorley 2006). This procedure converted variables to values over roughly the same range. Principal component loading values for each habitat variable are reported, as well as the percent variation explained by each PC. This analysis and all following multivariate statistical analyses were performed using PRIMER 6 unless otherwise indicated (Plymouth Routines In Multivariate Ecological Research) (2008 PRIMER-E Ltd).

In following analyses I included the seven most abundant *Etheostoma* species collected during the study; species occurring at < 25% of sites were not included. Furthermore, *Percina evides* was not included because I was most interested in habitat use among congenerics. Occurrence of each species of darter was overlaid onto quadrats of the PCA ordination (PC1 vs. PC2) to show habitat use of each species. This approach was favored because it illustrates the occurrence of each species in relation to all quadrats surveyed, while also describing variable influences along the component axes. Additionally, I include the PCA with convex hulls encompassing quadrats from each site. This was included to show relationships of habitat availability among the 15 sites (convex hulls were added using PC-ORD version 6).

To test for separation in habitat use among species I compiled habitat data for each individual collected from every quadrat where they occurred. Habitat data were coded by species names for inter-specific habitat use comparisons. The data were normalized across variables as above, and a Euclidean distance matrix was generated for species. A one-way analysis of similarity (ANOSIM) was employed to determine significant differences in habitat use among species. This was run using a maximum of 999 permutations to create the null distribution of R values, and global R value and associated P value were reported (Clarke 1993). Additionally, post test pair-wise comparisons of habitat use between species pairs are reported.

Because I was also interested in whether location along the stream gradient had an effect on species occurrence, a two-way ANOSIM was run to test for differences between species groups across sites, and differences between site groups across species. This was run using the same data treatments and procedures as above. This test allowed me to identify whether or not different species showed habitat use differences within sites, and whether or not individual species differed in habitat use from site to site. I used nonmetric multi-dimensional scaling (nMDS) to show habitat use relationships among species within each site. Species habitat use means (i.e., average habitat use of each species within each site) were used in the nMDS so that species relationships could be easily visualized in two-dimensional space. Furthermore, nMDS was implemented because relationships are generated from the same resemblance matrix as in ANOSIM, and therefore it acted as a visual compliment to the analysis.

***Habitat use in relation to evolutionary history* –** Hierarchical group average cluster analysis was used to examine overall relationships among species habitat use. This analysis was run from a Euclidean distance matrix based on mean habitat use of each species for each variable. Cluster analysis was paired with a similarity profile test (Simprof), which detected significant structure ($P \leq 0.05$) within the resulting dendrogram in relation to 999 randomly generated profiles (*see* Clarke et al. 2008 for further description).

A maximum likelihood molecular phylogeny was generated for the regional species pool using multiple nuclear and mitochondrial genes (cytochrome b [*Cyt b*], s7 intron 1 [*S7*], cytochrome oxidase 1 [*CO1*], NADH dehydrogenase 2 [*ND2*], and recombination activating gene 1 [*RAG1*]). Sequences were downloaded from the National Center for Biotechnology Information’s GenBank, and aligned using MUSCLE (Edgar 2004) (Appendix C). Randomized Axelerated Maximum Likelihood (RAxML) was run on the unpartitioned data set using BlackBox (Stamatakis 2006, Stamatakis et al. 2008). The general time reversible model of nucleotide evolution (GTR + γ + y) was selected for the analysis.

The resultant tree was pruned to the seven most abundant *Etheostoma* species, and a phylogenetic distance matrix (i.e., based on branch lengths) was then generated for comparisons with the habitat use dendrogram. I used RELATE analysis (a non-parametric version of a Mantel test) to test for correlation between the habitat use dendrogram and phylogeny (P value for RELATE analysis was based on 999 simulations) (2008 PRIMER-E Ltd).

Results

***Species habitat use* –** A total of 492 darters was collected from the Duck River in June 2012 (Table 1), including 12 *Etheostoma* and two *Percina* species. The number of darter species per site ranged from 2-8 (mean of 5.4 species per site). *Etheostoma zonale* (n= 165) and *E. rufilineatum* (n= 110) were the most abundant species in the study, whereas only one individual each of *E. cinereum* and *E. stigmaeum* was collected (Table 1).

The first three PCs explained 26.9%, 20.1%, and 17.9% of the variation among quadrats, respectively, and were interpretable based on the broken stick model (Jackson, 1993). The first PC represented a substrate gradient, where pebble % was positively associated, and cobble % was negatively associated (Table 2). Flow velocity and depth were both negatively associated with PC 2, and bedrock % and gravel % were positively associated with PC 3 (Table 2).

Species generally show non-random patterns of habitat use across available quadrats (Figure 2). *Etheostoma blennius* (2B), *E. tippecanoe* (2F), and *E. rufilineatum* (2D) preferred smaller substrate size (pebble), whereas *E. aquali* (2A), *E. blennioides* (2C), *E. simoterum* (2E), and *E. zonale* (2G) also occurred in quadrats containing larger substrate (cobble). *Etheostoma blennioides*, *E. simoterum,* and *E. zonale* occurred in slower flowing, shallower areas than other species (Figure 2). Additionally, convex hulls overlaid on the PCA show the high degree of overlap in habitat availability among the 15 sites sampled (Figure 3).

The resulting global R value from ANOSIM was 0.052 (P = 0.017), indicating that habitat use among *Etheostoma* species was structured (although the low global R value indicates overlap in habitat use among species). Pair-wise comparisons among species revealed seven significant differences ($P \leq 0.05$) in habitat use between species pairs (Table 3).

The two-way ANOSIM reported a global R value of 0.004 ($P = 0.42$) for differences between species across sites, indicating that consistent within-site differences in habitat use were not present. Furthermore, a global R of 0.419 ($P < 0.001$) was recovered for differences between sites across individual species habitat use. The nMDS depicts habitat use relationships found across sites, where plot points are mean habitat use of each species within each site, and are labeled by site (Figure 4a). Moreover, the same nMDS is presented with plot points labeled for species (Figure 4b). Figure 4a shows differences in structure among sites, which can be seen by the grouping of sites despite species.

***Habitat use in relation to evolutionary history* –** Cluster analysis coupled with the Simprof test recovered three distinct clusters based on species habitat use. *Etheostoma rufilineatum* was significantly distinct from *E. tippecanoe* and *E. blennius* ($P = 0.015$), and the two larger clusters were also significantly distinct ($P = 0.003$). The two main clusters included *E. tippecanoe*, *E. blennius*, *E. rufilineatum*, in one, and *E. aquali*, *E. zonale*, *E. blennioides*, *E. simoterum* in the other (Figure 5). Generally, *E. tippecanoe*, *E. blennius*, and *E. rufilineatum* preferred small particle substrate and higher flow velocities. In contrast, *E. aquali*, *E. zonale*, *E. blennioides*, and *E. simoterum* preferred larger substrate sizes and slower flow velocities (Table 4).

Two distinct clades, based on molecular data, were recovered among the seven most common *Etheostoma* species (the final likelihood value was -37217.02) (Figure 6). One clade included *E. tippecanoe*, *E. aquali*, and *E. rufilineatum*, and the other clade included *E. simoterum*, *E. zonale*, *E. blennius*, and *E. blennioides* (Figure 6). RELATE analysis including the seven most abundant species showed no overall significant correlation between habitat use relationships and phylogenetic relationships (Rho = 0.071, P = 0.22). Interestingly, sister species pairs on the phylogeny were displaced in habitat use, and occurred in opposing habitat clusters (Figure 5 & 6). Furthermore, habitat use relationships of the five most abundant species showed the same topology in the cluster analysis as in the phylogeny.

Discussion

Overall, I found structured habitat use among darter species across the Duck River system. My results show multiple inter-specific separations in habitat use among species despite the influence of habitat use differences between sites shown by the partitioned two-way analysis. Although individual species showed differences in habitat use across sites, several species were collected at the majority of sites sampled (e.g., *E. blennioides*, *E. rufilineatum*, *E. simoterum*, *E. zonale*), and showed preferences for specific habitat parameters despite site differences. For example, *E. rufilineatum* and *E. zonale* each occurred at ≥ 13 of the sites sampled (Table 1), and these two species were found to have significantly different habitat usage in one-way ANOSIM comparisons.

Combinations of habitat parameters (i.e., substrate, velocity, and depth) accounted for the observed habitat use differences. This was illustrated using PCA which depicted separations in habitat use among species, and was further tested using ANOSIM which found multiple pair-wise separations between species. Findings suggest that substrate size, specifically cobble% and pebble%, explained a large proportion of the variation across quadrats, and was a driver of differences among species. The influence of substrate composition on darter presence is not surprising, considering the benthic nature of *Etheostoma*. Species rely on substrate composition for protection, foraging and spawning (Schlosser & Toth 1984, Welsh & Perry 1998, Pratt & Lauer 2013), so it seems appropriate that many species would show separation in substrate use which could be a strategy to avoid competition for this valuable resource (e.g., Fischer 2000). Furthermore, I found less variance explained by depth and flow, however it should be noted that habitat variables may not be independent of one another making it difficult to directly weight each variables importance (e.g., higher flow may remove smaller substrate from an area). Although ANOSIM yielded a rather low global R value, some degree of significant community structure based on species habitat preference was detected. The low R value is most likely due to the high number of replicates included in the analysis, and indicates that there is a great degree of overlap in habitat use among species. Pair-wise comparisons yielded seven species pairs which differentiated in habitat use across quadrats sampled, and cluster analysis depicted the same significant separations in habitat use as were recovered in pair-wise comparisons (i.e., species found to be significantly different in ANOSIM occurred in opposing clusters). Interestingly, the two large significant clusters recovered contained three and four species each, indicating that separation in habitat use between groups of species may exist. The division of habitat resources among groups of species (rather than division among all individual species) may indicate a framework for maintaining community structure, and limiting levels of inter-specific competition in communities. Findings of group separation are interesting, and do not seem to corroborate predictions of niche theory, where species should completely segregate along at least one variable to co-exist (Vandermeer 1972). My results suggest strong overlap of habitat use within the two main clusters when including occurrences at all sites, however, it is possible that species may segregate at the microhabitat level, although I did not find consistent segregation among species within sites.

Comparisons of phylogenetic relationships with habitat use data demonstrate two potential processes of darter habitat trait evolution. First, the five most abundant species showed habitat use relationships which echoed phylogenetic relationships. In fact, when only considering the five most abundant species, the cluster dendrogram shared the same topology as the phylogeny. For these most common species, evolutionary history seems to play a strong part in determining ecological functioning, where closely related species have maintained similar habitat preferences across time. Under a Brownian motion model of evolution, this is the expected relationship between phylogeny and ecology (Blomberg et al. 2003), where distances between species in the phylogeny should directly correlate to the degree of difference in habitat use between species. However, I did not recover this relationship among all seven species, and thus the second potential process of habitat use evolution described is the displacement of habitat use traits among phylogenetically close species. Interestingly, closely related species (i.e., sister species within the seven species phylogeny) were found to have different habitat affinities from one another. This pattern was seen between *E. aquali* and *E. rufilineatum*, and between *E. blennius* and *E. blennioides* (Figure 5 & 6). Darter species that most recently diverged favored different habitats, which suggests some level of resource partitioning, or competitive displacement, between phylogenetically close species. Losos et al. (2003) found evolutionary divergence in habitat use among closely related *Anolis* species, where niche complementarity only occurred among distantly related taxa. Although the present study only recovered this pattern in the closest of relatives (i.e., most species showed conserved habitat use across the phylogeny), it is possible that natural selection favoring ecological divergence may have reduced inter-specific competition among "closer" relatives (Losos et al. 2003).

Future studies directly incorporating evolutionary relationships with habitat use data may be appropriate for gaining a better understanding of community structure and community assembly mechanisms (Webb et al. 2006, Vamosi et al. 2009). Furthermore, incorporating morphologic characteristics that reflect ecological functions may also have explanatory value in understanding habitat use among stream fish species (Douglas & Matthews 1992, Guill et al. 2003, Franssen et al. 2014). For example, Carlson et al. (2009) provides evidence that closely related, co-occurring *Percina* undergo more rapid morphologic diversification. Here I describe habitat use displacement among closely related co-occurring species, a pattern that may be further explained by ecologically relevant morphologic traits.

***Conclusions* –** I found separation in habitat use among groups of species in communities of closely related stream fishes. The division of habitat resources among groups of species (rather than a division among all individual species) suggests a framework by which community structure may be maintained, possibly through limiting levels of inter-specific resource competition. The shared pattern between phylogeny and habitat use in the five most abundant species suggests that ecological preferences may be strongly influenced by lineage among the most common community members. However, dissimilarity in habitat use between the most closely related species pairs indicates ecological displacement between genetically similar species. These findings are important for gaining a better understanding of ecological and evolutionary mechanisms that shape and maintain the co-occurrence of numerous congeneric species within stream communities.

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Chapter 2: **Phylogenetic and ecological factors influence temperate stream fish community structure**

Abstract

Both biotic and abiotic factors influence community assembly, but the relative influences of these interactions are not well established. Temperate stream fish communities present an opportunity to test the importance of assembly mechanisms, because phylogenetically closely related species often co-occur at fine spatial scales where inter-specific interactions are expected. I quantified phylogenetic relatedness, variance in habitat use, and variance in morphology, among co-occurring species of darters (Percidae: Etheostomatinae) in stream communities to elucidate mechanisms producing community structure. Additionally, I incorporated species abundances to assess density effects on patterns of phylogenetic structure. Communities showed consistent phylogenetic clustering both with and without abundance weighting, indicating habitat filtering assembly processes. Species' habitat use within communities also showed patterns of clustering, further identifying habitat filtering as an influential assembly mechanism. However, body shape among species within communities was even, suggesting high morphologic variance among species. Patterns of clustering indicated that habitat filtering was a main driver of darter community assembly, although I also found some degree of evolutionary convergence in habitat use among co-occurring species. Furthermore, body shape evenness within communities suggested levels of limiting similarity in morphology and some influence of competitive exclusion processes. The integration of phylogeny, habitat use, and morphologic traits allowed for a more complete examination of both habitat filtering and competitive exclusion

processes, and explained the roles that these processes play in community assembly. This integrated framework provided new insights because it linked evolutionary patterns to assembly processes through further examination of ecologically relevant traits.

Introduction

A fundamental goal of ecology is to determine the mechanisms responsible for the distributions and abundances of organisms. Because organisms occur in complex communities and are subject to both biotic and abiotic influences, processes thought to structure communities have been widely studied (e.g., Grossman 1982, Tilman 2004, Maherali & Klironomos 2007). Both biotic interactions (e.g., competition and predation) and abiotic factors (e.g., contemporary and historical habitat characteristics) have been demonstrated to affect community structure (e.g., Kelt et al. 1995, Poff 1997, Forsman et al. 2001), but uncertainties persist as to the relative importance of biotic and abiotic influences during community assembly (Webb et al. 2002, Kraft & Ackerly 2010, Godoy et al. 2014). While a focus on patterns of evolutionary relationships among community members has increased our understanding of assembly processes, there are still reservations about inferring ecological mechanisms from phylogenetic patterns alone (Barnagaud et al. 2014, Gerhold et al. 2015). Linking evolutionary patterns to ecological processes of community assembly requires integrating metrics of habitat use, functional morphological traits and phylogenetic patterns (Graham et al. 2012).

Phylogenetic approaches provide a tool to estimate the relative importance of biotic and abiotic interactions based on evolutionary relatedness of community members, given trait conservatism (Webb 2000). Trait conservatism is met when closely related species are more ecologically similar than expected from phylogenetic relationships (Losos 2008). These methods predict that inter-specific competition will produce communities containing species less closely related than expected by chance (i.e., phylogenetic evenness) due to competitive exclusion among closely related species competing for resources. Alternatively, habitat filtering is predicted to result in the coexistence of species that are more closely related than expected by chance (i.e., phylogenetic clustering) due to closely related species sharing similar habitat requirements (Weiher & Keddy 1995, Poff 1997, Webb et al. 2002). Non-random patterns of phylogenetic community structure have been demonstrated in a wide array of taxonomic groups, for example lake fishes (Helmus et al. 2007), fungi (Maherali & Klironomos 2007), birds (Gomez et al. 2010), mammals (Cardillo 2011) and plants (Webb 2000; Fine & Kembel 2011). Although such patterns are often recovered, identifying the processes (biotic and or abiotic) responsible for patterns is often difficult when habitat use and morphologic trait distributions among co-occurring species are not known (or included).

Because closely related species are predicted to be more ecologically similar than phylogenetically distant species, understanding species habitat use and morphologic relationships is key for a thorough interpretation of phylogenetic community structure patterns (Kraft et al. 2007, Graham et al. 2012, Barnagaud et al. 2014). Therefore, studies examining species’ ecologies in light of phylogenetic structure are needed to bridge the gap between contemporary and historical influences on community assembly. Phylogenetic patterns and distributions of habitat use and morphologic traits can be used to infer community assembly mechanisms across multiple scenarios, and moreover, identifying the relative influences of habitat filtering and competitive exclusion becomes more complex depending on the evolution of habitat use and morphology across the phylogeny (i.e., conserved and or convergent) (Figure 7).

Additionally, studies examining community assembly have relied on species presence/absence data when inferring phylogenetic structure. This approach is problematic because it ignores species' abundances which may contribute to community structure patterns. Including abundances when examining patterns of co-occurrence among species is important because of the possibility that assembly processes are heavily influenced, or masked, by more abundant species (Anderson et al. 2004; Hardy 2008; Vamosi et al. 2009). For example, competition may be important if the most abundant species in a community is also the most isolated species on the phylogeny. Without the incorporation of abundance, this signal could be missed.

I integrated genetic relatedness, morphologic trait structure, and habitat use data, among darter (Percidae: Etheostomatinae) community members to evaluate community structure patterns, and identify processes influencing community assembly in temperate U.S. streams. The objectives were *i*) to test for non-random phylogenetic structure among community members, while incorporating presence/absence and abundance weighted data, *ii*) test the degree of morphologic and habitat use similarity among co-occurring species within communities, and *iii*) examine the evolution of habitat use and morphologic traits among darter species present in the system to assess phylogenetic trait conservatism (i.e., conservatism of habitat use and morphology). Under trait conservatism, patterns of phylogenetic clustering will result from habitat filtering, and patterns of phylogenetic evenness will be the result of competitive interactions among co-occurring species. Furthermore, patterns of habitat use and morphologic trait structure that correlate with phylogenetic community structure patterns will link evolutionary relationships to ecological processes.

Study system – The southeastern United States harbors the most diverse temperate freshwater fish fauna in the world (Warren et al. 1997, Burr & Mayden 1992, Lundberg et al. 2000). At the center of this diversity are river systems included in the Tennessee River drainage (Warren et. al 1997). Large components of the ichthyofauna within these systems are darters (Percidae), a group that is endemic to North America and that contains an estimated 250 species (Scharpf 2008, Near et al. 2011). Darters are small bodied, benthic stream fishes that often occur in shallow areas with high flows (riffles). Additionally, darters rely on body shape characteristics to maintain position on the streambed and navigate without being displaced by high flows. Within the Duck River system (Lower Tennessee River drainage) ~30 species of darter are found and often co-exist in local communities (i.e., based on personal collections and museum records). Because these communities harbor great species diversity at small spatial scales, interactions between species within communities are plausible (Vamosi et al. 2009). Due to high levels of co-occurrence among congeners within stream communities, this is a model system for testing biotic and abiotic mechanisms of community assembly.

Materials & Methods

Data collection **–** In June 2012, I collected fish community data from 15 sites within the Duck River in Tennessee, U.S. (Figure 8). Sites were chosen based on the presence of riffle habitat which is preferred habitat for darter species. At each stream site, I collected darters and abiotic parameters from 18 quadrats (1.5m x 1.5m) distributed across three transects. Transects were established at the lower, intermediate, and upper portion of each site, and each transect contained six quadrats (n = 270 quadrats total). Transects spanned the width of the stream channel perpendicular to stream flow, and quadrats were evenly spaced across each transect to ensure unbiased sampling of available habitat (Figure 8). At each quadrat fishes were collected via kick seining using a weighted 1.8m seine net (3mm mesh). Because I was interested in community structure of stream sections, species collected from the 18 quadrats within each site were pooled prior to community analyses. Specimens were preserved in 10% formalin and later cataloged at the Sam Noble Oklahoma Museum of Natural History (SNOMNH), University of Oklahoma.

Abiotic data was collected from each quadrat and included: water depth, flow velocity (m/s), and substrate size. Within each quadrat, three flow velocity measures were taken using a FLO-MATE flowmeter (model 2000, Marsh-McBirney, Inc.). Total stream depth was measured at the same points as flow velocity. Substrate size was quantified by taking five random substrate grabs per quadrat (i.e., each grab consisted of an individual particle), and each particle was measured using a substrate sizer (AL-SCI Field Sieve). Substrate was categorized as gravel (2-16mm), pebble (16-64mm), cobble (64-256mm), boulder (> 256mm), and bedrock (Compton & Taylor 2013).

***Phylogeny* –** A maximum likelihood molecular phylogeny for the regional species pool was generated using multiple nuclear and mitochondrial genes (cytochrome b [*Cyt b*], s7 intron 1 [*S7*], cytochrome oxidase 1 [*CO1*], NADH dehydrogenase 2 [*ND2*], recombination activating gene 1 [*RAG1*]). The regional species pool was defined as species within the family Percidae occurring within the Duck River drainage (based on all available museum records accessed through the Fishnet2 Portal, www.fishnet2.net, 2015-02-15). I included 28 species in the regional pool, which excluded *P. shumardi*, *P. vigil*, and *E. histrio*, because collection records indicated these three species occur near the Duck and Buffalo River confluence, but do not typically occur upstream of this confluence in the Duck River. Sequences were downloaded from the National Center for Biotechnology Information's Genbank and aligned using MUSCLE (Edgar 2004) (Appendix C). Randomized Axelerated Maximum Likelihood (RAxML) was run on the unpartitioned data set using BlackBox (Stamatakis 2006, Stamatakis et al. 2008). The general time reversible model of nucleotide evolution (GTR + γ + y) was selected for the analysis. The ML tree was transformed to an ultrametric tree using the *chronopl* function in R (Ape package) (Sanderson 2002), which was required for phylogenetic community structure analyses (Hennequin et al. 2014).

***Phylogenetic community structure* –** Phylocom version 4.2 (Webb et al. 2008; Webb et al. 2011) was used to calculate metrics of phylogenetic community relatedness within each stream site based on the ultrametric tree. The *comstruct* function was used to calculate Mean Pairwise Distance (MPD) and Mean Nearest Phylogenetic Taxon

Distance (MNTD) for each assemblage. Observed MPD and MNTD values were compared to those of 999 randomly generated communities in order to determine significant phylogenetic structure ($P \leq 0.05$). Null communities maintained the species richness of the observed community, and species were randomly drawn without replacement from the phylogeny pool for each community. This model was chosen because of the assumption that species included in the regional phylogeny pool were not dispersal limited, and therefore it was plausible that all species had the potential to occur in each community. Two metrics were calculated for each community; the Net Relatedness Index (NRI) and the Nearest Taxon Index (NTI) (Equation 1 & 2).

Equation 1 $$NRI = -1 \times \frac{MPD_{sample} - MPD_{rndsample}}{sd(MPD_{rndsample})}$$

Equation 2 $$NTI = -1 \times \frac{MNTD_{sample} - MNTD_{rndsample}}{sd(MNTD_{rndsample})}$$

Net relatedness index (NRI) is calculated from the MPD, and measures the standard effect size of the mean phylogenetic branch length between all species pairs within a community. Nearest taxon index (NTI) is calculated from the MNTD and is the standard effect size of branch lengths separating each species from its closest relative within a community (i.e., nearest neighbor). Therefore NRI is sensitive to phylogeny-wide patterns of clustering or evenness, and NTI is sensitive to clustering or evenness patterns across the phylogeny tips (Webb 2000). Values (NRI or NTI) greater than zero indicate phylogenetic clustering, and values less than zero indicate phylogenetic evenness (Webb et al. 2002). All *comstruct* procedures were run using the presence/absence data set for each of the 15 communities, and were then additionally run incorporating the -*a* switch which included species abundance weighting in the analysis. Therefore, I examined patterns of phylogenetic community structure based on taxa present, and patterns based on relatedness among all individuals from each community (i.e., incorporating species abundances).

Morphology vs. phylogeny **–** Geometric morphometric techniques were used to quantify body shape for all darter species collected during the study period. Body shape is an important functional characteristic of darters, and it is suggested that fish body shape is an indicator of trophic niche and predator-prey interactions (Webb 1984, Guill et al. 2003). Twelve homologous landmarks were assigned to the left side of each specimen in order to quantify body shape. Landmarks were digitized for each individual using the software TPSDig version 2.12 (Rohlf 2008), and data for all specimens were combined using TPSUtil version 1.44 (Rohlf 2009). A Procrustes fit was performed on landmark data to correct for size and specimen position differences. Principal component analysis (PCA) was performed on landmark coordinate data using MorphoJ version 1.01b (Klingenberg 2008) to provide a good representation of species morphologic relationships within the species pool. To illustrate relationships between phylogeny and morphology, the molecular phylogeny was overlaid onto the morphologic relationships recovered in PCA (PC1 vs. PC2), and phylogenetic signal was assessed using a permutation test, which compared the resultant relationships against 10,000 randomized iterations. Principal component scores for the first three PC’s were interpretable based on the broken stick model (Jackson 1993), and were retained for tests of morphologic trait structure and conservatism in the following analyses.

Habitat use and morphologic trait structure – Trait variance within communities was examined using species habitat use data, as well as species morphologic data. Habitat use profiles were created for each species collected using the mean flow velocity, mean depth, and mean proportions of gravel, pebble, cobble, boulder, and bedrock from quadrats where each species occurred. Morphology was assessed by including PC loadings one through three for each species from above analysis of darter body shape. Patterns of clustering and or evenness were tested by asking whether co-occurring species showed more or less variance in their habitat use and or morphologies than expected in null communities generated from the regional species pool (i.e., based on 999 randomized runs). For these analyses, the species pool contained all species collected at all sites during the study period. Habitat use and morphologic trait patterns of clustering or evenness were measured using standard effect size of variance (SESvariance) for each habitat and morphologic parameter within each community (Equation 3). SESvariance values less than zero indicate that co-occurring darter species were more similar than at random, and values greater than zero indicate dissimilarity among co-occurring species. In essence, this is a measure of clustering or evenness of habitat use and body shape within communities. These measures were quantified using the *comtrait* function in Phylocom (Webb et al. 2011).

Equation 3

$$SES_{variance} = \frac{Variance_{obs} - Variance_{rnd}}{sd(Variance_{rnd})}$$

Analyses of trait evolution – Morphology and habitat use evolution were examined to assess whether these were evolutionarily conserved in darters. Using the *phylosignal* function in the R package Picante, the *K* statistic (Blomberg et al. 2003) for each trait was calculated to quantify conservatism of traits across the phylogeny in relation to traits evolving under a Brownian motion model. The *K* statistic represents the strength of phylogenetic signal for each trait, where values of one indicate that trait differences are proportional to tree branch lengths, and values greater than one indicate species traits are conserved. Therefore, traits show phylogenetic conservatism when species within clades were more similar ecologically than expected under Brownian motion evolution. Although *phylosignal* also outputs randomization tests for significant phylogenetic signal for each trait using independent contrasts, these values were not reported due to the low power of this analysis when examining trees with < 20 species (Blomberg et al. 2003). It should be noted that the *K* statistic is a standardized measure, and is not influenced by sample size. It is therefore an interpretable descriptor of trait conservatism despite number of species included (Blomberg et al. 2003).

Results

***Phylogenetic community structure* –** Communities showed a strong tendency towards phylogenetic clustering for all parameters and indices. A large proportion of communities were found to have positive SESmetric values both with and without the incorporation of species' abundances. *Comstruct* results based on taxa presence/absence recovered positive Net relatedness index (NRI) values for 10 of the 15 communities, and showed significant phylogentic clustering within three communities (Figure 9a). Moreover, Nearest taxon index (NTI) values were positive for 12 of the 15 communities, and significant clustering was recovered within five communities (Figure 9a). Similar results were recovered from the *comstruct* procedure, which included weighted abundances. NRI values were positive for 11 communities, and 13 communities had positive NTI values (two significantly clustered) (Figure 9b). The regional phylogeny is presented alongside presence and abundance data for each species within each community and shows the majority of species clustering within two clades (Figure 10).

***Morphology vs. Phylogeny* –** Principal components one through three combined explained 80.46% of variance in body shape. Principal component one (48.25% of variation) described variation in head length where positive values indicated species had larger heads in proportion to body length (Figure 11a). Principal component two (18.85%) described overall variation in body length proportions, with positive values representing species with longer caudal peduncles, and shorter body lengths (Figure 11b). Furthermore, PC three (13.37%) represented variation in head shape, and mouth position (Figure 11c). The test for phylogenetic signal showed significant signal in body shape ($P < 0.001$) among all PCs combined, and the relationships between phylogeny and body shape are depicted (Figure 12).

***Habitat use and morphologic trait structure* –** Co-occurring species within communities showed similar (i.e., clustered) habitat use for all habitat factors, with the exception of "boulder" as indicated by negative SESmetric values (Figure 13). The positive value for "boulder", however, is most likely due to the rarity of boulders in the system, as null communities would often have a variance of zero for boulder affinity due to a majority of species never occurring with boulders.

Darter body shape traits within communities were more varied than expected for PC1 and PC2, whereas PC3 showed less variance than expected. This indicated that species' morphologies within communities were more different than expected at random based on head size (PC1) and body/caudal peduncle length (PC2). However, species with similar head shapes and mouth position traits (PC3) co-occurred more frequently within communities than expected (Figure 13).

Trait evolution- Species body shape, based on PC1 (48.25% of morphologic variation), was conserved across the phylogeny, and yielded a Blomberg's $K > 1$ (Table 5). This was the only trait to show conservatism, although, several other habitat and morphologic traits yielded K values approaching one. K values close to one indicate phylogenetic distances among species are generally proportional to trait differences among species. Furthermore, PC3 had the lowest K value among all traits ($K = 0.493$), indicating possible convergence of head and mouth traits among more phylogenetically distant related taxa.

Discussion

Stream fish communities are a unique testing ground for implementing phylogenetic and trait based approaches for understand community assembly mechanisms. To my knowledge no studies have implemented this methodology in temperate stream communities, and few studies have examined assembly mechanisms in stream fish communities (*see* Peres-Neto 2004, Strecker & Olden 2014). Specifically, darter communities provided an opportunity to examine community dynamics among co-occurring, closely related species, where inter-specific interactions are plausible. Habitat filtering was identified as a driver of darter community assembly based on phylogenetic clustering and habitat use clustering among co-occurring species. Additionally, competitive exclusion was found to be of some importance based on patterns of morphologic evenness among co-occurring species. Assembly mechanisms were complex, and the integrated framework used was instrumental in understanding relative influences of both habitat filtering and competitive exclusion processes.

Patterns of phylogenetic structure – A strong tendency towards phylogenetic clustering within communities was found among species within stream sites, as well as among individuals within sites using both NRI and NTI. These analyses yielded similar results with and without abundance weighting, indicating that more abundant species were not more phylogenetically distant from close relatives within communities (i.e., the greater abundance of a species did not cause greater phylogenetic “repulsion”). These patterns including abundances across communities could be seen in Figure 10. The additional analyses including species abundances were important due to the possibility that competition among close relatives could have been masked using presence/absence data, but apparent when incorporating species' abundances (Anderson et al. 2004, Vamosi et al. 2009). This however, was not the case, and phylogenetic structure results were similar with and without the inclusion of species' abundances suggesting that recovered clustering patterns were representative of community member interactions. A greater amount of significant clustering was found using NTI, as compared to NRI, and therefore the patterns recovered were representative of clustering among nearest-neighbors at the "tips" of the phylogeny. Greater clustering across the "tips" of the phylogeny is expected with higher NTI values (Webb 2000, Kraft et al. 2007, Cardillo 2011). Clustering within communities mainly occurred in two clades (Figure 10). Although patterns of phylogenetic clustering are quite apparent among communities, it is interesting that the two main species clusters were relatively evenly dispersed across the phylogeny. Clustering within these clades indicates habitat filtering as an assembly mechanism; however, the phylogenetic distance between the two representative clades seems to depict phylogenetic evenness between the two lineages (Figure 10).

Habitat use and morphologic trait conservatism – Phylogenetic niche conservatism is assumed in order to properly infer assembly processes from phylogenetic patterns (Webb 2000). Here, conservatism was found for a large portion of darter body shape variation (PC1), however patterns of convergence were recovered for habitat use parameters. Habitat parameters and morphologic traits were identified *a priori* as being ecologically relevant for darter ecological interactions; however, trait evolution results suggest a lack of consensus between habitat use and morphology among species. This lack of consensus leads to the inevitable question, which traits are more ecologically important? Under trait conservatism, habitat filtering should yield clustered dispersion patterns, and if traits are convergent, processes of limiting similarity should yield patterns of clustering (Webb et al. 2002, Kraft et al. 2007). Although conservatism was not found for darter habitat use among species collected during the study, it should be noted that habitat use conservatism may have been recovered had data been available, and included, for all species in the regional pool. This may be the case considering sites were chosen based on similar habitat attributes (i.e., presence of riffles) and therefore species not collected during the study could be assumed to have quite different habitat use affinities from those included. That being said, habitat use and morphologic traits are probably both important indicators of ecological interactions, but they may be more or less important depending on the spatial scale at which communities are studied and the scale at which phylogenetic species pools are defined (Cavender-Bares et al. 2006, Cavender-Bares et al. 2009). Because phylogenetic clustering was most consistently observed across communities, and ecologically relevant traits were either conserved, or showed some level of convergence, it was necessary to examine the distribution patterns of habitat use and morphologic traits in communities to better identify assembly processes.

Patterns of habitat use and morphologic trait structure – Species with similar habitat use (i.e., substrate, depth, and flow) were more likely to co-occur together in communities. This suggested that habitat filtering was an acting assembly mechanism, and furthermore, patterns of habitat use clustering corroborated patterns of phylogenetic clustering in communities. The evolution of habitat use among species (for each habitat parameter) was not phylogenetically conserved, and therefore habitat use clustering patterns are likely the product of convergent evolution among species' habitat usage. In other words, species that shared similar habitat preference showed higher levels of co-occurrence, but were not always close phylogenetic relatives. A previous study examining darter habitat use as it relates to phylogeny, recovered some level of habitat use convergence among the closest phylogenetic relatives, although the majority of species tended to show phylogenetic signal for habitat use (Geheber & Frenette *in review*). Here again, it should be noted that in the present study habitat use was only quantified for species collected, whereas no habitat use data were available for additional species included in the regional species pool. Given that phylogenetic and habitat use clustering were recovered in riffle communities, interpreting these observed patterns in the light of "no habitat use conservatism" may be inappropriate due to the phylogenetic structure patterns observed. This reasoning can be visualized in figure 10, where species from the two main clades are represented in the majority of communities despite clades being distantly related, and overlap in habitat use between the clades likely represents why the assumption of conservatism was not met. Because of observed clustering patterns in habitat use and phylogenetic structure, habitat filtering was an apparent assembly mechanism in darter riffle communities despite habitat use convergence among clades.

Morphologies of co-occurring species were evenly distributed based on species body shape (PC1 and PC2), and body shape evolution was conserved among species. In short, species that have quite different body shapes are often found together within communities, and close phylogenetic relatives have similar body shapes. This result was interesting because it indicated that phylogenetic patterns alone, under trait conservatism, may not fully reflect acting assembly mechanisms. Rather, assembly is complex and multiple mechanisms may simultaneously influence observed community structure. Because phylogenetic clustering occurred most often in two relatively distant clades (Figure 10), evenness of species body shape likely represents some level of competitive exclusion among species within each of the two clades. However, multiple scenarios could be responsible for these patterns of evenness. First, evenness of darter body shape, a trait found to be conserved phylogenetically, may suggest there is more than one adaptive solution for functioning in riffle communities. Under this scenario species show similar habitat use within communities, but remain morphologically distinct (within clades) due to lack of selection pressure for an optimal body shape. It could be that no individual body shape is more beneficial than any other within riffles. Second, limiting similarity based on body shape may be important at the microhabitat scale. Although species within communities show habitat use similarity, it is possible that body shape characteristics are indicative of habitat use at micro-scales. Interestingly, it is predicted that at decreased spatial scales, habitat homogeneity will increase the strength of competitive interactions among closely related species, creating patterns of phylogentic and or trait evenness (Emerson & Gillespie 2008, Cavender-Bares et al. 2009). The prediction of increased competition at fine spatial scales could explain the patterns I recovered for darter body shape variance, where morphologies were evenly distributed among co-occurring darters. Although no habitat data were collected at scales finer than the quadrat (1.5x1.5m), it is feasible that darters may compete for habitat and food resources at extremely small scales considering their life history characteristics (e.g., use of specific substrates and micro-flows) (Matthews 1985, Kessler & Thorp 1993).

***Conclusions* –** I predicted that under phylogenetic trait conservatism phylogentically clustered communities would be assembled via habitat filtering processes and phylogenetically even communities would be assembled via competitive interactions. I found repeated patterns of phylogenetic clustering among darters in riffle communities, where species body shape was conserved. Habitat filtering was the predominant assembly mechanism as further suggested by patterns of habitat use clustering; however, these data alone did not give a complete picture of influential mechanisms. The integration of morphologic trait distributions aided in a more thorough investigation of assembly mechanisms, and allowed “untangling” of biotic and abiotic factors influencing community assembly (Graham et al. 2012). I illuminated unexpected relationships between phylogenetic structure patterns and trait structure patterns, including instances of morphologic limiting similarity among co-occurring darters, which suggested an underlying level of competitive exclusion among morphologically similar species likely occurring at the microhabitat scale. Had I examined patterns of phylogenetic structure in the absence of pertinent ecological data, mechanisms of assembly may have been improperly interpreted or over simplified.

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Chapter 3: **The effect of spatial scale on darter community assembly: Evolutionary relationships illustrate ecological processes**

Abstract

Understanding how biotic and abiotic processes influence community assembly is a fundamental theme in ecology. Although spatial scales at which communities are studied may affect the relative importance of such assembly processes, this influential aspect of community assembly has not been thoroughly addressed. I examined the effects of spatial scale on habitat filtering and competitive exclusion assembly processes in darter (Percidae: Etheostomatinae) communities across four temperate stream systems. I tested the hypothesis that in fine spatial scale communities, competitive exclusion will be influential on assembly, and habitat filtering will become more influential on assembly as spatial scale increases. It was assumed that habitat heterogeneity would increase with scale, and therefore would alleviate direct competitive exclusion acting at finer scales. Using a framework which incorporated genetic relatedness, morphologic traits, and habitat use among co-occurring darter species, I identified ecological and evolutionary patterns of structure to elucidate mechanisms of assembly. Based on phylogenetic structure, darter communities showed an increase in habitat filtering signal as I scaled up from fine to intermediate communities, and two of the four systems showed a further increase in habitat filtering signal at the broadest scale. Phylogenetic structure reported strong signal for competitive exclusion at the broadest spatial scale in one river system. Overall, evidence for both habitat filtering (based on phylogenetic structure and habitat use structure) and competitive exclusion (based on morphologic trait distributions) was

recovered, indicating that both assembly processes act simultaneously in communities. While habitat filtering was found to have high relative importance during assembly (i.e., and showed increased importance in larger scale communities), competitive exclusion likely acted as a secondary mechanism, limiting species similarity following habitat filtering processes. These results support increased habitat filtering influence at larger spatial scales. Moreover, results suggest that habitat filtering and competitive exclusion can act simultaneously during assembly, although the relative influence of each process may be spatial scale dependent. This study demonstrates the importance of understanding spatial scale and its effects on patterns and processes of diversity.

Introduction

Understanding the relative roles of biotic and abiotic processes in generating patterns of diversity within communities remains a fundamental theme in ecology. Although it has long been recognized that communities harbor nonrandom collections of co-occurring species (e.g., Connell & Slatyer 1977, Schlosser 1982, Silvertown 2004), the relative influences of processes that effect assembly are often hard to identify. Additionally, the spatial scales at which communities are viewed can influence the relative importance of assembly processes (Cavender-Bares et al. 2006, Kraft et al. 2007, Emerson & Gillespie 2008). Because recently diverged taxa are predicted to be similar ecologically (Darwin 1859), evolutionary relatedness among community members, and the ecological characteristics that community members possess, may provide a beneficial link for identifying assembly processes (Webb et al. 2002, Graham et al. 2012, Barnagaud et al. 2014). Using this framework, studies have indicated nonrandom patterns of evolutionary relatedness (*see* Webb et al. 2002 and Vamosi et al. 2009 for review), but few have examined such patterns and processes in a spatial scale-dependent context.

Generally, communities are thought to be structured by two main processes, competition and or habitat filtering. Competition is predicted to limit co-occurrence of species that are ecologically too similar through competitive exclusion, due to similar fundamental niches (Elton 1946, Hardin 1960, Diamond 1975, Webb et al. 2002). Alternatively, within a regional species pool, species with similar ecological requirements (e.g., species with similar trophic, physiological, and reproductive traits) are likely to co-occur where local conditions match these requirements (Weiher & Keddy 1995, Poff 1997). This process is habitat filtering, which operates by effectively eliminating species from the regional species pool that cannot persist under local environmental conditions. Although competitive exclusion and habitat filtering processes seem opposing, competitive exclusion is predicted to be more influential in fine scale communities due to limitation of resources that can be partitioned, and habitat filtering should be more apparent in larger scale communities due to greater habitat heterogeneity (i.e., an expanse in resource variation should alleviate direct competitive pressures) (Cavender-Bares et al. 2006, Emerson & Gillespie 2008, Cavender-Bares et al. 2009, Vamosi et al. 2009).

An increase in prevalence of phylogenetic available information and an increase in the number of studies incorporating species trait data have lead to a burgeoning field of community ecology which aims to understand assembly processes (e.g., Vamosi et al. 2009). Phylogenetic and trait based approaches provide a framework for estimating the relative importance of assembly processes based on patterns of relatedness (i.e., phylogenetic and or trait) among community members (Webb 2000). If ancestral traits related to resource use remain similar in descendents (i.e., phylogenetic trait conservatism), competitive exclusion should result in communities containing species less closely-related than in null communities. Alternatively, habitat filtering can result in the coexistence of species that are more closely related than at random due to closely related species sharing similar habitat requirements (Webb 2000, Webb et al. 2002). Moreover, this framework can be implemented across increasing spatial scales to assess ecological and evolutionary patterns in different sized communities (Emerson & Gillespie 2008, Gomez et al. 2010).

Temperate stream fish communities offer a unique opportunity to advance knowledge of assembly processes across differing spatial scales. Species co-occur within these communities in high densities at relatively fine scales where interactions among species are expected (Vamosi et al. 2009). Stream fish communities are known to exhibit patterns of community structure across time and space (Geheber & Piller 2012, Matthews et al. 2013), although it is not clear the extent to which these patterns are regulated by biotic and or abiotic processes (Grossman et al. 1982), and whether or not spatial scale affects the relative influences of such processes.

I examined evolutionary and ecological patterns to elucidate the relative importance of competitive exclusion and habitat filtering processes within darter (Percidae: Etheostomatinae) communities at multiple spatial scales. By incorporating genetic relatedness, phenotypic similarity, and habitat use data among co-occurring darters, I aimed to identify the relative influences of assembly processes at fine, intermediate, and broad spatial scales. I tested the hypothesis that in fine spatial scale communities competitive exclusion will be influential on assembly, and habitat filtering will become more influential on assembly as spatial scale increases.

***Study system* –** The southeastern United States has the most diverse temperate freshwater fish fauna in the world (Warren et al. 1997, Burr & Mayden 1992, Lundberg et al. 2000). River systems in the Tennessee River and Cumberland River drainages are near the center of this diversity (Warren et. al 1997). Large components of the ichthyofauna in these systems are darters (Percidae); a diverse group of small-bodied fishes endemic to North America, with an estimated 250 species (Scharpf 2008, Near et al. 2011). Darters are interesting ecologically in that most species lack swim bladders, and are benthic in nature. Due to this life history, stream bed characteristics (i.e., substrate size and type) and stream depth are important for their ecological functioning (e.g., feeding, reproduction, shelter, etc.). I examined darter communities in four river systems: The Duck, Buffalo, Harpeth, and Stones Rivers; all of which are included in the Highland Rim and or Nashville Basin located in central Tennessee (Etnier & Starnes 1993). Due to relatively high levels of co-occurrence among congeners within these stream communities (at all scales), darter communities are model systems for testing assembly mechanisms.

Materials & Methods

Field collections – During 2012 and 2013 stream fishes and abiotic parameters were sampled from 41 stream sites among four river systems in central Tennessee, USA. River systems included the Duck and Buffalo rivers (Tennessee River drainage), and the Harpeth and Stones rivers (Cumberland River drainage) (Figure 14). The Duck River was sampled in June 2012 and all others in June 2013.

In each stream site darters were collected in 18 quadrats (1.5m x 1.5m) that were distributed across three transects, each transect containing six quadrats (n = 738 quadrats total). Transects in each stream site were established approximately 10 meters either upstream or downstream from one another, and spanned the width of the stream channel perpendicular to stream flow (Figure 14). Quadrats were evenly spaced across each transect to ensure complete sampling of all available habitat. Darters were collected via kick seining using a weighted 1.8m seine net (3mm mesh), where the seine was positioned along the downstream edge of each quadrat and fishes were herded into the net by manual churning of substrate from the upstream quadrat edge. All collections were preserved, identified, and cataloged at the Sam Noble Oklahoma Museum of Natural History, University of Oklahoma.

Abiotic parameters including stream depth, stream flow (m/s), and substrate composition were collected from all quadrats directly following fish collections. Three velocity measures were collected from each quadrat using a FLO-MATE flowmeter (model 2000, Marsh-McBirney, Inc.). Total stream depth was measured at the same points as flow velocity in each quadrat. Substrate size was quantified by taking five random substrate grabs per quadrat (i.e., each grab consisted of an individual particle), and each particle was measured using a substrate sizer (AL-SCI Field Sieve). Substrate size classes were gravel (2-16mm), pebble (16-64mm), cobble (64-256mm), boulder (> 256mm), and bedrock.

***Phylogeny* –** I generated a maximum likelihood molecular phylogeny for all darter species known to occur among the four river systems, using multiple nuclear and mitochondrial genes (cytochrome b [*Cyt b*], s7 intron 1 [*S7*], cytochrome oxidase 1 [*CO1*], NADH dehydrogenase 2 [*ND2*], recombination activating gene 1 [*RAG1*]). Sequences were downloaded from National Center for Biotechnology Information's GenBank (Appendix D) and aligned using MUSCLE (Edgar 2004). Randomized Axelerated Maximum Likelihood (RAxML) was run on the unpartitioned data set using BlackBox (Stamatakis 2006; Stamatakis et al. 2008). The general time reversible model of nucleotide evolution (GTR + γ + y) was selected for the analysis. Four individual regional species pool phylogenies (i.e., one for each river system) were generated from this initial phylogeny through pruning the tree, so that each regional pool only included species known to occur in that river. Species included in each regional pool were determined by museum records of occurrence (based on all available museum records accessed through the Fishnet2 Portal, www.fishnet2.net, 2015-03-15). All ML trees were transformed to ultrametric trees using the *chronopl* (Sanderson 2002) function in the R package *ape* (Paradis et al. 2004, R Core Team 2015) prior to all community structure analyses.

Phylogenetic community structure **–** I quantified phylogenetic community structure within all communities using Phylocom version 4.2 (Webb et al. 2011). Phylogenetic community structure was examined at the quadrat, site, and entire river spatial scales for each river system. The *comstruct* function was used to calculate Mean Pairwise Distance (MPD) and Mean Nearest Phylogenetic Taxon Distance (MNTD) for each assemblage. Observed MPD and MNTD values were compared to those of 999 randomly generated communities in order to determine significant phylogenetic structure ($P \leq 0.05$). Null communities maintained the species richness of the observed community, and species were randomly drawn without replacement from the phylogeny pool for each community. This model was chosen because of the assumption that species included in each regional phylogeny pool were not dispersal limited, and therefore it was plausible that all species had the potential to occur in each community. Two metrics were calculated for each community; the Net Relatedness Index (NRI) and the Nearest Taxon Index (NTI) (Webb 2000). Net relatedness index (NRI) is calculated from the MPD, and measures the standard effect size of the mean phylogenetic branch length between all species pairs within an assemblage. Nearest taxon index (NTI) is calculated from the MNTD and is the standard effect size of branch lengths separating each species from its closest relative within an assemblage (i.e., nearest neighbor). Therefore NRI is sensitive to phylogeny-wide patterns of clustering or evenness, because the metric is derived from the mean of all pair-wise phylogenetic distances among community members. Moreover, NTI is sensitive to clustering or evenness patterns across the phylogeny tip labels due to its nearest neighbor approach (i.e., patterns are recognizable by viewing species presence at the tips of the phylogeny)

(Webb 2000, Webb et al. 2002). For both indices, values greater than zero indicate phylogenetic clustering, and values less than zero indicate phylogenetic evenness (i.e., “repulsion” of closest related species) (Webb et al. 2002). All *comstruct* runs used presence-absence data for communities at each spatial scale, and communities containing < 2 species were removed prior to the analyses (i.e., at least 2 species are required for community structure tests). Mann-Whitney’s U was used to test for differences in community structure metrics between quadrats and sites within each river. Additionally, species occurrence and abundance was depicted in relation to the regional species pool phylogenies for each river system.

***Morphologic & habitat use structure* –** Body shape is an ecologically relevant trait for stream fishes, and it is suggested that fish body shape is an indicator of trophic niche and predator-prey interactions (Webb 1984, Guill et al. 2003). Geometric morphometrics were used to quantify body shape for darter species collected from each of the four river systems. Twelve homologous landmarks were assigned to the left side of each specimen. Landmarks were digitized for each individual using the software TPSDig version 2.12 (Rohlf 2008), and data for all specimens were combined using TPSUtil version 1.44 (Rohlf 2009). A Procrustes fit was performed on landmark data to correct for size and specimen position differences. Principal component analysis (PCA) was performed on landmark coordinate data using MorphoJ version 1.01b (Klingenberg 2008), and the first three PC scores were retained for tests of morphologic trait structure and evolutionary conservatism in all following analyses.

Habitat use profiles were created for each species collected, using the mean flow velocity (m/s), mean depth (cm), and mean proportions of gravel, pebble, cobble, boulder, and bedrock from quadrats where each species occurred. Profiles were created for species within each river system independently, therefore a species that was collected in multiple systems would have habitat use profiles specific for each system in which it occurred.

Habitat use and morphologic trait structure based on distributions of co-occurring species were quantified in each of the four river systems at the quadrat and site scale. Morphologic and habitat use structure within full river communities was not reported in these analyses due to lack of habitat data for species not collected during the study, but were included in regional species pools. Both habitat use and morphologic trait structure were measured in communities using standard effect size of variance (SESvariance). This measure is similar to NRI and NTI, but uses habitat use and morphologic trait distributions instead of phylogenetic relatedness to calculate clustering or evenness among co-occurring species. SESvariance values less than zero indicate that co-occurring species have more similar habitat use or morphologies than at random (clustering), and values greater than zero indicate greater differences among species habitat use or morphologies than at random (evenness). These measures were quantified using the *comtrait* function in Phylocom, and null distributions were generated using 999 permutations, where habitat use and morphologic trait values were shuffled among species pool members.

Evolution of ecological factors and species co-occurrence – I measured evolution among darter habitat use and morphologic traits within each river system independently and tested for niche conservatism. Here I define niche conservatism as clades which retain their niches, or related ecological traits, and remain unchanged over time (Wiens & Graham 2005). Blomberg's *K* was implemented as a measure of phylogenetic niche conservatism (Blomberg et al. 2003), where values greater than one indicate phylogenetic conservatism, and values less than one indicate phylogenetic divergence or convergence. Blomberg's *K* values are based on a Brownian motion model of evolution; therefore a value of one indicates that evolutionary change in a trait or characteristic is directly proportional to phylogeny branch length (Blomberg et al. 2003).

To examine the relationships among Blomberg's *K*, habitat use, and morphologic trait distributions among co-occurring species, fingerprint regression (*fingerprint.regression*) was employed using the R package *pez* (Pearse et al. 2015, R Core Team 2015). This model used linear regression to determine the slope between trait similarity and species co-occurrence within communities. A negative slope shows high trait similarity among community members, and a positive slope indicates low trait similarity among community members. Fingerprint regression then plots the observed slope of each trait against its Blomberg's *K* value. The fingerprint regression procedure was run for stream site and quadrat communities to examine habitat use and morphologic similarity in relation to trait evolution at the two spatial scales. It is important to note that fingerprint regression depicts each trait (i.e., habitat use factors and morphologic traits) as a single data point derived from the aforementioned regression slopes, and it is similar to the methods used by Cavender-Bares et al. (2006).

Results

***Phylogenetic community structure* –** The regional pool phylogenies are depicted showing species' occurrences and abundances (square root transformed) within the entirety of each river system (Figure 15). The Stones River has a relatively evenly spaced community among clades, whereas the other river communities show species clustering on the phylogeny, and or clustering within clades (Figure 15). Furthermore, the most abundant species overall, *Etheostoma rufilineatum*, co-occurred with close relatives in all systems except the Stones River, where it was the only clade member collected. Additionally, strong representation of the clade containing *E. blennius*, *E. blennioides*, *E. zonale*, *E. simoterum*, and *E. flavum*, was seen in the Duck and Buffalo rivers (Figure 15).

***Phylogenetic community structure in relation to scale* –** Overall, quadrats showed a trend of phylogenetic clustering among species within quadrats (positive index values) although the signal was not strong (Figure 16). Scaling up from quadrats to sites, communities generally showed positive increases in index values for both the Net relatedness index (NRI) and Nearest taxon index (NTI) (Figure 16). The Duck and Buffalo Rivers had significant increase in index values going from quadrat to site communities (Table 6). Patterns of phylogenetic structure at the full river scale, however, differed among river systems and between indices (Figure 16). Net relatedness index (NRI) values were positive, and significantly structured in both the Buffalo and Harpeth Rivers, however, the Duck River yielded a slightly negative index value. For the most part, NTI revealed no change, or decrease in values moving from sites to the full river community scale, however, the Stones River had a significant negative NTI value at the river scale (Figure 16).

Habitat use and morphologic trait structure – I examined the distribution of habitat use and morphologic traits among species within communities. Generally, habitat use among co-occurring species was more similar than would be expected at random, in both quadrats and sites, for all rivers. This is indicated by negative SESvariance values, which mean that co-occurring species show clustering in their habitat usage. However, morphologic traits, specifically PC1, generally showed positive SESvariance values for co-occurring species, suggesting that co-occurring species had limiting similarity of morphology (Figure 17). Furthermore, quadrat and site communities had similar patterns of structure for both habitat use and morphology values, although the Harpeth and Stones Rivers did tend to show increased signal for morphologic evenness moving from quadrats to sites (Figure 17). All morphology PCs show increased positive values in the Harpeth sites, and PC1 shows an increase in Stones Sites.

Evolution of ecological factors and species co-occurrence – Phylogenetic niche conservatism ($K > 1$) was found in 13 instances among species in the four river systems (Table 7). The Buffalo River darters showed particularly high phylogenetic conservatism among habitat use and morphologic traits, and (PC 1) was highly conserved in three of the four rivers (Table 7). Fingerprint regressions showed the relationships between evolution and similarity of habitat use and morphologic traits based on species co-occurrence at the quadrat (Figure 18a) and site (Figure 18b) scales.

The majority of measured habitat use factors were found in the bottom left quadrant of the fingerprint regression, and showed high similarity (negative values) among co-occurring species and convergent evolution at both scales (Figure 18a & b). Here, convergence is assumed due to high similarity in habitat use despite the lack of phylogenetic conservatism for habitat use factors (i.e., traits in the bottom right quadrant would indicate a lack of convergence and a lack of phylogenetic conservatism). Habitat use and morphologic traits in the upper left quadrant are phylogenetically conserved, and show high similarity among co-occurring species. A majority of Buffalo River darter traits fell in this quadrant. The upper right quadrant included some of the most conserved morphologic traits from all river systems; however, these traits also had greater dissimilarity (positive values) among co-occurring species (Figure 18). In general, co-occurring species at the site spatial scale showed more variation in habitat use and morphologic trait similarity than that recovered at the quadrat spatial scale (Figure 18a vs. 18b).

Discussion

Under phylogenetic niche conservatism, one might predict that close relatives will co-occur in communities based on their similar ecological requirements (e.g., Lebrija-Trejos et al. 2010). However, this scenario becomes problematic when close relatives that are too similar (i.e., similar ecologically) are subject to competitive exclusion (Elton, 1946, Diamond 1975). I show that habitat filtering and competitive exclusion both act simultaneously at different “levels” of the phylogeny (species vs. clades) and may be influenced by the spatial scale at which communities are defined. This simultaneous influence was further illustrated by species habitat use and morphologic trait structure patterns which indicated filtering for habitat use and limiting similarity for morphologic traits in communities. Furthermore, in multiple cases, distantly related clades of species have converged on similar habitat use, but morphologic traits have remained highly conserved among darters.

Phylogenetic community structure in relation to scale – The hypothesis that competitive exclusion will dominate at fine scales and habitat filtering at more broad scales, predicts that communities will shift from phylogenetic evenness towards phylogenetic clustering with the increase in scale (Cavender-Bares et al. 2009). I found an increased signal for habitat filtering in darter communities moving from fine (quadrats) to intermediate (sites) scales within multiple river systems. However, broad scale communities revealed mixed influences of habitat filtering and competitive exclusion, suggesting that assembly processes may differ in influence form one stream system to the next. Habitat filtering was found to be an important assembly mechanism in the Buffalo and Harpeth rivers, based on significant index values and high evolutionary conservatism among habitat use and morphologic traits. Patterns of evenness in the Stones River community indicated that limiting similarity processes tended to be more influential during assembly. Furthermore, the Duck River community showed patterns of phylogenetic clustering within separate clades (Figure 15), however, these clades were distantly related. This nested pattern suggested that habitat filtering was an influential assembly mechanism acting on species within clades, and limiting similarity may occur between clades. Although, due to the high level of convergent habitat use within Duck River darters, patterns of evenness among clades may likely represent competitive exclusion at finer scales within larger communities (i.e., based on evenly distributed morphologic traits). Net relatedness and Nearest taxon indices yielded consistently similar results of increased phylogenetic clustering across the quadrat and site scales, but at the full river scale results between indices differed. Since NTI is based on patterns across the phylogeny tips (Webb 2000, Webb et al. 2002, Kraft et al. 2007), cases in which communities include patterns of clustering within clades and also include one or two evenly distributed distant relatives (e.g., Buffalo River Figure 15), the nearest neighbor approach of NTI is not well suited for identifying such clustering patterns. Using simulations, Kraft et al. (2007) found that NTI was much more powerful for identifying competitive exclusion, whereas NRI was more powerful for detecting habitat filtering in phylogeny pools similar in size to what was used in the present study.

Ecological structure & evolution – By incorporating patterns of habitat use and morphologic trait evolution and similarity in communities I was able to better untangle the relationships between phylogenetic structure patterns and ecological processes (Graham et al. 2012, Barnagaud et al. 2014). Habitat use clustering was apparent in darter communities at both fine and intermediate scales, and almost all habitat traits in all rivers showed low variance among species at both scales (i.e., darters with similar habitat affinities occurred together). It should be noted that because habitat use data for each species was collected at the quadrat scale, I predicted *a priori* that co-occurring species within quadrats should share similar habitat use based on no-independence. However, I included analyses of habitat use structure at the quadrat scale to act as a baseline for comparisons with habitat use structure in sites. Interestingly, I recovered patterns of habitat use clustering in larger scale communities similar to that found in quadrats. Overall, variation across all habitat and morphologic traits among co-occurring species did tend to increase in sites as compared with quadrats, as shown by fingerprint regressions. This is interesting, because it suggests that habitat use and morphologic trait relations among species become more extreme (i.e., greater similarity and or greater dissimilarity) in larger communities. The greater variation among species at sites as compared to quadrats, may be due to the assumed increase in habitat heterogeneity within larger areas (Cavender-Bares et al. 2006, Cavender-Bares et al. 2009). Communities with greater habitat heterogeneity should accommodate a greater range of species traits and characteristics, which might explain why several morphologic traits were dissimilar (i.e., evenly distributed) among species in site communities, and those same traits showed no apparent signal for clustering or evenness in quadrats (Figure 18a vs. 18b).

Without an understanding of how ecologically relevant traits have evolved, and are distributed among community members, it is difficult to properly interpret patterns of trait structure. I examined trait evolution in relation to trait distances among co-occurring darter species, which illustrated between species' trait distributions (within and among river communities) in an evolutionary context. This was based on the framework and approaches of Webb et al. (2002) and Cavender-Bares et al. (2004 & 2006). A high percentage of habitat use factors were phylogenetically convergent, and the majority of these factors were more similar among community members than expected at random. Due to the high incidence of co-occurring species that have converged on similar habitat use in communities (especially within the Duck, Harpeth, and Stones), it is likely that habitat filtering of convergent traits has played a large role in the basic assembly of these stream fish communities across all spatial scales examined. Furthermore, this prominence of convergent habitat use (as indicated in the lower left quadrant of Figure 18) may indicate that darter habitat use is rather plastic, or "quickly" evolving, in comparison to darter morphology, which was highly conserved in most cases. However, Buffalo River communities showed phylogenetic conservatism in a high number of habitat use and morphologic traits, and these communities were phylogenetically clustered and showed little convergence in habitat use among species. Darter body shape was highly conserved for PC1 (which described the majority of body shape variation) in the Duck, Buffalo, and Harpeth rivers. Interestingly, PC1 was evenly distributed among co-occurring darters at sites in these three rivers. This result indicates that competitive exclusion for darter body shape is occurring in communities, and this signal becomes more prevalent in the intermediate sized communities (Figure 18). However, stronger signal for clustering among numerous convergent habitat use traits was also found in intermediate communities, suggesting the influence of habitat filtering. This indicates that both habitat filtering and competitive exclusion processes may act simultaneously, although the number of clustered habitat use and morphologic traits are indicative of habitat filtering and suggest that it is a dominant process of darter community assembly across the scales studied.

***Conclusions* –** Darter communities were more greatly influenced by habitat filtering assembly processes as I scaled up from fine to intermediate spatial scales, and in two of the four systems I found further increases in signal for habitat filtering at the broadest scale. While habitat filtering was found to have high relative importance during assembly (i.e., and showed increased importance in larger scale communities), competitive exclusion likely acted as a secondary mechanism, limiting species morphologic similarity following habitat filtering processes. Moreover, it seems that habitat filtering and competitive exclusion can act simultaneously during assembly, although the relative influence of each process may be spatial scale dependent. This study demonstrates the importance of understanding spatial scale and its effects on processes which are responsible for patterns of diversity in freshwater stream systems.

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Appendix A

Table 1

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	***N***
E. aquali	0	1	2	2	0	1	0	0	2	1	0	0	0	3	0	**12**
E. blennius	0	6	0	0	9	6	2	0	0	0	0	0	0	0	0	**23**
E. blennioides	0	3	3	2	1	3	10	11	10	2	7	2	4	3	0	**61**
E. camurum	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	**6**
E. caeruleum	4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	**5**
E. cinereum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	**1**
E. flavum	9	0	0	0	0	0	1	0	0	0	0	0	0	0	0	**10**
E. rufilineatum	4	17	2	4	12	11	22	4	5	2	0	11	4	10	2	**110**
E. simoterum	1	1	5	0	0	0	1	6	6	2	0	3	1	0	1	**27**
E. stigmaeum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	**1**
E. tippecanoe	0	0	0	0	27	4	5	1	0	0	0	7	0	0	0	**44**
E. zonale	0	1	2	29	7	2	2	25	43	14	5	0	6	20	9	**165**
P. evides	0	0	0	0	0	0	0	0	0	0	0	2	13	3	1	**19**
P. caprodes	0	0	0	0	0	0	0	2	5	0	0	0	0	0	1	**8**
# of *Spp.*	**4**	**6**	**5**	**4**	**5**	**6**	**7**	**6**	**8**	**5**	**2**	**6**	**6**	**6**	**5**	

Table 2

Variable	PC1	PC2	PC3
velocity(m/s)	-0.065	**-0.609**	0.123
depth(cm)	-0.150	**-0.644**	0.235
gravel%	0.144	0.159	**0.608**
pebble%	**0.664**	-0.222	-0.234
cobble%	**-0.616**	0.096	-0.387
boulder%	-0.293	-0.304	0.003
bedrock%	-0.214	0.195	**0.596**

Table 3

	E. aquali	*E. blennius*	*E. blennioides*	*E. rufilineatum*	*E. simoterum*	*E. tippecanoe*
E. aquali						
E. blennius	**0.234**					
E. blennioides	-0.103	0.012				
E. rufilineatum	0.117	-0.057	**0.158**			
E. simoterum	0.052	**0.405**	-0.075	**0.249**		
E. tippecanoe	**0.263**	-0.037	0.049	-0.040	**0.391**	
E. zonale	-0.081	-0.006	0.034	**0.055**	0.008	0.016

Table 4

	gravel %	**pebble %**	**cobble %**	**boulder %**	**bedrock %**	**velocity (m/s)**	**depth (cm)**
E. aquali	1.81 (6.03)	60 (32.25)	34.55 (29.79)	0	3.64 (12.06)	0.61 (0.19)	30.39 (12.44)
E. blennius	4.44 (8.55)	92.22 (10.03)	3.33 (7.67)	0	0	0.72 (0.24)	30.52 (14.02)
E. blennioides	9.05 (17.22)	54.76 (33.66)	29.52 (33.13)	0	6.67 (19.08)	0.49 (0.21)	26.52 (12.54)
E. camurum	8 (17.89)	56 (38.47)	36 (38.47)	0	0	0.57 (0.23)	20.6 (7.24)
E. caeruleum	0	100	0	0	0	0.36 (0.22)	25.67 (7.07)
E. cinereum	0	80	20	0	0	0.28	40.33
E. flavum	0	90 (20)	10 (20)	0	0	0.37 (0.28)	24.92 (12.35)
E. rufilineatum	3.71 (7.83)	79.71 (22.46)	15.71 (23.32)	0.57 (3.36)	0.29 (2.39)	0.63 (0.26)	24.88 (12.85)
E. simoterum	5 (8.94)	57.5 (32.56)	35 (32.25)	0	3.75 (10.88)	0.33 (0.16)	28.73 (12.77)
E. stigmaeum	20	20	20	0	40	0.49	29.33
E. tippecanoe	7.2 (11.37)	90.4 (11.72)	2.4 (6.63)	0	0	0.68 (0.26)	29.08 (12.89)
E. zonale	4.21 (10.99)	56.84 (30.99)	33.95 (30.64)	0.53 (3.22)	4.21 (14.72)	0.59 (0.27)	26.58 (11.09)
P. caprodes	10 (11.55)	55 (30)	35 (30)	0	0	0.35 (0.21)	40.75 (11.50)
P. evides	18.18 (28.92)	72.73 (30.03)	9.09 (20.71)	0	0	0.66 (0.13)	29.33 (6.37)

Table 5

	K	**Trait evolution**
Habitat Use		
Gravel	0.851	labile
Pebble	0.646	labile
Cobble	0.591	labile
Boulder	0.584	labile
Bedrock	0.875	labile
Velocity	0.785	labile
Depth	0.839	labile
Morphology		
PC1	1.498	conserved
PC2	0.871	labile
PC3	0.494	labile

Table 6

Index Medians

NRI	**quadrat**	**site**	**U**	**P - value**
Duck	0.265	0.683	574	0.5
Buffalo	0.652	1.973	137	0.003*****
Harpeth	0.251	0.933	90	0.506
Stones	0.234	0.617	77	0.351
NTI				
Duck	0.545	1.453	383	0.012*****
Buffalo	0.917	1.062	278	0.364
Harpeth	0.490	1.112	86	0.415
Stones	-0.134	-0.212	102	0.95

Table 7

	Duck	**Buffalo**	**Harpeth**	**Stones**
Habitat use				
gravel	0.849	**1.322**	**1.025**	0.786
pebble	0.645	0.782	0.597	0.737
cobble	0.586	0.948	0.784	**1.690**
boulder	0.574	**1.021**	0.563	0.666
bedrock	0.880	**1.018**	0.618	0.746
velocity	0.782	**1.042**	0.930	0.954
depth	0.824	**1.406**	0.686	**1.037**
Morphology				
PC1	**1.494**	**1.765**	**1.344**	0.935
PC2	0.862	0.698	**1.144**	**1.347**
PC3	0.485	0.698	0.708	0.676

Appendix B

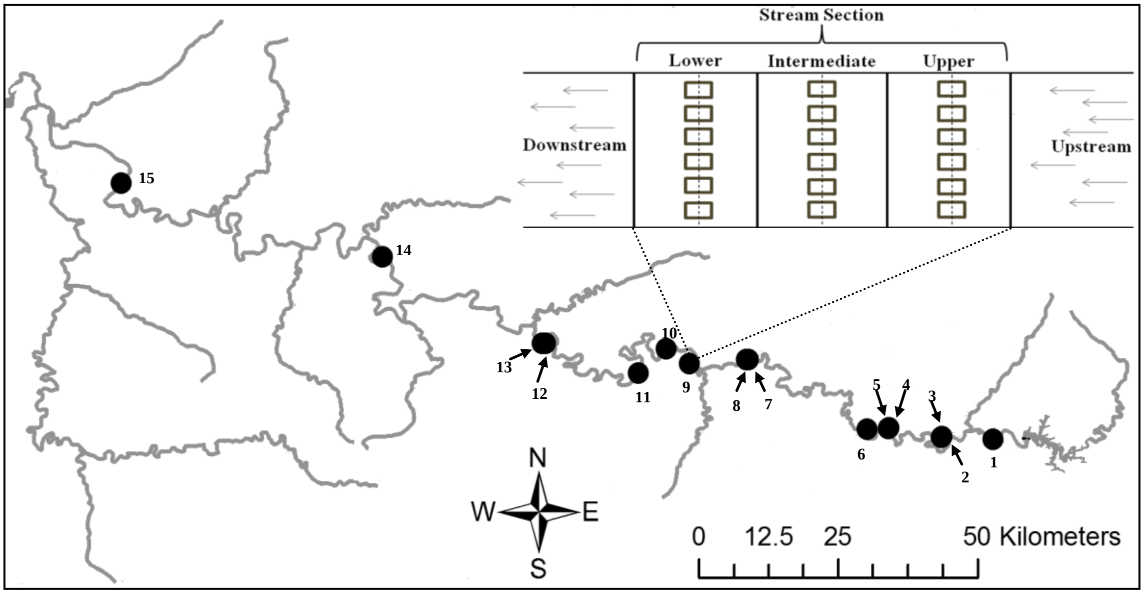


Figure 1

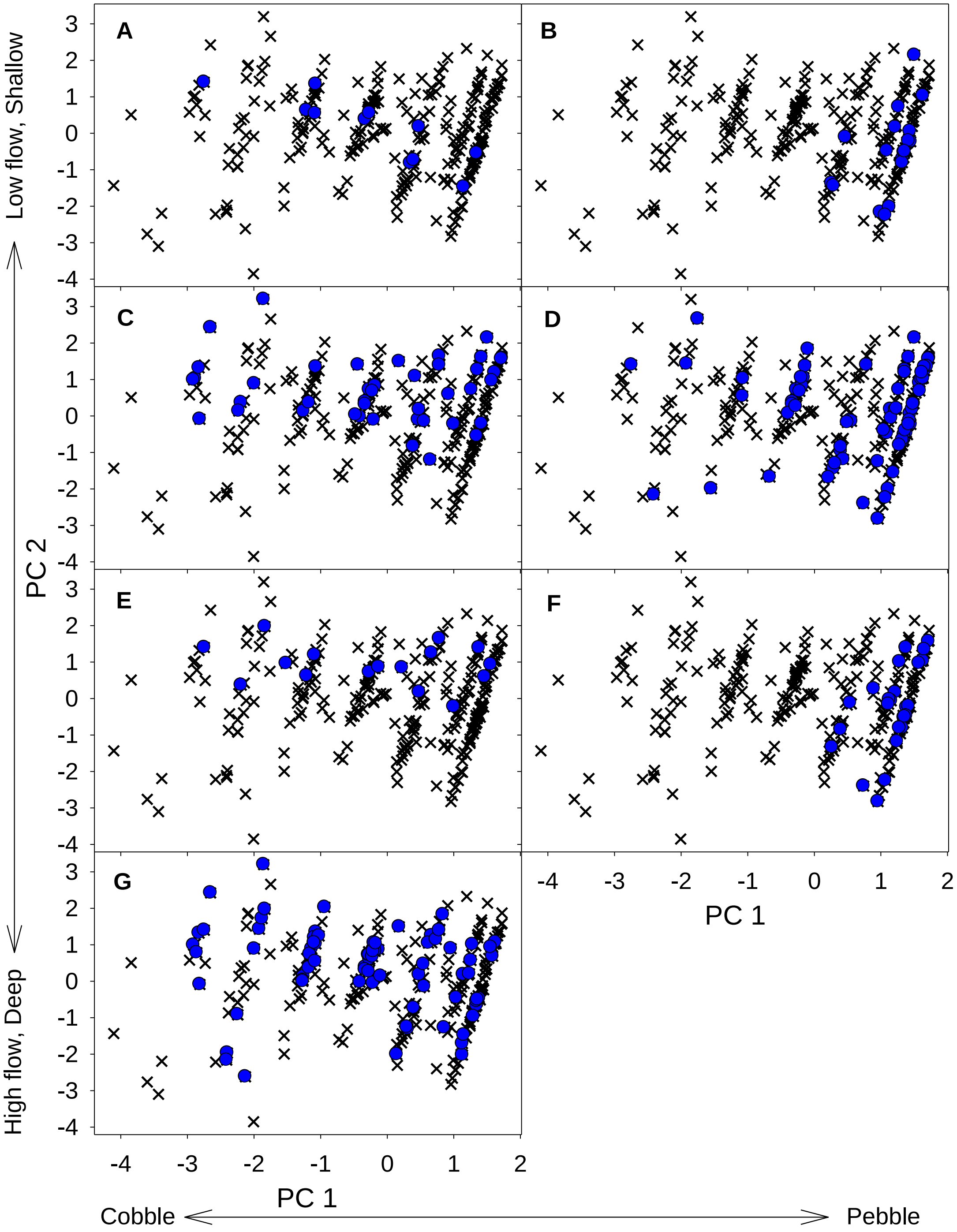

A
B
C
D
E
F
G
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
PC 2
PC 1
PC 1
Low flow, Shallow
High flow, Deep
Cobble
Pebble

Figure 2

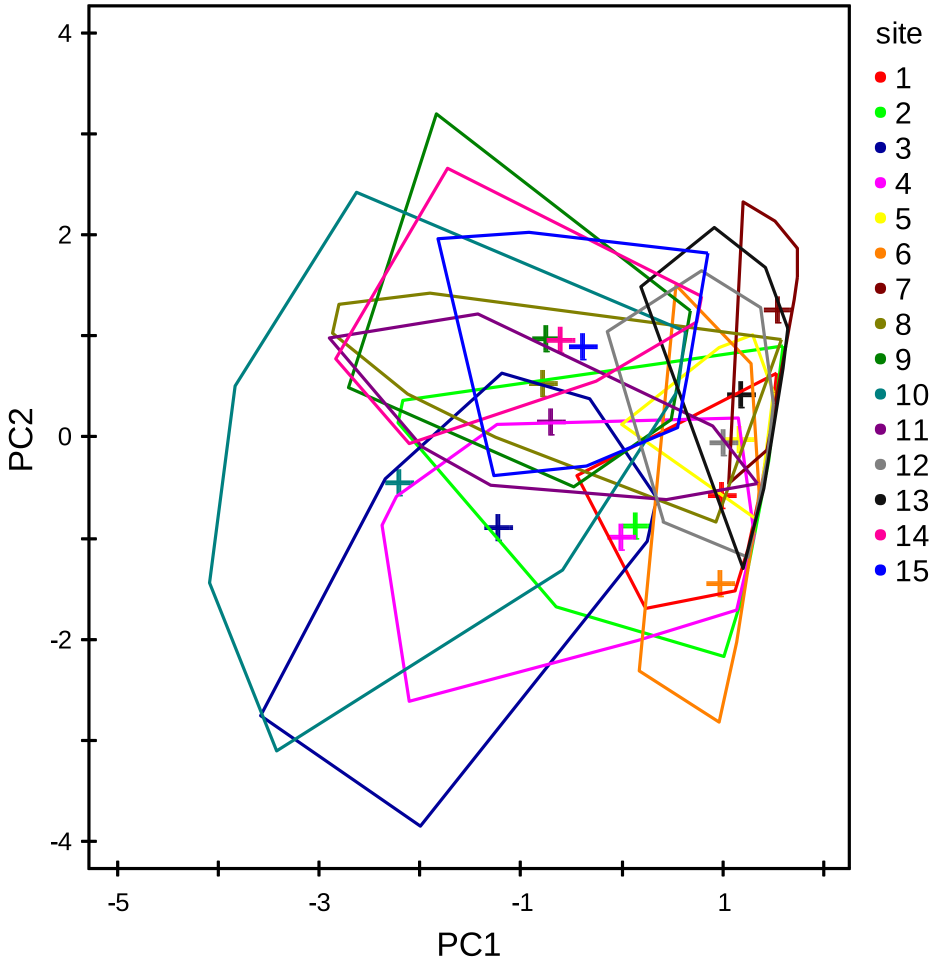


Figure 3

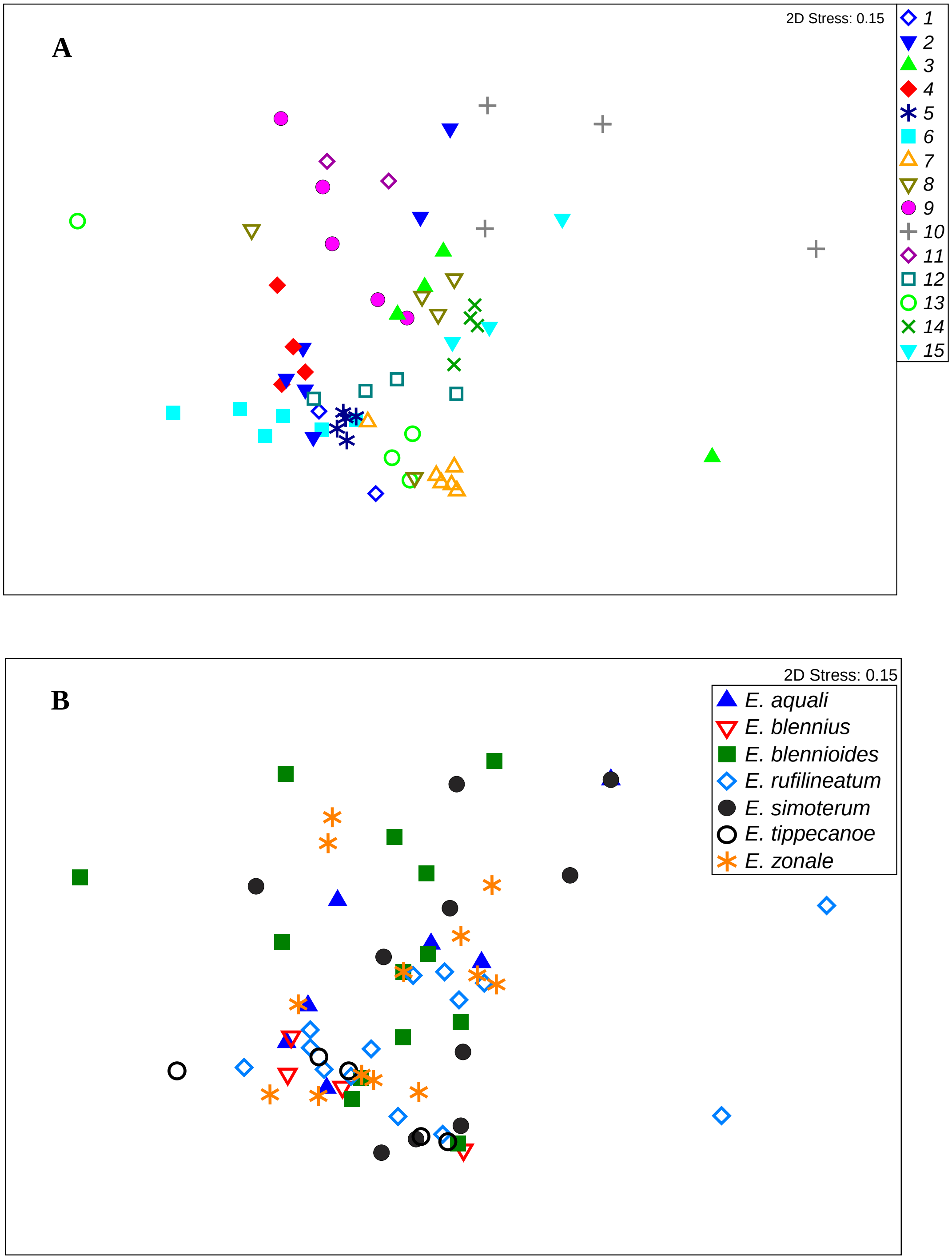

A
2D Stress: 0.15
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
B
2D Stress: 0.15
E. aquali
E. blennius
E. blennioides
E. rufilineatum
E. simoterum
E. tippecanoe
E. zonale

Figure 4

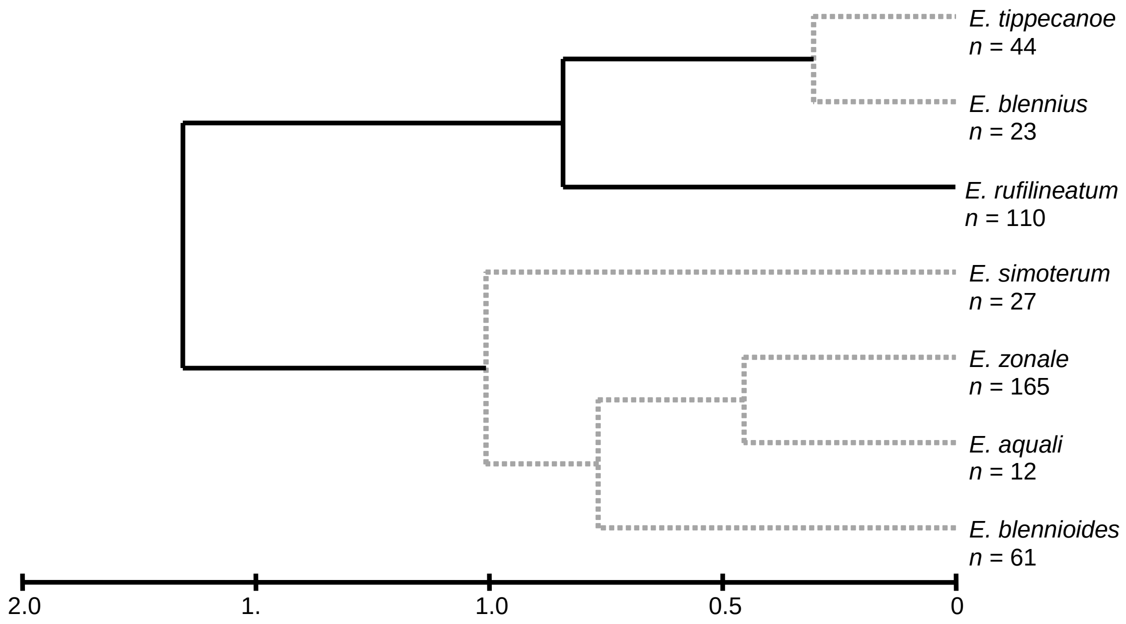

E. tippecanoe
n = 44
E. blennius
n = 23
E. rufilineatum
n = 110
E. simoterum
n = 27
E. zonale
n = 165
E. aquali
n = 12
E. blennioides
n = 61
2.0
1.
1.0
0.5
0

Figure 5

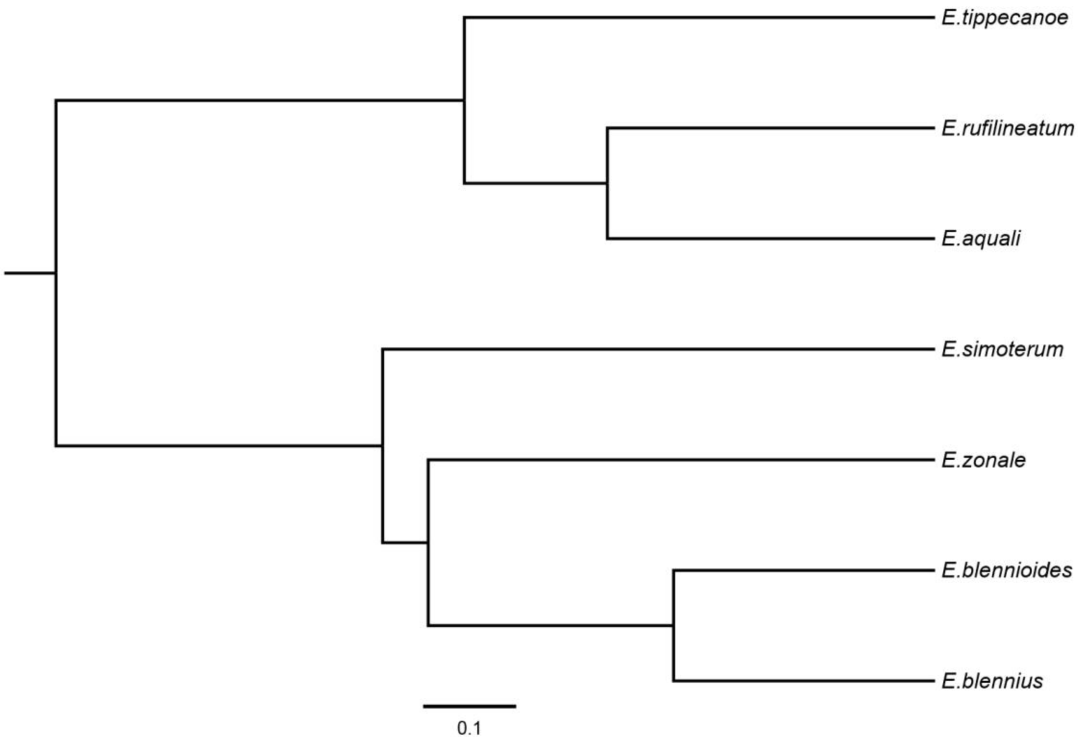

E.tippecanoe
E.rufilineatum
E.aquali
E.simoterum
E.zonale
E.blennioides
E.blennius
0.1

Figure 6

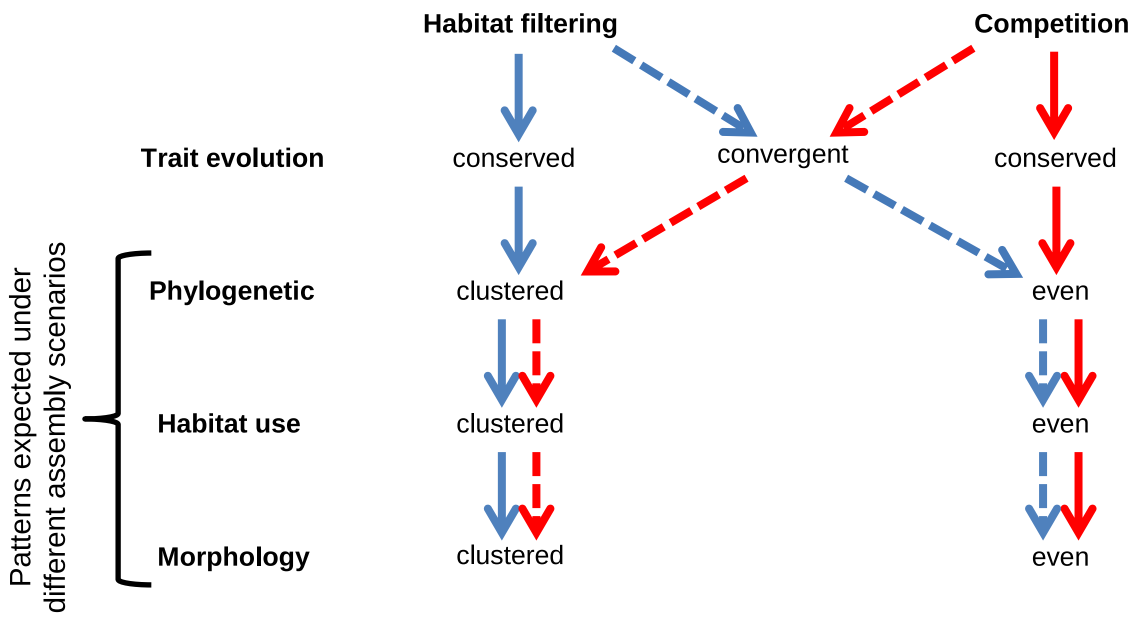

Habitat filtering
Competition
Trait evolution
conserved
convergent
conserved
Patterns expected under different assembly scenarios
Phylogenetic
clustered
even
Habitat use
clustered
even
Morphology
clustered
even

Figure 7

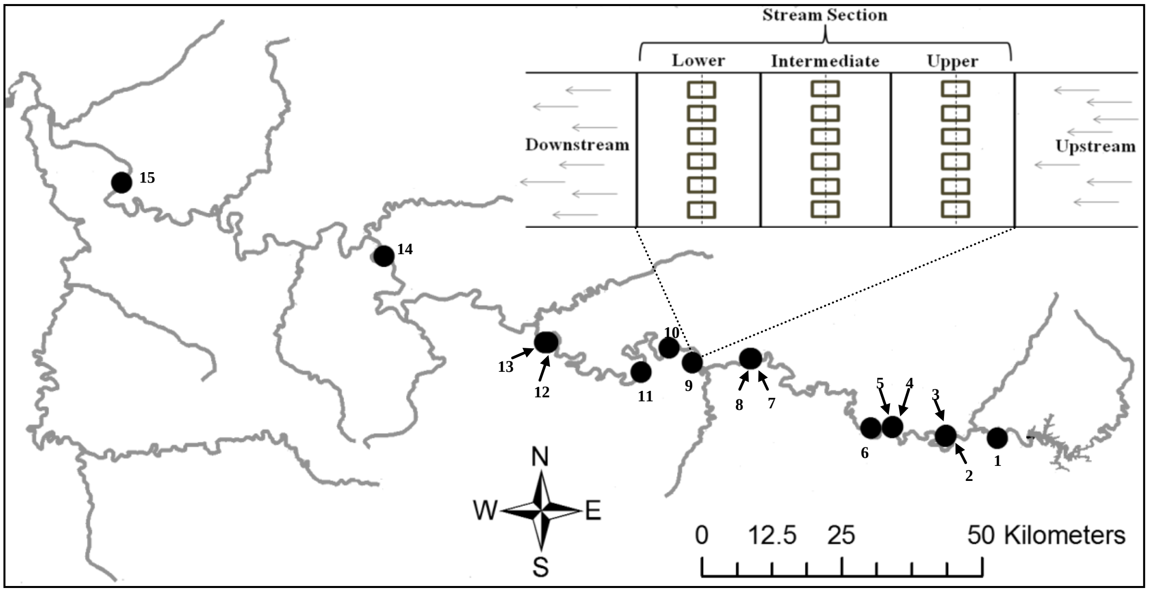
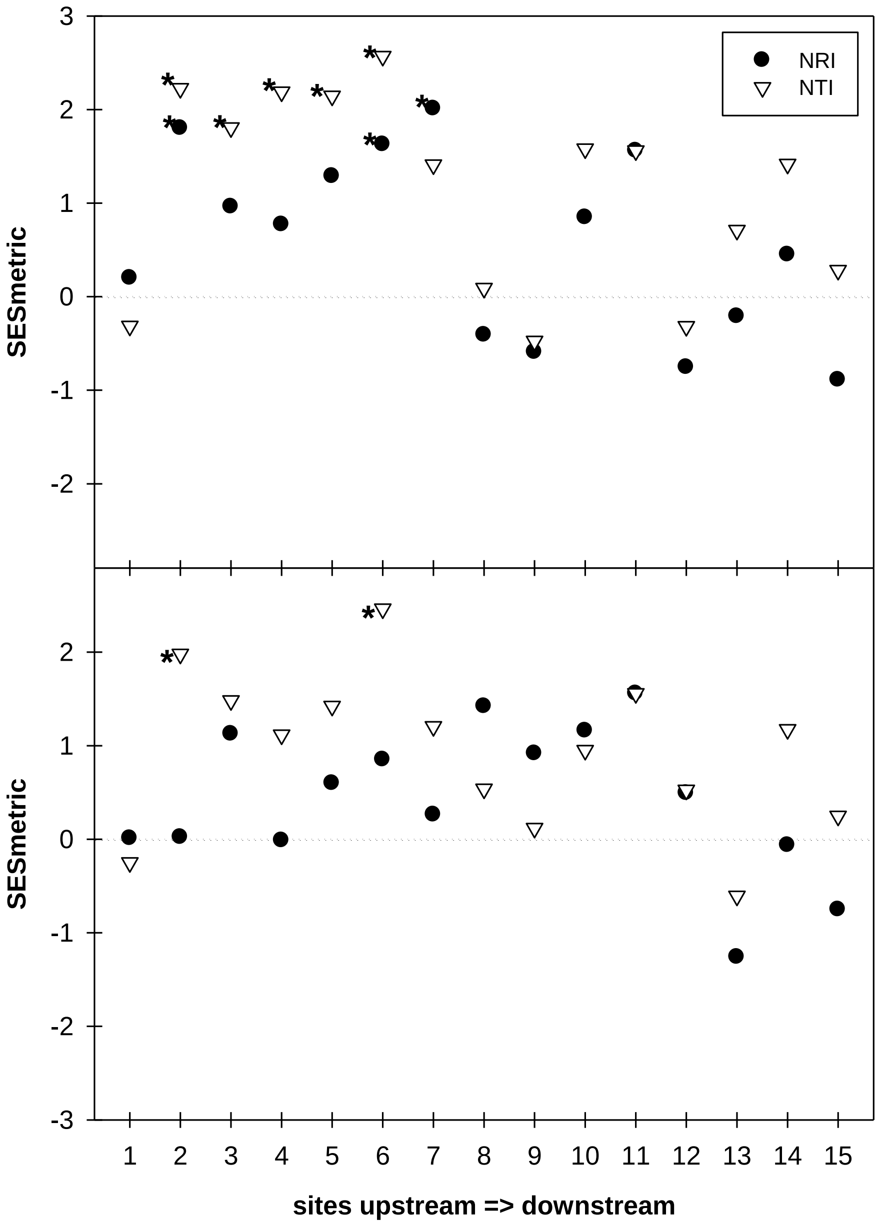

Stream Section
Lower
Intermediate
Upper
Downstream
Upstream
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
N
W
E
S
0
12.5
25
50 Kilometers

Figure 8



3
2
1
0
-1
-2
-3
SESmetric
SESmetric
NRI
NTI
*
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
sites upstream => downstream

Figure 9

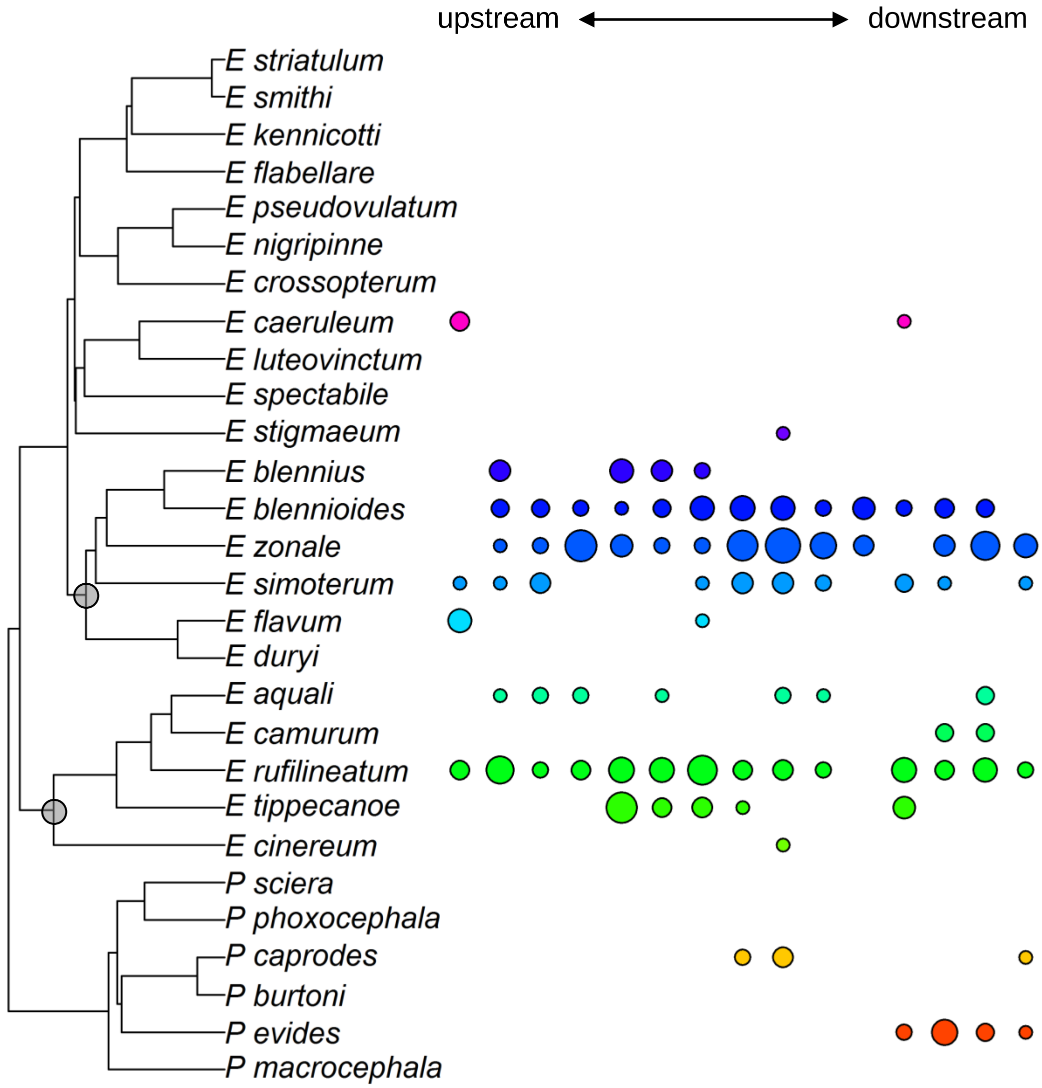

upstream
downstream
E striatulum
E smithi
E kennicotti
E flabellare
E pseudovulatum
E nigripinne
E crossopterum
E caeruleum
E luteovinctum
E spectabile
E stigmaeum
E blennius
E blennioides
E zonale
E simoterum
E flavum
E duryi
E aquali
E camurum
E rufilineatum
E tippecanoe
E cinereum
P sciera
P phoxocephala
P caprodes
P burtoni
P evides
P macrocephala

Figure 10

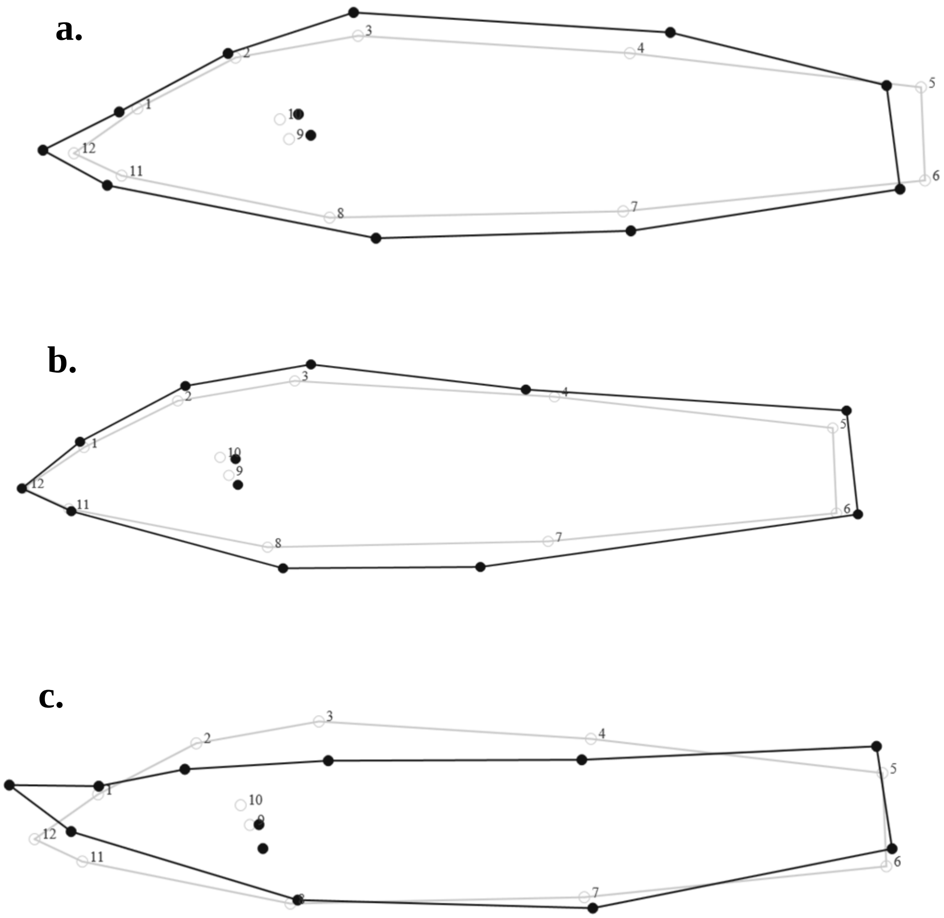


Figure 11

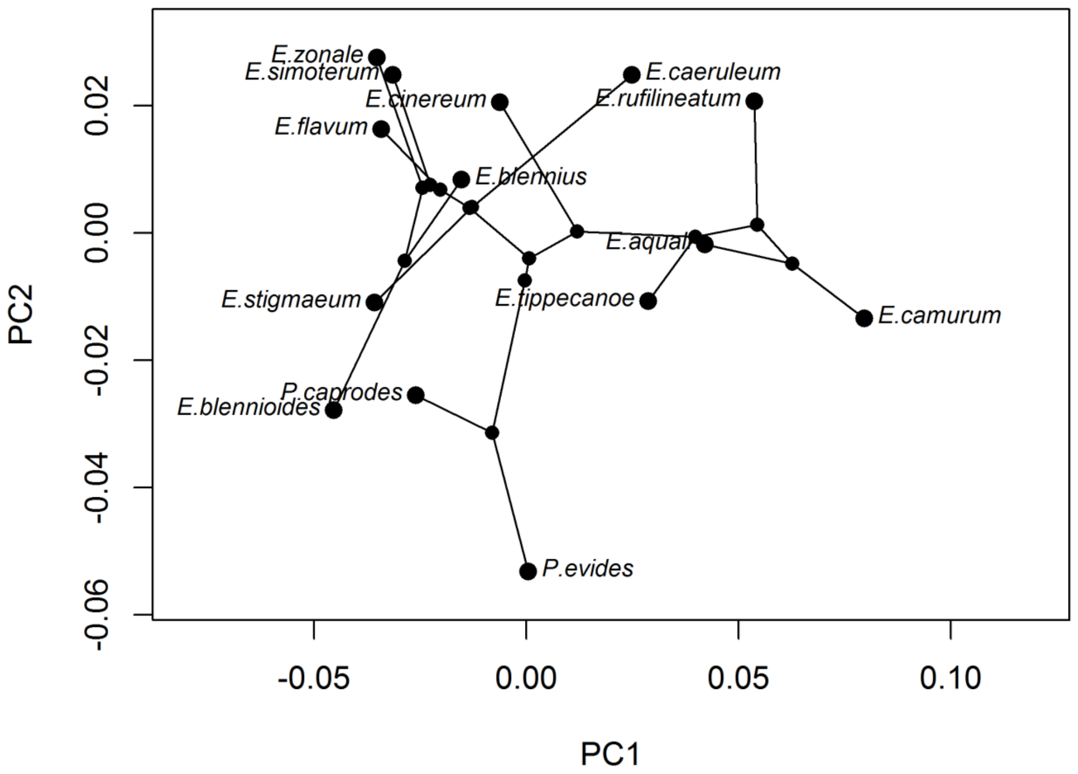

E.zonale
E.simoterum
E.cinereum
E.caeruleum
E.rufilineatum
E.flavum
E.blennius
E.aquali
E.stigmaeum
E.tippecanoe
E.camurum
P.caprodes
E.blennioides
P.evides
PC2
0.02
0.00
-0.02
-0.04
-0.06
-0.05
0.00
0.05
0.10
PC1

Figure 12

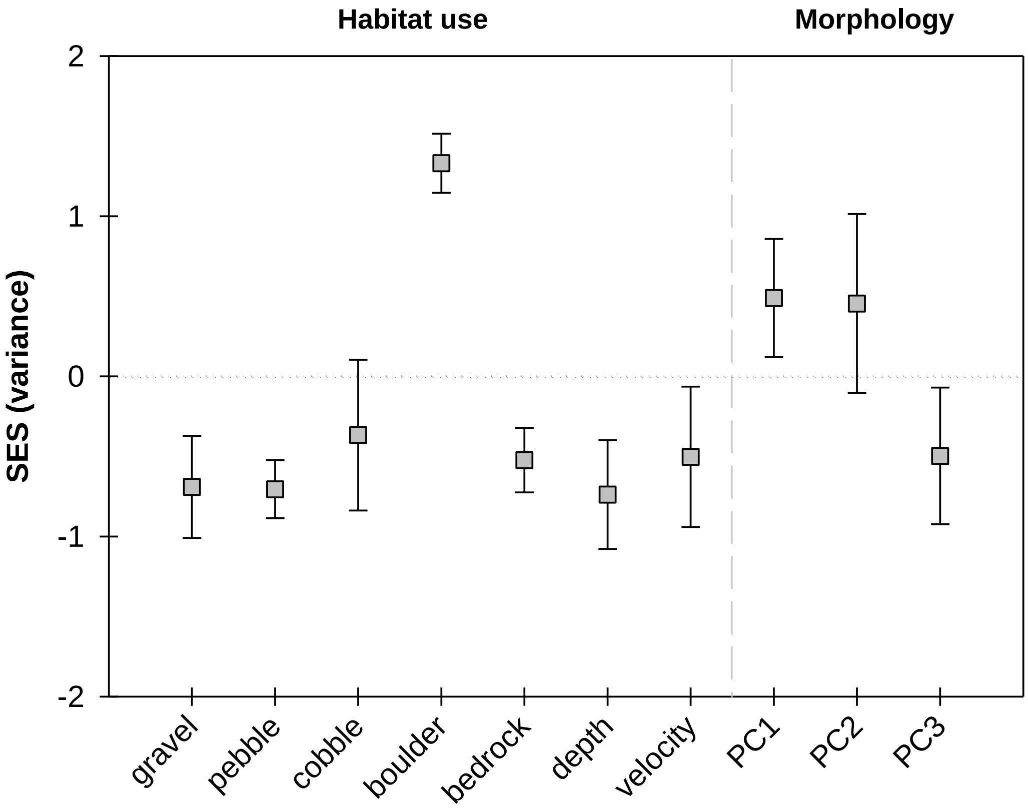

Habitat use
Morphology
SES (variance)
2
1
0
-1
-2
gravel
pebble
cobble
boulder
bedrock
depth
velocity
PC1
PC2
PC3

Figure 13

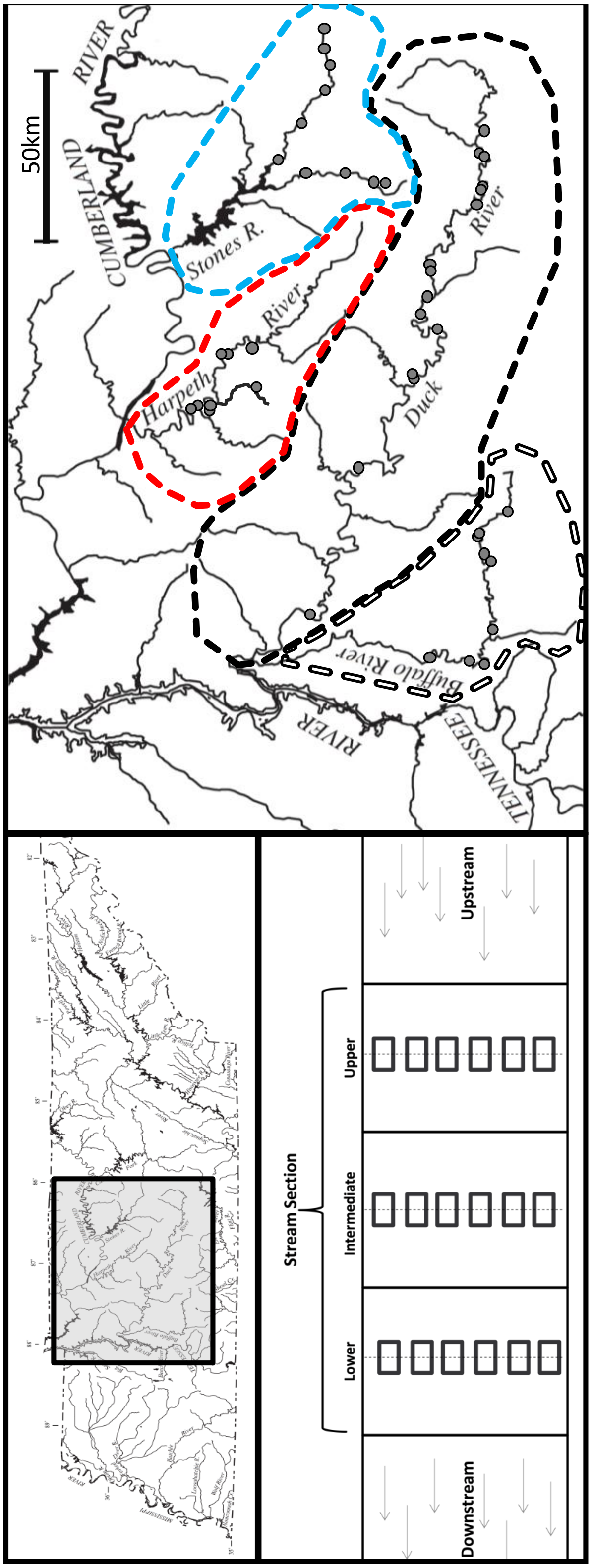

50km
CUMBERLAND
RIVER
Stones R.
Harpeth
River
Duck
River
Buffalo River
TENNESSEE
RIVER
Stream Section
Upstream
Upper
Intermediate
Lower
Downstream

Figure 14

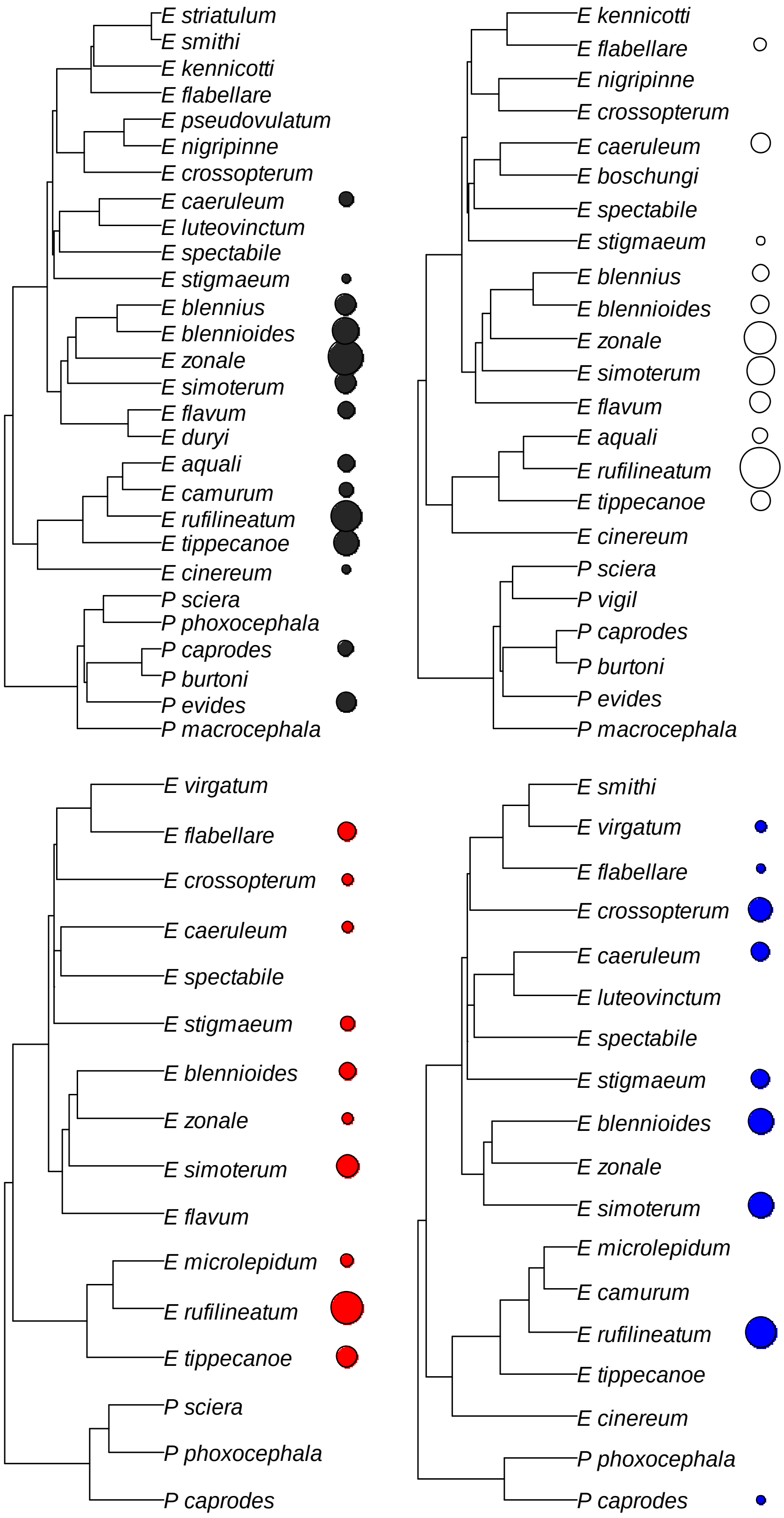

E striatulum
E smithi
E kennicotti
E flabellare
E pseudovulatum
E nigripinne
E crossopterum
E caeruleum
E luteovinctum
E spectabile
E stigmaeum
E blennius
E blennioides
E zonale
E simoterum
E flavum
E duryi
E aquali
E camurum
E rufilineatum
E tippecanoe
E cinereum
P sciera
P phoxocephala
P caprodes
P burtoni
P evides
P macrocephala
E kennicotti
E flabellare
E nigripinne
E crossopterum
E caeruleum
E boschungi
E spectabile
E stigmaeum
E blennius
E blennioides
E zonale
E simoterum
E flavum
E aquali
E rufilineatum
E tippecanoe
E cinereum
P sciera
P vigil
P caprodes
P burtoni
P evides
P macrocephala
E virgatum
E flabellare
E crossopterum
E caeruleum
E spectabile
E stigmaeum
E blennioides
E zonale
E simoterum
E flavum
E microlepidum
E rufilineatum
E tippecanoe
P sciera
P phoxocephala
P caprodes
E smithi
E virgatum
E flabellare
E crossopterum
E caeruleum
E luteovinctum
E spectabile
E stigmaeum
E blennioides
E zonale
E simoterum
E microlepidum
E camurum
E rufilineatum
E tippecanoe
E cinereum
P phoxocephala
P caprodes

Figure 15

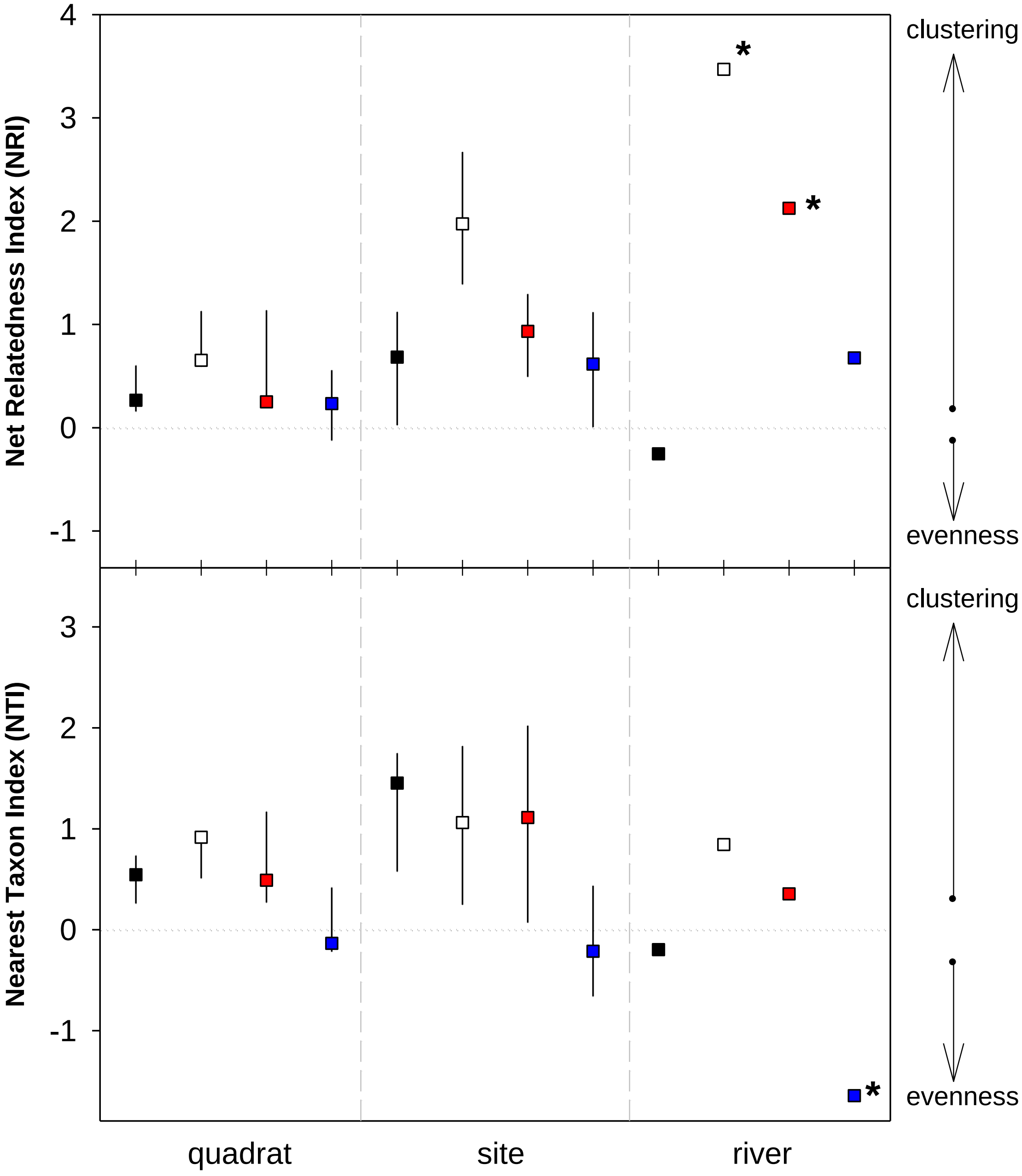

4
3
2
1
0
-1
Net Relatedness Index (NRI)
clustering
evenness
*
*
3
2
1
0
-1
Nearest Taxon Index (NTI)
clustering
evenness
*
quadrat
site
river

Figure 16

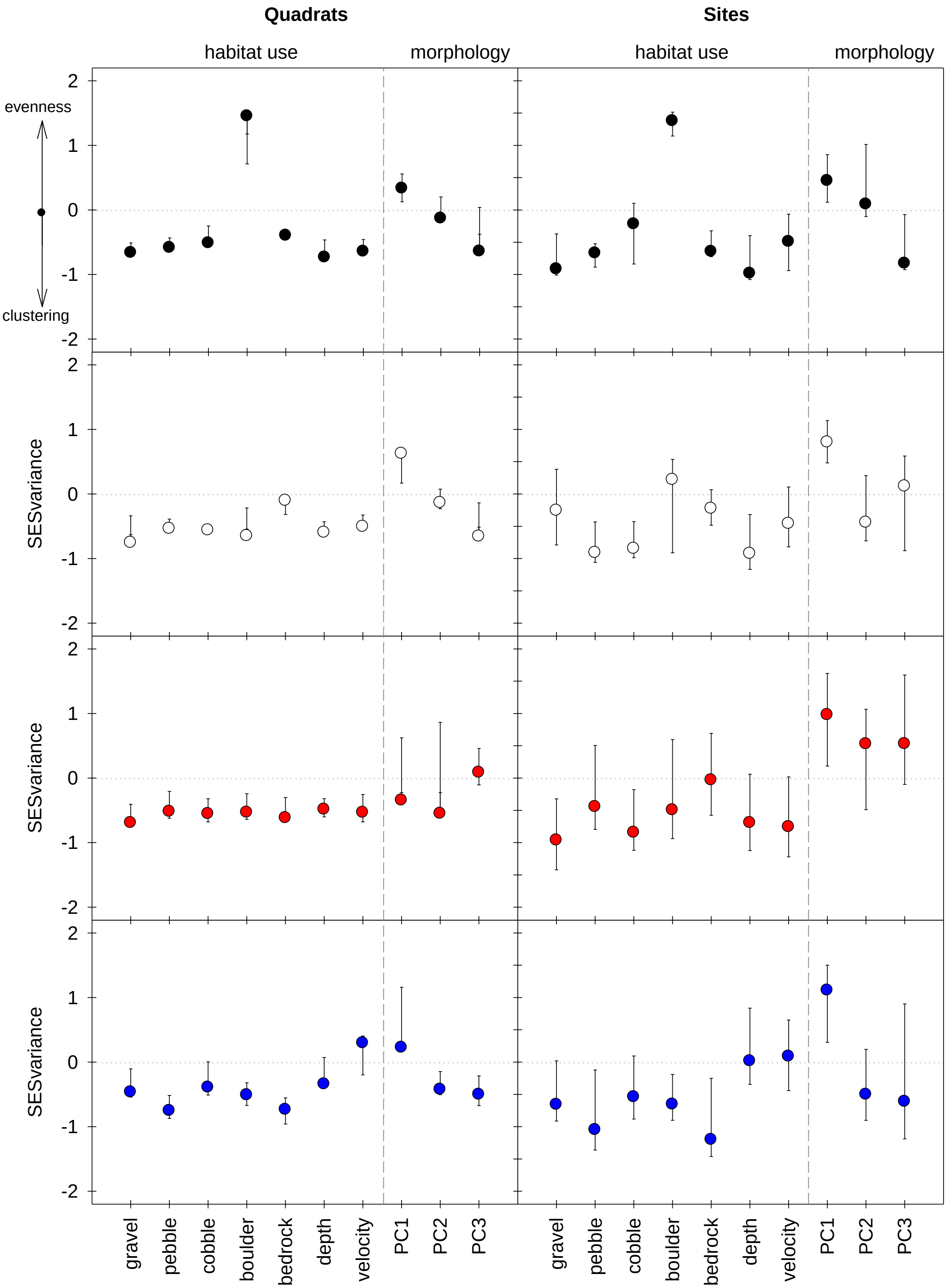

Quadrats
Sites
habitat use
morphology
habitat use
morphology
evenness
clustering
SESvariance
SESvariance
SESvariance
2
1
0
-1
-2
gravel
pebble
cobble
boulder
bedrock
depth
velocity
PC1
PC2
PC3

Figure 17

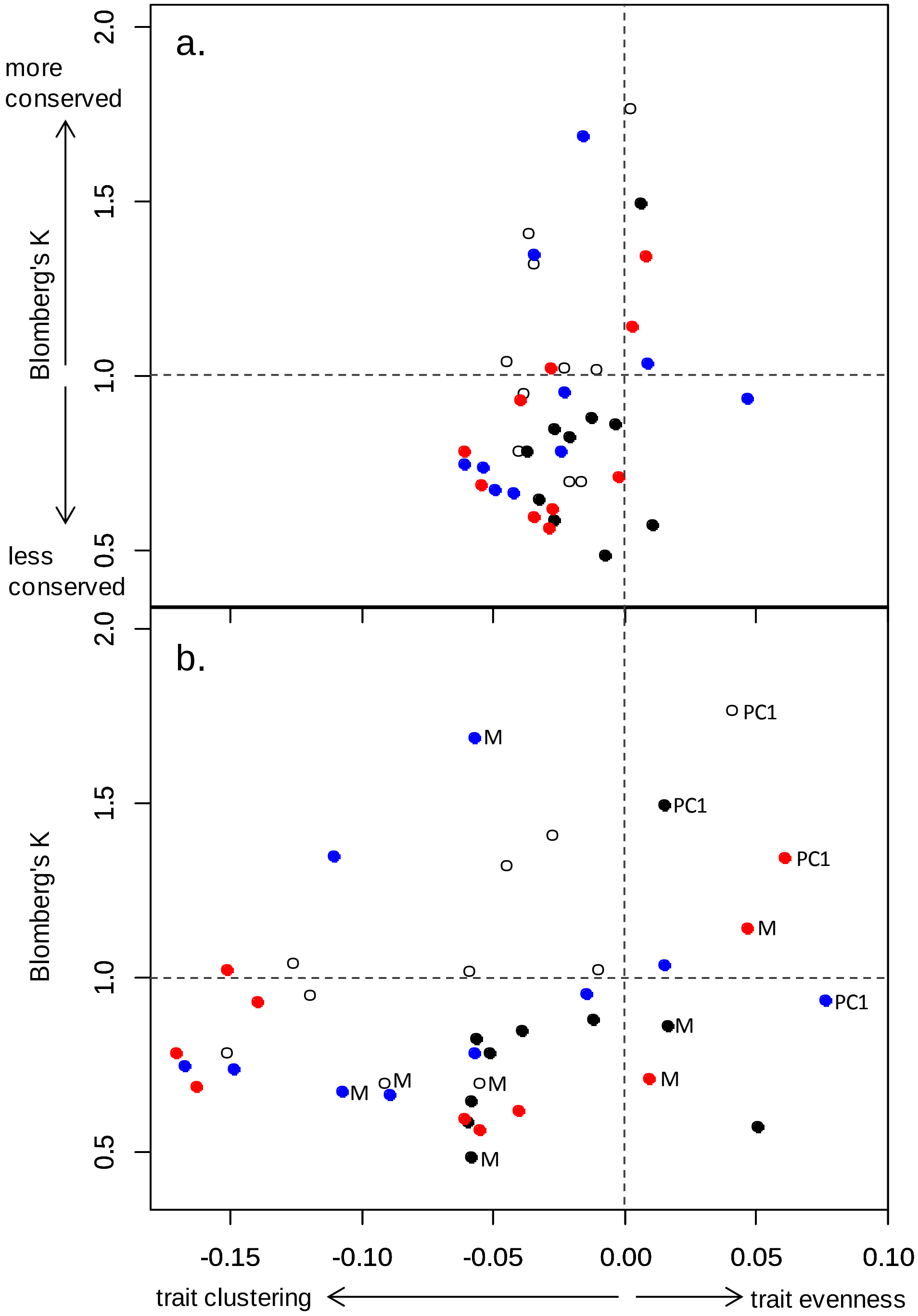

a.
b.
more conserved
less conserved
Blomberg's K
Blomberg's K
2.0
1.5
1.0
0.5
-0.15
-0.10
-0.05
0.00
0.05
0.10
trait clustering
trait evenness
PC1
PC1
PC1
PC1
M
M
M
M
M
M
M
M
M

Figure 18

Appendix C

	S7 Accession	**RAG1 accession**	**ND2 accession**	**CytB accession**	**CO1 accession**
Etheostoma aquali	EU094735	GU015638	JQ088507	AY374258	JN025481
Etheostoma blennioides	EU118897	HQ127777	JQ088514	AF288426	HQ579050
Etheostoma blennius	EU296723	HQ127778	JQ088515	AF288427	JN025639
Etheostoma caeruleum	EU046651	FJ381316	FJ381265	DQ465072	EU524020
Etheostoma camurum	EU094743	GU015642	EU814327	EU094665	JN025721
Etheostoma cinereum	HQ128332	HQ127795	EF027174	AF045349	JN025755
Etheostoma crossopterum	HQ128344	HQ127809	JQ088531	AF123031	JN025779
Etheostoma duryi	HQ128348	HQ127814	JQ088536	AF288432	JN025806
Etheostoma flabellare	AF412557	HQ127825	AF412540	AF045342	EU524031
Etheostoma flavum	HQ128359	HQ127826	JQ088541	HQ128132	JN025855
Etheostoma kennicotti	AF412558	HQ127851	AF412541	AF045341	JN025934
Etheostoma luteovinctum	FJ381353	FJ381313	EF027206	FJ381010	JN025973
Etheostoma nigripinne	HQ128414	HQ127884	JQ088560	AF123034	JN026032
Etheostoma pseudovulatum	HQ128435	HQ127913	JQ088572	AF123039	HQ579058
Etheostoma rufilineatum	EU094792	GU015730	JQ088578	AF274447	JN026293
Etheostoma simoterum	HQ128464	HQ127942	EF027222	AF288445	HQ579106
Etheostoma smithi	AF412562	HQ127946	AF412545	AF123040	HQ557429
Etheostoma spectabile	EU046628	FJ381333	FJ381287	AF045344	HQ557386
Etheostoma stigmaeum	HQ128472	JF742869	JQ088587	HQ128232	HQ579021
Etheostoma striatulum	AF412564	HQ127956	AF412547	AF123042	HQ557431
Etheostoma tippecanoe	EU094808	GU015838	EU814368	AF274448	JN026469
Etheostoma zonale	HQ128498	HQ127980	EF027233	AF288449	HQ579051
Percina burtoni	EU379110	HQ128003	AY770848	AY770840	JN027904
Percina caprodes	EU379112	HQ128005	AY770849	AF045354	EU524246
Percina evides	HQ128516	HQ128017	JQ088622	AF375938	JN027984
Percina macrocephala	HQ128519	HQ128022	DQ493546	AF386591	JN028019
Percina phoxocephala	HQ128536	HQ128045	AY770859	AF386563	HQ579002
Percina sciera	HQ128537	HQ128047	AY770862	AF386573	HQ557417

Appendix D

	S7 Accession	**RAG1 accession**	**ND2 accession**	**CytB accession**	**CO1 accession**
Etheostoma aquali	EU094735	GU015638	JQ088507	AY374258	JN025481
Etheostoma blennioides	EU118897	HQ127777	JQ088514	AF288426	HQ579050
Etheostoma blennius	EU296723	HQ127778	JQ088515	AF288427	JN025639
Etheostoma boschungi	EF035505	HQ127779	EF027185	HQ128095	JN025644
Etheostoma caeruleum	EU046651	FJ381316	FJ381265	DQ465072	EU524020
Etheostoma camurum	EU094743	GU015642	EU814327	EU094665	JN025721
Etheostoma cinereum	HQ128332	HQ127795	EF027174	AF045349	JN025755
Etheostoma crossopterum	HQ128344	HQ127809	JQ088531	AF123031	JN025779
Etheostoma duryi	HQ128348	HQ127814	JQ088536	AF288432	JN025806
Etheostoma flabellare	AF412557	HQ127825	AF412540	AF045342	EU524031
Etheostoma flavum	HQ128359	HQ127826	JQ088541	HQ128132	JN025855
Etheostoma kennicotti	AF412558	HQ127851	AF412541	AF045341	JN025934
Etheostoma luteovinctum	FJ381353	FJ381313	EF027206	FJ381010	JN025973
Etheostoma microlepidum	EU094782	HQ005692	JQ088556	AY742664	JN026008
Etheostoma nigripinne	HQ128414	HQ127884	JQ088560	AF123034	JN026032
Etheostoma pseudovulatum	HQ128435	HQ127913	JQ088572	AF123039	HQ579058
Etheostoma rufilineatum	EU094792	GU015730	JQ088578	AF274447	JN026293
Etheostoma simoterum	HQ128464	HQ127942	EF027222	AF288445	HQ579106
Etheostoma smithi	AF412562	HQ127946	AF412545	AF123040	HQ557429
Etheostoma spectabile	EU046628	FJ381333	FJ381287	AF045344	HQ557386
Etheostoma stigmaeum	HQ128472	JF742869	JQ088587	HQ128232	HQ579021
Etheostoma striatulum	AF412564	HQ127956	AF412547	AF123042	HQ557431
Etheostoma tippecanoe	EU094808	GU015838	EU814368	AF274448	JN026469
Etheostoma virgatum	AF412565	HQ127977	AF412548	AF123043	HQ579057
Etheostoma zonale	HQ128498	HQ127980	EF027233	AF288449	HQ579051
Percina burtoni	EU379110	HQ128003	AY770848	AY770840	JN027904
Percina caprodes	EU379112	HQ128005	AY770849	AF045354	EU524246
Percina evides	HQ128516	HQ128017	JQ088622	AF375938	JN027984
Percina macrocephala	HQ128519	HQ128022	DQ493546	AF386591	JN028019
Percina phoxocephala	HQ128536	HQ128045	AY770859	AF386563	HQ579002
Percina sciera	HQ128537	HQ128047	AY770862	AF386573	HQ557417
Percina shumardi	HQ128540	HQ128050	JQ088635	AF386571	JN028135
Percina vigil	HQ128551	HQ128061	AY770861	AF386569	JN028166